

# INDIAN COUNCIL OF MEDICAL RESEARCH



## CONSENSUS DOCUMENT FOR MANAGEMENT OF ACUTE MYELOID LEUKEMIA (AML)

*Prepared as an outcome of  
ICMR Subcommittee on Acute Myeloid Leukemia (AML)*



Division of Non Communicable Diseases  
Indian Council of Medical Research  
2019



# CONSENSUS DOCUMENT FOR MANAGEMENT OF ACUTE MYELOID LEUKEMIA (AML)

*Prepared as an outcome of  
ICMR Subcommittee on Acute Myeloid Leukemia (AML)*



Coordinated by  
Division of Non Communicable Diseases

Indian Council of Medical Research  
Ansari Nagar, New Delhi – 110029  
2019

### **Disclaimer**

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

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

Published in 2019

Head (Publication & Information) : Dr. Neeraj Tandon

Compiled & Edited by: Dr. Tanvir Kaur, Dr. Lalit Kumar

Production Controller : Mr. JN Mathur

Published by the Division of Publication and Information on behalf of the Secretary DHR & DG, ICMR, New Delhi.

## Foreword

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



Acute Myeloid Leukemia (AML) requires specialized multi-disciplinary care and treatment for better outcome. This document consolidates the modalities of treatment including the diagnosis, risk stratification and treatment. Hope that it would provide guidance to practicing doctors and researchers for the management of patients suffering from Acute Myeloid Leukemia (AML) and also focusing their research efforts in Indian context.

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

*Balram Bhargava*

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

## Message

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

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



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

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

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

## Preface

Acute myeloid leukaemia (AML) is most common leukaemia among adults. The morphology, cytogenetics and molecular abnormalities of leukemic cells (blasts) are currently used to classify AML into various risk categories. A better understanding of biology and effective therapeutic advances has led to the subset specific therapies with improved outcome. Acute promyelocytic leukaemia (APML, AML-M3) is the best example where treatment with all-trans retinoic acid (ATRA) in combination with arsenic trioxide (ATO) or Daunomycin results in long-term survival in the range of 85 to 90%. Apart from APML, concept of personalised therapy is now possible for treatment of FLT-3-positive AML with the tyrosine kinase inhibitors sorafenib or midostaurin. Hematopoietic stem cell transplantation is an option in the younger patients who are at higher risk of relapse and improves the survival further. Availability of hypomethylating agents (e.g. Azacitidine, Decitabine) have led to better management of AML in elderly patients and those who are unsuitable for chemotherapy.



A standard treatment approach is the key for appropriate management. While management of this disease is based on protocol based at most major tertiary cancer centres, it is not same in others. To develop uniformity across all centres, ICMR has taken initiative to develop standard guidelines. Consensus document for AML presented here focuses on management and provides recommendations on the work up, diagnostic evaluation and treatment options for younger (age <60 years) and older (age  $\geq$  60 years) adult patients. We hope this will be useful to busy residents and physicians involved in the care of such patients. These are broad guidelines and are not an attempt to overrule clinical judgment in an individual clinical condition.



(Lalit Kumar)  
Chairperson  
Subcommittee on Acute Myeloid, Lucknow

## Preface

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



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

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



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

## Acknowledgement

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



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

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

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

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

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

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

(Dr. Tanvir Kaur)  
Programme Officer & Coordinator

---

## Members of the Sub-Committee

---

### ***Chairperson***

**Dr. Lalit Kumar**

Prof. of Medical Oncology Institute of Rotary Cancer Hospital  
AIIMS, New Delhi

---

## Members

---

- |  |   |
|--|---|
| 1) Dr Prasanth Ganeshan<br>Addl. Professor, Department of Medical<br>Oncology JIPMER, Puducherry                           | 7) Dr Sumit Gujral<br>Department of Haematopathology,<br>Tata Memorial Hospital, Parel, Mumbai                        |
| 2) Dr Vikram Mathew<br>Professor of Haematology<br>Christian Medical College, Vellore, (TN)                                | 8) Dr Mammen Chandy<br>Director, Tata Medical Centre, Kolkata   |
| 3) Dr Sameer Bakhshi<br>Department of Medical Oncology<br>IRCH-AIIMS, New Delhi  | 9) Dr Smita Kayal<br>Associate Professor of Medical Oncology,<br>JIPMER, Puducherry                                   |
| 4) Dr Brijesh Aurora<br>(Formerly) Professor, Department of<br>Paediatric Oncology Tata Memorial<br>Hospital Parel, Mumbai | 10) Dr Navin Khattry<br>Professor of Medical Oncology and Incharge,<br>BMT, Tata Memorial Hospital, ACTREC,<br>Mumbai |
| 5) Dr Pankaj Malhotra<br>Professor of Medicine, PGIMER,<br>Chandigarh  | 11) Dr Tapan Saikia<br>Consultant, Prince Aly Khan Hospital<br>Mumbai   |
| 6) Dr Senthil Rajappa<br>Head, Department of Medical Oncology<br>Indo Americal Cancer Centre, Punjagutta,<br>Hyderabad     |   |

---

## CONTENTS

---

Foreword

Message from Chairperson

Preface (Chairperson of Subcommittee)

Preface

Acknowledgment

1.	Introduction	1
2.	Diagnosis	3
3.	Treatment : Induction Therapy	10
4.	Consolidation Therapy in AML	17
5.	Management of Elderly AML	21
6.	Prognostic Factors in Acute Myeloid Leukaemia	26
7.	Management of Acute Promyelocytic Leukemia	33
8.	Treatment of Granulocytic Sarcoma	40
9.	Down Syndrome & Acute Myeloid Leukemia	44
10.	Treatment of Secondary Acute Myeloid Leukemia.	48
11.	References	50
12.	List of Contributors	54



Acute Myeloid Leukemia (AML) is characterized by clonal neoplastic proliferation of myeloid precursor cells in the marrow and arrest of their maturation. The replacement of marrow by leukemic cells (blasts) leads to clinical features characterized by anemia, thrombocytopenia and granulocytopenia with or without leukocytosis. The resultant accumulation of these non-functional blast cells prevents normal hematopoiesis, which if untreated will result in bone marrow failure and the death of the affected individual. Its annual incidence is 2-3 per 100,000. Incidence increases with age. It is less than 1 per 100,000 under 30 years of age and 17 per 100,000 by 75 years. AML accounts for less than 10% of all leukemias in children less than 10 years of age, and for 25-30% between 10 and 15 years. In adults, AML accounts for 80-90% of cases of acute leukemia. The incidence is higher in males than in females. As per Delhi population based Cancer Registry AML constitutes about 3% of all cancers with a median age of 32 years.

**Etiology:**

Though in almost 90% of cases, etiology of AML is unknown. In the remaining, a number of risk factors have been implicated in the etiopathogenesis of AML. Many of these factors cause damage to DNA, a finding consistent with the notion that acute leukemia occurs on the basis of acquired genetic abnormalities in bone marrow cells. These include- genetic factors, radiation exposure, alkylating agents, drugs, cigarette smoking and electromagnetic radiation.

***Genetic Factors***

These include - disorders characterized by chromosomal abnormalities or instability e.g. Fanconi anemia, Ataxia Telangiectasia, Bloom syndrome, Diamond-Blackfan anemia, Down syndrome, Schwachman-Diamond syndrome, Li-Fraumeni syndrome, Neurofibromatosis type 1, Severe congenital neutropenia (Kostmann syndrome) etc.

Several chronic bone marrow disorders characterized by stem cell developmental defect are associated with an increased incidence of acute leukemia, these are - myeloproliferative disorders, paroxysmal nocturnal hemoglobinuria.

***Industrial Agents:*** Long term exposure to certain chemical solvents especially benzene is associated with higher risk of AML. Benzene is a solvent used in the rubber industry, oil refineries, chemical plants, shoe manufacturing, and gasoline-related industries, and is also found in cigarette smoke, gasoline and motor vehicle exhaust, and some glues, cleaning products, detergents, art supplies, and paints. Exposure to formaldehyde has also been linked to development of AML in some studies, but not in others. So far there is no conclusive link between workplace exposure to diesel, gasoline, exposure to herbicides or pesticides and development of AML.

### ***Radiation:***

Leukemogenic effect of ionizing radiations was established earlier when increased incidence of acute leukemia was noticed in radiologists and atomic bomb survivors in Hiroshima and Nagasaki; leukemia developing most often 6-8 years after exposure. Radiation and radio-isotope therapy has been associated with increased incidence of acute leukemia. The risk correlates with the radiation dose and age at exposure, with a more rapid peak in early life (less than 15 years). So far there is no conclusive evidence about exposure to electromagnetic radiation (people living near mobile towers) and AML

### ***Drugs:***

Alkylating agents, platinum (cisplatin, carboplatin) and topoisomerase II inhibitors are associated with development of AML. Alkylating agents cause point mutation which result in activation of oncogenes such as RAS as well as chromosomal deletions and unbalanced translocations involving chromosome 5 & 7. In such cases- there is a long latency period (5 to 7 years) between exposure and development of AML and in most cases there is preceding myelodysplastic phase. Common alkylating agents implicated are - cyclophosphamide, mechlorethamine, procarbazine, chlorambucil, melphalan, busulfan, and carmustine (BCNU).

Topoisomerase II inhibitor result in loss of critical enzyme involved in DNA replication leading to a balanced chromosomal translocation involving 11q23 or 21q22. Large cumulative dosage and prolonged courses have been implicated as increasing the risk of leukemia. There is short latency period (6 months to 5 years) with no preceding myelodysplastic phase and majority of cases are monoblastic (FAB-M5) or myelo-monocytic (FAB - M4). Common examples of topoisomerase II inhibitors include etoposide (VP-16), teniposide, mitoxantrone, epirubicin, and doxorubicin. Other drugs associated with AML are phenylbutazone, chloramphenicol, chloroquine, methoxypsoralens and LSD.

*Among Life style factors - Cigarette smoking* is associated with higher risk of AML in the epidemiological studies.

### a. Presenting features –

Symptoms are results of bone marrow failure, tissue infiltration by leukemic cells or may be due to circulating leukemic cells. Patient usually presents with fatigue and abnormal bleeding. Weight loss, fever, night sweats may be present. Bone pain and testicular enlargement are due to extra medullary involvement by the leukemic cells. Patient may have headache, vomiting, blurring of vision due to Central Nervous System involvement. On physical examination, pallor and features of thrombocytopenia like epistaxis, gingival bleed, petechial, ecchymotic patches may be obvious findings. Patient may have organomegaly-hepatosplenomegaly, Lymphadenopathy and gingival hypertrophy (usually in myelomonocytic and monocytic leukemia or acute promyelocytic leukemia). Rare features are skin involvement and arthritis.

### b. Investigations

**Table 1: Investigations checklist in a newly diagnosed patient of AML**

	Study	Remarks
1	CBC with differential, Peripheral smear	All cases
2	Biochemical (RFT, LFT, electrolytes, Uric acid)	All cases
3	Chest X ray	All cases
4	Bone marrow	All cases
5	Flow cytometry (Immunophenotyping)	All cases
6	Cytogenetics	Preferred
7	Molecular studies for prognosis	Preferred
8	HIV, HBsAg, Anti HCV ab	All cases
9	Blood grouping	All cases
10	HLA matching	In high and intermediate risk patients
11	Lumbar puncture	All children and symptomatic adults
12	Cardiac function evaluation (ECHO heart or MUGA scan)	All patients (patients 40 years and above and earlier if clinically indicated)

Peripheral and Bone marrow smears are examined after Wright –Giemsa's staining. At least 200 leukocytes and 500 nucleated cells on peripheral and BM smear are seen, respectively.

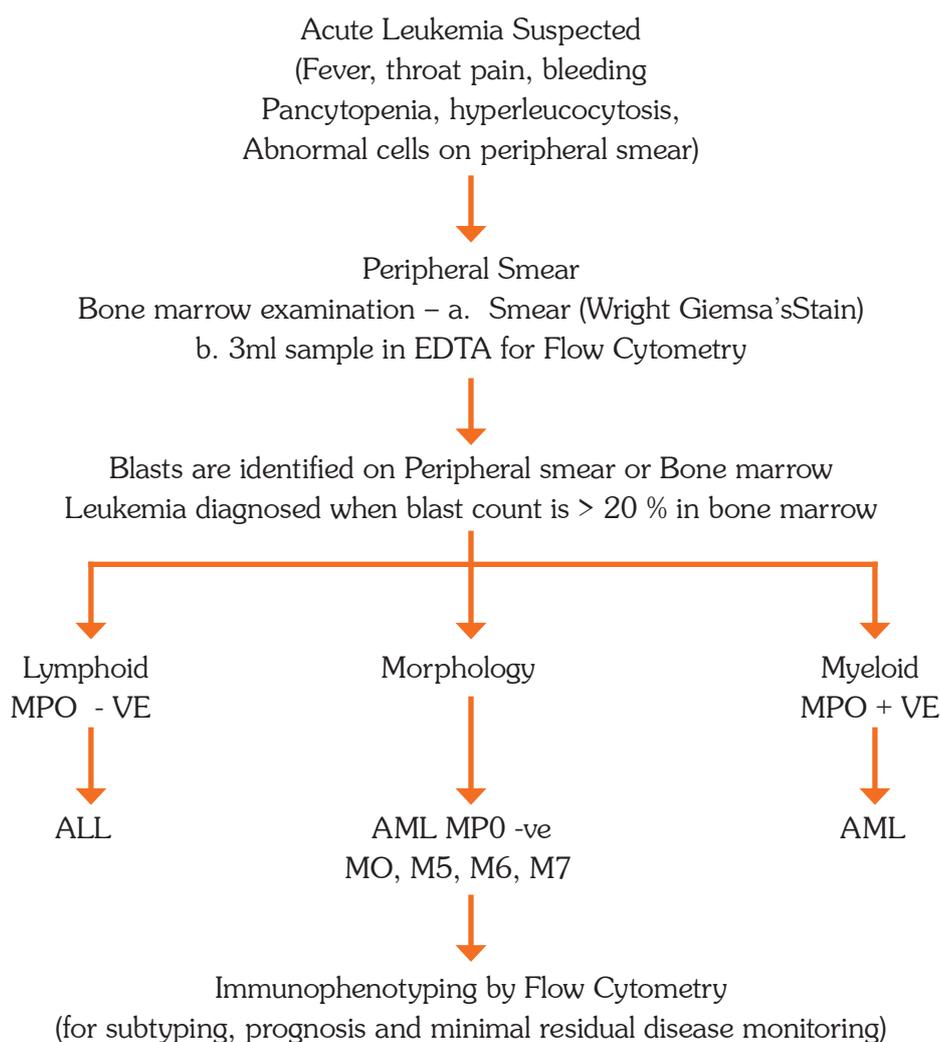
### Diagnosis: Criteria

The diagnosis of AML requires the presence of 20% blasts enumerated from all nucleated cells in the blood or bone marrow in most cases (blast count in peripheral blood and bone marrow may differ). In myelomonocytic and monocytic leukemias, promonocytes are considered comparable to blasts. The

blast count should be obtained from at least a 200-cell count of all nucleated cells in the blood and a 500-cell count of all nucleated cells in the bone marrow. For AML with t (15; 17), t (8; 21) and inv(16) do not require the arbitrary cut off of 20%. These recurrent genetic abnormalities are found only with leukemia. Similarly, the presence of a myeloid sarcoma is diagnostic of AML even if blasts are not significantly elevated in the blood or bone marrow. Additional tests such as Flow cytometry, cytogenetics conventional, FISH for specific translocations, PCR for FLT3 mutation and ITD, and NPM mutation will help to establish the prognostic subgroup. Bone Marrow Biopsy is not mandatory. The biopsy is done in cases of dry tap to further characterize the leukemia.

In few cases of AML, the diagnosis is not straightforward. > 3 % MPO positivity is considered as AML. In cases where MPO is negative, the role of further staining with different methods comes into play. Flow cytometry and Immunohistochemistry can detect the cytoplasmic MPO. MPO is negative in AML –M0, AML M6 (Erythroid leukemia), AML M7 (Megakaryocytic Leukemia) and in some cases of AML M5 (Monoblastic). Sudan black staining is less specific as around 5 % of ALL patients may have positivity with Sudan black. Other stains which have limited utility in making diagnosis are Non specific esterases, alpha naphthyl butyrate and alpha naphthyl acetate.

#### Flow diagram: Approach to a case suspected to have acute leukemia



## Cytogenetics

European Leukemia Group Net Classification System <sup>(Ref.1)</sup>	
Prognostic Group	Subset
Favorable	Inv (16), t (16;16), t (8;21) Normal cytogenetics with NPM mutation but no FLT3 -ITD Normal cytogenetics with mutated CEBPA
Intermediate 1	NPM -, ITD - NPM+, ITD - NPM -, ITD +
Intermediate 2	Cytogenetic abnormalities not considered best or worst, Including t (9;11)
Adverse	3 q abnormalities, t (6;9), deletion 7, deletion 5, del 5q, abnormal 11 q (other than t 9;11), abnormal 17p, Complex abnormalities

FLT3 – FMS like tyrosine kinases 3, ITD – internal tandem duplication, NPM – nucleophosmin, CEBPA- CCAAT enhancer binding protein alpha

### *Further readings*

1. Hartmut Dohner et al, Diagnosis and management of acute myeloid leukemia in adults: recommendations from an international expert panel, on behalf of the European Leukemia Net, Blood, 21 January 2010 Vol 115, Number 3.
2. James W. Vardiman et al, The 2008 revision of the World Health Organization (WHO) classification of myeloid neoplasms and acute leukemia: rationale and important changes, Blood, 30 July 2009 Volume 114, Number 5.
3. World Health Organization classification of tumors. In: Jaffe ES, Harris NL, Stein H, Vardiman JW, editors. Pathology and genetics of tumors of haematopoietic and lymphoid tissues. Lyons, France: IARC Press; 2008. p. 75-118.
4. Naresh KN, Lampert I et al. optimal processing of bone marrow trephine biopsy: the Hammersmith Protocol. J Clin Pathol 2006;59:903-11.
5. Gujral S, Subramanian PG, Dasgupta A et al, Report of proceedings of the national meeting on “Guidelines for Immunophenotyping of Hematolymphoid Neoplasms by Flow Cytometry”. Indian J Pathol Microbiol 2008; 51:161-6.

### ***Immunophenotyping :***

*Flow cytometry* has the ability to analyze in a short time, high numbers of cells and simultaneously measures multiple antigens on an individual cell. It has a well-established role in diagnosis, prognosis as well as in detecting Minimal Residual tic abnormalities in AML decide the cellular biology and its response.

### Figure : Flow cytometry diagnosis of AML

Screening Tube

CD 45, CD 34, cCD79a, cCD3, cMPO

(AML diagnosis is established once positive results obtained)

#### Myeloid Lineage

CD 45+, CD34+, cMPO+

- AML confirmed

Extended Panel

CD 13, CD 33, CD117,  
CD 65, CD11b, CD15

CD 13, CD 33, CD 117 - M5

CD 64, 14

CD 41, CD 61 - M7

CD 71 - M6

#### Lineage T cell (ALL)

CD 34+, CD 45+, CD 79a+

Further markers for subtyping and to rule out mixed phenotypes

CD 2, CD 5, CD 7, CD 4, CD 8, sCD3

CD19, CD 117, CD 13, CD 33, CD 1a,

CD 65, CD 34, CD45

#### Lineage B cell (ALL)

CD 19+, CD 20+, CD 22+

extended panel

CD 10, CD 38, CD13, CD33, CD 34, CD 45

#### Classification of AML

Acute myeloid leukemia is classified on the basis of morphology of the blast cells and on their cytochemical features. According to French-American-British (FAB) classification system AML is classified into 8 subtypes according to their extent of differentiation and cell type involved (table).

Table:- FAB Classification of AML<sup>(Ref)</sup>

AML subtype	Name	Frequency	Common cytochemical properties
M0	Undifferentiated acute leukemia	5%	MPO-
M1	AML, without maturation	15%	MPO+, NSE+
M2	AML with Maturation	25%	MPO+++ , NSE+
M3	Acute promyelocytic leukemia	10%	MPO+++ ,NSE+
M4	Acute myelomonocytic leukemia	20%	MPO±, NSE+++ with fluoride inhibition
M4E0	Acute myelomonocytic leukemia with eosinophilia	5%	
M5	Acute monoblastic leukemia	10%	MPO-, NSE+++ without fluoride inhibition
M6	Acute erythroid leukemia	5%	MPO±,NSE-,PAS+++
M7	Acute megakaryocytic leukemia	5%	MPO-

Abbreviations: MPO-myeloperoxidase, NSE-nonspecific esterase, PAS-Periodic acid Schiff

Ref. at <http://www.cancer.org/cancer/leukemia-acute-myeloid-aml/detailedguide/leukemia-acute-myeloid-myelogenous-classified>. December 9, 2014.

Recently WHO classification of AML has included molecular features into classification and four sub-classes (Table-).

### WHO classification of myeloid neoplasms and acute leukemia<sup>(Ref)</sup>

#### Acute myeloid leukemia (AML) and related neoplasms

AML with recurrent genetic abnormalities

AML with t(8;21) (q22;q22.1);RUNX1-RUNX1T1

AML with inv (16) (p13.1q22) or t(16;16)(p13.1;q22);CBFB-MYH11

APL with PML-RAR $\alpha$

AML with t(9;11) (p21.3;q23.3);MLLT3-KMT2A

AML with t(6;9) (p23;q34.1);DEK-NUP214

AML with inv (3) (q21.3q26.2) or t(3;3) (q21.3;q26.2); GATA2, MECOM

AML (megakaryoblastic) with t(1;22) (p13.3;q13.3); RBM15-MKL1

Provisional entity: AML with BCR-ABL1

AML with mutated NPM1

AML with biallelic mutations of CEBPA

Provisional entity: AML with mutated RUNX1

AML with myelodysplasia-related changes

Therapy-related myeloid neoplasms

AML, NOS

AML with minimal differentiation

AML with maturation

Acute myelomonocytic leukemia

Acute monoblastic/monocytic leukemia

Pure erythroid leukemia

Acute Megakaryoblastic leukemia

Acute panmyelosis with myelofibrosis

Myeloid sarcoma

Myeloid proliferations related to Down syndrome

Transient abnormal myelopoiesis (TAM)

Myeloid leukemia associated with Down syndrome

#### Blastic plasmacytoid dendritic cell neoplasm

#### Acute leukemias of ambiguous lineage

Acute undifferentiated leukemia

Mixed phenotype acute leukemia

Mixed phenotype acute leukemia (MPAL) with t(9;22) (q34.1;q11.2); BCR-ABL 1

MPAL with t(v;11qw23.3); knt2A rearranged

MPAL, B/myeloid, NOS

MPAL, T/myeloid, NOS

Ref. Vardiman JW, Harris NL, Brunning RD. The World Health Organization (WHO) classification of the myeloid neoplasm. *Blood*. 2002 Oct 1. 100(7):2292-302. [Medline].

### Clinical Features:

The clinical features are nonspecific but are directly attributable to bone marrow failure. Anemia leads to pallor, weakness and fatigue. Up to half of patients at diagnosis have petechiae, epistaxis, and easy bruising. Fever is seen in up to 80% of patients at diagnosis and is due to neutropenia; there could be evidence of infection especially pneumonia, sepsis and peri-rectal abscess. Leukemic infiltration of various tissues leads to hepatomegaly, splenomegaly, leukemia cutis, lymphadenopathy, bony pains, gingival hyperplasia and rarely cranial neuropathy. Hyperleucocytosis with count greater than 1,00,000/cmm can lead to ocular and cerebral dysfunction and bleeding. An isolated mass of leukemic blast is called granulocytic sarcoma and occurs in 2 to 14% of cases of AML. The tumors are often localized and involve bone periosteum, soft tissues, lymph nodes, or skin. Common sites are orbit, paranasal sinuses, but can also occur at other sites including GIT, gut, breast, cervix, salivary glands, mediastinum, pleura and peritoneum. These may occur at diagnosis or may precede the diagnosis. Testicular involvement is seen in 1-8% of cases of AML. Leukemia skin infiltration (Leukemia cutis) is seen in up to 10% of patients during the course of disease. The lesions are violaceous and nodular and are more common with AML-M4 or M5. Metabolic abnormalities include hyperuricemia, hypokalemia and rarely azotemia. CNS leukemia is less common in AML compared to ALL and has been reported in 5-20% of children and up to 15% of adults. CNS disease is associated with young age (less than 2 years), hyperleucocytosis, and monocytic (AML-M4 / M5) variants. It is often asymptomatic but may be associated with headache or cranial nerve palsies, particularly Vth and VII nerve. Intracerebral masses rarely co-exist with leukemic meningitis and have been reported with FAB-M4 EO. Clinical and laboratory features of 480 patients seen between 1991–1997 at our institute are given below.

**Table - Demographic, clinical and biological characteristics of AML patients at time of diagnosis (n = 480)**

#### Parameter

Median age (in years)	29 (range 8–60)
Pediatric patients (defined as age less than 18 years)	130 (27%)
Male : female ratio	3:2
Median duration of complaints (in months)	1.2 (range 0.2–8)
Performance status (ECOG)	
PS 1	156 (32.5%)
PS 2	140 (29.2%)
PS 3	75 (15.4%)
PS 4	46 (9.4%)
Not known	63 (13.4)
Clinical features	
Gum hypertrophy	120 (25%)
Lymph node enlargement	105 (21.9%)
Swellings (chloromas)	51 (10.6%)

Hepatosplenomegaly 144 (30%)

High TLC (>50 000/ $\mu$ L) 149 (31%)

Cytogenetic (n=394)

t(8;21) 79 (20%)

Normal cytogenetic 237 (60%)

Complex cytogenetic 25 (6.3%)

5q- 05 (1.2%)

7q- 05 (1.2%)

Indeterminate 21 (5.5%)

Others 22 (5.8%)

Risk stratification of AML in those assessed ( $n = 373$ )

Good risk 79 (21.6%)

Intermediate risk 259 (69.3%)

Poor risk 35 (9.1%)

FAB classification

M0 19 (04%)

M1 32 (6.7%)

M2 285 (59.4%)

M4 48 (10.2%)

M5 58 (12.1%)

M6 23 (4.8%)

M7 1 (0.2%)

Not available 14 (2.7%)

Ref. Bahl A, et al . Long-term outcomes for patients with acute myeloid leukemia: A single-center experience from AIIMS, India. Asia Pacific J Clin Oncology 2015; 11 (3): 242-52.

# 3

## TREATMENT PLAN

Once the diagnosis of acute leukemia is established, induction chemotherapy is given with the goal of rapidly restoring normal bone marrow function- called as complete remission (CR). The latter is defined as resolution of disease related sign and symptoms, absence of circulating blasts, reduction of BM blasts to <5% and recovery of peripheral blood cell counts. Most commonly used induction therapy is combination of 3 days of anthracycline given by short infusion with 7 days of continuous infusion of cytosine arabinoside (Ara-C), commonly known as 3+7 regimen. Daunorubicin is the most commonly used anthracycline, other similar drugs like idarubicin, doxorubicin and mitoxantrone have also been used. Approximately 60-70% patients achieve complete remission with initial therapy while remaining would require a repeat course of chemotherapy for the same. Attempts have been made to improve the results obtained by standard 3+7 regimen by adding a third drug like etoposide (VP-16) or 6-thioguanine; or by changing the schedule of drug infusion, or by increasing the dose of anthracycline. In most cases these variations of 3+7 regimen have produced comparable results. Severe myelo-suppression in the 2-3 weeks post induction is the most critical phase and requires close observation and prompt therapy for any signs of infection as well as blood and platelet support. Response assessment is done at 4 weeks of initiation of induction treatment. Patient attaining CR would proceed for post remission therapy called consolidation. Patients in poor risk subgroup are at higher risk of relapse, these are candidates for allogeneic bone marrow/stem cell transplantation.

**Patients with high TLC at presentation (>50,000/cmm):** For such patients - Hydroxyurea 2-3 G/day in 2-3 divided doses may be used with frequent monitoring of the counts and adequate prophylaxis against tumor lysis. Once chemotherapy is started then hydroxyurea may be stopped.

**Patients with Fever:** More than half of patients may have fever at the diagnosis. In addition to good history, all such patients must be carefully evaluated for any possible focus of infection with particular attention to oral cavity, axillae, perianal area and sinuses. Chest X ray and blood culture must be sent prior to starting broad spectrum antibiotics. In 5 to 10% of patients there may be invasive fungal infection and this may need additional imaging (HRCT chest) and therapy. Most physicians like to give 48 hours of antibiotics therapy before starting induction chemotherapy.

### *Induction Chemotherapy Treatment*

#### A. PRE-INDUCTION CHEMOTHERAPY CHECKLIST

- **Patient counseling and informed consent:**

Patients of AML while on induction therapy have severe neutropenia and thrombocytopenia lasting for almost 3 weeks. During this period there are associated problems of fever (requiring IV antibiotics, antifungals etc) and bleeding. It is preferable that treatment of such patients is undertaken at centres by experienced physicians. Significant morbidity and induction mortality 10% to 20% is seen at most centres. Hence, proper counseling of patient and their families must be done prior to initiation of therapy.

- **Financial issues:**

The treatment of AML is very expensive due to the requirement of supportive medications. It is important to be prepared for the financial burden of therapy before starting treatment.

- **Central venous access:**

Apart from chemotherapy, patient would need IV antibiotics, blood/platelets support, alimentation etc, it is important to have a good venous access. This can be either by a peripherally inserted (PICC) line or by subclavian or jugular venous access. Since most of the patients are thrombocytopenic at outset, the inherent risk of blind procedures involving the subclavian or jugular puncture remains and PICC line is preferred when possible to avoid these problems. However central insertions can be carried out after adequate platelet transfusions by experienced operators with minimum extra risk. Absolute sterile precautions must be followed.

- **Ensure availability of blood products.**

Motivate the patient's relative to donate adequate number of blood bags. It is better to be prepared for more than to run around when patient develops bleeding problems. Also, wherever facilities are available, screening of donors for single donor platelets must be done. These donors must be available for donation at short notice.

- **Infection prophylaxis**

- Antifungal prophylaxis: It is standard practice to start prophylactic antifungals in patients of AML. The used agent must be able to cover for molds. The options are:

- ◆ Voriconazole 200 mg PO BD\*
- ◆ Posaconazole 200 mg PO TID
- ◆ Amphotericin B 0.5mg/kg on alternate days

Voriconazole has very good action to prevent invasive aspergillus, however, it has poor action on mucor. So in centers where mucormycosis is a problem, it may be preferable to use amphotericin B or posaconazole.

- **Antibacterial prophylaxis: Routine antibiotic prophylaxis is not indicated.**

- **Antiviral prophylaxis:** Since there is high possibility of mucosal injury during 3+7 induction, use of Acyclovir 400 mg BD is recommended.

- Personal hygiene, steam inhalation, Sitz bath

- **Supportive care**

- **Hydration:** In the initial phase of treatment particular attention must be paid to maintain adequate hydration of the patient to prevent any consequence of tumor lysis. Intravenous fluids may be required where appropriate in patients who present with hyperleukocytosis or those with poor oral intake.

- **Allopurinol:** As prevention against tumor lysis syndrome in the first 1 week of therapy or till uric acid normalizes.

- **Norethisterone:** or any other suitable progesterone analog may be required in females in the reproductive age group to suppress menstrual cycles during the acute phases till recovery of platelet counts.
- **Growth factors: Routine use of G-CSF should be avoided.** May be considered in elderly, and patients with sepsis with delayed recovery of counts following induction therapy.
- Laxatives, antiemetics etc

**Treatment summary: Initial Supportive Treatment/Resuscitation**

- Correct metabolic disturbance
- Transfuse blood and platelets
- If fever start antibiotics as for neutropenic sepsis.
- Central Line or PICC line) – Ensure platelets counts as => 50,000 / cmm or give platelets prior to inserting central line.

Table : Checklist before starting induction therapy
DIAGNOSIS OF AML ESTABLISHED
Patient counselling & Informed consent
Financial aspects covered
Blood product availability checked
Antibiotic availability checked
Chemotherapy- Daunorubicin/ Cytarabine
Central venous access- subclavian/jugular or PICC
Supportive medications
Prophylaxis-fungal, viral

**# Induction chemotherapy**

(DNR: Ara-C : 3:7)

Inj Daunomycin                      60 mg/m<sup>2</sup> IV day 1 to day 3  
as infusion in normal saline over 2 hours daily x 3 days

Inj Cytosine Arabinoside        100 mg/m<sup>2</sup> IV day 1 to day 7  
as continuous infusion over 24 hours daily

Dose of Daunomycin : For patients aged 60 years and above, the dose of daunomycin is given as 45mg/m<sup>2</sup>. The cumulative dose of anthracycline ≥450mg/m<sup>2</sup> is important.

**# Consolidation Chemotherapy**

- Cycle #1    High dose cytosar
- # 2    High dose cytosar
- # 3    High dose cytosar

## Induction chemotherapy: Expected toxicity

Emesis – moderate

Alopecia-total

Myelosuppression – Severe & prolonged

### Other Toxicity

- Cardiomyopathy due to adriamycin/daunomycin
- Arrhythmia due to adriamycin / daunomycin
- Palmar desquamation (cytosine hands).

### Monitoring during Induction Chemotherapy

- **Daily rounds:** Once the induction chemotherapy is started patient needs to be closely monitored. Check daily for possible sites of infection, including inspection of the entire skin, respiratory system and oral cavity and mucosal areas. In addition, don't hesitate to examine the anal and perineal regions in a patient with symptoms or febrile Neutropenia because these are very common sites of infections.

Blood counts daily or alternate day

- Differential counts (absolute neutrophil count) thrice a week
- Blood sugar - Twice a week
- Liver function tests - Twice a week
- Renal functions - alternate day or earlier if indicated)
  - In the first 1 week of therapy monitoring for possible tumor lysis syndrome must be done.
  - In the later stages, Hypokalemia is a common problem in these patients. The cause of hypokalemia in these patients could be multifactorial. Hypokalemia can lead to generalized weakness and predisposes to cardiac arrhythmias.

**Table 4: Causes of hypokalemia during induction therapy of AML**

Poor oral intake, GI loss

Drugs causing potassium loss ( Amphotericin B, Azoles, Piperacillin)

Monocytic varieties of AML ( secrete lysozyme which causes potassium loss from the kidneys).

Pseudohypokalemia in patients with hyperleukocytosis due to “mopping up” of the potassium by the blast cells.

- **Febrile Neutropenia:** Standard guidelines for the management of high risk febrile neutropenia should be followed. Some patients may be febrile at presentation. Unless fully convinced about disease-related fever, it is best to empirically start antibiotics in these patients.

- **Blood transfusions:**

*RBC transfusions* must be given to maintain Hemoglobin  $\geq 8$  g3/dL.

*Platelet transfusions* aim at a platelet count  $>20,000$ /cmm in any patient with fever or overt bleeding. Otherwise a platelet count  $>10,000$ /cmm is a reasonably safe target. In patients with CNS bleeding or those undergoing induction for promyelocytic leukemia the platelet count target is 50,000/cmm.

All transfusions must be preferably leucodepleted. Single donor versus Random pooled platelets: Studies have shown no increase in alloimmunization with the use of pooled platelets from multiple donors (easily available from most blood banks) compared to that caused by the use of Single donor platelets.

- **Constipation:** Poor oral intake, lack of physical activity, hypokalemia, anti-emetics such as ondansetron are all causative factors. Can lead to further decrease in appetite and poor nutrition. Hard stools also aggravate hemorrhoids and fissures which are potential sources of infection in the neutropenic patients. Needs to be managed pro-actively with liberal use of laxatives.
- **Central line care:** meticulous care during the insertion followed by regular dressing change using sterile precautions as well as frequent inspections of the exit site can greatly prolong the life of central lines.
- **Use of Growth factors:** G-CSF has been used to decrease the duration of granulocytopenia. However, no study has shown survival advantage with the use of G-CSF during AML induction. Some studies have shown some advantage in those who are more than 60 years of age. Use of growth factors to try and augment the effects of chemotherapy has yielded conflicting results. Routine use of growth factors is not recommended.

#### D. SPECIAL SITUATIONS

- **Patient with severe infection at time of start of induction:** Some patients may present with infections like pneumonia/ sepsis initially. They are functionally neutropenic and there is fear/ danger of worsening their status by starting chemotherapy. Since there is very little evidence to support course of action in these situations, the decisions are individualised. The risk of worsening infection must be balanced against non-treatment of AML which will only delay the actual recovery of counts. Since high doses of anthracyclines are involved in AML induction, there is danger mucosal disruption during therapy which may aggravate risk of sepsis. In such a situation, it may be prudent to wait with broad spectrum antibiotics for 2-3 days before starting 3+7.
- **Patients presenting with hyperleucocytosis +/- features of leukostasis:** The best way to bring the counts down is to start chemotherapy as soon as possible. Sometimes there may be delay in establishing the diagnosis and patients may be manifesting features of leukostasis. Hydroxyurea may work in some patients to reduce the counts. Leukoreduction by pheresis is an option if facilities permit. But this is associated with significant risks of complications including bleeding and requires central venous access. These patients are at high risk of tumor lysis with therapy and rasburicase may be considered as prophylaxis / therapy.

#### E. END OF INDUCTION AND PLANNING DISCHARGE

- **When to do bone marrow examinations and how to establish remission status?**

Generally patient starts showing recovery of blood counts by the end of 3- weeks. As counts recover, all empirical antibiotics can be discontinued and blood product support decreases. Rebound fungal infections can occur during the period of neutrophil recovery.

Practically it is usual to wait until 3- 4weeks are completed or the patient has good recovery of counts before the 1<sup>st</sup> bone marrow examination is carried out. Demonstration of remission status involves not only control of the blasts but also demonstration of recovery of blood counts and tri-lineage differentiation in the bone marrow.

Use of growth factors can significantly confuse the interpretation of BM remission status. It is preferable to wait for at least 1 week after stopping growth factors to avoid false impressions.

- **What to do when the counts don't recover after 4 weeks?**

Some patients may have delayed recovery of counts. But mostly delayed recovery or non-recovery portends a marrow which still contains disease. Bone marrow studies will provide the answer.

- **What to do when the patient is not in remission?**

If there has been a significant reduction in the marrow blast percentage but remission has not been achieved it is reasonable to repeat the induction with the same protocol (use Dauno 45 this time if 90 has been used first time). A significant number of patients will achieve remission after this second course. Nevertheless these patients have a poorer prognosis which must be considered while planning their post-induction therapies. Those with minimal or no response to induction (persistent blasts >50%) rarely do well with repeating the standard chemotherapy strategies and are best considered for experimental therapies.

### Assessment for remission

Bone marrow between days 21-28.

On day 22

- (i) If both peripheral smear and BM shows blasts
- (ii) Consider re-induction chemotherapy
  - a) BM – severely hypocellular, no blasts withhold further chemotherapy, repeat another BM after one week, assess remission status, then decide.
  - b) BM – Normocellular or mildly hypocellular, No blasts i.e. complete remission, plan for consolidation chemotherapy
  - c) BM-cellular & shows significant number of blasts – possibly resistant disease, consider for re-induction chemotherapy or salvage chemotherapy or transplantation.

Table-3 **Overall survival in AML according to cytogenetic abnormalities** (Age Group: 16 to 59 years, Median age 43 years) : Data source MRC /NCRI AML Trials (adapted from)

	Cytogenetic abnormality	No of pts	Overall survival at 10 years (%)
Favorable	t(15;17)	759	73
	t(8;21)	401	61
Intermediate	Inv (16)(t(16;16)	266	55
	t(9;11)	56	39
	t(6;9)	34	26
Adverse	Inv (3)(t(3;3)	65	3
	t(9;22)	47	14
	Other t(11q23)	61	21
	AML with MDS related changes	797	16

Ref. David Grimwade et al

The Importance of Diagnostic Cytogenetics on Outcome in AML: Analysis of 1,612 Patients Entered Into the MRC AML 10 Trial Blood 1998 92:2322-2333

Genetic group	Subset
Favorable	t(8;21)(q22;q22); <i>RUNX1-RUNX1T1</i> inv(16)(p13.1q22) or t(16;16)(p13.1;q22); <i>CBFB-MYH11</i> Mutated <i>NPM1</i> without <i>FLT3-ITD</i> (normal karyotype) Mutated <i>CEBPA</i> (normal karyotype)
Intermediate -I	Mutated <i>NPM1</i> and <i>FLT3-ITD</i> (normal karyotype) Wild-type <i>NPM1</i> and <i>FLT3-ITD</i> (normal karyotype) Wild-type <i>NPM1</i> without <i>FLT3-ITD</i> (normal karyotype)
Intermediate -II	t(9;11)(p22;q23); <i>MLLT3-MLL</i> Cytogenetic abnormalities not classified as favorable or adverse
Adverse	inv(3)(q21q26.2) or t(3;3)(q21;q26.2); <i>RPN1-EVII</i> t(6;9)(p23;q34); <i>DEK-NUP214</i> t(v;11)(v;q23); <i>MLL</i> rearranged -5 or del(5q); -7; abn(17p); complex karyotype

### 3 : Prognostic factors predicting outcome in AML

Favorable factors	Unfavorable factors
Age <50	Age >60
Karnofsky score >60 percent	Karnofsky score <60 percent
MDR 1-negative phenotype	MDR 1-positive phenotype
No antecedent hematologic disorder or prior chemo/radiotherapy	Therapy-related AML, prior myelodysplastic syndrome, myeloproliferative or other hematologic disorder
t(8;21), inv(16)/t(16;16), t(15;17)	Complex karyotypic abnormalities, -5, -7, t(6;9), 11q23 aberrations except for t(9;11), "monosomal karyotype"
<i>NPM1</i> mutation, <i>CEBPA</i> mutation	<i>FLT3/ITD</i> mutation, <i>MLL</i> partial tandem duplication, mutations in <i>IDH1</i> and/or <i>IDH2</i>

Post-CR (complete remission) therapy aims to destroy leukemia cells that survived induction chemotherapy but are undetectable by conventional studies.

There are three basic options for post-remission therapy:

Consolidation chemotherapy,

Autologous hematopoietic stem cell transplantation (HSCT), or

Allogeneic HSCT.

### RISK STRATIFICATION

Favorable risk (20 percent):	Intermediate risk (60 percent):	Unfavorable risk (20 percent):
<ul style="list-style-type: none"> <li>• t(8;21); RUNX1-RUNX1T1</li> <li>• inv(16), t(16;16), CBFB-MYH11, whether alone or in conjunction with other abnormalities</li> <li>• NK ,NPM mutated, FLT3 wild</li> <li>• NK displaying mutations in the CCAAT/enhancer binding protein alpha (CEBPA) gene</li> </ul>	<ul style="list-style-type: none"> <li>• Normal cytogenetics, except for those described in favorable risk subgroup</li> <li>• Normal cytogenetics with NPM1 mutations and FLT3-ITD mutations</li> <li>• Normal cytogenetics with wild-type NPM1 and FLT3-ITD</li> <li>• t(9;11), MLLT3-MLL</li> <li>• Other cytogenetic abnormalities not described as either favorable or unfavorable</li> </ul>	<ul style="list-style-type: none"> <li>• 3(q21q26) abnormalities, RPN1-EVI1</li> <li>• del(5q), del(7q), t(6;9), other 11q23 abnormalities, 17p abnormalities, or complex aberrant karyotypes described as at least 3 unrelated abnormalities excluding cases with t(8;21), inv(16), and t(15;17)</li> </ul>

### FAVORABLE RISK DISEASE —

Consolidation chemotherapy with high dose cytarabine (HIDAC) is the standard approach and provides the best survival for most patients with favorable risk disease. This approach results in overall survival rates at four years of 60 to 75 percent. In patients with favourable cytogenetics, stem cell transplantation is reserved for use at the time of relapse.

High dose cytarabine produces high intracellular drug concentrations, which saturate the deaminating metabolic enzyme pathway, leading to increased levels of the active agent ara-cytidine triphosphate. In this way, HDAC can often effectively eliminate minimal residual disease that survived induction with cytarabine containing regimens.

### IDEAL DOSE:

While it is clear that higher doses of cytarabine are preferable to lower doses, the ideal dose remains unknown and there is likely a dose above which additional cytarabine is not of benefit.

**NUMBER OF CYCLES—** The ideal number of post-remission chemotherapy cycles is not known, but patients who receive at least three cycles appear to do better than those who receive fewer.

**IRCH-AIIMS PROTOCOL FOR FAVOURABLE RISK: 3 COURSES OF HDAC @ 3G/M2 BD D1, 3, 5 FOLLOWED BY OBSERVATION.**

***Administration of consolidation HDAC :***

Consolidation is begun after confirmation of a complete remission and resolution of all toxicities from induction therapy.

***3 courses of Cytarabine 3 g/m<sup>2</sup> administered over three hours twice per day on days 1, 3, and 5 for a total of six doses per course (TOTAL =18 G/M<sup>2</sup>)***

***Patients older than 60 years typically receive 2 g/m<sup>2</sup> and those older than 70 years 1.5 g/m<sup>2</sup>.***

**Supportive medications :**

1. Low dose Dexamethasone 0.1% eye drops should be administered to both eyes 2-times daily until 5 days after completion of the HDAC infusion ( total =10 days) to prevent chemical conjunctivitis due to ara C in the tears.
2. Tab Pyridoxine (Benadon) 40 mg once a day for 10 days beginning day 1.
3. I V fluids SOS
4. Comprehensive Cerebellar evaluations for Horizontal Nystagmus , Dysdiadochokinesis or Past-pointing should be performed at least every 12 hours during therapy.
5. Twice Daily hand writing assessment on paper.

**Other recommendations:**

1. Sequential courses are administered no sooner than every 28 days or 1 week after marrow recovery as documented by an absolute neutrophil count 1000 and platelets 100,000 without transfusion.
- 2.

CYTARABINE Dose	
CREATININE CLEARANCE	% DOSE
>60 ml/mt	100%
46-60	60% DOSE
31-45	50% DOSE
<30	withhold
BILIRUBIN	
<2 mg/dl	100%
>2 mg/dl	50%

**Toxicities —**

1. This regimen is usually administered as an inpatient and requires close monitoring for toxicities. courses can also be safely given from day care also with admission during neutropenia.
2. Treatment related mortality, mostly due to infection, is approximately 5 percent.
3. Common major side effects of HDAC include - pancytopenia, infection (62 percent), central nervous system toxicity (13 percent), hyperbilirubinemia (11 percent), and fatigue (10 percent).

4. Serious central nervous system toxicities (eg, somnolence and confusion, and rarely seizures, cerebral dysfunction, and acute cerebellar syndrome) can be seen in approximately 12 percent of patients overall. This percentage rises to approximately 30 percent in patients over the age of 60 years. ; 40 percent of whom may be left with permanent disability.

**Management of cerebellar toxicity:**

- Signs of cerebellar dysfunction usually emerge between the third and eighth day from start of treatment.
- In most patients the symptoms resolve in 3-10 days, but in others symptoms persist and can be debilitating or even fatal.
- The pathophysiology of HiDAC-induced neurotoxicity is poorly understood and the main goal is prevention since there is no effective treatment.
- Retrospective studies report increasing age and cumulative dose are the main risk factors, but others identify renal insufficiency and liver dysfunction. Other possible risk factors are rate of administration, CNS involvement, previous CNS pathology, concurrent medications (such as antiemetic drugs) and previous cytarabine-related neurotoxicity.
- CNS toxicity can occur at any age and even among patients with normal organ function.
- Examine for horizontal nystagmus, a subtle early sign of the syndrome, as part of a daily comprehensive cerebellar examination.
- When treatment is initiated, any suspicion of cerebellar dysfunction mandates prompt discontinuation.
- Computerized tomography (CT) and magnetic resonance imaging (MRI) scans are usually normal, at least initially.
- The risk of recurrent neurotoxicity is unknown.
- The decision to reinstitute such therapy after complete resolution of neurotoxicity, where there is a compelling rationale to do so, may be possible.

**INTERMEDIATE RISK DISEASE —**

Post-remission therapy options for patients with intermediate risk disease include consolidation chemotherapy with high dose cytarabine (HDAC) as given for favorable risk disease, HDAC followed by autologous hematopoietic cell transplantation (HCT), or allogeneic HCT alone:

Treatment	TRM	4 yr DFS
HDAC	5%	30%
AUTO HSCT	<6%	30-50%
ALLO HSCT	20%	48%

**EVIDENCE :**

**Three large meta-analyses (Yanada et al 2005, Cornellisen et al 2007, Koreth J et al 2009)**

In an intention-to-treat analysis, patients “genetically assigned” to matched related donor HSCT (ie, those who had an HLA-matched sibling) had superior rates of four-year disease-free survival for intermediate risk subgroup. While overall survival did not reach statistical significance in the first meta-analysis,

*combination of the data from these three meta-analyses demonstrated a benefit in overall survival implying that allogeneic HCT was beneficial for patients in this risk group overall.* Such results were particularly noticeable for patients younger than age 35 years who tolerate the morbidity from HSCT better.

*A decision regarding the use of allogeneic HCT in first CR must also consider the estimated rate of relapse and the efficacy of available treatments at the time of relapse.*

In a study by **Burnett AK et al, 2013**, in the IR subgroup, there was a similar difference in survival outcome for patients who had allogeneic HSCT in first CR and for those who did not undergo HSCT in first CR but underwent a late HSCT for salvage after relapse of leukemia. The choice of post-remission therapy for an individual with intermediate risk disease depends upon the patient's age and co-morbidities.

#### **UNFAVORABLE RISK DISEASE —**

These patients do poorly when treated with high dose Ara-C based consolidation chemotherapy alone after attainment of a complete remission; rates of five-year overall survival with this approach are 15 to 30 %. In contrast, treatment with allogeneic hematopoietic cell transplantation (HCT) provides an additional graft-versus-leukemia (GVL) effect together with myeloablative chemotherapy, *resulting in superior survival rates of approximately 40 % at four years.*

Unfortunately, the beneficial GVL response is closely associated with acute and chronic graft-versus-host disease (GVHD), while intensive myeloablative chemotherapy is associated with life-threatening cytopenias.

In order to offer these patients the best chance of a successful HSCT before relapse, planning for HSCT should begin as early as possible with HLA typing and a donor search.

#### **AIIMS PROTOCOL FOR HIGH RISK:**

Allogenic Haemopoietic stem cell transplant for those with an HLA-identical sibling match.

3 courses of HIDAC for those without an HLA-identical sibling match.

Management of Acute myeloid leukemia in the elderly (60 years and above) presents certain unique challenges. These factors include- (i) an increased incidence of unfavorable cytogenetic abnormalities and mutations (ii) AML in elderly patients often arises in a background of myelodysplasia or post chemotherapy (iii) a proportion of elderly patients have pre-existing co-morbidities which may increase morbidity and mortality following treatment.

In view of the above features, decision regarding choice of treatment is much more complicated than in the young where the prognosis is much better. A host of multi-factorial risk algorithms, which include patient factors (age, ECOG performance status, co-morbidities, presence of infection e.g. pneumonia) disease factors (blood parameters, albumin, creatinine, molecular abnormalities) have been devised to aid in decision-making regarding the intensity and intent of treatment.<sup>[2-5]</sup> Most of these algorithms have been derived from trials of intensive therapy and hence may not be applicable to a vast majority of patients.

Hence, choosing the optimal treatment is a complex process and should take into account patient factors and disease biology and exercise one's clinical judgment to decide on the most appropriate strategy. Those with poor prognostic characteristics like over expression of the oncogene EVI-1, ASXL1 gene mutations, biallelic FLT3-ITDs, p53 gene mutations, and complex and/or monosomal karyotypes should not be subjected to intensive treatment unless an allogeneic stem cell transplant (SCT) is feasible. Enrollment in a clinical trial should be done wherever feasible. Those being considered for allogeneic stem cell transplant may be considered for non-myeloablative conditioning; outcome of such patients is not very different from that of a myeloablative allotransplant. Further, any decision should also include socio economic status, support systems and patient's wishes.

**Elderly patients with AML can be broadly categorized into:**

1. *Candidate for intensive therapy:*

- De novo AML without unfavorable cytogenetic or molecular characteristics
- Secondary AML and those associated with unfavorable cytogenetic and molecular characteristics

2. *Not a candidate for intensive therapy*

**Treatment options:**

**Induction therapy:**

1. *Fit patient with favorable disease characteristics and those with unfavorable disease characteristics and a matched donor for allogenic SCT:*

- Standard dose cytarabine 100 mg/m<sup>2</sup> continuous infusion for 7 days with Inj.Idarubicin 12 mg/m<sup>2</sup> or Inj.Daunomycin 60 mg/m<sup>2</sup> or Inj.Mitoxantrone 12 mg/m<sup>2</sup> for 3 days
- Low intensity therapy with hypomethylating agents (5 azacytidine and decitabine).

The complete remission rates with the cytosine arabinoside plus daunomycin regimens range between 40-50%. The French ALFA trials 9801 & 9803 showed that the CR rates with Idarubicin were higher compared to Daunomycin. Combined analysis of these trials showed a median OS of 14.2 months and a 5 year OS rate of 15.3%.<sup>[7]</sup>

There is conflicting data regarding the addition of Gemtuzumab ozagomycin (GO) to the standard induction chemotherapy. Two of the three trials showed a reduction in the relapse rates while the third showed an increase in the induction mortality without increase in CR rates.<sup>[8-10]</sup> Similarly, the role of clofarabine remains undefined.<sup>[11-12]</sup>

Hypomethylating agents may be an option for a patient who is unwilling to undergo intensive induction. A fraction of patients with unfavorable disease characteristics will achieve remission with this approach.

## 2. *Fit patient with unfavorable disease characteristics without a matched donor for allogeneic SCT:*

- Low intensity therapy with hypomethylating agents (5 azacytidine or decitabine) or low dose cytosine arabinoside
- ## 3. Not a candidate for intensive therapy
- Low intensity therapy with hypomethylating agents (5 azacytidine or decitabine) or low dose cytosine arabinoside
  - Best supportive care (hydroxyurea and transfusion support)

In a subset of 113 patients with blasts between 20-30%, who were part of a randomized trial of patients with MDS, there was a survival benefit for azacytidine compared to either best supportive care or low dose cytosine arabinoside. The 2 year OS was 50 vs 16% in favor of azacytidine.<sup>[13]</sup>

Similarly, in a post hoc analysis of a randomized trial comparing decitabine to best supportive care or low dose cytosine arabinoside, there was an 18% reduction in the risk of death favoring the decitabine arm. The CR rate was higher with decitabine (18 vs 8%).<sup>[14]</sup>

The survival advantage of low dose cytosine arabinoside (20 mg twice daily for 10 days every 4-6 weeks) over best supportive care was restricted to those with favorable or normal karyotype AML. There was 26% induction mortality denoting that tolerance to even low dose chemotherapy is poor in this elderly unfit group.<sup>[15]</sup>

## **Post induction therapy**

Similar to younger patients, a bone marrow is done to ascertain remission status 21-28 days after induction therapy. Patients in remission but with hypoplasia should ideally wait for the marrow to recover before starting post remission therapy. Some patients, who have a partial remission (low volume residue) without hypoplasia, may enter remission without any further therapy. For the others, options include, repeat induction with anthracycline or mitoxantrone with cytosine arabinoside, hypomethylating agents, allogeneic stem cell transplantation or best supportive care.

## Post remission therapy

### 1. *Fit patient with favorable disease characteristics*

- Cytosine arabinoside 1-1.5G/m<sup>2</sup>, 4- 6 doses for 1-2 cycles in those with good PS, normal renal function
- Standard dose Cytosine arabinoside with anthracycline (idarubicin or daunorubicin) for 1 - 2 cycles
- Maintenance therapy with 5 azacytidine or decitabine every 4-6 weeks until progression

Alternatively, patients who have had significant complications while on induction therapy may be offered observation and best supportive care.

### 2. *Fit patient with unfavorable disease characteristics and a matched donor for allogeneic SCT*

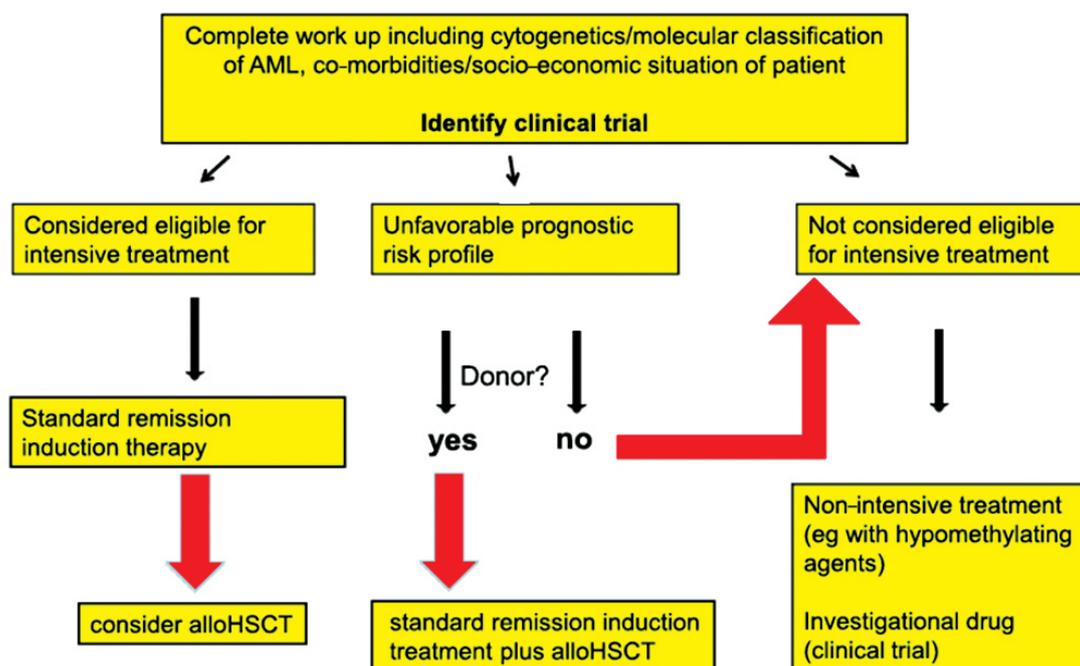
- Those who are fit and have a matched donor and accept the morbidity and mortality should be subjected to a reduced intensity conditioning allogeneic SCT if feasible. Long-term data with non-myeloablative allogeneic SCT has similar anti leukemic effectiveness compared to myeloablative allogeneic SCT. Although the leukemia relapse rates with allotransplant are much lower compared to conventional chemotherapy, the mortality rates with a non-myeloablative allogeneic SCT in the elderly range between 20-35%.<sup>[16]</sup>
- For patients who decline an allogeneic SCT or in whom it cannot be undertaken, any of the post remission options specified for those with favorable characteristics may be chosen

### 3. *Not a candidate for intensive therapy*

- Best supportive care with hydroxyurea and transfusion
- Continuation of hypomethylating agents or low dose cytosine arabinoside until progression or intolerance

## Algorithm for the treatment of elderly AML

(reproduced from Ossenkoppele et al, Blood 2015;125(5):767-774)<sup>[17]</sup>



## REFERENCES

1. Smith M, Barnett M, Bassan R, et al. Adult acute myeloid leukaemia. *Crit Rev OncolHematol* 2004;50:197-222.
2. Wheatley K, Brookes CL, Howman AJ, et al; United Kingdom National Cancer Research Institute Haematological Oncology Clinical Studies Group and Acute Myeloid Leukaemia Subgroup. Prognostic factor analysis of the survival of elderly patients with AML in the MRC AML11 and LRF AML14 trials. *Br J Haematol* 2009;145 (5):598-605.
3. Kantarjian H, O'brien S, Cortes J, et al. Results of intensive chemotherapy in 998 patients age 65 years or older with acute myeloid leukemia or high-risk myelodysplastic syndrome: predictive prognostic models for outcome. *Cancer* 2006;106 (5):1090-1098.
4. Malfuson JV, Etienne A, Turlure P, et al; Acute Leukemia French Association (ALFA). Risk factors and decision criteria for intensive chemotherapy in older patients with acute myeloid leukemia. *Haematologica* 2008;93 (12):1806-1813.
5. Krug U, Rollig C, Koschmieder A, et al. Complete remission and early death after intensive chemotherapy in patients aged 60 years or older with acute myeloid leukaemia: a web-based application for prediction of outcomes. *Lancet* 2010;376:2000-8.
6. Pollyea DA, Kohrt HE, Medeiros BC. Acute myeloid leukaemia in the elderly: a review. *Br J Haematol* 2011;152 (5):524-42.
7. Gardin C, Chevret S, Pautas C, et al. Superior long-term outcome with idarubicin compared with high-dose daunorubicin in patients with acute myeloid leukemia age 50 years and older. *J Clin Oncol* 2013;31:321-27.
8. Burnett AK, Russell NH, Hills RK, et al. Addition of GemtuzumabOzogamicin to Induction Chemotherapy Improves Survival in Older Patients With Acute Myeloid Leukemia. *J Clin Oncol* 2012;30:3924-31.
9. Castaigne S, Pautas C, Terre C, et al. Effect of gemtuzumabozogamicin on survival of adult patients with de-novo acute myeloid leukaemia (ALFA-0701): a randomised, open-label, phase 3 study. *Lancet* 2012;379:1508-16.
10. Amadori S, Suci S, Stasi R, et al. Sequential combination of gemtuzumabozogamicin and standard chemotherapy in older patients with newly diagnosed acute myeloid leukemia: results of a randomized phase III trial by the EORTC and GIMEMA consortium (AML-17). *J Clin Oncol* 2013;31:4424-30.
11. Faderl S, Ravandi F, Huang X, et al. A randomized study of clofarabine versus clofarabine plus low-dose cytarabine as front-line therapy for patients aged 60 years and older with acute myeloid leukemia and high-risk myelodysplastic syndrome. *Blood* 2008;112:1638-45.
12. Burnett AK, Russell NH, Hunter AE, et al. Clofarabine doubles the response rate in older patients with acute myeloid leukemia but does not improve survival. *Blood* 2013;122:1384-94.
13. Fenaux P, Mufti GJ, Hellstrom-Lindberg E, et al. Azacitidineprolongs overall survival compared with conventional care regimens in elderly patients with low bone marrow blast count acute myeloid leukemia. *J Clin Oncol* 2010;28:562-69.
14. Kantarjian HM, Thomas XG, Dmoszynska A, et al. Multicenter, Randomized, Open-Label, Phase III Trial of Decitabine Versus Patient Choice, With Physician Advice, of Either Supportive Care or Low-Dose Cytarabine for the Treatment of Older Patients With Newly Diagnosed Acute Myeloid Leukemia. *J Clin Oncol* 2012;30:2670-77.
15. Burnett AK, Milligan D, Prentice AG, et al. A comparison of low dose cytarabine and hydroxyurea with or without all-trans retinoic acid for acute myeloid leukemia and high-risk myelodysplastic syndrome in patients not considered fit for intensive treatment. *Cancer* 2007;109:1114-24.
16. Farag SS, Maharry K, Zhang MJ, et al. Comparison of reduced intensity hematopoietic cell transplantation with chemotherapy in patients age 60-70 years with acute myelogenous leukemia in first remission. *Biol Blood Marrow Transplant* 2011;17:1796-1803
17. Ossenkoppele G, Löwenberg B. How I treat the older patient with acute myeloid leukemia. *Blood* 2015;125(5):767-74.

**PROGNOSTIC FACTORS IN ACUTE MYELOID LEUKAEMIA  
SUMMARY OF THE PROGNOSTIC MARKERS IN AML**

Risk Category	Patient related	Disease Related				Treatment Responses				MRD	
		Genetic	Molecular	Secondary Leukemias	Scoring	Peripheral blast reduction	ALC recovery	Early BM assessment	Leukemic Stem cell Frequency	Leukemia associated IPT	Mutation specific PCR
<b>Favourable</b>	Younger age Financial Reimbursement	t(8;21), Inv (16)	NPM1 without FLT3/ITD; CEBPA		PINA-OS PINA-RFS	<3d (Microscopic) <4d (FCM – 90% reduction)	ALC>500 at D15/22/28				MRD2 – 3log reduction (CBF Leukemia) PCR negativity post induction / completion of therapy
<b>Intermediate</b>		Cytogenetically Normal AML	NPM1 with wFLT3/ITD			3-6 d (Microscopic) 5d (FCM – 90% reduction)					
<b>Adverse</b>	65y ECOG 2 Financial constraints High ADL score (geriatric patients)	abn(3q) inv(3) (q21q26) t(3;3) (q21;q26) abn(5q)/del (5q), -5, -7, abn(7q)/del(7q), t(11q23) complex cytogenetic aberrations		t-AML (secondary to chemotherapy/radiotherapy) secondary to MDS		>6d (Microscopic) >5d (FCM – 90% reduction)	ALC<500 at D15/22/28 In patients <21y ALC<350 at (D15,21,28)	BM blasts >10% at D15	Positive expression of CD34, CD25, CD47, CD56 on leukemic blasts	MRD > 0.1%	>1 mutant NPM1 copies/ 100 ABL1 copies Postchemo>10 mutant NPM1 copies/ 100 ABL1 copies after Allo HSCT

Mandatory       Optional

Current guidelines on prognostication of AML are based on the review of the latest literature available on the subject, with few tests/ laboratory evaluation marked as mandatory and others as optional. We would like to reiterate the fact that these are mere guidelines and may be modified wherever necessary based on the local needs/ available logistics as deemed fit in the best interest of the patient owing to the resource constraints in our settings. It is important to differentiate the thin line between prognostic factors versus predictive factors. Prognostic factors help in predicting prognosis in the background of a standard therapy, whereas predictive factors, predicts the modality of therapy with best outcome. Biomarkers sometimes can both be used as prognostic or predictive markers depending on the clinical circumstances.

Prognostic factors are classified into patient related factors, disease related factors and MRD analysis as enumerated in figure 1. Patient related factors predict early death, whereas the disease related factors are markers of disease relapse and MRD analysis suggests the disease free relapse and overall survival.

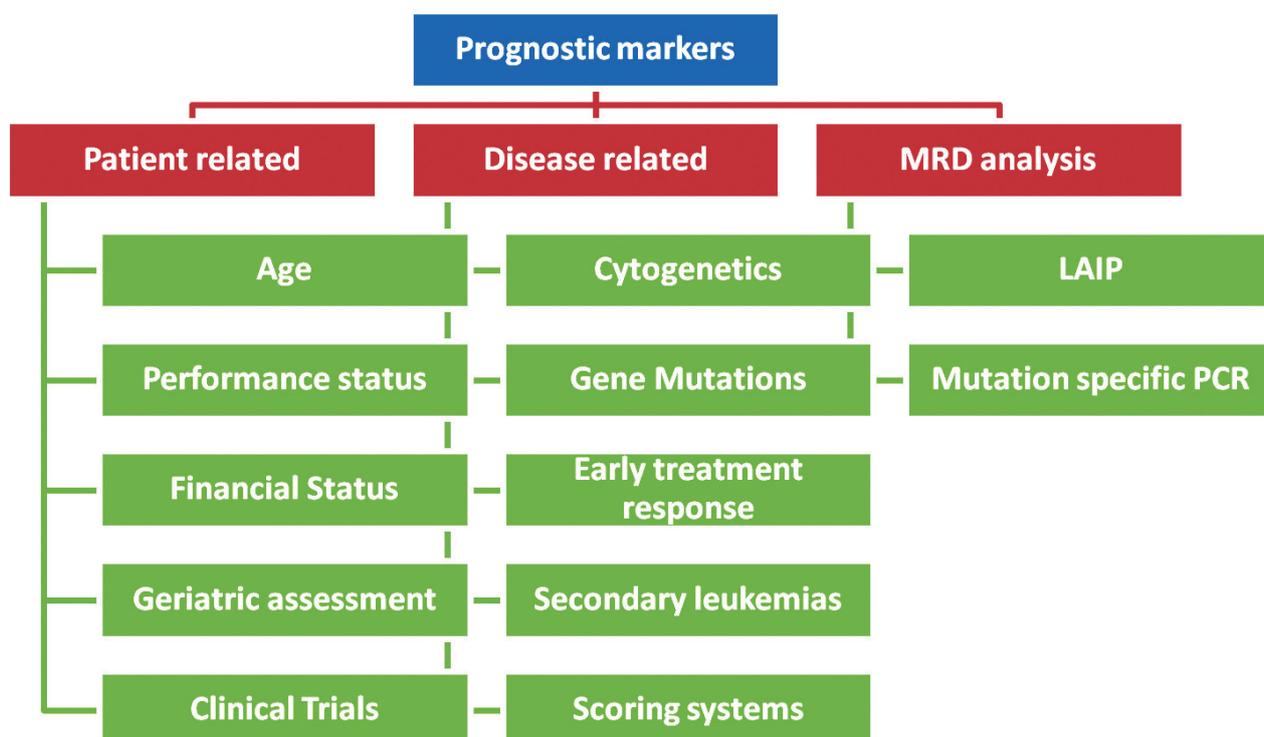


Figure 1: Classification of the prognostic markers

## PATIENT RELATED FACTORS:

- **Age**
  - Younger patients do better than older at any age.<sup>1</sup>
  - Elder patients do poorly due to increased co-morbidities, inability to tolerate intensive chemotherapy regimens, higher probability of decreased PS, increased chances of secondary AML (Post MDS, t-AML), and increased chances of poor genetic factors.
  - Age > 65y have dismal prognosis irrespective of other factors.<sup>2</sup>
  - Influence of age on prognosis is evident either above the age of 50 years<sup>3</sup> and below the age of 30 years.<sup>4</sup> Biological age is more important than the physical age.
- **Performance status (PS)**
  - Eastern Cooperative Oncology Group (ECOG) performance status > 2 have poor outcomes irrespective of the age of the patient.<sup>5</sup>
  - Combination of age with PS is a good predictor of poor outcomes in elderly and predicts death in first 28 days of induction therapy.<sup>6</sup>
  - Data from elderly underestimate this risk factor owing to the selection bias in larger trial (where patients with organ dysfunction, poor PS are excluded), physician decision to exclude these patients from intensive therapies that they wouldn't tolerate/ benefit from treatment and many elderly patients refusing treatment.
  - Also PS in elderly is a dynamic feature owing to less body reserves and physicians should desist from therapeutic decisions based on one time PS.
- **Financial status/ reimbursement status**
  - It plays a major role in Indian scenarios with data from our country suggestive of statistically significant differences in affordable patients as compared to patients with poor financial status and with no reimbursement.<sup>7,8</sup>
- **Geriatric Assessment** – ADL scoring.
- **Clinical trials:** Entering carefully planned prospective clinical trials is the most positive prognostic factor irrespective of age of the patient/ type of AML.

## DISEASE RELATED FACTORS

- **Cytogenetics** - Recurrent cytogenetic abnormalities are classified into three prognostic risk groups. It is presently mandatory to evaluate for cytogenetic anomalies as part of management plan of AML.
  - Favourable – t(8;21), Inv (16) – CBF-AML
    - ◆ Role of CBF
      - It has an independent prognostic impact
      - Concurrent genetic abnormalities abbreviates the favourable risk
      - It can be used as MRD marker (discussed below).
      - In all patients with CBF leukemia, KIT mutation analysis should be done.

- Intermediate – Cytogenetically normal (CN) –AML
- Poor - abn(3q) (excluding t(3;5)(q25;q34)), inv(3)(q21q26)/t(3;3) (q21;q26), abn(5q)/del (5q), -5, -7, abn(7q)/del(7q), t(11q23) (excluding t(9;11) (p21–22;q23) and t(11;19)(q23;p13)), t(9;22) (q34;q11), -17,abn(17p) and complex cytogenetic aberrations with at least three aberrations excluding good-risk abnormalities.
- **Gene mutations:**
  - Multiple genetic mutations/ molecular markers have been studied for their prognostic impact as enumerated in table 2. Lately various targeted therapies against adverse genetic mutations are underproduction and in different stages of clinical trials. We also have enumerated the molecular markers with an impact on the therapeutic decision in table 2.
- **Secondary Leukemias**
  - AML occurring post therapy (Chemo, Radiotherapy), exposure to radiation, post MDS/ MPN/ CMML are labelled as secondary leukemias.
  - They carry as a group a poor prognosis owing to the commonly associated complex/ poor cytogenetic abnormalities, expression of efflux pumps/ MDR receptors on leukemic cells and prior exposure to variable chemotherapeutic agents.
- **Common Laboratory parameters**
  - LDH, B2M, Hb, Platelets, High TLC: Prognostic impact small when compared to age and cytogenetic risk factors.
- **Genetic risk scores**
  - As it is clear that the AML is caused by more than one molecular/ genetic aberration, investigators have tried to incorporate multiple factors as scoring systems.
  - Some incorporated only the genetic mutation levels whereas others incorporated both genetic and clinical features (IPRS)
  - Major deficiency –
    - ◆ lack of standardisation in reporting and testing the genetic aberrations
    - ◆ Except NPM1, FLT3/ITD and CEBPA – evaluation of other molecular markers currently not recommended.
- **Scoring systems to incorporate clinical and genetic factors**
  - PINA<sub>OS</sub> – Prognostic scoring in CN-AML – Overall survival
  - PINA<sub>RFS</sub> –Prognostic scoring in CN-AML – Relapse free survival
  - Advantages
    - ◆ Only incorporates recommended molecular markers
    - ◆ Also incorporated clinical features with highest impact
    - ◆ Externally validated

## EARLY TREATMENT RESPONSE AS PROGNOSTIC MARKERS FOR OS/RFS

- **Morphology:** Minimal residual disease (MRD) detection by cytomorphology and cytochemistry (MPO, NSE) is very limited. Complete remission at defined time points, as described below, both on peripheral smears and bone marrow have been studied. Definition of complete remission needs revision (e.g., **CRp** – CR with platelets < 100000/ $\mu$ L, **CRi** – less than 5% blasts on marrow provided > 200 cells enumerated on marrow irrespective of peripheral blood counts).<sup>9</sup>
- 1. **Peripheral blood blast clearance:** Blast clearance as assessed by *microscopic* examination (Cytomorphology)
  - i. Three risk groups have been studied based on blasts clearance <3d, 4-5d, >6d.<sup>10</sup>
  - ii. Blast clearance from periphery within 6d post induction therapy is an independent predictor.<sup>11</sup>
- 2. **Absolute lymphocyte recovery**
  - a. ALC > 500/ $\mu$ L at all defined time points (D15, 22, 28) post-induction predicts improved survival<sup>12</sup>
  - b. In patients younger than 21y, ALC <350/ $\mu$ L at all time points (D15, 21,28) post induction predicts poor survival<sup>13</sup>
- 3. **Early bone marrow assessment** is defined as BMA between D12-D16 from onset of induction.
  - a. D-15 BM blasts > 10% predicts poor survival (German AMLCG group)<sup>14</sup>
  - b. In patients with D-15 BM blasts > 40%, benefit in CR rate is noted with HiDAC over StandardAra-C consolidation.<sup>15</sup>
- Flow cytometric analysis(FCM)
- 1. **Blast clearance** –
  - a. Blast clearance on FCM as early as Day+2 post-induction can differentiate responders from non-responders<sup>16</sup>
  - b. Patients with >90% reduction of blasts on FCM into three groups as <4d, 5d, >5d can also identify the prognostic risk groups<sup>17</sup>
- 2. **Leukemic stem cell frequency**
  - a. Increased leukemic stem cells in the periphery – Confer resistance to therapy
  - b. Expression of CD34, CD32, CD25, CD47, CD96, CD123 and CD56 – LSC
  - c. Flourescent activated stem cell sorting – FACS – positive expression of CD34, CD25, CD47, CD56 on leukemic blasts – Poor prognosis.
  - d. CD56 – Associated with t(8:21)
- **WT1 transcript levels**
  - Aberrantly overexpressed in AML. <sup>18,19</sup>
  - Ratio of Day1/Day5 WT1 transcripts less than 5.82 (suggesting slow fall of WT1 post therapy) is suggestive of poor survival<sup>20</sup>
  - >2 log reduction after induction is associated with reduced relapse rate<sup>21</sup>

## MRD ANALYSIS AS PROGNOSTIC MARKERS FOR OS/ RFS

- MRD analysis is an extremely important tool to predict the outcomes post therapy. At present we do not alter the therapy based on post induction MRD, but studies to implicate the same are underway.
- ***Leukemia associated Immunophenotype (LAIP)***
  - The advent of multicolour FCM and increasing availability of newer fluorochrome labelled monoclonal antibodies in comparison to standard 3-colour approach have increased the sensitivity of the MRD.<sup>22</sup> The time points of MRD analysis differs in various studies. Positive MRD status is associated with poor outcomes irrespective of time points.
  - Studies on LAIP were conducted primarily in children and young adults with only one study in adults with good/intermediate risk CG showing correlation of MRD analysis post-consolidation with survival.<sup>23</sup>
  - Patients in morphological CR with a MRD > 0.1% have higher chances of relapse.<sup>24</sup>
  - Positive flow MRD status prior to Allo-HSCT in patients both in CR and incomplete CR were associated with poor outcomes (OS).<sup>25</sup>
- ***Mutation specific PCR***
  - Most of the AML patients are associated with genetic mutations/ chromosomal abnormalities which can potentially be quantified, but the sensitivities and specificities need to be determined in long term studies.<sup>26</sup> The major limitations are lack of standardisation/ type of assay used with only few of these (as described below) being standardised.<sup>27</sup>

### ***a. Patients with core binding factor Leukemias***

- i. In patients with core binding factor leukemias, major molecular remission is defined as 3 log reduction of the transcripts after MRD2 (Induction + 1 Consolidation). Prospective study by investigators of French Intergroup trial suggested this as the only independent risk factor for relapse.<sup>28</sup> It didn't translate into improved survival in different observational studies due to fallacies in study designs as patients not achieving MMR are transplanted rather than continuing with two further HiDAC consolidations.
- ii. Persistence/ reoccurrence of positive MRD after induction or first consolidation was associated with poor outcomes in multiple studies.<sup>29,30</sup>

### ***b. NPM1***

- i. In AMLSG group, PCR negativity post induction/ completion of therapy was associated with improved OS. This group used PCR cutoff of more than 2 mutant NPM1 copies/ 100 ABL1 copies in patients post Allo HSCT for predicting relapse.<sup>31</sup>
- ii. SAL group used different PCR cutoffs in post chemotherapy and post AlloHSCT scenarios for predicting poor survival of more than 1 mutant NPM1 copies/ 100 ABL1 copies and more than 10 mutant NPM1 copies/ 100 ABL1 copies respectively.<sup>32</sup>
- iii. One major fallacy in using NPM1 as a MRD marker is that 10% of the patients loose NPM1 at relapse despite positivity at diagnosis.<sup>33</sup>

**c. Other genetic alterations**

- i. Lately there is increasing literature on utility of FLT3-ITD/ CEBPA/ MLL-PTD as MRD markers. But data is limited to case series with larger studies needed to prove there clinical benefit in clinical practise.
- ii. Utility of FLT3-ITD is increasing with the advent of FLT3 tyrosine kinase inhibitors (lestaurtinib, sorafenib, midostaurin and quizartinib) which can be administered in MRD positive individuals. But inherently FLT3-ITD estimation has two major disadvantages: Firstly, as seen in NPM1, there is loss of this mutation during relapse/ disease progression. Secondly, the limitations with FLT3 mutation testing.
- iii. The major limitation with FLT3 mutation testing includes lack of sensitivity. The sensitivities of PCR assays used for other gene fusions in AML can be increased by increasing the number of cycles. But in FLT3/ITD assay PCR primers used to amplify the mutant allele also amplify the WT allele, with shorter WT allele having a competitive advantage over the mutant allele thus decreasing sensitivity on increasing the number of cycles. Also as the FLT3/ITD evolves from diagnosis to relapse, the mutant to WT allelic burden and length of mutant allele also typically evolves thus making it an unsuitable marker for MRD.<sup>34</sup>

**SUGGESTED GUIDELINES FOR THE INVESTIGATORS DESIGNING STUDIES ON PROGNOSTICATION IN AML**

<b>Various outcomes which can be utilised in the studies designed for prognostication</b>
Overall survival (Most ideal) – Should definitely comment on this Relapse free survival, Relapse rate, Cumulative incidence of relapse MRD by FCM/ specific molecular PCR Rate of CR following 1st Induction
<b>Utility of scoring systems – Major advantages on scoring systems</b>
Incorporate multiple factors simultaneously Inter-relation of the various factors can be studied Wholesome picture – rather than piece meal picture Enables fine-tuning of the prognostic impact of individual factors

**Table 2: Gene mutations and their relevance in prognostication of AML**

Factor	Comments	Implications on therapeutic decisions
Mandatory molecular markers evaluation		
NPM1 mutational status/FLT3-ITD <sup>35,36</sup>	Predicts favourable prognosis of mutated NPM1 and No FLT3-ITD in CN-AML after induction/ consolidation. Most common molecular aberration seen in ~ 50% in CN-AML and 9-20% in Adult AML. 36-50% of NPM1 positive cases have associated FLT3/ITD. Independent prognostic factor irrespective of FLT3/ITD. Prognostic, but no clear predictive value for Post-remission treatment.	Under debate

CEBPA mutations <sup>37, 38</sup>	Favorable prognosis of biallelic mutations in CN-AML after induction/ consolidation Positive prognostic impact restricted to bi-allelic variant This positive prognostic impact of mutation can also be seen in intermediate risk cytogenetic abnormalities. FLT3/ITD neutralises the positive impact of CEBPA	No
KIT mutations <sup>39, 40</sup>	Adverse in CBF leukaemias when present in a high mutant to wildtype allelic ratio Difference between NCCN and ELN guidelines as KIT mutations is mandatory as per NCCN Especially in co-occurrence with CBF – 15-46% Activating mutation in tyrosine kinase – Exon 8 Use sequential method rather than the parallel for KIT mutation Basis for dasatinib in CBF-AML	No
FLT3/ITD	Occurs in 35% of adult AML Adverse prognostic impact It neutralizes the positive impact of favourable factors. Complex interactions with concurrent genetic abnormalities. Difficulties in testing due to lack of standardization of testing. Relevance of ITD size/ location in prognosis is unclear. Only prognostic relevance of FLT3 with internal tandem duplication and not for tyrosine kinase domain mutation	Yes/ Debatable
Gene mutation with probable prognostic impact and not mandatory for evaluation		
DNMT3A mutations <sup>41</sup>	Considered negative prognostic marker by most authors/ groups but literature is divided on verdict. This discrepancy could be due to the influence of anthracycline dose in induction on prognostic impact of DNMT3A Better outcome of with higher doses of Daunorubicin and hypomethylating agents (decitabine/ Azacytidine)	Yes
TET2 mutations <sup>42</sup>	Negative prognostic in CN-AML and favourable genetic aberrations but no independent prognostic significance Higher response rate after 5-azacytidine in patients with 20-30% BM blasts	Yes
RUNX1 mutations <sup>43</sup>	Occur in 6-26% of AML Adverse outcomes	No
MLL-PTD <sup>44</sup>	Adverse to no impact PTD seen in 5% of AML	No
ASXL1 mutations <sup>45, 46</sup>	Adverse in CN-AML patients and older patients with favorable genetic risk	No
TP53 mutations <sup>47</sup>	Associated with complex cytogenetic aberrations No independent prognostic value among all AML Predicts poor survival in AML patients with complex cytogenetic aberrations outweighing impact of monosomal karyotype	No
WT1 mutations	Adverse to no impact in CN-AML patients	No
RAS mutations <sup>48</sup>	12-27 % of AML patients No prognostic impact except one study showing prolonged remission for CR patients treated with HiDaCconsolidation	Yes/ Debatable
Microenvironment molecular markers – Being studied for prognosis – Only in trial settings		
Osteopontin <sup>49</sup>	Increased expression: adverse	No
CXCR4 <sup>50, 51</sup>	Increased expression: adverse	No
MN1, MECP2, ERG, BAALC, ID1 <sup>52, 53</sup>	Increased expression: adverse	No
KMT2E <sup>54</sup>	Increased expression: favourable	No

**Introduction:**

APL is a particularly aggressive subtype of AML, comprising approximately 10% of AML cases. Acute promyelocytic leukemia (APL) is a subtype of acute myeloid leukemia (AML) with distinct molecular and clinical features and characterized by the presence of a reciprocal translocation involving a portion of the retinoic acid receptor alpha gene (RAR ) on chromosome 17 and a portion of a partner gene which in 95% of cases is the PML gene on chromosome 15, denoted as t(15;17)(q22;q21)<sup>1</sup>. However, APL is biologically, clinically and prognostically distinctively different from other subtypes of AML. The most dramatic change in the management of this condition came with the introduction of all-trans retinoic acid (ATRA) by Chinese investigators in 1986<sup>2</sup>. Subsequently through a series of clinical trials the importance of combining anthracycline with ATRA in induction, intensification of the chemotherapy schedule with repeated cycles of myelotoxic combination chemotherapy along with a two year maintenance schedule was established and lead to long term survival rates exceeding 80% even in high risk subsets (WBC count at presentation  $>10 \times 10^9/Lt$ ).

In parallel and again from China, in the late 1970's, arsenic trioxide (ATO) was found to be effective in the management of APL with the publication by Shen et al on its role in the management of relapsed and refractory APL<sup>3</sup>. Subsequent work drew attention to synergistic effect between ATRA and ATO in eradicating leukemic cells in APL<sup>2</sup>. This approach was soon validated in a clinical trial by the Shanghai group, though concerns remain about the increased incidence of late relapses on long term follow up, especially in the high risk subset<sup>4</sup>.

The first large multi-centre Phase II study that reported on the synergistic effect of ATRA and ATO combination with a conventional anthracycline administration being limited to induction therapy came from the Australasian Leukaemia and Lymphoma Group (ALLG) APL4 study which was initially published in 2012<sup>8</sup> and long term follow up data of this study was recently reported. Along the same lines but in a randomized controlled trial design and with a therapy structure that completely eliminated chemotherapeutic agents and maintenance therapy was reported by the combined Gruppo Italiano Malattie Ematologiche dell'Adulto, German–Austrian Acute Myeloid Leukaemia Study Group, and the Study Alliance Leukaemia (GIMEMA-AMLSG-SAL) APL 0406 study in 2013<sup>9</sup>. The latter study was limited to low and intermediate risk patients which account for two third to three quarters of patients with APL. It demonstrated that the combination of ATRA and ATO without chemotherapy in induction and consolidation (4 courses) without maintenance therapy was superior to a conventional ATRA plus chemotherapy induction with repeated cycles of myelotoxic chemotherapy and two years of maintenance therapy.

An increased focus needs to be given to early diagnosis and supportive care in induction where the

maximum mortality is likely to occur. This is evidenced by early death rate below 10% reported for patients enrolled in clinical trials compared to the general population where early mortality rates are still high. (Lehmann S et al, Leukemia 2011; 25(7): 1128-34)

**Diagnosis:** A high index of suspicion is needed to make a diagnosis of APL. It is mandatory to establish diagnosis either using karyotype for t (15;17) or using FISH and RT-PCR for PML/RARA at the earliest. A high index of suspicion must be maintained even or the morphology is not classical in the presence of the classical immunophenotype and in the presence of coagulopathy.

**Initiation of therapy:** One should consider referring all patients with this diagnosis to a tertiary referral centre for further management. It is very important to start therapy as soon as the diagnosis is suspected. Initial therapy should start with ATRA as soon as a diagnosis is suspected. Along with this supportive care for coagulopathy should be initiated at the earliest (see latter under supportive care). Once definitive molecular diagnosis is established the definitive regimen that one intends to use based on the risk stratification needs to be started.

**Risk stratification:** Conventional risk stratification is based on a combination of the white cell count and platelet count at presentation<sup>10</sup>.

Good risk: WBC <10,000/mm<sup>3</sup> and platelet count > 40,000/mm<sup>3</sup>

Intermediate risk: WBC <10,000/mm<sup>3</sup> and platelet count <40,000/mm<sup>3</sup>

High risk: WBC >10,000/mm<sup>3</sup>

Good and intermediate risk patients account for 60% to 75% of patients with APL.

In the context of India it may also be important to recognize a subset of patients with a WBC count of <5000/mm<sup>3</sup> and platelet count of >20,000/mm<sup>3</sup> that represent a very good risk subset that does not have a risk of coagulopathy related deaths with standard supportive care and has a 100% overall survival with single agent arsenic trioxide<sup>7</sup>.

There is no substantial evidence that the use of additional molecular markers such as FLT3-ITD contribute significantly to risk stratification or prognostication with current combined ATO+ATRA based regimens.

**Treatment regimen of good and intermediate risk patients:** Based on the randomized study comparing ATO+ATRA versus conventional intensive ATRA + combination chemotherapy it would be reasonable to consider the regimen of ATO+ATRA as standard of care<sup>9</sup>. The regimen used is summarized below in

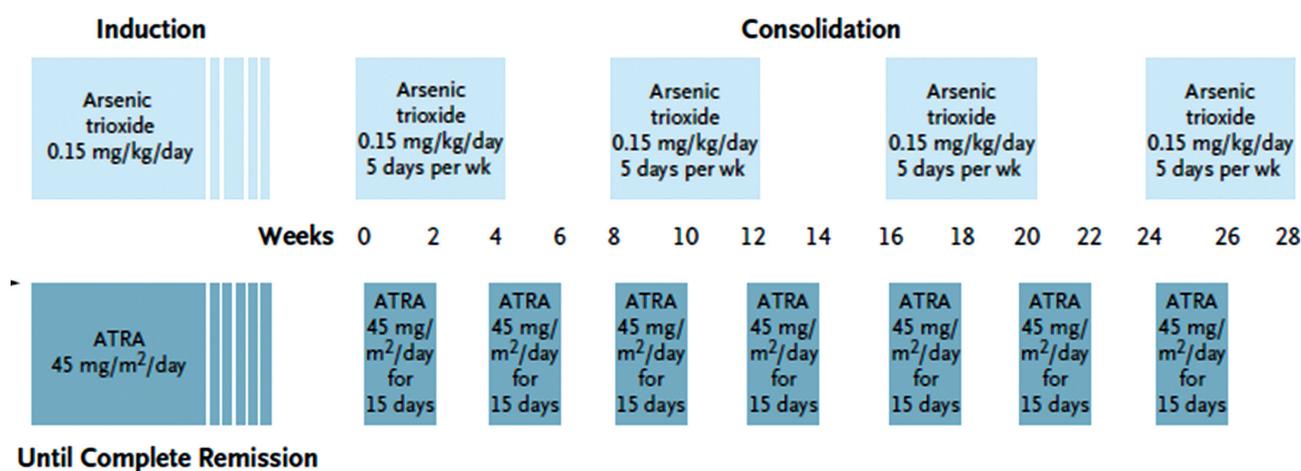


Figure 1: Overview of combination regimen for treatment of good and intermediate risk APL

Figure 1:

Some elements that need to be highlighted with this regimen is (i) the absence of a maintenance course with this regimen (ii) use of prophylactic steroids (iii) dose of ATRA at full dose (iv) administration of ATO at standard doses but only 5 days/week.

It is important to note that in the above regimen there is no maintenance therapy. Several groups have published large trials with excellent outcomes. However to achieve the expected results, one needs to use the regimen consistently through all components and not mix induction from one trial with consolidation from another.

**Treatment of High risk Patients:** Based on the recently published Phase II Australasian Leukaemia and Lymphoma Group (ALLG) APLM4 study and the long term follow up data it would be reasonable to consider this regimen for the high risk subset<sup>8</sup>. This regimen includes ATO and ATRA along with an anthracycline and is less chemotherapy intensive than previously reported studies which were all Phase II studies as well<sup>11,12</sup> and has results which are comparable if not superior. The regimen is summarized in Figure 2. The important elements of this regimen are the use of age adjusted dose of Idarubicin that is limited to induction therapy, absence of cytarabine in any phase of treatment, combination of ATO and ATRA in all phases and only two courses of consolidation therapy in contrast to the GIMEMA-AMLSG-SAL APL 0406 study. It is important to note that the maintenance component of the regimen, albeit modified, is retained.

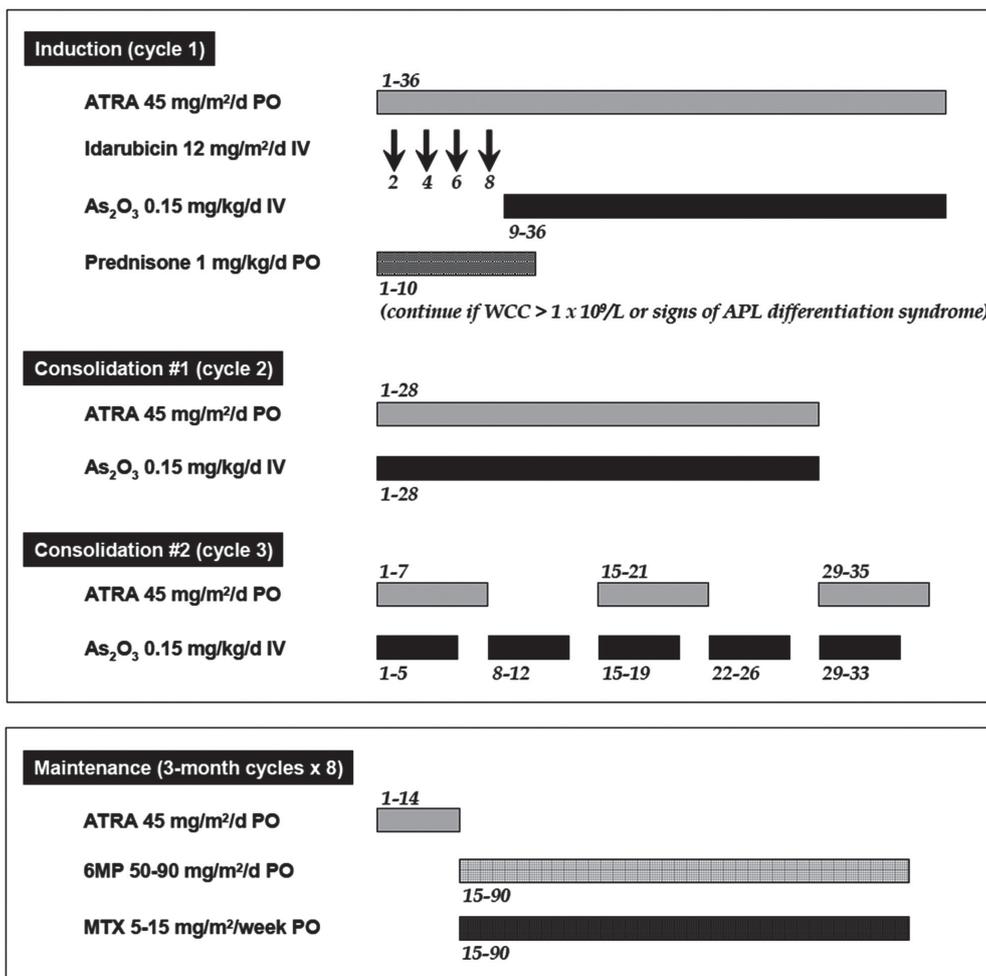


Figure 2: Regimen to treat High risk APL.

**Role of low cost single agent ATO regimen:** There is a need to explore the possibility of single agent ATO in a subset of low risk patients. Previously published literature<sup>6,7</sup> and experience would suggest that it would be possible to cure APL in about 40-60% of patients with single agent ATO, for the remaining low and intermediate risk patients probably a combination of ATRA and ATO would suffice as demonstrated in the GIMEMA-AMLSG-SAL APL 0406 study, without maintenance therapy in either of these hypothetical subsets. For the truly high risk patients addition of an anthracycline, at least, in induction is probably warranted and the role of maintenance therapy remains to be further evaluated.

**Supportive care:** This is a critical component in the management of APL especially in the induction phase<sup>12</sup>.



**Figure 1. Regimen of single-agent arsenic trioxide.** Arsenic trioxide was administered intravenously at a dose of 10 mg for adults and 0.15 mg/kg for pediatric patients until CR. Another 4-week course (consolidation) was administered after a 4-week interval for those achieving CR. Subsequently, after a second 4-week interval it was administered for 10 days/month for 6 months (maintenance).

Figure 3: Potential regimen to consider for a very good risk subset of patients defined by WBC count of  $<5000/\text{mm}^3$  and platelet count of  $>20,000/\text{mm}^3$ .

**During induction:** Monitor counts daily till stable, followed by 2 to 3 times/week till recovery from cytopenia. Monitor PT, APTT and fibrinogen at admission and daily for the first week. Second week, if stable, parameters normal and no bleeding: monitor on alternate days (daily if abnormal, symptoms of bleeding). If PT / APTT abnormal  $\pm$  active bleeding transfuse fresh frozen plasma [FFP] 15 ml/kg, if active bleeding repeat till normal. If Fibrinogen  $<140\text{mg}\%$  transfuse 1-2 units of cryoprecipitate / 10kg and recheck fibrinogen the next day (more frequently if active bleed). Transfuse Platelet concentrates to maintain platelet count  $>30,000/\text{mm}^3$  in the absence of bleeding and  $>50,000/\text{mm}^3$  in the presence of bleeding.

Day 1 if platelet count  $<30,000/\text{mm}^3$  transfuse 2 Platelet Rich Concentrate (PRC) twice daily, after first infusion check platelet count after 1 - 4 hours, based on this value increase PRC transfusion support to achieve the above target (if possible). A high clinical suspicion of intra-cranial bleeding is to be maintained till recovery from cytopenia. If patient complains of headache transfuse 4 PRC urgently and an urgent CT scan must be done (do not wait for CT scan to be done or reported). If a bleed is present transfusion support should be increased to target a platelet count of  $50,000/\text{mm}^3$  and transfuse FFP's/Croprecipitate to maintain normal hemostatic parameters. There is no role for the use of tranexamic acid or heparin in induction. There are numerous case reports of significant adverse effects, including life threatening complication with the use of tranexamic acid in induction. Factor VIIa concentrate can be used for severe life threatening hemaostatic emergencies though there is limited data on its role in this situation.

Leukocytosis can occur with this therapy which can result in DIC, leukostasis and a differentiation syndrome. Hydroxyurea will be administered to control leucocytosis if required.

A broad guideline for use of hydroxyures in addition to standard drugs for control of leucocytosis is as follows:

WBC > 5000 - 10000/mm <sup>3</sup>	Hydroxyurea 500 mg OD
WBC > 10000 - 15000/mm <sup>3</sup>	Hydroxyurea 500 mg BD
WBC > 15000 - 20000/mm <sup>3</sup>	Hydroxyurea 500 mg TID
WBC > 20000 - 50000/mm <sup>3</sup>	Hydroxyurea 500 mg QID
WBC > 50000/mm <sup>3</sup>	Hydroxyurea 1.0 gm QID

**Electrolytes:** Monitor alternate day (more frequently if required). Maintain K<sup>+</sup>>4mEq/Lt and Mg<sup>+</sup> >2mg% (through induction)

**ECG:** Monitor once a week during induction only (more frequently if required). Consider stopping ATO if corrected Qtc>500mSec (Note: Bazetts formula over corrects in Fridericia or Framingham (used in Lo-coco trial) formula is preferred the setting of tachycardia due to any etiology. More significant if Qtc prolonged with HR < 100 / min)

**Differentiation Syndrome:** Important points to note about differentiation syndrome is as follows: The presence of three or more features is sufficient for confident clinical diagnosis of DS in the absence of another cause.

- Fever 38C
- Weight gain > 5 Kg
- Hypotension
- Dyspnea
- Radiographic abnormalities

1. Differentiation syndrome develops after a median of 11 days of Rx [range: 2 - 45].
2. Clinical diagnosis - there is no definite diagnostic criteria.
3. Major manifestations [>10% incidence] include:
  - Respiratory distress
  - Hypotension
  - Fever
  - Pulmonary oedema, pulmonary infiltrates
  - Headache
  - Pleural or pericardial effusion
  - Congestive cardiac failure
  - Acute renal failure

For patients at high risk (WBC count > 10,000/mcL) of developing differentiation, syndrome, initiate prophylaxis with corticosteroids either prednisone (0.5 mg/kg) from day 1 or dexamethasone 10 mg every 12 hour. The steroid dose should be tapered over a period of several days. It is recommended that the prophylaxis regimen follow the specific treatment protocol used.

## Treatment of Differentiation syndrome

If using prophylactic steroids use guidelines based on regimen you are using. If not using prophylactic steroids or if differentiation syndrome develops in spite of prophylaxis consider doing the following:

Stop ATO and ATRA if severe (continue if mild with steroid cover)

Inj Dexamethasone 10 mg IV twice daily 3 - 5 days.

If syndrome resolves re-institute ATRA and ATO at 50% of the initial dose and escalate to the full dose in 3 - 5 days if the syndrome does not recur. Preferably re-institute the drugs one at a time till back to full combination.

**During consolidation and maintenance:** There is no need to monitor coagulopathy, ECG or electrolytes on a regular basis in these phases unless clinically indicated or dictated by additional co-morbidities. Monitor blood counts once a week during consolidation and once in two weeks during maintenance. React appropriately to  $ANC < 1000/mm^3$ . Consider stopping therapy for a period of 5-7 days in such a situation. Re-challenge with full doses and consider dose reductions if recurs.

Marrow evaluation is not recommended until recovery of blood counts, usually 4 to 6 weeks after induction. Cytogenetic analysis is usually normal by this point but molecular remission often requires at least 2 cycles of consolidation.

**Molecular monitoring:** Ideally molecular monitoring should be done following the first consolidation on a bone marrow specimen based on the increased sensitivity of a bone marrow aspirate compared to a peripheral blood sample<sup>13</sup>. Subsequent monitoring can be done on peripheral blood samples once in 3 months until two years from completion of the therapy. It is not practical to do bone marrow MRD monitoring in our setting. In fact, in the current scenario, it needs to be stressed that with currently used regimens including the GIMEMA-AMLSG-SAL APL 0406 study and the ALLG APL4 study the probability of demonstrating a survival advantage is practically non-existent with any level of MRD monitoring.

### Special situations:

1. Elderly or patients with significant co-morbidities: consider use of single agent ATO based regimen for these patients.
2. Pregnant women. If in first trimester recommend urgent MTP with appropriate supportive care and start treatment at the earliest. Both ATO and ATRA are teratogenic and should not be administered in this phase unless an MTP is planned. If patient refuses MTP consider management with single agent anthracycline. If in second or third trimester start treatment with ATRA and chemotherapy. There is insufficient data with ATO to recommend it at any stage of pregnancy.
3. Paediatric patients: Data suggest that lower doses of ATRA ( $25mg/m^2$ ) in any stage divided doses until clinical remission may be used in children and adolescents. (Kutny MA et al, Best Pract Res Clin Haematol. 2014 Mar;27(1) : 69-78). Arsenic trioxide consolidation allows anthracycline dose reduction for paediatric patients with acute promyelocytic leukemia (Kutny MA et al. JCO 2017; 35 (26); 3021-29).

Treatment related APL: APL may be de novo or therapy-related. Some of the following attributes of therapy-related APL (t-APL) were highlighted in a systematic review.

1. The average age of diagnosis is 47 years with high incidence in women.
2. The risk significantly declines 2 years after completion of treatment for the primary antecedent disease;
3. Breast cancer, hematologic malignancy, other solid tumors and multiple sclerosis.

#### References:

1. Sirulnik A, Melnick A, Zelent A, Licht JD. Molecular pathogenesis of acute promyelocytic leukaemia and APL variants. *Best Pract Res Clin Haematol.* 2003;16:387-408.
2. Wang ZY, Chen Z. Acute promyelocytic leukemia: from highly fatal to highly curable. *Blood.* 2008;111:2505-2515.
3. Shen ZX, Chen GQ, Ni JH, et al. Use of arsenic trioxide (As<sub>2</sub>O<sub>3</sub>) in the treatment of acute promyelocytic leukemia (APL): II. Clinical efficacy and pharmacokinetics in relapsed patients. *Blood.* 1997;89:3354-3360.
4. Zhu H, Hu J, Li X, et al. All-trans retinoic acid and arsenic combination therapy benefits low-to-intermediate-risk patients with newly diagnosed acute promyelocytic leukaemia: a long-term follow-up based on multivariate analysis. *Br J Haematol.* 2015.
5. Lehmann S, Ravn A, Carlsson L, et al. Continuing high early death rate in acute promyelocytic leukemia: a population-based report from the Swedish Adult Acute Leukemia Registry. *Leukemia.* 2011;25:1128-1134.
6. Estey E, Koller C, Cortes J, et al. Treatment of newly-diagnosed acute promyelocytic leukemia with liposomal all-trans retinoic acid. *Leuk Lymphoma.* 2001;42:309-316.
7. Mathews V, George B, Chendamarai E, et al. Single-agent arsenic trioxide in the treatment of newly diagnosed acute promyelocytic leukemia: long-term follow-up data. *J Clin Oncol.* 2010;28:3866-3871.
8. Iland HJ, Bradstock K, Supple SG, et al. All-trans-retinoic acid, idarubicin, and IV arsenic trioxide as initial therapy in acute promyelocytic leukemia (APML4). *Blood.* 2012;120:1570-1580; quiz 1752.
9. Lo-Coco F, Avvisati G, Vignetti M, et al. Retinoic acid and arsenic trioxide for acute promyelocytic leukemia. *N Engl J Med.* 2013;369:111-121.
10. Sanz MA, Martin G, Gonzalez M, et al. Risk-adapted treatment of acute promyelocytic leukemia with all-trans-retinoic acid and anthracycline monochemotherapy: a multicenter study by the PETHEMA group. *Blood.* 2004;103:1237-1243.
11. Sanz MA, Montesinos P, Vellenga E, et al. Risk-adapted treatment of acute promyelocytic leukemia with all-trans retinoic acid and anthracycline monochemotherapy: long-term outcome of the LPA 99 multicenter study by the PETHEMA Group. *Blood.* 2008;112:3130-3134.
12. Sanz MA, Grimwade D, Tallman MS, et al. Management of acute promyelocytic leukemia: recommendations from an expert panel on behalf of the European LeukemiaNet. *Blood.* 2009;113:1875-1891.
13. Grimwade D, Jovanovic JV, Hills RK, et al. Prospective minimal residual disease monitoring to predict relapse of acute promyelocytic leukemia and to direct pre-emptive arsenic trioxide therapy. *J Clin Oncol.* 2009;27:3650-3658.

**Definition**

Granulocytic sarcoma (GS) also called as myeloid sarcoma, chloroma (owing to its green colour attributed to the enzyme myeloperoxidase), myeloblastoma and extramedullary myeloid cell tumor is a rare extramedullary tumorous aggregate of malignant myeloid precursor cells that disrupts the normal architecture of the tissue in which it is found. (1). GS is included as one of the major subgroups of myeloid neoplasms and acute leukemia in the WHO classification. It may also occur as a de novo isolated leukemic tumor or precede the appearance of blood or bone marrow (BM) disease. Less often, GS may occur in association with a myeloproliferative neoplasm (MPN) or myelodysplastic disorder (MDS). Owing to the rarity of this disorder, the literature is mainly composed of small case series and case reports.

**Prevalence**

Primary form of de novo GS is relatively rare. On the other hand, secondary GS (defined as the occurrence of GS manifestation in patients with previous or current bone marrow involvement by AML, MDS, or MPD) occurs in approximately 1.4% to 9% of patients (2,3). The incidence of patients developing GS after allogeneic hematopoietic cell transplantation (HCT), manifesting as an isolated disease or accompanying BM relapse, has been reported to be 0.2%-1.3% with poor overall survival (4,5).

**Pathogenesis**

GS and other extra-medullary (EM) manifestations may result from aberrant homing signals for the leukemic blasts precluding the more common bone marrow localization. This may represent a subclone of an original AML clone in cases of concurrent presentation or in relapse. The chemokine receptors implicated in the homing and retention of AML blasts in extra-medullary sites include CCR5, CXCR4, CXCR7, CX3CR1 and also interaction between the matrix metalloproteinase (MMP)-9 and the leukocyte surface beta (2) integrin(6,7).

**Clinical presentation**

The most common locations include the soft tissue, bone, periosteum, and lymph nodes; however, this can also occur in central nervous system (CNS), oral and nasal mucosa, breast, genitourinary tract, chest wall, pleura, retroperitoneum, gastrointestinal tract and testis(8,9). In children with newly diagnosed AML, extramedullary involvement was most common in the skin (in 54%) with orbital involvement being the second most common site(10). GS is often initially misdiagnosed, in about 50% of cases when immune histochemistry is not used (11), the most common alternative diagnoses being lymphoma, small round cell tumors (neuroblastoma, rhabdomyosarcoma, Ewing sarcoma, and medulloblastoma) undifferentiated cancer, malignant melanoma, extramedullary hematopoiesis and inflammation (9,12,13).

## **Evaluation : Morphological diagnosis**

The diagnosis of GS in patients with an established leukemia is relatively straightforward and should always be included in the differential diagnosis of patients with AML who develop a soft tissue mass. Attempt should be made to obtain a tissue sample to confirm the diagnosis if the risks of biopsy are reasonable. In the absence of a clinical history of leukemia, a diagnosis of GS can be difficult; every effort to obtain a tissue diagnosis should be done.

The morphologic appearance of GS typically consists of a diffuse and infiltrative population of myeloblasts and granulocytic cell components. The malignant cells are typically large with abundant cytoplasm and large nuclei. Importantly, the neoplastic cell lineage should be consistent with the underlying leukemia. Immuno-histochemistry and immunophenotyping are crucial for the accurate diagnosis of GS. According to the WHO 2008 classification, cytochemical stains should include chloroacetate esterase, myeloperoxidase (MPO) and nonspecific esterase. MPO staining is very often positive in the malignant cells of GS, which is a quick way for establishing the diagnosis and ruling out other tumors. Immunophenotyping can be done either in paraffin section or via flow cytometry analysis on cell suspension derived from the tumor. The most common positive markers in paraffin sections include CD68/KP1, MPO, CD117, CD 99, CD 68/PG-M1, lysozyme, CD34, TdT, CD56, CD61, CD30, glycophorin and CD4. CD13, CD33, CD117 and MPO are the most common markers in flow cytometric analysis in tumors with myeloid differentiation and CD14, CD163 and CD11c in tumors with monoblastic differentiation. B- and T-lineage markers, in particular CD20, CD45RO, CD79a and CD3, should be added to the panel in order to exclude other differential diagnoses (15).

Additionally, once the EM mass is established as leukemia, a bone marrow aspirate and biopsy is also performed, which is sent for identical studies.

## **Cytogenetic and molecular characteristics**

A variety of chromosomal abnormalities have been reported in patients with AML with EM involvement, most commonly reported association is with core binding factor (CBF) leukemias. The prevalence of GS in patients with translocation t(8;21) in different studies ranges between 9% and 35%. On the other hand, the reported rate of t(8;21) in GS patients ranges from 3.3% to 43%. In children, it has been associated with orbital GS (16,17). The inv(16) is another cytogenetic abnormality with a higher incidence of EM involvement, particularly in the abdomen and characterised by a microscopic appearance of plasmacytoid monocyte clusters (12,18). EM involvement in infants has been associated with 11q23, which has a characteristic MLL rearrangement (19). Other reported abnormalities in GS include t(9;11), del(16q), t(8;17), t(8;16), and t(1;11). Abnormalities of chromosome 8 have also been associated with the development of leukemia cutis in patients with AML. NPM1 mutations have been reported in 14% of GS in one study of 181 GS samples (22). In a small pathologic series, FLT3 mutations were identified in 15% of GS cases (23).

## **Imaging**

GS often appears as a soft-tissue mass best suited to imaging by computed tomography. Given the wide variety of sites in which GS develops, imaging can facilitate diagnosis and monitor treatment response. Magnetic resonance imaging (gadolinium enhanced) is useful for CNS GS.

## **Prognosis**

Owing to the rarity of GS there are no large studies analyzing prognostic factors in these patients. Few reports compare the prognosis of isolated GS with patients with either GS with concomitant AML

or AML presenting without GS, making the contributing effect of GS on prognosis difficult to assess. Although the presence of EM disease may be associated with a poor prognosis and shorter survival(9), 5-year survival rates for patients with GS range between 20% and 30%, which appear similar to AML in general(2,14). The prognostic significance of cytogenetic alterations in the presence of GS is not fully understood. Although the presence of translocation t(8;21) is associated with a relatively good prognosis when treated with standard induction and intensive consolidation chemotherapy, it remains unclear whether this favorable prognosis remains in the presence of EM disease because there are conflicting reports(25,26).

## Treatment

Since randomized prospective trials are lacking, there is no consensus on the treatment of GS. The current recommended treatment regimen is conventional AML-type chemotherapeutic protocols in patients presenting with isolated GS or GS presenting concomitantly with AML. In isolated GS patients treated with AML-based induction regimens, CR rates are comparable with AML without GS with similar prognostic features, and prolonged disease-free survival (DFS), from 3.5 to 16 years, has been reported(2,28).

The role of RT in addition to systemic chemotherapy is not established, although it is often given. Several study groups have not found any significant difference in outcome with or without radiotherapy (10,14). Given the favorable results from retrospective studies(12,30), treatment intensification with allogeneic HCT can be considered for patients with GS and concurrent marrow involvement after evaluation of other patient-related factors, including standard age and cytogenetic and molecular based risk profiling.

## Relapsed GS

Isolated GS at relapse is rare and often heralds systemic relapse. The median time to marrow relapse in this setting is approximately 7 months(31). For patients who have relapsed after chemotherapy alone, options of treatment include reinduction chemotherapy and RT to the tumor. There is no standard chemotherapy regimen for relapsed GS, a regimen is selected that would have applied to relapsed AML. HCT is often recommended, although its potential benefits in this setting are unclear. However, rare cases of isolated GS after HCT have been described (32).

## References

1. Vardiman JW, Thiele J, Arber DA, Brunning RD, Borowitz MJ, Porwit A, et al. The 2008 revision of the World Health Organization (WHO) classification of myeloid neoplasms and acute leukemia: rationale and important changes. *Blood*. 2009 Jul 30;114(5):937-51.
2. Tsimberidou A-M, Kantarjian HM, Estey E, Cortes JE, Verstovsek S, Faderl S, et al. Outcome in patients with nonleukemic granulocytic sarcoma treated with chemotherapy with or without radiotherapy. *Leukemia*. 2003 Jun;17(6):1100-3.
3. Neiman RS, Barcos M, Berard C, Bonner H, Mann R, Rydell RE, et al. Granulocytic sarcoma: a clinicopathologic study of 61 biopsied cases. *Cancer*. 1981 Sep 15;48(6):1426-37.
4. Szomor A, Passweg JR, Tichelli A, Hoffmann T, Speck B, Gratwohl A. Myeloid leukemia and myelodysplastic syndrome relapsing as granulocytic sarcoma (chloroma) after allogeneic bone marrow transplantation. *Ann Hematol*. 1997 Dec;75(5-6):239-41.
5. Békássy AN, Hermans J, Gorin NC, Gratwohl A. Granulocytic sarcoma after allogeneic bone marrow transplantation: a retrospective European multicenter survey. Acute and Chronic Leukemia Working Parties of the European Group for Blood and Marrow Transplantation. *Bone Marrow Transplant*. 1996 May;17(5):801-8.
6. Faaij CMJM, Willemze AJ, Révész T, Balzarolo M, Tensen CP, Hoogeboom M, et al. Chemokine/chemokine receptor interactions in extramedullary leukaemia of the skin in childhood AML: differential roles for CCR2, CCR5, CXCR4 and CXCR7. *Pediatr Blood Cancer*. 2010 Aug;55(2):344-8.
7. Stefanidakis M, Karjalainen K, Jaalouk DE, Gahmberg CG, O'Brien S, Pasqualini R, et al. Role of leukemia cell invadosome in extramedullary infiltration. *Blood*. 2009 Oct 1;114(14):3008-17.
8. Byrd JC, Edenfield WJ, Shields DJ, Dawson NA. Extramedullary myeloid cell tumors in acute nonlymphocytic leukemia: a clinical review. *J Clin Oncol Off J Am Soc Clin Oncol*. 1995 Jul;13(7):1800-16.

9. Paydas S, Zorludemir S, Ergin M. Granulocytic sarcoma: 32 cases and review of the literature. *Leuk Lymphoma*. 2006 Dec;47(12):2527–41.
10. Dusenbery KE, Howells WB, Arthur DC, Alonzo T, Lee JW, Kobrinsky N, et al. Extramedullary leukemia in children with newly diagnosed acute myeloid leukemia: a report from the Children's Cancer Group. *J Pediatr Hematol Oncol*. 2003 Oct;25(10):760–8.
11. Audouin J, Comperat E, Le Tourneau A, Camilleri-Broët S, Adida C, Molina T, et al. Myeloid sarcoma: clinical and morphologic criteria useful for diagnosis. *Int J Surg Pathol*. 2003 Oct;11(4):271–82.
12. Pileri SA, Ascani S, Cox MC, Campidelli C, Bacci F, Piccioli M, et al. Myeloid sarcoma: clinico-pathologic, phenotypic and cytogenetic analysis of 92 adult patients. *Leukemia*. 2007 Feb;21(2):340–50.
13. Suh YK, Shin HJ. Fine-needle aspiration biopsy of granulocytic sarcoma: a clinicopathologic study of 27 cases. *Cancer*. 2000 Dec 25;90(6):364–72.
14. Lan T-Y, Lin D-T, Tien H-F, Yang R-S, Chen C-Y, Wu K. Prognostic factors of treatment outcomes in patients with granulocytic sarcoma. *Acta Haematol*. 2009;122(4):238–46.
15. Avni B, Koren-Michowitz M. Myeloid sarcoma: current approach and therapeutic options. *Ther Adv Hematol*. 2011 Oct;2(5):309–16.
16. Bönig H, Göbel U, Nürnberger W. Bilateral exophthalmus due to retro-orbital chloromas in a boy with t(8;21)- positive acute myeloblastic acute leukemia. *Pediatr Hematol Oncol*. 2002 Dec;19(8):597–600.
17. Schwyzer R, Sherman GG, Cohn RJ, Poole JE, Willem P. Granulocytic sarcoma in children with acute myeloblastic leukemia and t(8;21). *Med Pediatr Oncol*. 1998 Sep;31(3):144–9.
18. Zhang X-H, Zhang R, Li Y. Granulocytic sarcoma of abdomen in acute myeloid leukemia patient with inv(16) and t(6;17) abnormal chromosome: case report and review of literature. *Leuk Res*. 2010 Jul;34(7):958–61.
19. Leblanc T, Le Coniat M, Flexor M, Baruchel A, Daniel MT, Berger R. An interstitial 11q23 deletion proven to be a rearrangement interrupting the MLL gene in an infant with acute myeloblastic leukemia. *Leukemia*. 1996 Nov;10(11):1844–6.
20. Evans GD, Grimwade DJ. Extramedullary disease in acute promyelocytic leukemia. *Leuk Lymphoma*. 1999 Apr;33(3-4):219–29.
21. Montesinos P, Díaz-Mediavilla J, Debén G, Prates V, Tormo M, Rubio V, et al. Central nervous system involvement at first relapse in patients with acute promyelocytic leukemia treated with all-trans retinoic acid and anthracycline monochemotherapy without intrathecal prophylaxis. *Haematologica*. 2009 Sep;94(9):1242–9.
22. Falini B, Lenze D, Hasserjian R, Coupland S, Jaehne D, Soupier C, et al. Cytoplasmic mutated nucleophosmin (NPM) defines the molecular status of a significant fraction of myeloid sarcomas. *Leukemia*. 2007 Jul;21(7):1566–70.
23. Ansari-Lari MA, Yang C-F, Tinawi-Aljundi R, Cooper L, Long P, Allan RH, et al. FLT3 mutations in myeloid sarcoma. *Br J Haematol*. 2004 Sep;126(6):785–91.
24. Fritz J, Vogel W, Bares R, Horger M. Radiologic spectrum of extramedullary relapse of myelogenous leukemia in adults. *AJR Am J Roentgenol*. 2007 Jul;189(1):209–18.
25. Byrd JC, Weiss RB, Arthur DC, Lawrence D, Baer MR, Davey F, et al. Extramedullary leukemia adversely affects hematologic complete remission rate and overall survival in patients with t(8;21)(q22;q22): results from Cancer and Leukemia Group B 8461. *J Clin Oncol Off J Am Soc Clin Oncol*. 1997 Feb;15(2):466–75.
26. Ruiz-Argüelles GJ, Morales-Toquero A, Manzano C, Ruiz-Delgado GJ, Jaramillo P, Gonzalez-Carrillo ML, et al. t(8;21) (q22;q22) acute myelogenous leukemia in Mexico: a single institution experience. *Hematol Amst Neth*. 2006 Aug;11(4):235–8.
27. Yamauchi K, Yasuda M. Comparison in treatments of nonleukemic granulocytic sarcoma: report of two cases and a review of 72 cases in the literature. *Cancer*. 2002 Mar 15;94(6):1739–46.
28. Meis JM, Butler JJ, Osborne BM, Manning JT. Granulocytic sarcoma in nonleukemic patients. *Cancer*. 1986 Dec 15;58(12):2697–709.
29. Bakst RL, Tallman MS, Douer D, Yahalom J. How I treat extramedullary acute myeloid leukemia. *Blood*. 2011 Oct 6;118(14):3785–93.
30. Chevallier P, Mohty M, Lioure B, Michel G, Contentin N, Deconinck E, et al. Allogeneic hematopoietic stem-cell transplantation for myeloid sarcoma: a retrospective study from the SFGM-TC. *J Clin Oncol Off J Am Soc Clin Oncol*. 2008 Oct 20;26(30):4940–3.
31. Byrd JC, Weiss RB. Recurrent granulocytic sarcoma. An unusual variation of acute myelogenous leukemia associated with 8;21 chromosomal translocation and blast expression of the neural cell adhesion molecule. *Cancer*. 1994 Apr 15;73(8):2107–12.
32. Au WY, Chan AC, Lie AK, Chen FE, Liang R, Kwong YL. Recurrent isolated extramedullary relapses as granulocytic sarcomas following allogeneic bone marrow transplantation for acute myeloid leukemia. *Bone Marrow Transplant*. 1998 Jan;21(2):205–8.

Leukemia in Down syndrome (DS) patients offers unique models for our understanding of the genetic basis of oncogenesis and leukemogenesis, as well as mechanisms of chemotherapy sensitivity.

### Epidemiology

Children with DS are at an increased risk both of acute megakaryocyte-erythroid leukemia (known as myeloid leukemia of DS [ML-DS]) by 150-fold and of acute B-lineage lymphoblastic leukemia by 33-fold compared with children without DS. Approximately, 1:100 to 1:150 DS children will develop acute leukemia.

DS-AML constitutes 15% of childhood AML cases. Majority of cases of DS-AML (approx. 90%) occur within 4 years of age. No sex predilection is observed.

Acute megakaryoblastic leukemia (AMkL) is the most common FAB subtype in DS-AML patients, as reported by multiple pediatric cooperative groups, constituting 90% of them. This is in stark contrast to non-DS-AML patients where AMkL subtype comprises <10%.

### DS and TMD

Transient Myeloproliferative Disorder (TMD) is an AMkL-related disorder diagnosed in 10% of newborn DS babies. However, 7% to 16% of TMD cases have mosaic T21. The current definition of TMD is imprecise. It is defined as the presence of circulating blood blasts in a baby with typical clinical features of TMD who may or may not have hematologic abnormalities. Recently, to avoid over diagnosis of TMD, it has been suggested to define it as blasts greater than 10% and a GATA1 mutation detected by conventional sequencing and/or DHPLC. The median age of presentation of TMD is 3–7 days. The clinical presentation of neonates with TMD ranges from a healthy appearance to bruising, respiratory distress, fulminant hepatic failure, hydrops fetalis or even death in 15–20% of cases. Majority of cases resolve spontaneously with normal blood counts at a mean of 84 days. Approximately 20%–30% of DS patients with a previous history of TMD will develop AMkL and is often preceded by a myelodysplastic phase.

**Diagnosis:** Laboratory tests show either thrombocytosis or thrombocytopenia accompanied by elevated white blood cell count (WBC) with excess of blasts. The blood smear may show nucleated red cells, giant platelets and megakaryocyte fragments, and, most significantly, megakaryocytic blasts with characteristic blebs. Flow cytometry reveals that the blasts are positive for CD34, CD33, CD41, CD61, glycophorin A, and quite often CD7 and CD36. The blasts of TMD usually show mutations in GATA1.

**Prognosis & Treatment of TMD:** The natural history of TMD is one of spontaneous regression over several months. Mild cases do not require treatment, whereas symptomatic conventional AML-type chemotherapeutic protocols cases may require supportive care measures and occasionally chemotherapy. Four large studies from the United States, Japan, and Europe have recently reported the natural course of TMD in 264 infants with DS. These studies confirmed the transient course of this disease that usually

resolved spontaneously within the first 3 months of life. However, these studies revealed that the disease is not benign, as early death was reported in 15% to 20% of infants with clinically evident TMD. Poor prognostic factors included high WBC count, prematurity, severe liver failure manifested by increasing jaundice, bleeding diatheses, and failure of spontaneous remission within the first 3 months. (Table-1) Recently, TMD patients were stratified in 3 risk groups by COG A2971 study. Patients, who presented with early evidence of acute life threatening symptoms (LTS; Table-1) were classified as a high risk group, had a 3-year EFS of 30%. TMD patients who had any evidence of hepatomegaly and hepatic dysfunction, but had no evidence of LTS were classified as an intermediate risk group and had a 3-year EFS of 55.2%. The remaining patients were classified as being low risk and had a 3-year EFS of 83%.

**Table-1: High risk factors in TMD**

Clinical factors	
1	Prematurity
2	Ascites
3	Bleeding*
4	Signs of hyperviscosity*
5	Hepatosplenomegaly causing respiratory compromise*,
6	Heart failure (ejection fraction <47% or shortening fraction <27%) not directly the result of a congenital heart defect*
7	Hydrops fetalis*
Laboratory factors	
1	White blood cells/Blast count > 100 10 <sup>9</sup> /L*
2	Severe coagulopathy*
3	Progressive renal/liver failure*
4	No complete remission by 3 mo

\* Acute Life threatening symptoms (LTS) as per COG A2971

AML-BFM study suggested that low-dose cytarabine (0.5-1.5 mg/kg for 3-12 days) improved outcome in patients with high risk factors. Among children at risk for early death, 5-year EFS was superior in those receiving treatment versus not (52 ± 12% versus 28 ± 11%). The higher dose of cytarabine (3.33 mg/kg/24 h given by continuous infusion for 5 days) used in the COG A2971 study was associated with significant toxicity. Hence, low dose cytarabine (ara-C) administered subcutaneously or intravenously every 12 hours for 5 to 7 days may be sufficient for patients with high risk features.

### DS and AML

Up to 30% of neonates with clinical TMD will subsequently develop AML at a variable tempo usually after a latency period of 1-4 years. Before the diagnosis of AML, most DS patients develop signs of myelodysplasia, characterized by progressive anemia and thrombocytopenia, dysplastic erythroid cells and megakaryocytes in the bone marrow that may be difficult to aspirate due to the presence of myelofibrosis. This myelodysplastic phase frequently precedes the development of AML, and the spectrum of both myelodysplastic syndrome (MDS) and AML have been known collectively as ML-DS. Rarely ML-DS can progress directly from aggressive TMD.

To differentiate from non-resolving TMD, a bone marrow aspirate and trephine can be helpful to confirm greater than 30% blasts. On flow cytometry blasts express the same megakaryoblastic phenotype in the majority of cases as TMD blasts. High expression of CD36 marker distinguishes ML-DS from most cases of de novo AMKL. The cytogenetics of DS AML cases differs significantly from non-DS patients with

AML. DS children with AML more frequently have trisomies 8, 11, and 21, dup(1p),del(6q), del(7p), dup(7q), and del(16q). Translocations commonly seen in non-DS AML [eg, t(8;21); t(15;17);inv(16), 11q23 rearrangements] are rare in patients with DS. Furthermore, neonates and children with DS developing ML-DS usually do not acquire the specific non-DS childhood AMKL cytogenetic abnormality, t(1;22) or the cryptic inversion of chromosome 16 that produces the CBFA2T3-GLIS2 fusion gene.

### **Treatment considerations of DS-AML**

DS children with AML, and in particular children with the AMkL phenotype, have exceptionally high cure rates of >80% with contemporary DS specific protocols incorporating ara-C (Table-2). Older studies using standard dose & intensity of therapy in DS demonstrated a high complete remission rate, low induction failure rate, and a relatively low relapse rate but higher treatment related complications. The time-intensive strategy for treating pediatric AML (without evidence of full marrow recovery from neutropenia and thrombocytopenia) in CCG-2861/2891 studies and standard dose regimens of Medical Research Council AM 10/12 studies were found to be too toxic for patients with ML-DS with 32% and 27% treatment-associated mortality respectively. Further, in POG 9421 study (which used a high total cumulative anthracycline dose); 21% of children developed late-onset congestive heart failure, including several treatment-associated (and non-leukemic) deaths. Hence, despite using similar drugs, current treatment strategies differ significantly between the DS and non-DS patient groups and, in particular, balance curative therapy against the risk of treatment-associated morbidity and mortality by using reduced treatment intensity regimens with significant dose-reductions for cytarabine and anthracyclines. The potential for reduced treatment intensity is based on the unique hypersensitivity of myeloid leukemia cells of DS patients to chemotherapy, when compared with AML cells from non-DS individuals, as demonstrated by various investigators. This increased sensitivity to chemotherapy extends to agents with different mechanisms of action. DS myeloid blasts are significantly more sensitive to cytarabine (median, 12-fold), anthracyclines (2- to 7-fold), mitoxantrone (9-fold), amsacrine (16-fold), etoposide (20-fold), 6-thioguanine (3-fold), busulfan (5-fold) and vincristine (23-fold), than non-DS AML cells. Furthermore, due to excellent outcome with chemotherapy alone and rarity of CNS disease, DS patients do not receive maintenance therapy, nor cranial radiation, nor stem-cell transplantation.

### **Reasons for heightened chemo-sensitivity and toxicity in DS-AML**

*In vitro* studies have demonstrated that DS leukemia blasts are more sensitive to a number of different chemotherapy agents than non-DS leukemia blasts. The reasons put forth were

1. The trans-sulfuration pathway enzyme, cystathionine-synthase (CBS): Localized to chromosome 21q22.3, this enzyme is involved in cysteine metabolism and its increased activity is associated with significantly lower levels of homocysteine, methionine and S-adenosyl methionine. This causes a reduced folate metabolism and increases the susceptibility of cells to nucleoside analogs such as Ara-C.
2. Somatic mutation in GATA1: Acquired somatic mutations of X-linked transcription factor gene GATA1 is seen in nearly all cases of DS-TMD and AMkL. The GATA1 protein is a zinc finger transcription factor essential for normal erythroid and megakaryocytic differentiation. Mutations in GATA1 in DS leads to a truncated protein with altered transactivation activity. One target gene is cytidine deaminase (CDA), which deaminates ara-C to its inactive metabolite. CDA transcripts, measured by real-time PCR, were a median 5-fold lower in DS-megakaryoblasts than in non-DS AML blasts. This is postulated to cause increased ara-C exposure to leukemic blasts.

3. Increased intracellular levels of Ara-CTP: DS AMkL blasts also generated significantly higher levels of the active intracellular ara-C metabolite, ara-CTP, than non-DS AML blasts following in vitro incubations with 3H-ara-C, indicating that the metabolism of ara-C in DS AMkL cells is altered.
4. Sensitivity to Daunorubicin: Chromosome 21 localized carbonyl reductase is involved in the metabolism of daunorubicin, converting the parent drug to a less potent but longer lasting daunorubicinol.

The net effect of these changes is a heightened chemosensitivity of DS-AML blasts especially to Ara-C and Daunorubicin at the expense of increased toxicity at the conventional doses used in treatment of non DS-AML.

**CNS prophylaxis:** CNS involvement is rare in DS AMkL. Recent Japanese protocols have eliminated CNS prophylaxis due to the rarity of CNS involvement in DS AML;

**Stem cell transplantation:** The CCG AML trial 2891 showed lower post-remission disease-free survival (DFS) of ML-DS when randomized to allogeneic bone marrow transplant (BMT) or autologous BMT compared to chemotherapy, with DFS of 33%, 67%, and 89% respectively. In view of excessive toxicity with SCT and excellent outcomes with chemotherapy alone, SCT is not recommended in first remission.

**Treatment recommendations for ML-DS:** Children with ML-DS should be treated in large centres with sufficient experience and good supportive care. Appropriate therapy for younger children (aged 4 years) with ML-DS is any of the reduced intensity contemporary DS specific protocols (Table-2) such as COG AAML0431, European BFM-98 or MRC-AML protocols. In centres with limited supportive care Japanese AML99DS protocol may be feasible. Hematopoietic stem cell transplant is not indicated in first remission. Also, maintenance therapy, and cranial irradiation are not recommended. Recent collaborative efforts are directed toward better identification of risk groups by making use of GATA1MRD and other genomic markers.

**Table-2 Contemporary protocols & outcomes for ML-DS**

Protocol	No of patients	EFS (%)	Relapse (%)	death in CCR (%)	Cytarabine (g/m <sup>2</sup> )	Daunorubicin (mg/m <sup>2</sup> )
POG9421	57	77 (5y)	7	14	20.7	135
CCG2891	161	77 (6y)	14	4	15.8	320
COG-A2971	132	79 (5y)	11	3	24.8	80
MRC-AML10/12	46	74 (5y)	3	15	7.8	300
AML-BFM98	67	89 (3y)	6	5	23-29	Ida; 26-36
AML99 DS	72	83 (4y)	12.5	1.4	3.5	Pirarubicin; 250
NOPHO-AML93	41	85 (8y)	7	5	49.6	150

**Table-3 Adverse Prognostic factors in ML-DS**

1	Age >4 years
2	Normal Karotype
3	White blood cell count of 20,000/mL
4	No Prior TMD
5	Monosomy 7
6	High MRD

# 10 TREATMENT OF SECONDARY ACUTE MYELOID LEUKEMIA

Secondary acute myeloid leukemia (sAML) may arise from the previous clonal disorder of hematopoiesis, usually from myelodysplastic syndrome (MDS) or from chronic myeloproliferative neoplasia (cMPN) or after exposure to a leukemogenic agent.

Cytotoxic agents implicated in secondary AML/ therapy related AML:

- Alkylating agents – Melphalan, cyclophosphamide, nitrogen mustard, chlorambucil, busulfan, dacarbazine, procarbazine, carmustine, mitomycin C, thiotepa.
- Topoisomerase II inhibitors – Etoposide, teniposide, doxorubicin, Daunorubicin, epirubicin, mitoxantrone, actinomycin D.
- Antimetabolites – Thiopurines, mycophenolae and Fludarabine.
- Ionizing radiation therapy given as large fields that include the bone marrow.

### Prognosis:

These patients are usually older patients and carry poor risk cytogenetic (50% of therapy related AML have high risk cytogenetic changes), thus making them poor responders to conventional induction chemotherapy regimens. However, patients with favourable risk cytogenetics respond to conventional chemotherapy and have better survival.

### Treatment:

Two major factors affect the treatment decision in secondary AML:

1. Performance status: Patients with poor performance status are not candidates for intensive induction regimen. Most patients are either previously treated (t AML) or have previous MDS/MPN, so the tolerance to intensive chemotherapy is poor in such patients.
2. Cytogenetic risk category:
  - Favourable group: Patients with favourable risk cytogenetics have better outcome, however they constitute small subset of patients. These patients should be offered intensive chemotherapy regimen. The CR rates in this group are 60-65% with median survival of 27 months (1–3).
  - Intermediate group: Patients who have normal karyotype or karyotype not fitting into favourable or unfavourable risk cytogenetics are grouped under this group. The data in this group is limited however fit patients can be considered for intensive induction regimen followed by allogeneic stem cell transplant if match is available. Median overall survival time for patients with intermediate-risk karyotype treated with conventional chemotherapy of 13 months(3).

- Unfavourable group: Patients with unfavorable karyotypes include those with 3q21q26 abnormalities, del 5, del 7, monosomies 5 or 7, 11q23 abnormalities, 12p abnormalities, 17p abnormalities, or a complex aberrant karyotypes described as at least 3 abnormalities. Median overall survival with conventional chemotherapy is only six months. These patients have poor response to intensive chemotherapy regimen and high treatment related mortality. Less intensive regimen such as DNA methyl transferase inhibitors (eg, azacitidine, decitabine) can be considered in patients with poor performance status. Young and fit patients can be considered for intensive chemotherapy followed by allogeneic stem cell transplant(3).

#### References:

1. Slovak ML, Bedell V, Popplewell L, Arber DA, Schoch C, Slater R. 21q22 balanced chromosome aberrations in therapy-related hematopoietic disorders: report from an international workshop. *Genes Chromosomes Cancer*. 2002 Apr;33(4):379-94.
2. Andersen MK, Larson RA, Mauritzson N, Schnittger S, Jhanwar SC, Pedersen-Bjergaard J. Balanced chromosome abnormalities inv(16) and t(15;17) in therapy-related myelodysplastic syndromes and acute leukemia: report from an international workshop. *Genes Chromosomes Cancer*. 2002 Apr;33(4):395-400.
3. Kern W, Haferlach T, Schnittger S, Hiddemann W, Schoch C. Prognosis in therapy-related acute myeloid leukemia and impact of karyotype. *J Clin Oncol Off J Am Soc Clin Oncol*. 2004 Jun 15;22(12):2510-1.

- 1 Shah A, Andersson TM, Racht B, Björkholm M, Lambert PC. Survival and cure of acute myeloid leukaemia in England, 1971-2006: a population-based study. *Br J Haematol*. 2013 Aug;162(4):509-16. doi: 10.1111/bjh.12425. Epub 2013 Jun 21. PubMed PMID: 23786647.
- 2 Schoch, C., Kern, W., Schnittger, S., Hiddemann, W. & Haferlach, T. (2004b) Karyotype is an independent prognostic parameter in therapyrelated acute myeloid leukemia (t-AML): an analysis of 93 patients with t-AML in comparison to 1091 patients with de novo AML. *Leukemia*, 18, 120–125.
- 3 Juliusson, G., Antunovic, P., Derolf, A., Lehmann, S., Mollgard, L., Stockelberg, D., Tidefelt, U., Wahlin, A. & Hoglund, M. (2009) Age and acute myeloid leukemia: real world data on decision to treat and outcomes from the Swedish Acute Leukemia Registry. *Blood*, 113, 4179–4187
- 4 Creutzig, U., Buchner, T., Sauerland, M.C., Zimmermann, M., Reinhardt, D., Dohner, H. & Schlenk, R.F. (2008) Significance of age in acute myeloid leukemia patients younger than 30 years: a common analysis of the pediatric trials AML-BFM 93/98 and the adult trials AMLCG 92/99 and AMLSG HD93/98A. *Cancer*, 112, 562–571.
- 5 Juliusson, G., Antunovic, P., Derolf, A., Lehmann, S., Mollgard, L., Stockelberg, D., Tidefelt, U., Wahlin, A. & Hoglund, M. (2009) Age and acute myeloid leukemia: real world data on decision to treat and outcomes from the Swedish Acute Leukemia Registry. *Blood*, 113, 4179–4187
- 6 Estey EH. Therapeutic options for acute myelogenous leukemia. *Cancer*. 2001 Sep 1;92(5):1059-73. Review. PubMed PMID: 11571716.
- 7 Kulkarni KP, Marwaha RK. Childhood acute myeloid leukemia: an Indian perspective. *Pediatr Hematol Oncol*. 2011 May;28(4):257-68. doi: 10.3109/08880018.2010.531521. Epub 2011 Jan 8. PubMed PMID: 21214409.
- 8 Bahl A, Sharma A, Raina V, Kumar L, Bakhshi S, Gupta R, Kumar R. Long-term outcomes for patients with acute myeloid leukemia: A single-center experience from AIIMS, India. *Asia Pac J Clin Oncol*. 2015 Sep;11(3):242-52. doi: 10.1111/ajco.12333. Epub 2015 Jan 30. PubMed PMID: 25639656.
- 9 Cheson BD, Bennet JM, Kopecky KJ, Büchner T, Willman CL, Estey EH, Schiffer CA, Doehner H, Tallman MS, Lister TA, Lo-Coco F, Willemze R, Biondi A, Hiddemann W, Larson RA, Löwenberg B, Sanz MA, Head DR, Ohno R, Bloomfield CD; International Working Group for Diagnosis, Standardization of Response Criteria, Treatment Outcomes, and Reporting Standards for Therapeutic Trials in Acute Myeloid Leukemia. Revised recommendations of the International Working Group for Diagnosis, Standardization of Response Criteria, Treatment Outcomes, and Reporting Standards for Therapeutic Trials in Acute Myeloid Leukemia. *J Clin Oncol*. 2003 Dec 15;21(24):4642-9. Erratum in: *J Clin Oncol*. 2004 Feb 1;22(3):576. LoCocco, Francesco [corrected to Lo-Coco, Francesco]. PubMed PMID: 14673054.
- 10 Elliott, M.A., Litzow, M.R., Letendre, L.L., Wolf, R.C., Hanson, C.A., Tefferi, A. & Tallman, M.S. (2007) Early peripheral blood blast clearance during induction chemotherapy for acute myeloid leukemia predicts superior relapse-free survival. *Blood*, 110, 4172–4174
- 11 Arellano, M., Pakkala, S., Langston, A., Tighiouart, M., Pan, L., Chen, Z., Heffner, L.T., Lonial, S., Winton, E. & Khoury, H.J. (2012) Early clearance of peripheral blood blasts predicts response to induction chemotherapy in acute myeloid leukemia. *Cancer*, 118, 5278–5282.
- 12 Behl, D., Porrata, L.F., Markovic, S.N., Letendre, L., Pruthi, R.K., Hook, C.C., Tefferi, A., Elliot, M.A., Kaufmann, S.H., Mesa, R.A. & Litzow, M.R. (2006) Absolute lymphocyte count recovery after induction chemotherapy predicts superior survival in acute myelogenous leukemia. *Leukemia*, 20, 29–34.
- 13 De Angulo, G., Yuen, C., Palla, S.L., Anderson, P.M. & Zweidler-McKay, P.A. (2008) Absolute lymphocyte count is a novel prognostic indicator in ALL and AML: implications for risk stratification and future studies. *Cancer*, 112, 407–415

- 14 Kern, W., Haferlach, T., Schoch, C., Loffler, H., Gassmann, W., Heinecke, A., Sauerland, M.C., Berdel, W., Buchner, T. & Hiddemann, W. (2003) Early blast clearance by remission induction therapy is a major independent prognostic factor for both achievement of complete remission and long-term outcome in acute myeloid leukemia: data from the German AML Cooperative Group (AMLCG) 1992 Trial. *Blood*, 101, 64–70.
- 15 Buchner, T., Hiddemann, W., Wormann, B., Loffler, H., Gassmann, W., Haferlach, T., Fonatsch, C., Haase, D., Schoch, C., Hossfeld, D., Lengfelder, E., Aul, C., Heyll, A., Maschmeyer, G., Ludwig, W.D., Sauerland, M.C. & Heinecke, A. (1999) Double induction strategy for acute myeloid leukemia: the effect of highdose cytarabine with mitoxantrone instead of standard-dose cytarabine with daunorubicin and 6-thioguanine: a randomized trial by the German AML Cooperative Group. *Blood*, 93, 4116–4124.
- 16 Gianfaldoni, G., Mannelli, F., Baccini, M., Antonioli, E., Leoni, F. & Bosi, A. (2006) Clearance of leukaemic blasts from peripheral blood during standard induction treatment predicts the bone marrow response in acute myeloid leukaemia: a pilot study. *British Journal of Haematology*, 134, 54–57.
- 17 Lacombe, F., Arnoulet, C., Maynadie, M., Lippert, E., Luquet, I., Pigneux, A., Vey, N., Casasnovas, O., Witz, F. & Bene, M.C. (2009) Early clearance of peripheral blasts measured by flow cytometry during the first week of AML induction therapy as a new independent prognostic factor: a GOELAMS study. *Leukemia*, 23, 350–357
- 18 Miwa, H., Beran, M. & Saunders, G.F. (1992) Expression of the Wilms' tumor gene (WT1) in human leukemias. *Leukemia*, 6, 405–409.
- 19 Cilloni, D., Renneville, A., Hermitte, F., Hills, R.K., Daly, S., Jovanovic, J.V., Gottardi, E., Fava, M., Schnittger, S., Weiss, T., Izzo, B., Nomdedeu, J., van der Heijden, A., van der Reijden, B.A., Jansen, J.H., van der Velden, V.H., Ommen, H., Preudhomme, C., Saglio, G. & Grimwade, D. (2009) Real-time quantitative polymerase chain reaction detection of minimal residual disease by standardized WT1 assay to enhance risk stratification in acute myeloid leukemia: a European LeukemiaNet study. *Journal of Clinical Oncology*, 27, 5195–5201
- 20 Gianfaldoni, G., Mannelli, F., Ponziani, V., Longo, G., Bencini, S., Bosi, A. & Vannucchi, A.M. (2010) Early reduction of WT1 transcripts during induction chemotherapy predicts for longer disease free and overall survival in acute myeloid leukemia. *Haematologica*, 95, 833–836.
- 21 Gianfaldoni, G., Mannelli, F., Ponziani, V., Longo, G., Bencini, S., Bosi, A. & Vannucchi, A.M. (2010) Early reduction of WT1 transcripts during induction chemotherapy predicts for longer disease free and overall survival in acute myeloid leukemia. *Haematologica*, 95, 833–836.
- 22 Bjorklund, E., Gruber, A., Mazur, J., Martensson, A., Hansson, M. & Porwit, A. (2009) CD34 + cell subpopulations detected by 8-color flow cytometry in bone marrow and in peripheral blood stem cell collections: application for MRD detection in leukemia patients. *International Journal of Hematology*, 90, 292–302.
- 23 Rubnitz, J.E., Inaba, H., Dahl, G., Ribeiro, R.C., Bowman, W.P., Taub, J., Pounds, S., Razzouk, B.I., Lacayo, N.J., Cao, X., Meshinchi, S., Degar, B., Airewele, G., Raimondi, S.C., Onciu, M., Coustan-Smith, E., Downing, J.R., Leung, W., Pui, C.H. & Campana, D. (2010) Minimal residual disease-directed therapy for childhood acute myeloid leukaemia: results of the AML02 multicentre trial. *Lancet Oncology*, 11, 543–552. ; van der Velden, V.H., van der Sluijs-Geling, A., Gibson, B.E., te Marvelde, J.G., Hoogeveen, P.G., Hop, W.C., Wheatley, K., Bierings, M.B., Schuurhuis, G.J., de Graaf, S.S., van Wering, E.R. & van Dongen, J.J. (2010) Clinical significance of flowcytometric minimal residual disease detection in pediatric acute myeloid leukemia patients treated according to the DCOG ANLL97/MRC AML12 protocol. *Leukemia*, 24, 1599–1606.; Loken, M.R., Alonzo, T.A., Pardo, L., Gerbing, R.B., Raimondi, S.C., Hirsch, B.A., Ho, P.A., Franklin, J., Cooper, T.M., Gamis, A.S. & Meshinchi, S. (2012) Residual disease detected by multidimensional flow cytometry signifies high relapse risk in patients with de novo acute myeloid leukemia: a report from Children's Oncology Group. *Blood*, 120, 1581–1588.
- 24 Terwijn, M., van Putten, W.L., Kelder, A., van der Velden, V.H., Brooimans, R.A., Pabst, T., Maertens, J., Boeckx, N., de Greef, G.E., Valk, P.J., Preijers, F.W., Huijgens, P.C., Drager, A.M., Schanz, U., Jongen-Lavrecic, M., Biemond, B.J., Passweg, J.R., van Gelder, M., Wijermans, P., Graux, C., Bargetzi, M., Legdeur, M.C., Kuball, J., de Weerd, O., Chalandon, Y., Hess, U., Verdonck, L.F., Gratama, J.W., Oussoren, Y.J., Scholten, W.J., Slomp, J., Snel, A.N., Vekemans, M.C., L Owenberg, B., Ossenkoppele, G.J. & Schuurhuis, G.J. (2013) High prognostic impact of flow cytometric minimal residual disease detection in acute myeloid leukemia: data from
- 25 Walter, R.B., Gooley, T.A., Wood, B.L., Milano, F., Fang, M., Sorror, M.L., Estey, E.H., Salter, A.I., Lansverk, E., Chien, J.W., Gopal, A.K., Appelbaum, F.R. & Pagel, J.M. (2011) Impact of pretransplantation minimal residual disease, as detected by multiparametric flow cytometry, on outcome of myeloablative hematopoietic cell transplantation for acute myeloid leukemia. *Journal of Clinical Oncology*, 29, 1190–1197.

- 26 Patel, J.P., Gonen, M., Figueroa, M.E., Fernandez, H., Sun, Z., Racevskis, J., Van Vlierberghe, P., Dolgalev, I., Thomas, S., Aminova, O., Huberman, K., Cheng, J., Viale, A., Socci, N.D., Heguy, A., Cherry, A., Vance, G., Higgins, R.R., Ketterling, R.P., Gallagher, R.E., Litzow, M., van den Brink, M.R., Lazarus, H.M., Rowe, J.M., Luger, S., Ferrando, A., Paietta, E., Tallman, M.S., Melnick, A., Abdel-Wahab, O. & Levine, R.L. (2012) Prognostic relevance of integrated genetic profiling in acute myeloid leukemia. *New England Journal of Medicine*, 366, 1079– 1089.
- 27 Patel, J.P., Gonen, M., Figueroa, M.E., Fernandez, H., Sun, Z., Racevskis, J., Van Vlierberghe, P., Dolgalev, I., Thomas, S., Aminova, O., Huberman, K., Cheng, J., Viale, A., Socci, N.D., Heguy, A., Cherry, A., Vance, G., Higgins, R.R., Ketterling, R.P., Gallagher, R.E., Litzow, M., van den Brink, M.R., Lazarus, H.M., Rowe, J.M., Luger, S., Ferrando, A., Paietta, E., Tallman, M.S., Melnick, A., Abdel-Wahab, O. & Levine, R.L. (2012) Prognostic relevance of integrated genetic profiling in acute myeloid leukemia. *New England Journal of Medicine*, 366, 1079– 1089.
- 28 Jourdan, E., Boissel, N., Chevret, S., Delabesse, E., Renneville, A., Cornillet, P., Blanchet, O., Cayuela, J.M., Recher, C., Raffoux, E., Delaunay, J., Pigneux, A., Bulabois, C.E., Berthon, C., Pautas, C., Vey, N., Lioure, B., Thomas, X., Luquet, I., Terre, C., Guardiola, P., Bene, M.C., Preudhomme, C., Ifrah, N., Dombret, H. & French, A.M.L.I. (2013) Prospective evaluation of gene mutations and minimal residual disease in patients with core binding factor acute myeloid leukemia. *Blood*, 121, 2213–2223.
- 29 Yin, J.A., O'Brien, M.A., Hills, R.K., Daly, S.B., Wheatley, K. & Burnett, A.K. (2012) Minimal residual disease monitoring by quantitative RTPCR in core binding factor AML allows risk stratification and predicts relapse: results of the United Kingdom MRC AML-15 trial. *Blood*, 120, 2826–2835.
- 30 Weisser, M., Haferlach, C., Hiddemann, W. & Schnittger, S. (2007) The quality of molecular response to chemotherapy is predictive for the outcome of AML1-ETO-positive AML and is independent of pretreatment risk factors. *Leukemia*, 21, 1177–1182.
- 31 Kronke, J., Schlenk, R.F., Jensen, K.O., Tschurtz, F., Corbacioglu, A., Gaidzik, V.I., Paschka, P., Onken, S., Eiwen, K., Habdank, M., Spath, D., Lubbert, M., Wattad, M., Kindler, T., Salih, H.R., Held, G., Nachbaur, D., von Lilienfeld-Toal, M., Germing, U., Haase, D., Mergenthaler, H.G., Krauter, J., Ganser, A., Gohring, G., Schlegelberger, B., Dohner, H. & Dohner, K. (2011) Monitoring of minimal residual disease in NPM1-mutated acute myeloid leukemia: a study from the German-Austrian acute myeloid leukemia study group. *Journal of Clinical Oncology*, 29, 2709–2716.
- 32 Shayegi, N., Kramer, M., Bornhauser, M., Schaich, M., Schetelig, J., Platzbecker, U., Röllig, C., Heiderich, C., Landt, O., Ehninger, G. & Thiede, C. (2013) The level of residual disease based on mutant NPM1 is an independent prognostic factor for relapse and survival in AML. *Blood*, 122, 83–92.
- 33 Papadaki, C., Dufour, A., Seibl, M., Schneider, S., Bohlander, S.K., Zellmeier, E., Mellert, G., Hiddemann, W. & Spiekermann, K. (2009) Monitoring minimal residual disease in acute myeloid leukaemia with NPM1 mutations by quantitative PCR: clonal evolution is a limiting factor. *British Journal of Haematology*, 144, 517–523
- 34 Shih LY, Hunag CF, Wu JH et al. Internal tandem duplication of FLT3 in relapsed acute myeloid leukemia: a comparative analysis of bone marrow samples from 108 adult patients at diagnosis and relapse. *Blood*. 2002; 100(7): 2387-2392.
- 35 Becker, H., Marcucci, G., Maharry, K., Radmacher, M.D., Mrozek, K., Margeson, D., Whitman, S.P., Wu, Y.Z., Schwind, S., Paschka, P., Powell, B.L., Carter, T.H., Kolitz, J.E., Wetzler, M., Carroll, A.J., Baer, M.R., Caligiuri, M.A., Larson, R.A. & Bloomfield, C.D. (2010) Favorable prognostic impact of NPM1 mutations in older patients with cytogenetically normal de novo acute myeloid leukemia and associated gene and microRNA-expression signatures: a Cancer and Leukemia Group B study. *Journal of Clinical Oncology*, 28, 596–604
- 36 Daver, N., Liu Dumlao, T., Ravandi, F., Pierce, S., Borthakur, G., Pemmaraju, N., Nazha, A., Faderl, S., Jabbour, E., Garcia-Manero, G., Cortes, J., Kantarjian, H. & Quintas-Cardama, A. (2013) Effect of NPM1 and FLT3 mutations on the outcomes of elderly patients with acute myeloid leukemia receiving standard chemotherapy. *Clinical Lymphoma Myeloma and Leukemia*, 13, 435–440.
- 37 Dufour, A., Schneider, F., Metzeler, K.H., Hoster, E., Schneider, S., Zellmeier, E., Benthaus, T., Sauerland, M.C., Berdel, W.E., Buchner, T., Wormann, B., Braess, J., Hiddemann, W., Bohlander, S.K. & Spiekermann, K. (2010) Acute myeloid leukemia with biallelic CEBPA gene mutations and normal karyotype represents a distinct genetic entity associated with a favorable clinical outcome. *Journal of Clinical Oncology*, 28, 570–577.
- 38 Green, C.L., Evans, C.M., Zhao, L., Hills, R.K., Burnett, A.K., Linch, D.C. & Gale, R.E. (2011) The prognostic significance of IDH2 mutations in AML depends on the location of the mutation. *Blood*, 118, 409–412.
- 39 Allen, C., Hills, R.K., Lamb, K., Evans, C., Tinsley, S., Sellar, R., O'Brien, M., Yin, J.L., Burnett, A.K., Linch, D.C. & Gale, R.E. (2013) The importance of relative mutant level for evaluating impact on outcome of KIT, FLT3 and CBL mutations in core-binding factor acute myeloid leukemia. *Leukemia*, 27, 1891–1901.

- 40 Paschka, P., Du, J., Schlenk, R.F., Gaidzik, V.I., Bullinger, L., Corbacioglu, A., Spath, D., Kayser, S., Schlegelberger, B., Krauter, J., Ganser, A., Kohne, C.H., Held, G., von Lilienfeld-Toal, M., Kirchen, H., Rummel, M., Gotze, K., Horst, H.A., Ringhoffer, M., Lubbert, M., Wattad, M., Salih, H.R., Kundgen, A., Dohner, H. & Dohner, K. (2013) Secondary genetic lesions in acute myeloid leukemia with inv(16) or t(16;16): a study of the German-Austrian AML StudyGroup (AMLSG). *Blood*, 121, 170–177.
- 41 Patel, J.P., Gonen, M., Figueroa, M.E., Fernandez, H., Sun, Z., Racevskis, J., Van Vlierberghe, P., Dolgalev, I., Thomas, S., Aminova, O., Huberman, K., Cheng, J., Viale, A., Socci, N.D., Heguy, A., Cherry, A., Vance, G., Higgins, R.R., Ketterling, R.P., Gallagher, R.E., Litzow, M., vanden Brink, M.R., Lazarus, H.M., Rowe, J.M., Luger, S., Ferrando, A., Paietta, E., Tallman, M.S., Melnick, A., Abdel-Wahab, O. & Levine, R.L. (2012) Prognostic relevance of integrated genetic profiling in acute myeloid leukemia. *New England Journal of Medicine*, 366, 1079–1089.
- 42 Itzykson, R., Kosmider, O., Cluzeau, T., Mansat-De Mas, V., Dreyfus, F., Beyne-Rauzy, O., Quesnel, B., Vey, N., Gelsi-Boyer, V., Raynaud, S., Preudhomme, C., Ades, L., Fenaux, P., Fontenay, M. & Groupe Francophone des M. (2011) Impact of TET2 mutations on response rate to azacitidine in myelodysplastic syndromes and low blast count acute myeloid leukemias. *Leukemia*, 25, 1147–1152.
- 43 Schnittger, S., Dicker, F., Kern, W., Wendland, N., Sundermann, J., Alpermann, T., Haferlach, C. & Haferlach, T. (2011b) RUNX1 mutations are frequent in de novo AML with noncomplex karyotype and confer an unfavorable prognosis. *Blood*, 117, 2348–2357.
- 44 Bacher, U., Kern, W., Schnittger, S., Hiddemann, W., Haferlach, T. & Schoch, C. (2005) Population-based age-specific incidences of cytogenetic subgroups of acute myeloid leukemia. *Haematologica*, 90, 1502–1510.
- 45 Chou, W.C., Huang, H.H., Hou, H.A., Chen, C.Y., Tang, J.L., Yao, M., Tsay, W., Ko, B.S., Wu, S.J., Huang, S.Y., Hsu, S.C., Chen, Y.C., Huang, Y.N., Chang, Y.C., Lee, F.Y., Liu, M.C., Liu, C.W., Tseng, M.H., Huang, C.F. & Tien, H.F. (2010b) Distinct clinical and biological features
- 46 Schnittger, S., Eder, C., Jeromin, S., Alpermann, T., Fasan, A., Grossmann, V., Kohlmann, A., Illig, T., Klopp, N., Wichmann, H.E., Kreuzer, K.A., Schmid, C., Staib, P., Peceny, R., Schmitz, N., Kern, W., Haferlach, C. & Haferlach, T. (2013) ASXL1 exon 12 mutations are frequent in AML with intermediate risk karyotype and are independently associated with an adverse outcome. *Leukemia*, 27, 82–91.
- 47 Rucker, F.G., Schlenk, R.F., Bullinger, L., Kayser, S., Teleanu, V., Kett, H., Habdank, M., Kugler, C.M., Holzmann, K., Gaidzik, V.I., Paschka, P., Held, G., von Lilienfeld-Toal, M., Lubbert, M., Frohling, S., Zenz, T., Krauter, J., Schlegelberger, B., Ganser, A., Lichter, P., Dohner, K. & Dohner, H. (2012) TP53 alterations in acute myeloid leukemia with complex karyotype correlate with specific copy number alterations, monosomal karyotype, and dismal outcome. *Blood*, 119, 2114–2121.
- 48 Neubauer, A., Maharry, K., Mrozek, K., Thiede, C., Marcucci, G., Paschka, P., Mayer, R.J., Larson, R.A., Liu, E.T. & Bloomfield, C.D. (2008) Patients with acute myeloid leukemia and RAS mutations benefit most from postremission high-dose cytarabine: a Cancer and Leukemia Group B study. *Journal of Clinical Oncology*, 26, 4603–4609.
- 49 Liersch, R., Gerss, J., Schliemann, C., Bayer, M., Schwoppe, C., Biermann, C., Appellmann, I., Kessler, T., Lowenberg, B., Buchner, T., Hiddemann, W., Muller-Tidow, C., Berdel, W.E. & Mesters, R. (2012) Osteopontin is a prognostic factor for survival of acute myeloid leukemia patients. *Blood*, 119, 5215–5220.
- 50 Konoplev, S., Rassidakis, G.Z., Estey, E., Kantarjian, H., Liakou, C.I., Huang, X., Xiao, L., Andreeff, M., Konopleva, M. & Medeiros, L.J. (2007) Overexpression of CXCR4 predicts adverse overall and event-free survival in patients with unmutated FLT3 acute myeloid leukemia with normal karyotype. *Cancer*, 109, 1152–1156.
- 51 Rombouts, E.J., Pavic, B., Lowenberg, B. & Ploemacher, R.E. (2004) Relation between CXCR-4 expression, Flt3 mutations, and unfavorable prognosis of adult acute myeloid leukemia. *Blood*, 104, 550–557.
- 52 Tang, R., Hirsch, P., Fava, F., Lapusan, S., Marzac, C., Teyssandier, I., Pardo, J., Marie, J.P. & Legrand, O. (2009b) High Id1 expression is associated with poor prognosis in 237 patients with acute myeloid leukemia. *Blood*, 114, 2993–3000.
- 53 Marcucci, G., Baldus, C.D., Ruppert, A.S., Radmacher, M.D., Mrozek, K., Whitman, S.P., Kolitz, J.E., Edwards, C.G., Vardiman, J.W., Powell, B.L., Baer, M.R., Moore, J.O., Perrotti, D., Caligiuri, M.A., Carroll, A.J., Larson, R.A., de la Chapelle, A. & Bloomfield, C.D. (2005) Overexpression of the ETS-related gene, ERG, predicts a worse outcome in acute myeloid leukemia with normal karyotype: a Cancer and Leukemia Group B study. *Journal of Clinical Oncology*, 23, 9234–9242.
- 54 Damm, F., Heuser, M., Morgan, M., Wagner, K., Gorlich, K., Grosshennig, A., Hamwi, I., Thol, F., Surdziel, E., Fiedler, W., Lubbert, M., Kanz, L., Reuter, C., Heil, G., Delwel, R., Lowenberg, B., Valk, P.J., Krauter, J. & Ganser, A. (2011a) Integrative prognostic risk score in acute myeloid leukemia with normal karyotype. *Blood*, 117, 4561–4568

# 12

## LIST OF MEMBERS WHO PARTICIPATED AND CONTRIBUTED FOR THE CONSENSUS DOCUMENT ON AML.

1. Dr Prasanth Ganeshan, MD;DM  
Addl Professor  
Department of Medical Oncology  
JIPMER, Puducherry  
E mail : pg1980@gmail.com
2. Vikram Mathew, MD;DM  
Professor of Haematology  
Christian Medical College, Vellore, (TN)  
E mail : vikram@cmcvellore.ac.in
3. Sameer Bakhshi, MD  
Professor,  
Department of Medical Oncology  
IRCH-AIIMS, New Delhi 110029  
E Mail : sambakh@hotmail.com
4. Brijesh Aurora,MD;DM  
(formerly) Professor  
Department of Paediatric Oncology  
Tata Memorial Hospital  
Parel, Mumbai-400012  
E Mail : brijesh.aurora@gmail.com
5. Pankaj Malhotra,MD  
Professor of Medicine  
PGIMER, Chandigarh  
E mail : malhotrapankaj@hotmail.com
6. Senthil Rajappa, MD;DM  
Head, Department of Medical Oncology  
Indo American Cancer Centre  
Punjagutta, Hyderabad  
E mail : senthilrajappa@gmail.com
7. Sumit Gujral,MD  
Department of Haematopathology  
Tata Memorial Hospital  
Parel, Mumbai 400012  
E mail : flowtmh@gmail.com
8. Mammen Chandy, MD  
Director  
Tata Medical Centre, Kolkata  
E Mail : mammen.chandy@gmail.com
9. Smita Kayal, MD;DM  
Associate professor of medical Oncology  
JIPMER, Puducherry  
E mail : kayalsmita@gmail.com
10. Navin Khattry,MD;DM  
Professor of medical Oncology and Incharge,  
BMT  
Tata Memorial Hospital, ACTREC,  
Mumbai  
E mail : nkhatry@gmail.com
11. Tapan Saikia,MD  
Consultant, Prince Aly Khan Hospital  
Mumbai  
E Mail : tapan.saikia@gmail.com





**icmr**  
INDIAN COUNCIL OF  
MEDICAL RESEARCH  
Serving the nation since 1911

**INDIAN COUNCIL OF MEDICAL RESEARCH**

**Department of Health Research**

(Ministry of Health & Family Welfare)

V. Ramalingaswami Bhawan, Post Box 4911,  
Ansari Nagar, New Delhi-110029

Phone: 91-11-26588980, 26589794, 26588895

E-mail: [icmrhqds@sansad.nic.in](mailto:icmrhqds@sansad.nic.in)

Website: <http://www.icmr.nic.in>