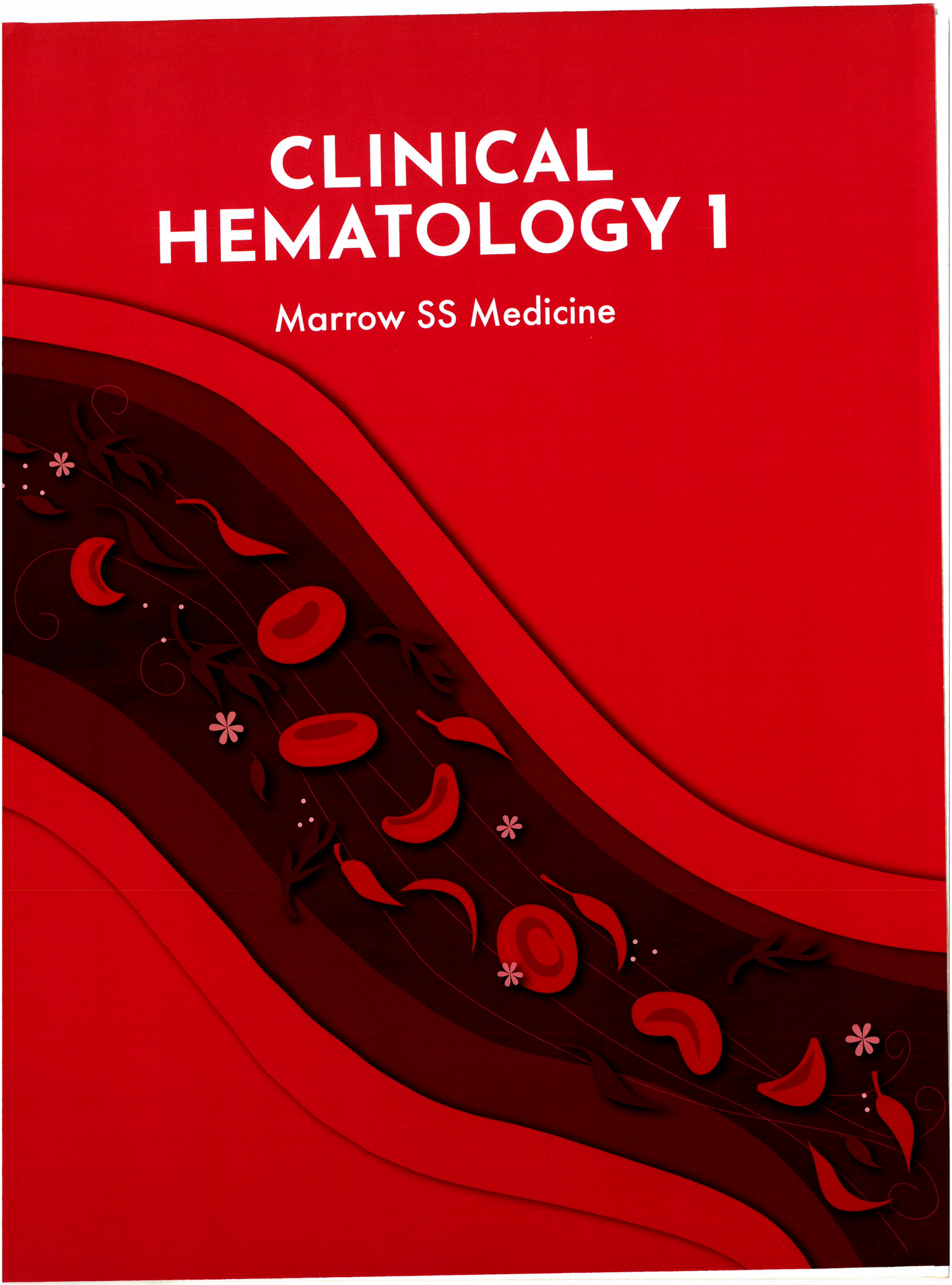
CLINICAL HEMATOLOGY 1

Marrow SS Medicine



Instructions

- The notes must be used in conjunction with the Marrow SS Medicine videos and should not be considered standalone material.

Please note:

- The information in this book has been printed based on the transcript of the Marrow SS Medicine videos.
- The information contained in this book is for educational purposes only. The content provided is not intended to substitute for professional medical advice, diagnosis or treatment.
- This book cannot be sold separately. It has been made available to only select eligible users who have an active subscription to Marrow SS Medicine videos.
- The text, images, slides, and other materials used in this book have been contributed by the faculty, who are subject matter experts. We have merely reproduced them as video transcripts in this book.
- The notes have been consciously designed in a way that is concise and revisable. To ensure this, we have intentionally added only the most relevant modules and images that are needed for you.
- Reasonable care has been taken to ensure the accuracy of the information provided in this book. Neither the faculty nor Marrow takes any responsibility for any liability or damages resulting from applying the information provided in this book.
- This set contains notes of all main videos published in the app until Jan 2025. You can find notes for any additional videos published after this date within the app under the videos section.

//ME801SS-SAP

Contents

Volume - 1

Plasma Cell Dyscrasias

Myeloproliferative Neoplasms

Hematopoietic Stem Cell Transplantation

Volume - 2

Anemia

Hemolytic Anemia

Hemoglobinopathies

Platelet Disorders

Coagulation Disorders

Thrombosis

Fungal Infections

Special topics

ACUTE MYELOID LEUKEMIA

----- Active space -----

Introduction

00:00:15

Features :

Acute myeloid leukemia (AML) is a neoplasm characterized by :

- Infiltration of the blood, bone marrow, and other tissues.
- Proliferative, clonal, poorly differentiated cells of hematopoietic system.
- 1.3% of all cancers.
- 31% of all acute leukemias.
- But, 62% of all leukemic deaths.
- AML is the most common acute leukemia in older patients, with a median age at diagnosis of 67 years.

Causes :

- Idiopathic (most common).
- Genetic predisposition.
- Radiation.
- Chemicals/other occupational exposures → Benzene, chloramphenicol, phenylbutazone.
- Drugs :
  i. Alkylating agents → Latency period of 5 yrs (Chr 5 & 7).
  ii. Topoisomerase-II inhibitors → Latency period of 1-3 yrs (Chr 11q23).

Classification of AML :

WHO 2016 myeloid neoplasms with germline predisposition :

MN with germline predisposition :

- AML with germline CEBPA.
- MN with germline DDX41.

MN with germline predisposition + pre-existing platelet disorder :

- MN with germline RUNX1.
- MN with germline ANKRD26.
- MN with germline ETV6.

----- Active space -----

MN with germline predisposition + other organ dysfunction :

- MN with germline GATA2.
- MN with BM failure syndromes.
- MN with telomere biology disorders.
- MN with Down/Noonan syndrome.

Down syndrome associated AML :

- Age <4 years.
- Acute megakaryoblastic type.
- Mutation of GATA 1 gene.

Pre malignant states :

	CHIP or ARCH	IDUS	ICUS
Mutations	+	-	-
Dysplasia	-	+	-
Cytopenias	-	-	+

- Mutations in DNMT3A, TET2, ASXL1,DTA has 10x risk of haematological malignancy and increased risk of CV mortality.
- ARCH : Age-related clonal hematopoiesis.
- CHIP : Clonal hematopoiesis of indeterminate potential.
- ICUS : Idiopathic cytopenia of undetermined significance.
- IDUS : Idiopathic dysplasia of undetermined significance.

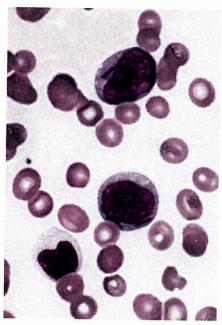
Diagnosis of AML

00:04:39

Case scenario :

A 50 year old man with fatigue and gum bleed since 2 weeks, CBC :7.0 / 1.25 lac / 15,000, peripheral smear reports 80% atypical cells (Shown in panel on right),- flow cytometry shows cells +ve for CD45 dim, cMPO+, CD34+, CD13+, CD33+, CD117+.

Diagnosis is AML.

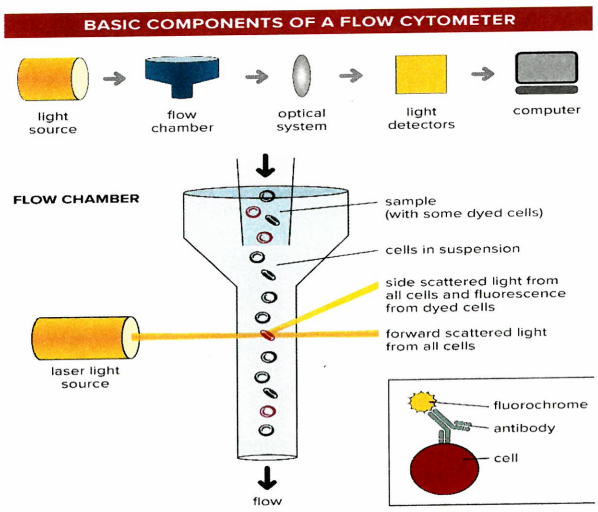


Peripheral smear

Flow cytometry/immunophenotyping :

----- Active space -----

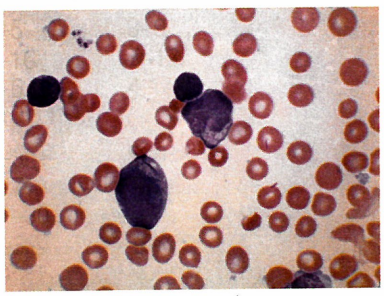
- Uses antibodies against specific proteins on surface of cells to differentiate leukemic cells from normal cells.
- Side scattered indicates granularity, forward scattered indicates size.



Flow cytometry

Auer rods :

- Auer rods are rod-shaped crystalline structures derived from the primary granules of myeloid cells.
- Auer rods are of considerable diagnostic importance since they indicate both the lineage and the neoplastic nature of the condition observed.



Auer rods

Assignment of lineage :

WHO 2008 Acute leukemias of ambiguous lineage :

Lineage	Requirements
Myeloid.	Myeloperoxidase (flow cytometry, immunohistochemistry or cytochemistry) or Monocytic differentiation (Diffuse positivity for NSE or atleast two of the following : CD11c,CD14,CD36,CD64,lysozyme.
T.	Cytoplasmic CD3 (Flow cytometry with antibodies to CD3 epsilon chain) or Surface CD3.
B.	Strong CD19 and strong expression of atleast one of the following : CD79a, cytoplasmic CD22, CD10. or Weak CD19 and strong expresion of atleast two of the following : CD79a, cytoplasmic CD22, CD10.

----- Active space -----

WHO 2016 classification :

It is based on :

- Clinical presentation.
- Morphology.
- Cytogenetics.
- Molecular features.

Diagnosis :

BM (or blood) blast count ≥20%, except for AML with recurrent genetic abnormalities.

i. t(15;17).
ii. t(8;21).
iii. Inv(16) or t(16;16).

Acute myeloid leukemia (AML) with recurrent genetic abnormalities
AML with t(8;21)(q22;q22); *RUNX1-RUNX1T1*
AML with inv(16)(p13.1q22) or t(16;16)(p13.1;q22); *CBFB-MYH11*
Acute promyelocytic leukemia with *PML-RARA*
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, not otherwise specified (NOS)
AML with minimal differentiation
AML without maturation
AML with maturation
Acute myelomonocytic leukemia
Acute monoblastic/monocytic leukemia
Pure erythroid leukemia
Acute megakaryoblastic leukemia
Acute basophilic leukemia
Acute panmyelosis with myelofibrosis
Myeloid sarcoma
Myeloid proliferations related to Down syndrome
Transient abnormal myelopoiesis (TAM)
Myeloid leukemia associated with Down syndrome

AML classification

Association of chromosomal abnormalities with specific features :

- Inv(16) (p13.1q22) with abnormal BM eosinophils.
- t(8;21) (q22;q22) : Slender Auer rods, CD19+, increased normal eosinophils, myeloid sarcomas.
- t(15:17) : DIC.
- t(9;11) (p22;q23), other 11q23 abn : monocytic features, EM involvement.

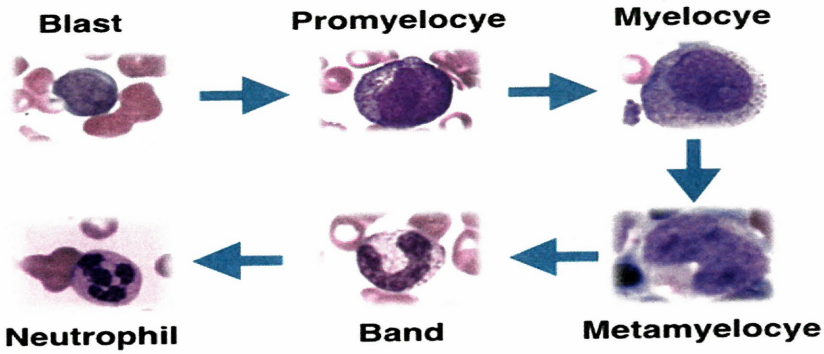
- NPM1 mutation especially when co-occurring with FLT3 mutation : "Cup-shaped" nuclear morphology, high WBC count.
- t(8;21) and t(15;17) : Younger age.
- Del5q, del7q, TP53 mutation : Older age.

----- Active space -----

APML

00:14:48

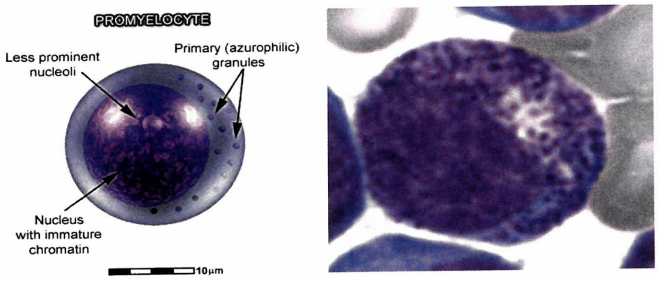
A subtype of AML. There is block in myeloid differentiation at the promyelocyte level.



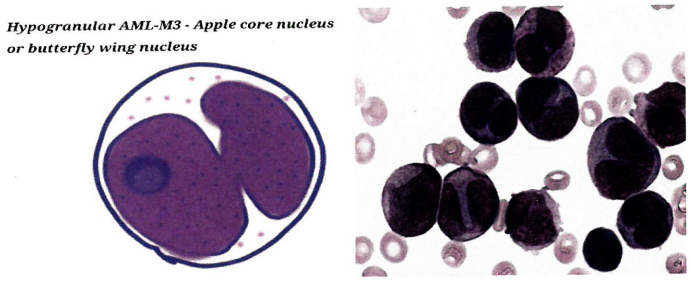
myeloid differentiation

Peripheral smear findings :

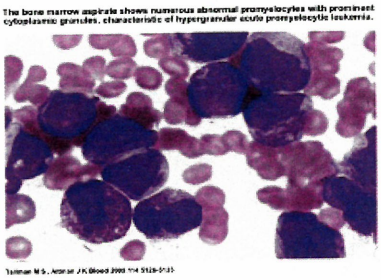
- Normal promyelocyte : Less prominent nucleoli, Primary (azurophilic) granules, nucleus with immature chromatin.
- Abnormal promyelocyte : Apple core/butterfly wing nucleus, prominent violet granules in cytoplasm.
- Pathognomic finding of APML : Faggot cells → Bunch of auer rods.



Normal promyelocyte



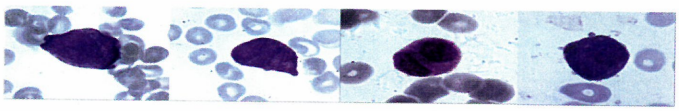
Abnormal promyelocyte

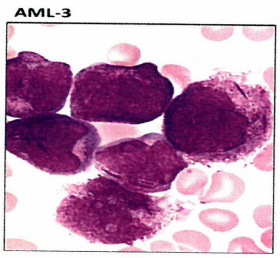


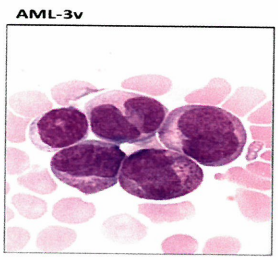
Faggot cell

----- Active space -----

Hypergranular or typical APL (m3).	Hypogranular or microgranular APL (m3v).
60 to 70% of cases.	Leukocytosis.
Low white blood cell count.	Numerous abnormal promyelocytes readily identified on aperipheral blood smear.
Abnormal promyelocytes with numerous red to purple cytoplasmic granules that are typically darker and larger than normal neutrophil granules.	Irregular nucleus and granulations sparser and finer compared with the hypergranular form.
Identifiable faggot/matchstick cells with numerous auer rods.	Faggot cells with multiple auer rods less commonly seen.



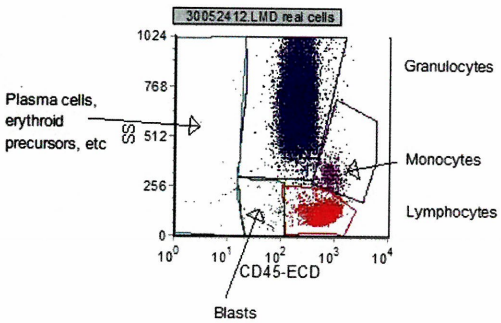


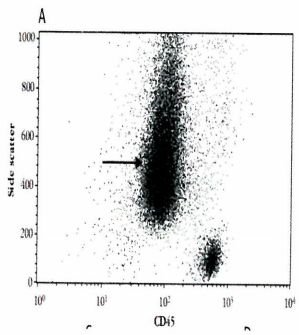


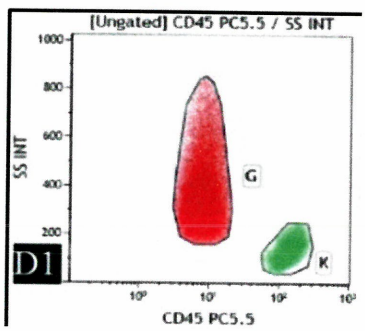
APM

Typical characteristics in flow cytometry :

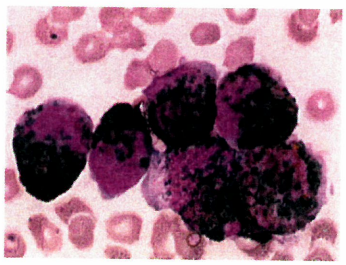
- Intense MPO positivity.
- Autofluorescence on CD45 on X axis and side scattered cells on the y axis.





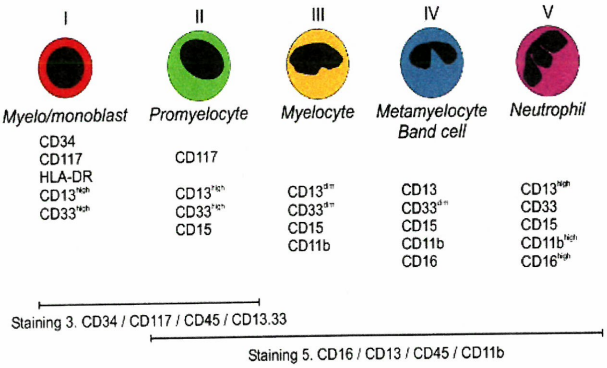


Autofluorescence



MPO positivity

- Flow cytometry pattern in APML :
  i. HLA DR CD34-.
  ii. CD13+ CD33+ CD117+.
  iii. CD11b-.



Flow cytometry in APML

----- Active space -----

Pathogenesis :

Pathognomic of APML is 15/17 translocation/PML RARA.

This translocation was given by Janet rowley M.D. (1925 - 2013).

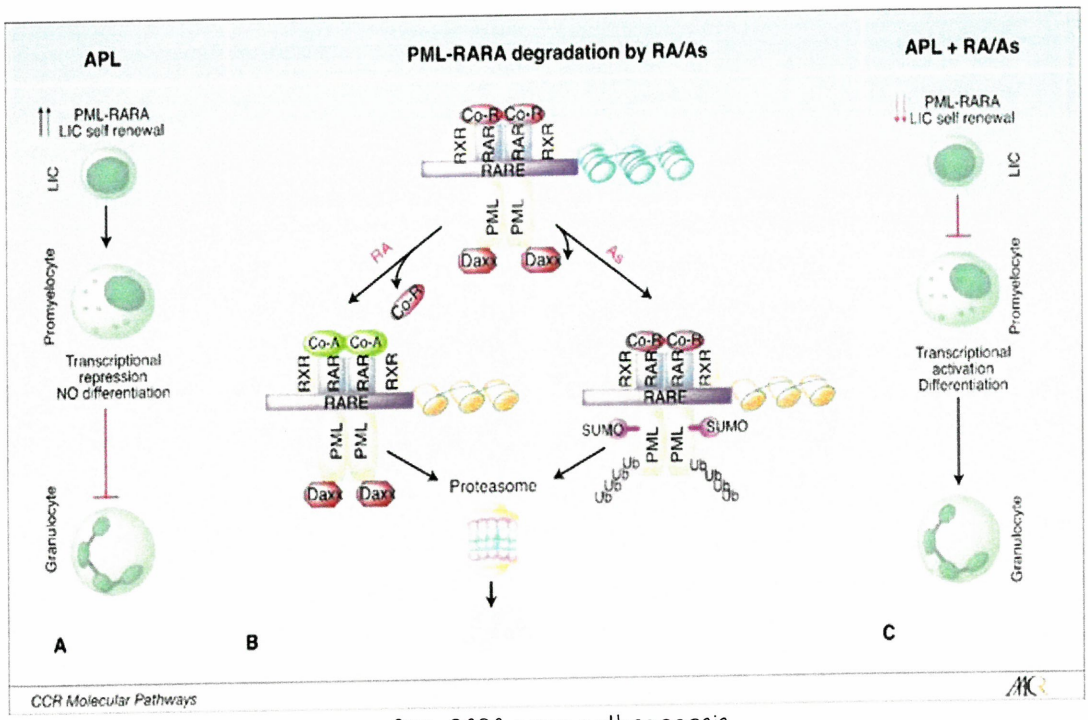
Normal cellular functions :

RARA :

- RAR-alpha interacts with RXR .
- Normal RAR-a-RXR heterodimer recruits corepressor (CoR) or coactivators (CoA) complexes at the chromatin level to differentially regulate transcription of its target genes.

PML :

- Present in specific nuclear structures called nuclear bodies (NBs).
- Promotes apoptosis, and acts as a tumor suppressor.



PML RARA gene pathogenesis

----- Active space -----

Mutations :

- NPM1, biallelic CEBPA → Good prognosis.
- FLT3 mutations → Either ITD (bad prognosis) or TKD (uncertain prognosis).
- FLT3-ITD → Occurs preferentially in CN-AML.
- FLT3 allelic ratio (number of mutated alleles to wild-type alleles) is more relevant (Allelic ratio is determined by DNA-fragment length analysis. AUC of FLT3-ITD / AUC of FLT3-wild type).
- Ratio <0.5 – low allelic ratio (AR) → Good prognosis.
- Ratio >0.5 – high allelic ratio → Bad prognosis.

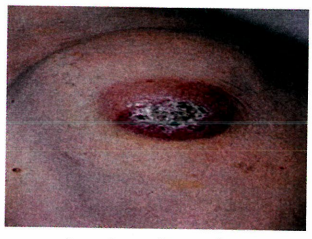
Clinical features of AML

00:26:44

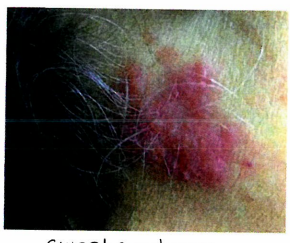
Salient features :

- Fatigue (50%).
- Anorexia and weight loss .
- Fever with or without an identifiable infection (10%).
- Signs of abnormal hemostasis (5%) :
- Bleeding , easy bruising.
- Petechiae.
- Bone pain.
- Lymphadenopathy.

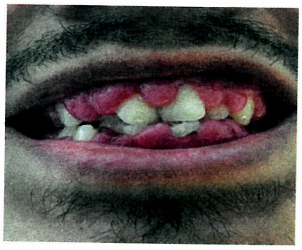
	Leukemia cutis.	Sweet syndrome.
Location.	Trunk.	Face,neck,arms.
Tenderness.	No.	Yes.
Biopsy.	Infiltrate of blasts; Diffuse dermal infiltrate with sparing of upper dermis (Grenz zone).	Infiltrate of neutrophils; Diffuse/ band like dermal infiltrate.
Rx.	Same as underlying disease.	Steroids.

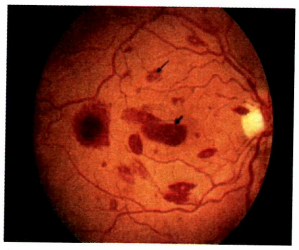


Leukemia cutis



Sweet syndrome





AML features

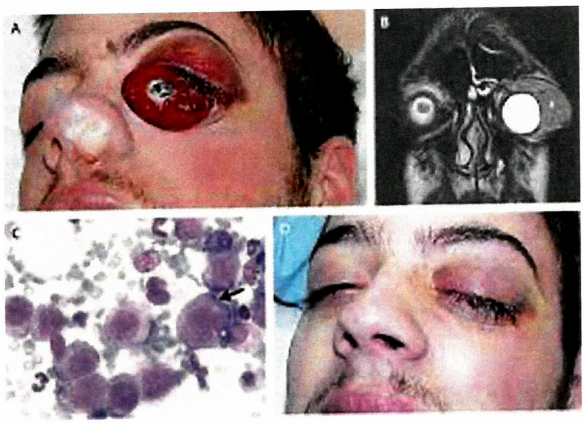
Myeloid sarcoma

00:28:54

----- Active space -----

General features :

- Extramedullary collections of myeloblasts.
- Skin, LN, GIT, soft tissue, and testis.
- Association with monosomy 7, trisomy 4, trisomy 8, 11q23 rearrangement, inversion[16], t[8;21].
- may precede or coincide with blood and/or BM involvement by AML.
- MS patients typically develop blood and/or BM involvement quickly thereafter and cannot be cured with local Rx (radiation or surgery) alone.



myeloid sarcoma

Risk stratification of AML

00:30:03

ELN 2017 risk stratification of AML :

Complex CTG :

>=3 unrelated CTG abn [in absence of t(8;21), inv 16, t(6;9), t(9;11), inv(3), AML with BCR-ABL1].

Monosomal karyotype :

- 1 monosomy (except loss of X or Y) + 1 additional monosomy.
- 1 monosomy + 1 additional structural CTG abn (except CBF AML).

t(9;11) + adverse risk mutations : Count it as t(9;11)

RUNX1, ASXL1, TP53 should not be counted for prognostication, if they occur with favourable risk CTG.

----- Active space -----

RISK CATEGORY[b]	GENETIC ABNORMALITY
Favorable	t(8;21)(q22;q22); *RUNX1-RUNX1T1*
	inv(16)(p13.1q22) or t(16;16)(p13.1;q22); *CBFB-MYH11*
	Mutated *NPM1* without *FLT3*-ITD or with *FLT3*-ITD[low(c)]
	Biallelic mutated *CEBPA*
Intermediate	Mutated *NPM1* and *FLT3*-ITD[high(c)]
	Wild-type *NPM1* without *FLT3*-ITD or with *FLT3*-ITD[low(c)] (w/o adverse-risk genetic lesions)
	t(9;11)(p21.3;q23.3); *MLLT3-KMT2A*[d]
	Cytogenetic abnormalities not classified as favorable or adverse
Adverse	t(6;9)(p23;q34.1); *DEK-NUP214*
	t(v;11q23.3); *KMT2A* rearranged
	t(9;22)(q34.1;q11.2); *BCR-ABL1*
	inv(3)(q21.3q26.2) or t(3;3)(q21.3;q26.2); *GATA2, MECOM(EVI1)*
	–5 or del(5q); –7; –17/abn(17p)
	Complex karyotype,[e] monosomal karyotype[f]
	Wild type *NPM1* and *FLT3*-ITD[high(c)]
	Mutated *RUNX1*[g]
	Mutated *ASXL1*[g]
	Mutated *TP53*[h]

Prognostic factors :

- Patient related.
- Age.
    i. Comorbidities : Inability to give intensive chemo.
    ii. Intrinsically more resistant disease : Higher number of mutations, CHIP & MDS.
- Poor ECOG-PS.

Disease related :

1. Cytogenetics :
    - t(15;17) have a very good prognosis (85% cured).
    - CBF-AML – t(8;21), inv(16) have a good prognosis (55% cured) (exception – KIT mutations).
    - No CTG abnormality → 40% cured.
    - TP53 mutation, complex karyotype, t(6;9), inv(3), or –7 → very poor prognosis.

2. Antecedent MDS/MPN.

3. Cytopenia duration :
    - Lesser CR rate in patients who have had anemia, leukopenia, and/or thrombocytopenia for >3 months before the diagnosis of AML.
4. Hyperleukocytosis (>100,000/microL) : Risk of early CNS bleeding and pulmonary leukostasis.

----- Active space -----

molecular prognostic markers :

Prognostic molecular markers in AML are not mutually exclusive & often occur concurrently (>80% patients have at least >=2 prognostic gene mutations), the likelihood that distinct marker combinations may be more informative than single markers

	NPM1	FLT3-ITD
Favorable	+	-
Intermediate	+	+
	-	-
Bad	-	+

GENE SYMBOL	GENE LOCATION	PROGNOSTIC IMPACT
Genes Included in the WHO Classification and ELN Reporting System		
NPM1 mutations	5q35.1	Favorable
CEBPA mutations	19q13.1	Favorable
FLT3-ITD	13q12	Depends on allelic ratio and *NPM1* mutational status
Genes Encoding Receptor Tyrosine Kinases		
KIT mutation	4q12	Adverse
FLT3-TKD	13q12	Unclear
Genes Encoding Transcription Factors		
RUNX1 mutations	21q22.12	Adverse
WT1 mutations	11p13	Adverse
Genes Encoding Epigenetic Modifiers		
ASXL1 mutations	20q11.21	Adverse
DNMT3A mutations	2p23.3	Adverse
IDH mutations (*IDH1* and *IDH2*)	2q34 & 15q26.1	Adverse
KMT2A-PTD	11q23	Adverse
TET2 mutations	4q24	Adverse
Deregulated Genes		
BAALC overexpression	8q22.3	Adverse
ERG overexpression	21q22.3	Adverse
MN1 overexpression	22q12.1	Adverse
EVI1 overexpression	3q26.2	Adverse
Deregulated MicroRNAs		
miR-155 overexpression	21q21.3	Adverse
miR-3151 overexpression	8q22.3	Adverse
miR-181a overexpression	1q32.1 and 9q33.3	Favorable

*This table excludes acute promyelocytic leukemia.

Abbreviations: AML, acute myeloid leukemia; ELN, European LeukemiaNet; ITD, internal tandem duplication; PTD, partial tandem duplication; TKD, tyrosine kinase domain; WHO, World Health Organization.

Treatment

00:35:35

Pre treatment evaluation :

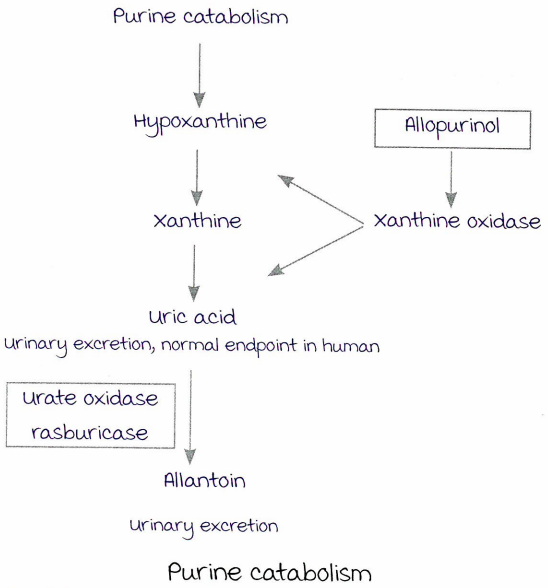
Diagnosis :

- CBC : median WBC 15,000, 1/3rd - <5K, 20% - >1lac, <5% → Aleukemic leukemia.
- Platelet <1lac in 75% (1/4th with <25K).
- BM aspirate, biopsy, flow-cytometry, CTG.

----- Active space -----

Fitness :

- LFT, RFT (especially uric acid, calcium, creat, phosphorus).
- Coagulation – PT, PTT, Fibrinogen, D-dimer.
- Cardiac – Echo or MUGA scan.
- Viral markers.
- Blood grouping.
- Future : HLA matching, cryopreservation of leukemia cells, sperm cryopreservation.



Purine catabolism

Response assesment :

Definition of CR :

- Achievement of CR is associated with better outcome and longer survival.
- ANC ≥1000/micro L.
- Platelet count ≥100,000/micro L (Hb is not considered in determining CR).
- Bm blasts <5%, without Auer rods.
- Circulating blasts should be absent. Although rare blasts may be detected in the blood during marrow regeneration, they should disappear on successive studies.
- Extramedullary leukemia should not be present.

Principles of treatment :

- Rx divided in 2 parts : Induction & Consolidation.
- Induction therapy : To induce CR.
- Consolidation chemotherapy : Prolong survival and achieve cure.

Induction chemotherapy :

Intensive chemotherapy (ICT) → 7+3 regimen :

- 7 days Ara-C (100-200mg/m2/d continuous infusion) + 3 days Daunomycin (60mg/m2/d).
- CR rates : Young (<60yrs) → 60-80%, Elderly fit → 30-60% CR.
- Rest have induction deaths or drug resistant leukemia.
- Cytarabine : Cell cycle specific (S-phase) becomes phosphorylated intracellularly to an active triphosphate form .Interferes with DNA synthesis.
- Anthracyclines : Cell-cycle non-specific (DNA intercalators),Inhibition of topoisomerase II, leading to DNA breaks.

----- Active space -----

Consolidation or Post-remission Rx in favourable risk AML : IDAC/HIDAC

- Durable CR-1 is critical to long-term survival in AML.
- Without further therapy after induction, virtually all patients relapse.
- Consolidation Rx is given to eradicate residual leukemic cells to prevent relapse.
- HiDAC (3 g/m2, every 12 h on D 1, 3, 5) or IDAC (1-1.5 g/m2).
- Number of cycles : 2-4.

Consolidation or post-remission treatment in Intermediate or high-risk AML :

- Allogeneic HCT is the best relapse-prevention strategy currently available for AML.
- Why transplant ?
  i. Once AML relapses, it is typically resistant to chemotherapy.
  ii. Hence, allogeneic HCT in CR1 is a favoured strategy.
- Why allogeneic HSCT, if autologous HSCT has less transplant-related mortality ? Relapse rate is less due to graft-versus-leukemia effect.

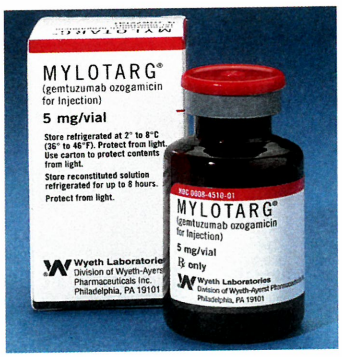
Novel agents :

FDA approvals in 2017 :

- Gemtuzumab ozogamicin.
- Enasidenib.
- Midostaurin.
- CPX351.

Gemtuzumab ozogamicin :

- 1 SEP/ 2017, FDA approved.
- Indications :
  i. Adults with newly diagnosed CD33+ AML.
  ii. >2 yrs with CD33+ AML relapsed / refractory.

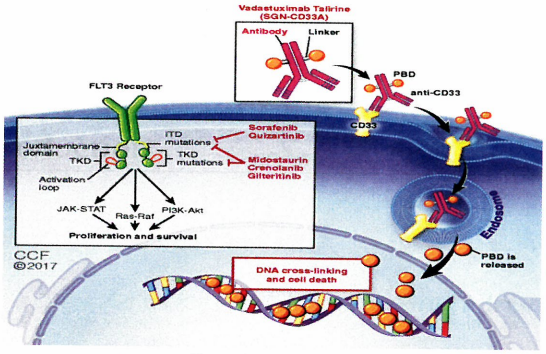


Gemtuzumab ozogamicin

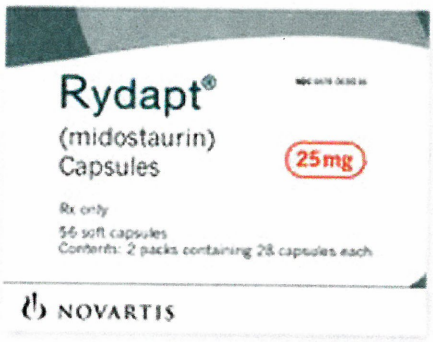
----- Active space -----

Midostaurin :

FLT3 inhibitor.



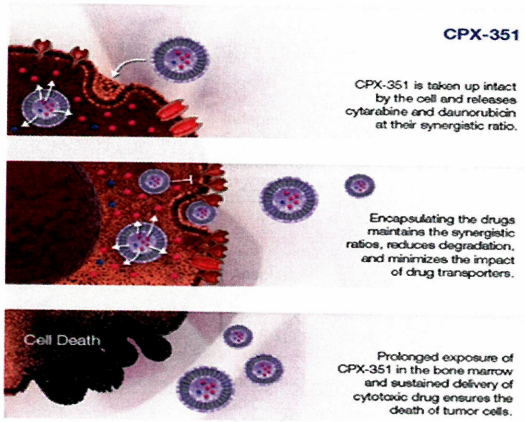
MOA of midostaurin



Midostaurin tablet

CPX-35 I: VYXEOS :

- FDA approved for elderly (60-75 years) fit patients with t- AML or AML - MRC.
- A nano scale liposomal co formulation of cytarabine and daunorubicin at a synergistic 5:1 molar ratio.
- Taken up preferentially by human leukemia cells, ensuring intracellular delivery of the optimal dose.
- Hypothesized that preferential uptake into leukemia cells boosts efficacy while maintaining a very favorable non hematological toxicity profile.



CPX 35

Elderly AML :

Elderly vulnerable : Consider lower intensity therapy.

- Hypomethylating agent (decitabine or azacitidine) + Venetoclax.
- Low dose cytarabine + Venetoclax.
- IDH1 or IDH2 inhibitor, if mutated.
- Gemtuzumab single agent.
- preferably investigational therapy.

Elderly frail : Best supportive care/palliative care.

Supportive care :

- Maintain a platelet count above 10,000/micro L.
  i. >20000/micro L - febrile patients and during episodes of active bleeding.
  ii. >50000/microL - hyperleukocytosis.