Advances in the Pathology and Biology of Acute Myeloid Leukemia

Carlos E. Bueso-Ramos

Department of Hematopathology
The University of Texas M. D. Anderson Cancer Center
Houston, Texas

THE UNIVERSITY OF TEXAS
MD Anderson Cancer Center
Making Cancer History®

Disclosures

Consultant, Incyte

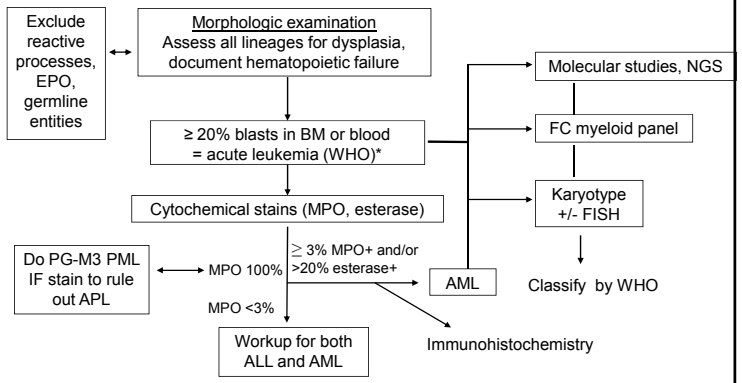
Outline

- Diagnosis
- Classification, 2016 WHO revision
- Cytogenetic/Genetic studies
- Novel somatic gene mutations in AML
- Conclusions

AML

- Genetically heterogeneous clonal disorder
- Characterized by the somatic acquisition of genetic and epigenetic alterations in hematopoietic progenitor cells
- Disrupts normal mechanisms of self-renewal, proliferation, and differentiation

Algorithmic Approach to AML Diagnosis



WHO Classification updates

2008 → **2016**

AML with recurrent genetic abnormalities

Entities with NO CHANGE

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*

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

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

WHO Classification updates

2008 → **2016**

AML with recurrent genetic abnormalities

Updated name

APL with *PML-RARA*

(previous APL with t(15;17)(q22;q12), *PML-RARA)*

Updates in genes names

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

(Previous *MLLT3-MLL*)

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

(previous *RPN1-EVI1*)

WHO Classification updates

2008 → **2016**

AML with recurrent genetic abnormalities

New entities

AML with mutated *NPM1*

AML with biallelic mutations of *CEBPA*

New Provisional entities

AML with *BCR-ABL1*

AML with mutated *RUNX1*

2016 WHO Classification of AML

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-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

The 20% blasts rule does not apply (WHO)*

2016 WHO Classification of AML

AML with myelodysplasia-related changes

- Morphological features of MDS
  Except if mutation of NPM1or Biallelic mutation of CEBPA is present
- Prior history of MDS or MDS/MPN
- MDS-related cytogenetic abnormalities
  Except del(9q)
- Absence of AML with recurrent genetic abnormalities
- No prior history of cytotoxic or radiation Rx

Cytogenetic Abnormalities to Diagnose AML with MDS-Related Features

Complex karyotype	
Unbalanced abnormalities	**Balanced Abnormalities**
-7/del(7q)	t(11;16)(q23;p13.3)
-5/del(5q)	t(3;21)(q26.2;q22.1)
i(17q)/t(17p)	t(1;3)(p36;3q21.1)
-13/del(13q)	t(2;11)(p21;q23)
del(11q)	t(5;12)(q33;p12)
del(12p)/t(12p)	t(5;7)(q33;q11.2)
del(9q) Not longer included	t(5;10)(q33;q21)
idic(X)(q13)	t(3;5)(q25;q34)

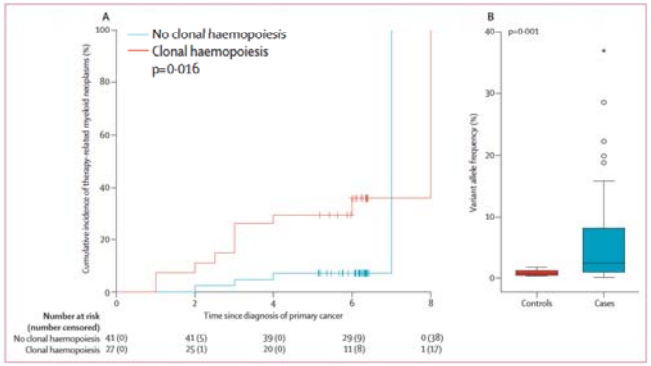
2016 WHO Classification of AML

Therapy-related myeloid neoplasms

- t-AML (blasts 20%+)
- t-MDS (blasts <20%)
- t-MDS/MPN

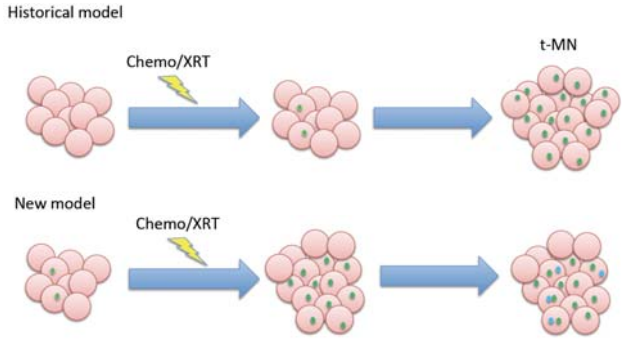
Do **NOT** include **transformation of MPN**

Preleukemia clonal hemopoiesis is a predictive marker of therapy-related myeloid neoplasms in patients with cancer



Takahashi et al. Lancet Oncol 2017; 18: 100–11

New model of therapy-related myeloid neoplasms



Takahashi et al. Lancet Oncol 2017; 18: 100–11

WHO Classification updates

2008 → **2016**

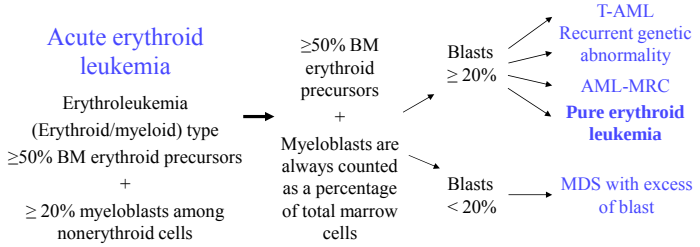
AML not otherwise categorized

- 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

WHO Classification updates

2008 → **2016**

AML not otherwise categorized



WHO Classification updates

2008 → **2016**

Myeloid neoplasms with germ line predisposition

Without a preexisting disorder or organ dysfunction
- AML with germ line CEBPA mutation
- Myeloid neoplasms with germ line DDX41 mutation*

With preexisting platelet disorders
- RUNX1 mutation*
- ANKRD26 mutation*
- ETV6 mutation*

With other organ dysfunction
- GATA2 mutation
- Associated with BM failure syndromes
- Associated with telomere biology disorders
- JMML associated with neurofibromatosis, Noonan syndrome or Noonan syndrome-like disorders
- Myeloid neoplasms associated with Down syndrome*

Arber at al. Blood. Blood. 2016;127(20):2391-2405
The University of Chicago Hematopoietic Malignancies Cancer Risk Team. Blood. 2016;128(14):1800-1813

Recommended Workups for Various Stages of AML

	Morphology Immunophenotyping	Cytogenetics FISH
New diagnosis	• Morphology, cytochemical stains • Flow immunophenotyping (FC) to evaluate blasts and • background hematopoiesis for dysplasia	• Conventional karyotype • FISH for translocations, as needed
MRD assessment	• Kinetics of blasts during induction • MRD by FC with acquisition of at least 100,000 cells	• FISH for AML-specific change (if molecular test is not available)
Overt relapse	• Morphology, cytochemical stains Extended FC panel	• Conventional karyotype (or CGH or SNP array)

Recommended Workups for Various Stages of AML

	Molecular Testing
New diagnosis	• NGS 82 gene panel • FLT3 • NPM1 (if intermediate-risk) • KIT (if CBF leukemias) • CEBPA • Chromosome translocation screen
MRD assessment	• AML-specific translocation • Specific gene mutation

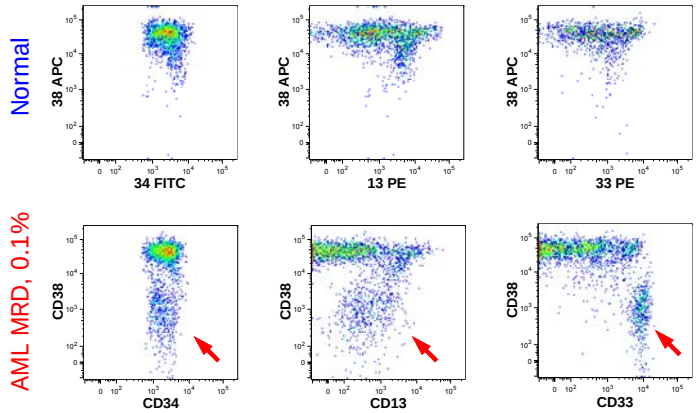
MRD-test results refine risk-stratification for patients in morphologic remission

Positive MRD-test: associated with higher relapse risk and/or shorter survival at cohort level

- Day 15 of first cycle of chemotherapy
- After first or second cycle of chemotherapy
- During/after post-remission chemotherapy
- Before autologous or allogeneic transplantation
- After transplantation

Luger. Lymphoma, Myeloma & Leukemia. Vol2, S2 Sept 2017 SOHO meeting

AML MRD vs. Normal CD34+



Courtesy of Dr. Jeffrey Jorgensen

Ravandi F, Jorgensen JL, JNCCN 2012; 10:1029-36

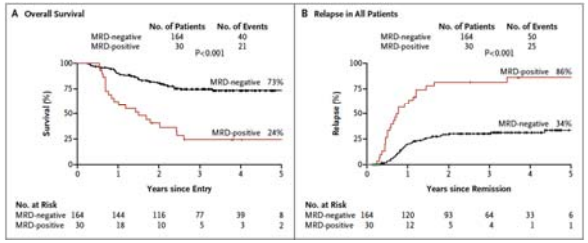
Molecular monitoring of MRD

- APL (*PML-RARA)*: MRD assessment has been introduced as a component of the standard response criteria
- CBF leukemias:
  - >3 log reduction in *RUNX1-RUNX1* transcripts in BM after 1 cycle of induction therapy was the strongest prognostic variable for relapse
  - *CBF-MYH11*: MRD level is a predictor of relapse. Then serial monitoring should be performed at 3-month intervals during follow-up.

Ossenkoppele, et al. Hematology 2016

Molecular monitoring of MRD

The presence of minimal residual disease, as determined by quantitation of *NPM1*-mutated transcripts, provided powerful prognostic information independent of other risk factors (*FLT3* and *DNMT3A*)



Ivey et al. NEJM. 2016;374:422-33.

Molecular monitoring of MRD

- *MLL-MLLT3:* 2% of AMLs (adults). MRD assessment is useful to predict outcome
- *FLT3-ITD*: Technical limitations and instability of the marker, the clinical applicability for MRD assessment is currently limited
- *WT1* gene expression: lack of specificity and limited sensitivity

Ossenkoppele, et al. Hematology 2016

European Leukemia Net (ELN) Prognostic System Younger Adults with AML

2010 → 2017

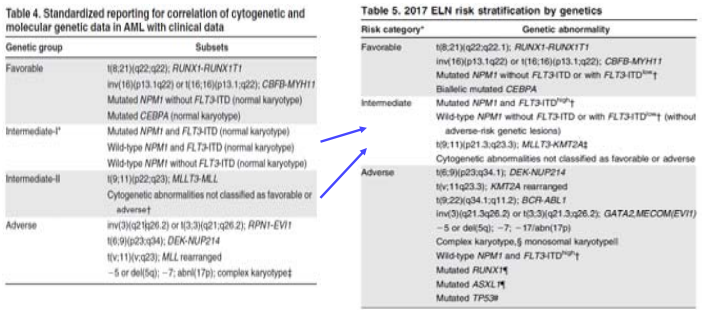


Table 4. Standardized reporting for correlation of cytogenetic and molecular genetic data in AML with clinical data

Genetic group	Subsets
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 (normal karyotype) Mutated *CEBPA* (normal karyotype)
Intermediate-I*	Mutated *NPM1* and *FLT3*-ITD (normal karyotype) Wild-type *NPM1* and *FLT3*-ITD (normal karyotype) Wild-type *NPM1* without *FLT3*-ITD (normal karyotype)
Intermediate-II	t(9;11)(p22;q23); *MLLT3-MLL* Cytogenetic abnormalities not classified as favorable or adverse†
Adverse	inv(3)(q21q26.2) or t(3;3)(q21;q26.2); *RPN1-EVI1* t(6;9)(p23;q34); *DEK-NUP214* t(v;11)(v;q23); *MLL* rearranged −5 or del(5q); −7; abnl(17p); complex karyotype‡

Table 5. 2017 ELN risk stratification by genetics

Risk category*	Genetic abnormality
Favorable	t(8;21)(q22;q22.1); *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}† Biallelic mutated *CEBPA*
Intermediate	Mutated *NPM1* and *FLT3*-ITD^{high}† Wild-type *NPM1* without *FLT3*-ITD or with *FLT3*-ITD^{low}† (without adverse-risk genetic lesions) t(9;11)(p21.3;q23.3); *MLLT3-KMT2A*‡ 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,§ monosomal karyotype‖ Wild-type *NPM1* and *FLT3*-ITD^{high}† Mutated *RUNX1*¶ Mutated *ASXL1*¶ Mutated *TP53*#

Döhner, H. et al. Blood 2010;115:453-474
Döhner, H. et al. Blood 2017;129(4):424-447

European Leukemia Net (ELN) Prognostic System Younger Adults with AML

2010 → 2017

Genetic group	Subset
Favorable	• t(15;17)(q22;q12-21) • t(8;21)(q22;q22.1); 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}* • Biallelic mutated CEBPA

*FLT3-ITD^{low}: allelic ratio <0.5

Döhner, H. et al. Blood 2010;115:453-474
Döhner, H. et al. Blood 2017;129(4):424-447

European Leukemia Net (ELN) Prognostic System Younger Adults with AML

2010 → 2017

Genetic group	Subset
Intermediate	• Mutated NPM1 and FLT3-ITD^{high}* • Wild-type NPM1 without FLT3-ITD or with FLT3-ITD^{low}* (without adverse-risk genetic lesions) • t(9;11)(p21.3;q23.3); MLLT3-KMT2A • Cytogenetic abnormalities not classified as favorable or adverse

*FLT3-ITD^{low}: allelic ratio <0.5
*FLT3-ITD^{high}: allelic ratio >0.5

Döhner, H. et al. Blood 2010;115:453-474
Döhner, H. et al. Blood 2017;129(4):424-447

European Leukemia Net (ELN) Prognostic System Younger Adults with AML

2010 → 2017

Genetic group	Subset
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, monosomal karyotype • Wild-type NPM1 and FLT3-ITD^{high} • Mutated RUNX1 • Mutated ASXL1 • Mutated TP53

*FLT3-ITD^{high}: allelic ratio >0.5

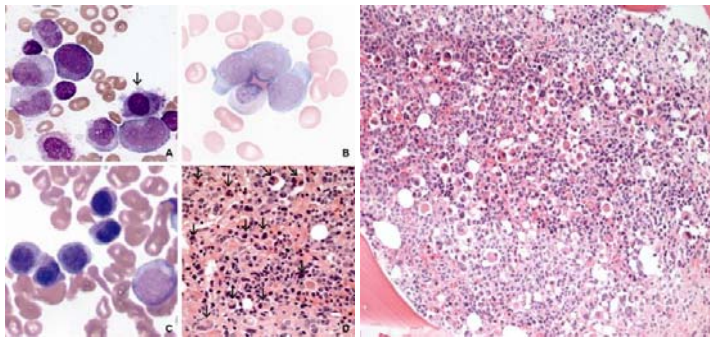
Döhner, H. et al. Blood 2010;115:453-474
Döhner, H. et al. Blood 2017;129(4):424-447

NCCN guidelines Version 3. 2017 for AML

RISK STATUS	CYTOGENETICS	MOLECULAR ABNORMALITIES
Favorable-risk	Core binding factor: inv(16)[2,3,4] or t(16;16)[2,3,4] or t(8;21)[2,4] or t(15;17)[4]	Normal cytogenetics: NPM1 mutation in the absence of FLT3-ITD or isolated biallelic (double) CEBPA mutation
Intermediate-risk	Normal cytogenetics +8 alone t(9;11) Other non-defined	Core binding factor with KIT mutation[2]
Poor-risk	Complex (≥3 clonal chromosomal abnormalities) Monosomal karyotype -5, 5q-, -7, 7q- 11q23 - non t(9;11) inv(3), t(3;3) t(6;9) t(9;22)[5]	Normal cytogenetics: with FLT3-ITD mutation[6] TP53 mutation

O'Donnell et al. NCCN guidelines. June 2017

De novo AML with Inv(3)(q21q26.1) or t(3;3)(q21;q26.2) Is An Aggressive Type of Leukemia



Sun, J. et al. Mod Pathol 2011;24:384

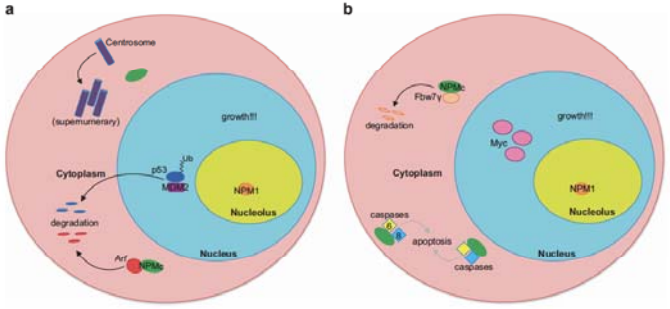
Molecular Lesions AML Normal Cytogenetics

Affected gene	Molecular defect	Chromosomal location	Incidence (%)
FLT3	Internal tandem duplication	13q12	25–37
NPM1	4-base pair duplication	5q35	30
RUNX1	N-terminal mutation	21q22	5
MLL	Partial tandem duplication	11q23	11
DNMT3A	Mutations	2p23.3	23
CEBPA	C-terminal and N-terminal mutations	19q13.1	9
KIT	Point mutations	4q11	6
WT1	Over-expression	11p13	8
NRAS	Point mutations	1p13.2	10
TET2	Mutations, alters methylation	4q24	8
IDH1, IDH2	Mutations, alters methylation	2q 33.3 & 15q 26.1	7-8%

Nucleophosmin (NPM1) in AML

- NPM1 exon-12 mutation in 50% to 60% of adult AML with normal karyotype.
- Predictor of favorable response.
- Cytoplasmic NPM1 IHC on biopsies predicts NPM1 mutations.
- NPM1 is not mutated in CML & CBF AML

NPM1 and leukemogenesis



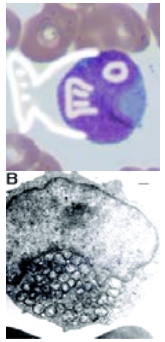
Arf degradation
p53 degradation
supernumerary centrosomes

Fbw7γ degradation (Myc increase)
Interacts with and inhibits caspase-6/-8

Heath et al. Leukemia (2017) 798 – 807

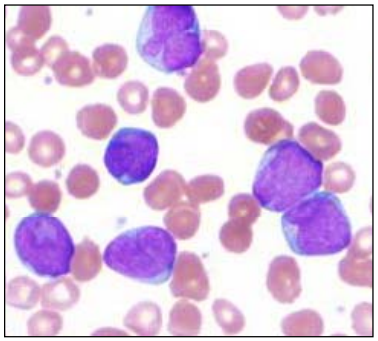
Clinical features related to *NPM1* mutated in AML

- Female sex
- Lack of CD34, HLA-DR
- Normal cytogenetics
- Cuplike nuclei "fish mouth"
- Frequent NPM1 and fms-like tyrosine kinase 3 mutations (FLT-3, 86%)
- Higher D-dimer levels

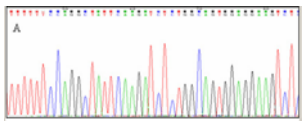


Chen, W. Cancer 2009;115:5481

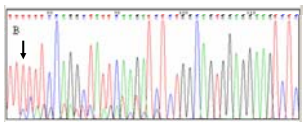
De Novo Ph+ AML with NPM1 Mutation



Wild –type NPM1



Mutant NPM1



Konoplev et al Leuk Lymphoma 2012 Early Online:1-7

Characterization of De Novo Ph+ AML at MDACC

Ph+ AML is distinct from CML-BP

Molecular

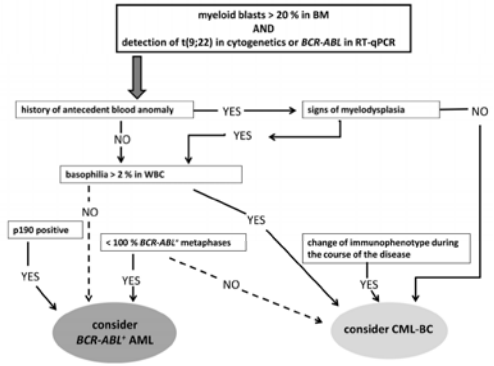
- Carried NPM1 at a similar to that in AML patients in general
- Lacked ABL1 mutations

Clinical presentation

- less splenomegaly and peripheral blood basophilia
- Lower bone marrow cellularity and myeloid/erythroid ratios

Konoplev et al. Leuk Lymphoma 2013. 54(1): 138–144
Soupir et al. Am J Clin Pathol 2007;127:642-650

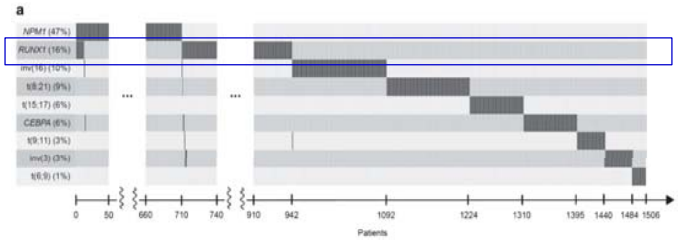
Clinical path for the differential diagnosis of BCRABL+ AML and CML blast crisis



Neuendorff, et al.Ann Hematol (2016) 95:1211–1221

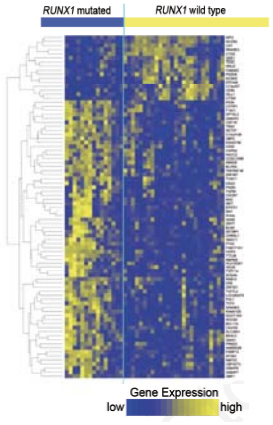
AML with mutated RUNX1

Mutually exclusive of AML with recurrent genetic abnormalities



Gainzik et al. Leukemia. Leukemia (2016) 30, 2160–2168

AML with mutated RUNX1



Gene expression signature showed 85 differentially expressed genes

The most prominently up-regulated genes are lymphoid genes:

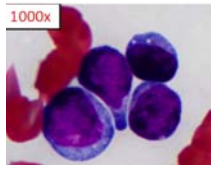
- *HOPX*
- *DNTT*
- *BLNK*

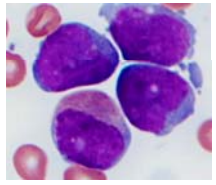
Greif et al. Hematologica. 2012; 97(12)

AML with mutated RUNX1

Clinico-pathologic and genetic features:

- Older age (16–59 years: 8.5%; ⩾60 years: 15.1%)
- Male gender
- More immature morphology
- Secondary AML evolving from myelodysplastic syndrome





Gainzik et al. Leukemia. Leukemia (2016) 30, 2160–2168

AML with mutated RUNX1

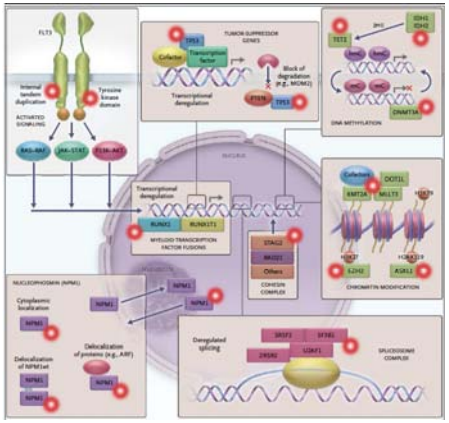
- Frequently co-occurred with a complex pattern of gene mutations
  - Bad outcome
    - RUNX1mut/ASXL1mut (OS, P = 0.004)
    - RUNX1mut/SRSF2mut (OS, P = 0.007)
    - RUNX1mut/PHF6mut (OS, P = 0.03)
  - Good outcome
    - RUNX1mut/IDH2mut (OS, P = 0.04)

Gainzik et al. Leukemia. Leukemia (2016) 30, 2160–2168

AML Features Mapped to Molecular Defects

- AML, a mosaic of multiple genomes (the cancer genome atlas, 2010)
- Abnormal Signaling Pathways
- Abnormal Gene-Expression Signatures
- Loss of p53 Function
- Epigenetic Silencing of Genes
- Post-Transcriptional Regulation, microRNA

Functional categories of genes that are commonly mutated in AML



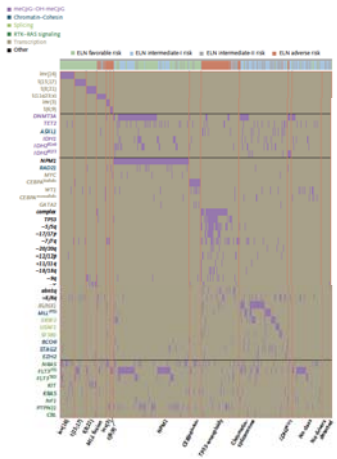
Döhner et al. N Engl J Med 2015;373:1136-52.

Functional categories of genes that are commonly mutated in AML

Functional class	Specific example mutations
Signaling and kinase pathway	*FLT3, KRAS, NRAS, KIT, PTPN11,* and *NF1*
Epigenetic modifiers (DNA methylation and chromatin modification)	*DNMT3A, IDH1, IDH2, TET2, ASXL1, EZH2,* and *MLL/KMT2A*
Nucleophosmin	*NPM1*
Transcription factors	*CEBPA, RUNX1,* and *GATA2*
Tumor suppressors	*TP53*
Spliceosome complex	*SRSF2, U2AF1, SF3B1,* and *ZRSR2*
Cohesin complex*	*RAD21, STAG1, STAG2, SMC1A,* and *SMC3*

DiNardo, et al. Hematology 2016

Mutational Complexity of AML



14 classes of AML with distinct diagnostic features and clinical outcomes. (11 already identified and 3 new)

- AML with mutations in genes encoding chromatin, RNA splicing regulators, or both
- AML with *TP53* mutations, chromosomal aneuploidies, or both
- AML with *IDH2*R172 mutations

Papaemmanuil et al. N Engl J Med 2016;374:2209-21

Genomic classification of AML

Genomic Subgroup	Frequency in the Study Cohort (N=1540)	Most Frequently Mutated Genes*
	no. of patients (%)	*gene (%)*
AML with *NPM1* mutation	418 (27)	***NPM1*** (100), *DNMT3A* (54), *FLT3*^{ITD} (39), *NRAS* (19), *TET2* (16), *PTPN11* (15)
AML with mutated chromatin, RNA-splicing genes, or both†	275 (18)	***RUNX1*** (39), ***MLL***^{PTD} (25), ***SRSF2*** (22), *DNMT3A* (20), ***ASXL1*** (17), ***STAG2*** (16), *NRAS* (16), *TET2* (15), *FLT3*^{ITD} (15)
AML with *TP53* mutations, chromosomal aneuploidy, or both‡	199 (13)	**Complex karyotype** (68), **–5/5q** (47), **–7/7q** (44), ***TP53*** (44), **–17/17p** (31), **–12/12p** (17), +8/8q (16)
AML with inv(16)(p13.1q22) or t(16;16)(p13.1;q22); *CBFB–MYH11*	81 (5)	**inv(16)** (100), *NRAS* (53), +8/8q (16), +22 (16), *KIT* (15), *FLT3*^{ITD} (15)
AML with biallelic *CEBPA* mutations	66 (4)	***CEBPA***^{biallelic} (100), *NRAS* (30), *WT1* (21), *GATA2* (20)
AML with t(15;17)(q22;q12); *PML–RARA*	60 (4)	**t(15;17)** (100), *FLT3*^{ITD} (35), *WT1* (17)
AML with t(8;21)(q22;q22); *RUNX1–RUNX1T1*	60 (4)	**t(8;21)** (100), *KIT* (38), –Y (33), –9q (18)
AML with *MLL* fusion genes: t(x;11)(x;q23)§	44 (3)	**t(x;11q23)** (100), *NRAS* (23)
AML with inv(3)(q21q26.2) or t(3;3)(q21;q26.2); *GATA2, MECOM(EVI1)*	20 (1)	**inv(3)** (100), –7 (85), *KRAS* (30), *NRAS* (30), *PTPN11* (30), *ETV6* (15), *PHF6* (15), *SF3B1* (15)
AML with *IDH2*^{R172} mutations and no other class-defining lesions	18 (1)	***IDH2***^{R172} (100), *DNMT3A* (67), +8/8q (17)
AML with t(6;9)(p23;q34); *DEK–NUP214*	15 (1)	**t(6;9)** (100), *FLT3*^{ITD} (80), *KRAS* (20)
AML with driver mutations but no detected class-defining lesions	166 (11)	*FLT3*^{ITD} (39), *DNMT3A* (16)
AML with no detected driver mutations	62 (4)	
AML meeting criteria for ≥2 genomic subgroups	56 (4)	

Papaemmanuil et al. N Engl J Med 2016;374:2209-21

Conclusions

- Phenotypic defects of AML can be mapped to specific molecular anomalies
- Whole genome sequencing will help identify similarities and differences between de novo and secondary AML
- Development of AML involves multiple genetic lesions that complement each other
- Aberrant signal transduction enhances the survival and proliferation of Leukemic Cells
- Critical functional dependencies of Leukemic Cells result in activation of limited effector pathways