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par

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Étude du profil mutationnel en association avec le profil clinique, biologique et phénotypique dans la leucémie aiguë myéloïde

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RÉSUMÉ

Introduction : Les leucémies aiguës myéloïdes (LAM) sont des hémopathies malignes caractérisées par une prolifération clonale de progéniteurs myéloïdes immatures. Le diagnostic de LAM est confirmé par l'examen cytologique d'une aspiration médullaire révélant la présence d'au moins 20% de blastes présentant une morphologie d'allure myéloïde. L'examen cytologique est complété par une analyse du phénotype des blastes afin de caractériser plus précisément l'origine de la cellule tumorale. Les analyses cytogénétique et la recherche de mutation acquise par *Next Generation Sequencing* (NGS) permettent d'identifier les patients à haut risque de rechute. Plusieurs études ont décrit les mutations présentes au diagnostic de LAM. Peu décrivent les associations entre gènes mutés et les caractéristiques cliniques, phénotypiques, cytogénétiques. La majorité des patients présente une ou plusieurs mutations génétiques. Il existe des groupes de co-mutations comme la co-mutation NPM1/FLT3 qui sont décrites comme associées à un pronostic favorable. Depuis fin 2017, l'analyse NGS est réalisé pour la majorité des patients diagnostiqué d'une LAM. L'objectif de la présente étude, est de décrire les caractéristiques cliniques, cytogénétiques, phénotypiques et moléculaires des patients ayant été pris en charge au CHU de Tours. L'objectif secondaire est d'étudier les associations entre ces caractéristiques afin de déterminer si les profils mutationnels peuvent être associés à une présentation clinique, un profil cytogénétique ou phénotypique spécifiques.

Méthodes : Nous avons conduit une étude monocentrique rétrospective chez des patients diagnostiqués d'une LAM au CHU de Tours et ayant débuté un traitement entre 2018 et 2024 ayant eu une analyse génétique par la technique NGS au diagnostic retrouvant des mutations génétiques (étude COMUTAML CNIL #2024-099, IRB #2024-051).

Résultats : Nous avons inclus 152 patients. L'âge médian était de 64 [IQR 53 ;71] ans au diagnostic, 45% étaient des femmes, 77% avaient un score ECOG à 0 ou 1 au diagnostic, 39% avaient une comorbidité cardiovasculaire comme hypertension artérielle, troubles du rythme ou coronaropathies, 22% des patients avaient une hyperleucocytose supérieure à 50 G/L. Le taux médian de blastes circulants était de 30% au diagnostic. 12,5% de patients n'avaient pas de blastes circulants, 47% des patients avaient une LAM secondaire et 51% étaient de risque défavorable selon la classification ELN 2022, 14% des patients présentaient un risque favorable dont 63% avait une mutation NPM1 et 32% avaient une mutation impliquant le CBF. L'analyse cytogénétique a retrouvé un caryotype complexe dans 13% des cas. L'analyse mutationnelle a révélé des altérations fréquentes dans des gènes impliqués dans la leucémogenèse, notamment NPM1 (28 %), DNMT3A (26 %), RUNX1 (24 %), FLT3-ITD (22 %) et TET2 (20 %). D'autres mutations fréquentes concernaient SRSF2 (16 %), ASXL1 (16 %), NRAS (16 %), IDH1 (12%), IDH2 (12%) et TP53 (10 %). Les co-mutations les plus souvent associées étaient FLT3-ITD et NPM1 pour 23 patients (15%), DNMT3A et NPM 1 pour 22 patients (14%) et ASXL1 et RUNX1 pour 18 patients (12%).

Discussion : Cette étude décrit les caractéristiques cliniques, cytogénétiques, phénotypiques et moléculaires au diagnostic de LAM au CHU de Tours. Ces caractéristiques sont comparables avec d'autres études épidémiologiques retrouvées dans la littérature. On observe cependant une prévalence importante de la mutation RUNX1 dans notre population. Des analyses complémentaires permettront de décrire les associations entre anomalies chromosomiques, phénotypiques et moléculaires.

Mots-clés : Leucémie aiguë myéloïde, biologie moléculaire, cytométrie en flux, analyse cytogénétique, co-mutations

Study of mutation profile in association with clinical, biological, and phenotypical profiles in Acute Myeloid leukemia

ABSTRACT

Introduction: Acute myeloid leukemia (AML) is a malignant hematologic disorder characterized by the clonal proliferation of immature myeloid progenitors. The diagnosis of AML is confirmed by cytological examination of a bone marrow aspiration revealing the presence of at least 20% blasts with a myeloid morphology. Cytological analysis is complemented by immunophenotyping to more precisely characterize the origin of the tumor cell. Cytogenetic analysis and the detection of acquired mutations using Next Generation Sequencing (NGS) help identify patients at high risk of relapse. Several studies have described the mutations present at AML diagnosis. However, few have explored the associations between mutated genes and clinical, phenotypic, and cytogenetic characteristics. The majority of patients present with one or more genetic mutations. Some co-mutation groups, such as the NPM1/FLT3 co-mutation, have been described as being associated with a favorable prognosis. Since late 2017, NGS analysis has been performed for most patients diagnosed with AML. The objective of this study is to describe the clinical, cytogenetic, phenotypic, and molecular characteristics of patients treated at the University Hospital of Tours. A secondary objective is to investigate associations between these characteristics to determine whether mutational profiles can be linked to specific clinical presentations, cytogenetic profiles, or immunophenotypic features.

Methods: We conducted a retrospective, single-center study of patients diagnosed with AML at the University Hospital of Tours who started treatment between 2018 and 2024 and underwent genetic analysis using NGS at diagnosis, revealing genetic mutations (study COMUTAML CNIL #2024-099, IRB #2024-051).

Results: We included 152 patients. The median age at diagnosis was 64 years [IQR 53;71], 45% were women, and 77% had an ECOG score of 0 or 1 at diagnosis. Additionally, 39% had a cardiovascular comorbidity such as hypertension, arrhythmias, or coronary artery disease, and 22% of patients had leukocytosis exceeding 50 G/L. The median circulating blast percentage at diagnosis was 30%. Notably, 12.5% of patients had no circulating blasts. Secondary AML was observed in 47% of patients, and 51% were classified as having an adverse risk according to the 2022 ELN classification. A favorable risk was identified in 14% of patients, of whom 63% had an NPM1 mutation and 32% had a mutation involving the CBF. Cytogenetic analysis identified a complex karyotype in 13% of cases. Mutational analysis revealed frequent alterations in genes involved in leukemogenesis, notably NPM1 (28%), DNMT3A (26%), RUNX1 (24%), FLT3-ITD (22%), and TET2 (20%). Other frequently mutated genes included SRSF2 (16%), ASXL1 (16%), NRAS (16%), IDH1 (12%), IDH2 (12%), and TP53 (10%). The most common co-mutations were FLT3-ITD and NPM1 in 23 patients (15%), DNMT3A and NPM1 in 22 patients (14%), and ASXL1 and RUNX1 in 18 patients (12%).

Discussion: This study describes the clinical, cytogenetic, phenotypic, and molecular characteristics of AML at the University Hospital of Tours. These findings are comparable to those reported in other epidemiological studies. However, we observed a high prevalence of RUNX1 mutations in our cohort. Further analyses will be conducted to explore the associations between chromosomal, phenotypic, and molecular abnormalities.

Keywords : Acute myeloid leukemia, molecular biology, flow cytometry, co-occurrence of mutations

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SERMENT D'HIPPOCRATE

En présence des Maîtres de cette Faculté,
de mes chers condisciples
et selon la tradition d'Hippocrate,
je promets et je jure d'être fidèle
aux lois de l'honneur et de la probité
dans l'exercice de la Médecine.

Je donnerai mes soins gratuits à l'indigent,
et n'exigerai jamais un salaire au-dessus de mon travail.

Admise dans l'intérieur des maisons,
mes yeux ne verront pas ce qui s'y passe,
ma langue taira les secrets qui me seront confiés
et mon état ne servira pas à corrompre les mœurs
ni à favoriser le crime.

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je rendrai à leurs enfants
l'instruction que j'ai reçue de leurs parents.

Que les personnes m'accordent leur estime
si je suis fidèle à mes promesses.
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et méprisée de mes confrères et consœurs
si j'y manque.

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LIST OF EMPLOYED ABREVIATIONS

AML	Acute Myeloid Leukaemia
AML-MRC	Acute Myeloid Leukaemia with Myelodysplasia Related Changes
BM	Bone Marrow
CHIP	Clonal hematopoiesis of indeterminate potential
CNIL	Comission Nationale de l'Informatique et des Libertés
CRi	Complete Remission with Incomplete hematologic recovery
DfN	Different from Normal
ELN	European Leukemia Net
FAB	French American British association
ICC	International Consensus Classification
IQR	Interquartile Range
LAIP	Leukaemia-Associated Immunophenotypes
MDN	Myelodysplastic Neoplasm
MFC	Multiparameter Flow Cytometry
MRD	Minimal Residual Disease
NGS	New Generation Sequencing
PCR	Polymerase Chain Reaction
VAF	Variant Allele Frequency
WHO	World Health Organization

INTRODUCTION

Acute Myeloid Leukemia (AML) is a malignant disease originated from hematopoietic stem cells and resulting to a clonal expansion and accumulation of abnormally differentiated blasts of myeloid lineage.

It is the most common acute type of leukemia in adults, accounting for approximately 1% of new cancer cases and responsible for approximately 1.8% of all deaths related to cancer (1). The rate of new cases was 4.2 per 100,000 men and women per year based on USA data on 2017–2021 cases. (1) In France, according to data of the National Cancer Institut, approximately 3400 patients were diagnosed with AML in 2018 , corresponding to an incidence of approximately 3 per 100,000 (2). AML incidence seems to be increasing overtime based on different studies conducted in the USA, UK and other European countries, Canada and Australia. Data suggests that males are 1.2 to 1.6 times more likely to be diagnosed with AML in their lifetime in comparison to women. The highest incidence rates were observed in Caucasians and the lowest in Hispanic and Pacific Islanders/Alaskan populations (1,3–6).

Acute myeloid leukemia is most frequently diagnosed among people aged from 65 to 74 with a median age of 69 years (1). Death rates from AML are higher among older adults and the highest among people aged from 75 to 84 with a median age at death of 74 years. The 5-years survival rate is estimated at 31,9% (1). Several conditions, such as myelodysplastic Neoplasm (MDN) or myeloproliferative disease, environmental exposures, previous chemo- or radiation therapy, and genetic predispositions, have been identified as predisposing in the development of AML. For example, there is increasing evidence to support the role of chronic benzene exposure as a risk factor of acute myeloid leukemia (7). A large number of germline mutations increasing the risk of developing AML has been identified such as mutations in the *RUNX1*, *DDX41* or *GATA2* genes as well as numerous syndromes namely: Li Fraumeni, Fanconi anemia or telomere disorders. These germline mutations lead to an evolution in AML mostly in children or young adults, but they can be observed even at a more advanced age as acquired mutations.

Another factor predisposing to the development of AML is the exposure to chemotherapy, and in particular alkylating agents or topoisomerase inhibitors or to ionising radiation. These AML are referred as also known therapy related acute myeloid leukemia. (8)

Diagnosis usually follows clinical manifestations but can occur more rarely after a routine blood test. Constitutional symptoms such as weight loss, fever and night sweats might be present. The proliferation of immature myeloid cells results to the impairment of normal hematopoiesis, leading thus to cytopenias. Anemia might present as extreme fatigue or dyspnea, thrombopenia can lead to easy bruising and bleeding, neutropenia can be the cause of severe infections. Some patients might also present with extramedullary disease, including involvement of the central nervous system, skin lesions, gingival hypertrophy, or hepatosplenomegaly. Urgent treatment might be indicated in the presence of tumor lysis syndrome, disseminated intravascular coagulation or leukostasis.

AML diagnosis is based on the presence of immature cells called blasts in a bone marrow aspiration. A percentage of at least 20% blasts (or 10% in the presence of recurrent genetic abnormalities or certain type of mutations/karyotype abnormalities) is necessary for the diagnosis. A diagnosis immunophenotyping assay allows to identify cell surface and intracellular proteins expressed by blasts, and also identify markers for subsequent Minimal Residual Disease (MRD) monitoring. The are two current approaches for MRD monitoring using flow cytometry: the first one uses the identification of blasts expressing leukemia-associated immunophenotypes (LAIP) that distinguish AML cells from normal hematopoietic cells in each patient. The second one uses 'different from normal' (DfN) aberrant differentiation/maturation profiles compared to that seen in normal hematopoietic precursor cells. The DfN approach allows both following clones present at diagnosis and searching for new emerging clones during the follow up period (9–11).

The first widely used classification was the French American British classification (FAB), first published in 1976 and revised in 1985. It was based on morphology and cytochemical staining (12). The FAB classification distinguished seven types of AML as seen in **Appendix 1**:

The World Health Organization (WHO) introduced a new classification in 1999, incorporating cytogenetics, molecular genetics, and clinical features, rather than just morphology(8). Updates were published in 2001, 2008, 2016 et 2022 with addition of different cytogenetic and molecular abnormalities as our understanding of AML biology advanced. The latest WHO classification published in 2022 recognizes different subtypes of AML as seen in **Appendix 2**.

In 2022, the International Consensus Classification (ICC) was introduced, separate from WHO, refining AML categories based on genetic drivers (13). One of the main changes in both classifications is the limit of 20% blasts required for the diagnosis. There is no cut off in the

2022 WHO criteria in the presence of recurrent genetic abnormalities whereas in the ICC there is still a limit of 10%. In the ICC classification, the entity of MDS/AML has also been introduced when blasts are between 10 et 20%. The cut off of 20% is still necessary for the diagnosis of all other AML categories. New recurrent genetic abnormalities have been incorporated into the diagnostic criteria, for example translocations affecting the *RARA*, *KMT2A* and *MECOM* genes.

In a study conducted in Italy a total of 1001 patients with AML were reclassified based on the new WHO and ICC classifications. The overall diagnostic changes between the WHO 2016 and the WHO 2022 and ICC classifications were 22.8% and 23.7%, respectively, with a 13.1% difference in patients' distribution between ICC and WHO 2022. The 2022 ICC "not otherwise specified" and WHO "defined by differentiation" AML category sizes shrank when compared with that in WHO 2016 (24.1% and 26.8% respectively, vs 38.7%) most probably because of NGS analyses permitting to categorize previously NOS cases in the MDS related category (14).

Before the advent of cytogenetics and molecular testing, AML was primarily classified using morphology with the FAB classification. However, this system had limited value in predicting the risk of death or relapse. Later, in the 1980s and 1990s, researchers identified chromosomal abnormalities affecting prognosis. The most important prognostic abnormalities described were: t(8;21)(q22;q22) , inv(16)(p13q22) or t(16;16) and t(15;17)(q22;q12) being correlated with a favorable risk , as well as complex karyotypes (≥3 abnormalities), monosomies 5 et 7, 11q23 rearrangements (KMT2A fusions) being associated with an adverse risk. Cytogenetic risk stratification became progressively widely used, leading to risk-adjusted treatment approaches especially for high-risk patients (15,16).

Increased understanding of the biology of acute myeloid leukemia with advances in molecular biology, particularly polymerase chain reaction (PCR) and next-generation sequencing (NGS) has led to a more precise risk assessment now including a growing number of recurrent molecular aberrations. At the end of 1990s, FLT3-ITD mutations were identified and associated with poor prognosis (17). Later, NPM1 and CEBPA mutations were identifier and associated with favorable outcomes, whereas TP53, ASXL1, and RUNX1 mutations identified as high-risk markers. The vast majority of patients have at least one genetic mutation (18). It is also known that some co-mutations such as *NPM1-FLT3* have an impact on

prognosis (19). However, few studies describe frequency of partners and explore the impact of less frequent co-mutations.

These findings led to the European Leukemia Net (ELN) risk stratification, the latest version being ELN2022, showed in **Appendix 3** (10). Compared to ELN2017, key changes were: all AML with an FLT3 ITD mutation are now in the intermediate risk group without considering the allelic ratio or the presence of a NPM1 mutation (20). Myelodysplasia related gene mutations in AML are now in the adverse risk group and they include not only *ASXL* and *RUNX1* genes but also *BCOR*, *EZH2*, *SF3B1*, *SRSF2*, *STAG2*, *U2AF1* and *ZRSR2* genes. AML with an *NPM1* mutation and adverse risk cytogenetics are now classified in the adverse risk group. The role of coexistence of *NPM1* and myelodysplasia related mutations is yet to be defined. In frame basic leucine zipper domain (bZIP) *CEBPA* mutations are considered of favorable prognosis as biallelic or monoallelic mutations alike. The addition of new anomalies in the adverse cytogenetics group such as t(3q26.2;v) and t(8;16)(p11.2;p13.3). And, the hyperdiploid karyotypes with multiple trisomies or polysomies are now extracted from the adverse group (10,21).

Treatment strategies can vary depending on age, overall health, genetic mutations, and risk stratification. Induction therapy is the first line of treatment aimed at achieving remission. For fit patients, chemotherapy with cytarabine and an anthracycline is typically used, while for patients with multiple comorbidities, advanced age, or other factors contraindicating chemotherapy, hypomethylating agents like azacitidine may be used. After achieving remission with induction therapy, consolidation therapy aims to eradicate any remaining leukaemia cells in the body to prevent relapse. Consolidation options include high-dose chemotherapy or hematopoietic stem cell transplantation, depending on relapse risk, age, and comorbidities. Other treatment options include BCL2 inhibitors (e.g., venetoclax) or targeted therapies for specific gene mutations, such as midostaurin for FLT3 mutations or ivosidenib for IDH1 mutations (22–24). Gilteritinib is another FLT3 inhibitor approved for use in relapse/refractory AML with recent data suggesting that it can be also used as a first line treatment for patients ineligible for intensive chemotherapy (25,26). Moreover, ongoing clinical trials are evaluating the use of menin inhibitors as a treatment for patients with NPM1 mutations or with KMT2A rearrangements (27,28).

As a good knowledge of the prognosis impact of clinical, cytogenetic, phenotypic and molecular features is essential to determine the therapeutic strategy, it is crucial to

understand them perfectly. As the individual impact of each identified feature is widely detailed in literature, data is missing regarding the impact of the association of features.

The aim of this study is to describe the clinical, cytogenetic, phenotypic and molecular characteristics of patients in the university hospital of Tours. The secondary objective is to study the associations between those characteristics to determine if mutational profiles can be associated with specific clinical presentations, cytogenetic profiles, and phenotypic profiles.

METHODS

Research type and environment

This study was retrospective monocentric study in the Tours University Hospital, France, over a period of six years, between January 2018 and November 2023.

Inclusion criteria

Patients were identified by administration of specific AML treatment: induction with chemotherapy or azacitidine. We included patients aged over 18 years-old diagnosed with AML according to the WHO 2022 classification in which a NGS assay based on a blood or bone marrow sample at diagnosis and resulting in at least one detected mutation or more. We excluded patients with promyelocytic leukemia, patients previously treated for AML and patients diagnosed with a bi-phenotypic AML. Patients were screened based on treatment data in our centre: all patients treated using AML treatment protocols between 2018 and 2023.

Collected data

The collected data were patient characteristics at diagnosis, blood sample results at diagnosis such as blood cell count, blast count, biochemistry and coagulation results, bone marrow (BM) aspiration, karyotypical analysis, immunophenotype analysis using multiparameter flow cytometry (MFC), New Generation Sequencing (NGS). Treatments, response to treatments and survival data were also collected.

Outcomes

The primary outcomes were the clinical, phenotypic, cytogenetic and molecular characteristics of patients. Secondary outcomes were the co-mutational profiles and the association of phenotypic profiles with the clinical, cytogenetic and molecular characteristics of patients.

Statistical analysis

Descriptive statistics were expressed using percentages, medians and interquartile ranges (IQR). For analytic researches of associations, we used univariate logistic regressions. Data were analysed using R Studio v. 4.4.3.

Ethics and legislation

The study was approved by the ethical committee of the Centre-Val-de-Loire region in France (IRB #2024-051) and was registered in the informatic record of Tours University Hospital (n° 2024_099) following the rules of the Commission Nationale de l'Informatique et des Libertés (CNIL) for research that do not implicate human person.

RESULTS

Patients characteristics

Among the 279 screened patients, 152 patients were included, 127 patients meeting exclusion criteria **(Figure 1)**. We could find no information for one patient, and no information on the NGS data for 9 patients because they were either diagnosed or treated in another hospital.

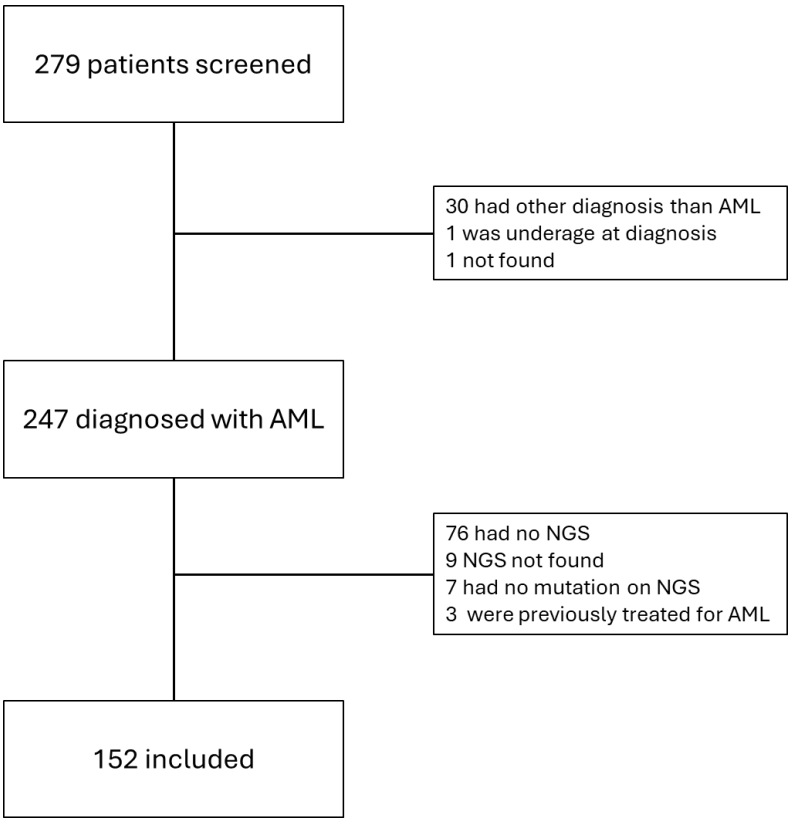


Figure 1 – Flow chart of inclusion

Baseline characteristics of population are detailed in **Table 1.** Patients median age at diagnosis was 64 [IQR 53; 71] years. Gender ratio (male/female) was 1,28. Seventy-seven percent had an ECOG score of 0 or 1 at diagnosis, and 39% had a cardiovascular comorbidity such as hypertension, arrhythmias, or coronary artery disease. Fourty-seven percent of patients had AML with Myelodysplasia Related Changes AML-MRC, and 7% had extramedullary disease. Fifty-one percent of patients were stratified in the adverse risk group according to ELN2022. Twenty-two percent of patients had hyperleukocytosis > 50 G/L and 88% had blasts present in peripheral blood at diagnosis.

Characteristic	n = 152
GENERAL CHARACTERISTICS	
Female gender – no. (%)	69 (45%)
Age at diagnosis – yr	64 [53 – 71]
ECOG score – no. (%)	
0	40 (26%)
1	77 (51%)
2	27 (18%)
3	7 (5%)
Cardiovascular comorbidity – no. (%)	60 (39%)
Neurovascular comorbidity – no. (%)	4 (3%)
Ischemic cardiopathy – no. (%)	10 (7%)
BLOOD WORKUP AT DIAGNOSIS	
Hemoglobin – g/dL	9,0 [7,6 – 10,8]
< 8 g/dL – no. (%)	50 (33%)
8 - 10 g/dL – no. (%)	47 (31%)
> 10 g/dL – no (%)	55 (36%)
Platelets – G/L	73 [121 – 85]
Thrombocytopenia – no. (%)	123 (81%)
20 - 50 G/L – no. (%)	41 (27%)
< 20 G/L – no. (%)	18 (12%)
Leukocytes – G/L	7,7 [2,6 – 46,3]
< 50 G/L – no. (%)	119 (78%)
> 50 G/L – no. (%)	33 (22%)
Granulocytes – G/L	1,2 [0,4 – 3,8]
Neutropenia < 1.5 G/L – no. (%)	84 (56%)
Grade 4 neutropenia < 0.5 G/L – no. (%)	41 (27%)
Lymphocytes – G/L	1,9 [1,1 – 3,8]
Lymphopenia < 1 G/L – no. (%)	32 (21%)
Monocytes – G/L	0,15 [0,02 – 1,52]
Monocytosis > 1 G/L – no. (%)	48 (32 %)
Monocytopenia < 0 –1 G/L – no. (%)	57 (38 %)
Blasts in peripheral blood– %	30 [5 – 69]
Blasts present in peripheral blood – no. (%)	134 (88%)
Creatinine – µmol/L	77 [64 – 100]
Urea – mmol/L	5,4 [4,0 – 7,2]
Serum potassium – mmol/L	4 [3,7 – 4,3]
Serum phosphate – mmol/L	1,03 [0,87 – 1,20]
Uric acid – µmol/L	313 [246 – 417]
LDH > 240 UI/L – no. (%)	108 (75%)
AST or ALT elevation > 35 UI/L – no. (%)	55 (38%)
PT < 60% – no. (%)	23 (17%)
Fibrinogen < 2 g/L – no. (%)	10 (7%)

Table 1 – Baseline characteristics – General and Blood workup

BONE MARROW ASPIRATE	
Cellularity – no. (%)	
I	10/142 (7%)
II	28/142 (20%)
III	54/142 (38%)
IV	50/142 (35%)
Megakaryocytes observed – no. (%)	96/146 (66%)
Blasts – median %	56 [28 – 83]
Fab classification – no. (%)	
0	7 (5%)
1	13 (9%)
2	29 (19%)
4	15 (10%)
5	26 (17%)
6	5 (3%)
7	1 (1%)
unknown	59 (39%)
AML-MRC – no. (%)	71 (47%)
Extramedullary disease – no. (%)	10 (7%)
ELN Risk stratification (2017) – no. (%)	
Favorable	32 (21%)
Intermediate	55 (36%)
Adverse	65 (43%)
ELN Risk stratification (2022) – no. (%)	
Favorable	22 (14%)
Intermediate	53 (35%)
Adverse	77 (51%)
Mutation profile – no. (%)	
Transcription factors	54/146 (36%)
NPM1	43/146 (28%)
Tumor suppressor genes	18/146 (12%)
DNA methylation-related genes	61/146 (40%)
Metabolic enzymes	35/146 (23%)
Signaling genes	86/146 (57%)
Chromatin-modifying genes	39/146 (26%)
Cohesin-complex genes	21/146 (14%)
Spliceosome-complex genes	42/146 (28%)
Other	22/146 (14%)
Values in brackets are medians [IQR]. Values following a slash indicate the total number of patients composing a subgroup after exclusion of missing data)	

Table 2 – Baseline characteristics – Bone marrow analysis

Treatments and evolution

Characteristics describing treatments and evolution are listed in **Table 3.** 59% of patients were treated with intensive chemotherapy as a first line treatment, using an Aracytine and Anthracycline based regimen in combination or not with a targeted therapy, while 35% received other treatments including an hypomethylating agent, anti BCL2 or therapies targeting *IDH* mutations. Of all the treated patients, 70% achieved complete remission after first line treatment, of which 42% received an allogenic stem cell transplant. 38% of the patients relapsed with a median of 12 months between diagnosis and relapse. At the moment of data collection in July 2024, 55% patients of our population were deceased.

	n = 147*
Intensive chemotherapy – no. (%)	90/147 (59%)
Bone marrow biopsy repeated at day 15 – no. (% of patients receiving intensive chemotherapy)	58/90 (64%)
Blast percentage at day 15 – no. (% of patients biopsied at day 15)	
0	40/58 (69%)
0 - 5	1/58 (2%)
> 5	17/58 (29%)
Complete remission after intensive chemotherapy – no. (%)	73/83 (88%)
Other treatment than intensive chemotherapy – no. (%)	52/147 (35%)
Azacitidine-Venetoclax – no. (%)	31/52 (60%)
Azacitidine monotherapy – no. (%)	10/52 (19%)
Azacitidine-Cusatuzumab – no. (%)	3/52 (6%)
Azacitidine-Ivosidenib – no. (%)	2/52 (4%)
Other	6/52 (11%)
Complete remission or CRi¤ with other treatment– no. (%)	22/52 (42%)
Palliative treatment – no. (%)	5/147 (3%)
Complete remission after first line treatment (CR1) – no. (% of patients who had a treatment)	95/135 (70%)
HSCT – no. (% of patients with CR1)	40/95 (42%)
Relapse after CR1* – no. (%)	27/71 (38%)
Months between diagnosis and relapse – median	12 [8 – 16]
CR2 – no. (%)	9/26 (35%)
HSCT CR2 – no. (% of patients with CR2)	15/26 (58%)
Death – no. (%)	81/146 (55%)
Months between diagnosis and death – median	7 [2 – 14]

Plus-minus values are observed means ± SD. Values in brackets are medians [IQR]. Values following a slash indicate the total number of patients composing a subgroup after exclusion of missing data).
*Treatment data was missing for 5 patients.
¤ CRi, Complete remission with incomplete hematologic recovery

Table 3 – Treatments and evolution

Phenotypical analysis (MFC)

Phenotypical analysis data using MFC were found and retrieved for 131 of our patients **(Table 4)**. The most extensive panel for MFC used 34 markers. 106 patients had an MFC using this extensive panel of cell markers.

MFC percent of positive blast cells – median [IQR]	N = 131
HLADR	86 [68 - 96]
CD10	0 [0 - 2]
CD34	92 [17 - 100]
CD56	3 [0 - 15]
CD11B	9 [2 - 44]
CD13	90 [70 - 96]
CD14	1 [0 - 8]
CD15	15,5 [4 - 50]
CD16	2 [0 - 6]
CD33	97 [86 - 100]
CD36	9 [2 - 28]
CD41A	0 [0 - 2]
CD71	72 [38 - 91]
CD117	50 [16 - 74]
GLYA	0 [0 - 2]
CD2	0 [0 - 3]
CD4	19 [1 - 68]
CD7	1 [0 - 23]

Table 4 – MFC markers expression

Cytogenetic

Cytogenetic analysis by karyotype was found for all but two patients **(Table 5).** Karyotypical analysis found no abnormality in 46% of patients whereas 13% of karyotypes were complex (3 or more abnormalities) thus associated with a poor prognosis. Other poor prognosis related anomalies observed were monosomy 7 in 7% of patients, monosomy 5 and del5q in 2% and 5% respectively. Accumulated incidence of good prognosis karyotype anomalies including t(8;21), inv(16) and t(16;16) were 6%. Trisomy 8 was also frequently observed (10%).

KARYOTYPE – no. (%)	**n = 150***
Normal	69 (46%)
Complex karyotype	20 (13%)
Trisomy 8	15 (10%)
Other hyperdiploidy	14 (9%)
-7	11 (7%)
Other monosomy	11 (7%)
del7q	10 (7%)
del5q	8 (5%)
45 -Y	5 (3%)
t (8;21) (q22; q22.1)	4 (3%)
del20q	4 (3%)
inv(16) (p13.1;q22)	3 (2%)
-5	3 (2%)
-17	3 (2%)
t (9;11) (p21.3;q23.3)	3 (2%)
t (6;11) (q27; q23)	2 (1%)
del3q	2 (1%)
del9q	2 (1%)
del11q	2 (1%)
del12p	2 (1%)
add7q	2 (1%)
add17p	2 (1%)
t (16;16) (p13.1; q22)	1 (1%)
t (3;3) (q21; q26)	1 (1%)
t (16 ;21)	1 (1%)
t (7;12) (q36; p13)	1 (1%)
Other deletion	4 (3%)
Other	3 (2%)

*2 patients had no available karyotype.

Table 5 – Karyotype

NGS analysis

We performed an analysis using only the mutated genes with a Variant Allele Frequency (VAF) greater than 5%, as seen bellow. The most frequent mutation detected was on the *NPM1* gene (28%), the second being *DNMT3A* (25%) , followed by *FLT3* and *RUNX1* with a frequency of 24% each. **(Figure 2)** Among FLT3 mutations, 31 were *FLT3*-ITD and 6 were *FLT*-TKD.

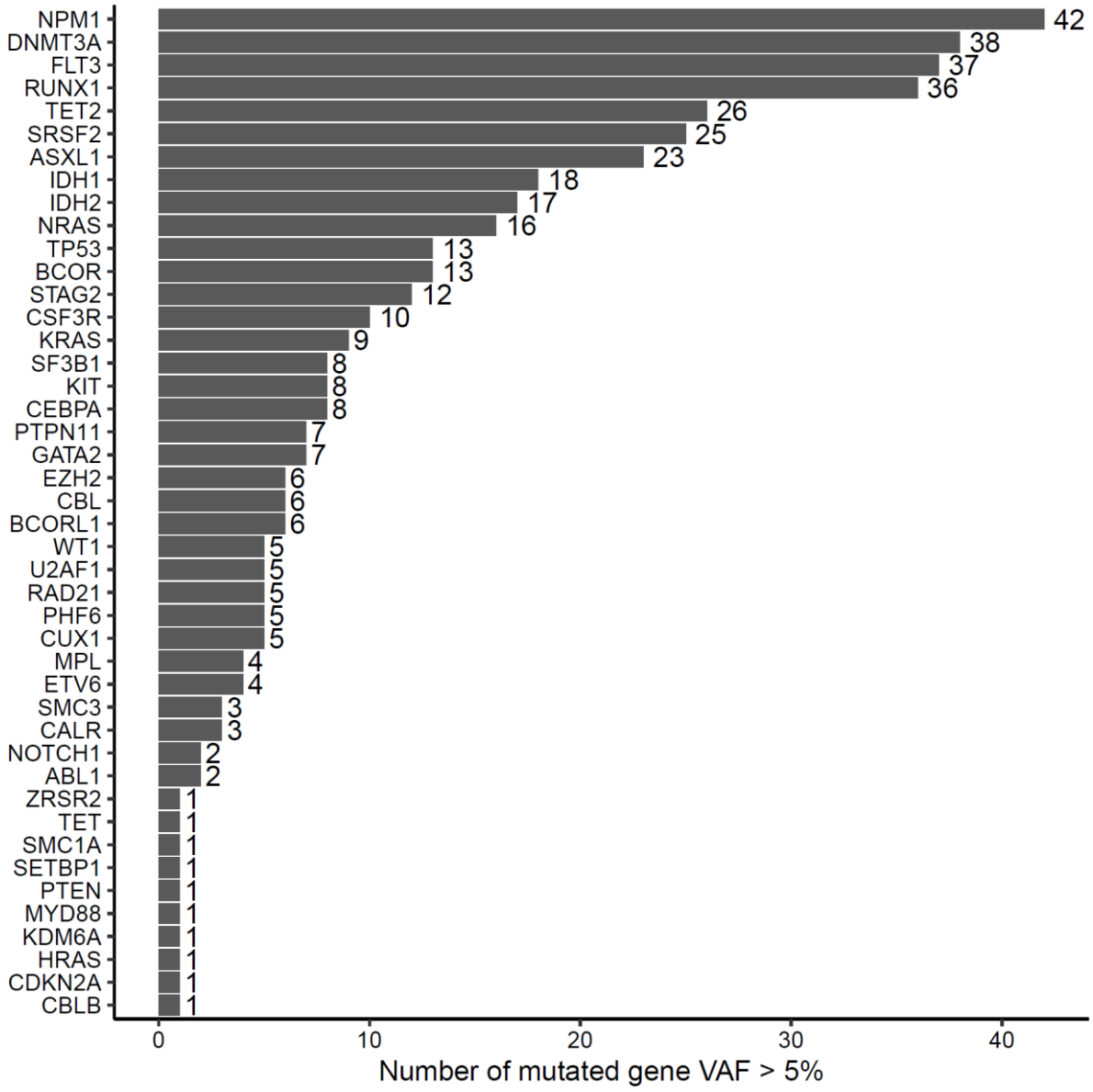


Figure 2 – Barplot depicting the number of patients with a mutation (VAF >5%)

Higher VAF mutations were observed in genes *DNMT3A*, *TET2*, *ASXL1*, *RUNX1* and *SRSF2* while mutations in *NPM1*, *FLT3 KRAS* showed a broad range of Variant Allele Frequency (VAF) **(Figure 3)**.

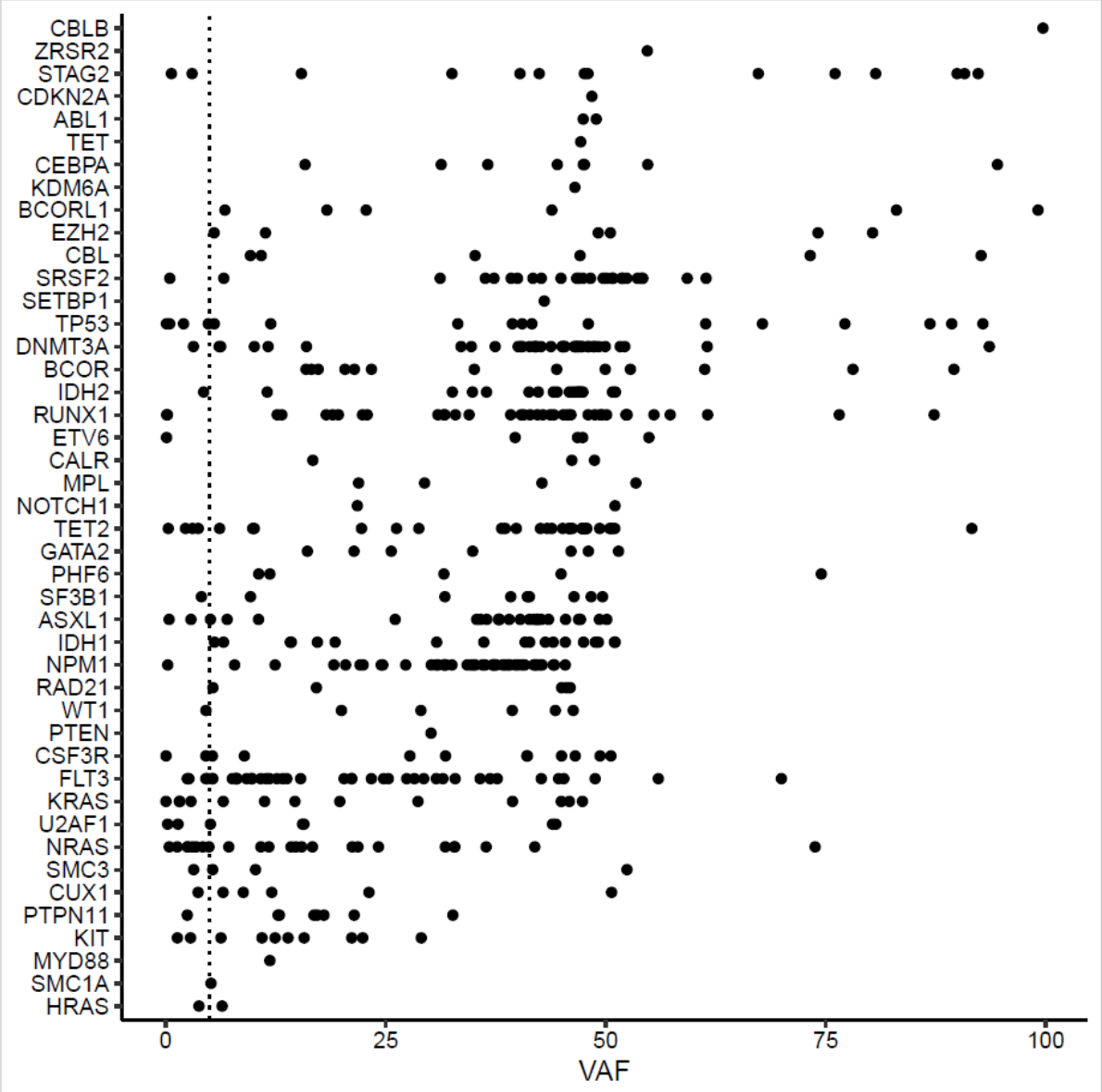


Figure 3 – VAF per mutation

We then evaluated the frequency of co-mutations in our population **(Figure 4);** The mutations most frequently associated were *NPM1/FLT3* in 25 (16%) patients, *NPM1/DNMT3A* in 22 (14%) patients, *RUNX1/ASX*L1 in 19 (13%) patients, *RUNX1/SRSF2* and *FLT3/DNMT3A* in 16 (11%) patients each.

We observed a high concurrence with other mutations for certain genes such as *RUNX1*, *SRSF2*, *ASXL1*, *NPM1*, *FLT3* whereas some mutations were only found in pairs. For example, the only co mutation of *CBL* was with *ASXL1* and *KIT* with *NRAS*. **(Figure 5)**

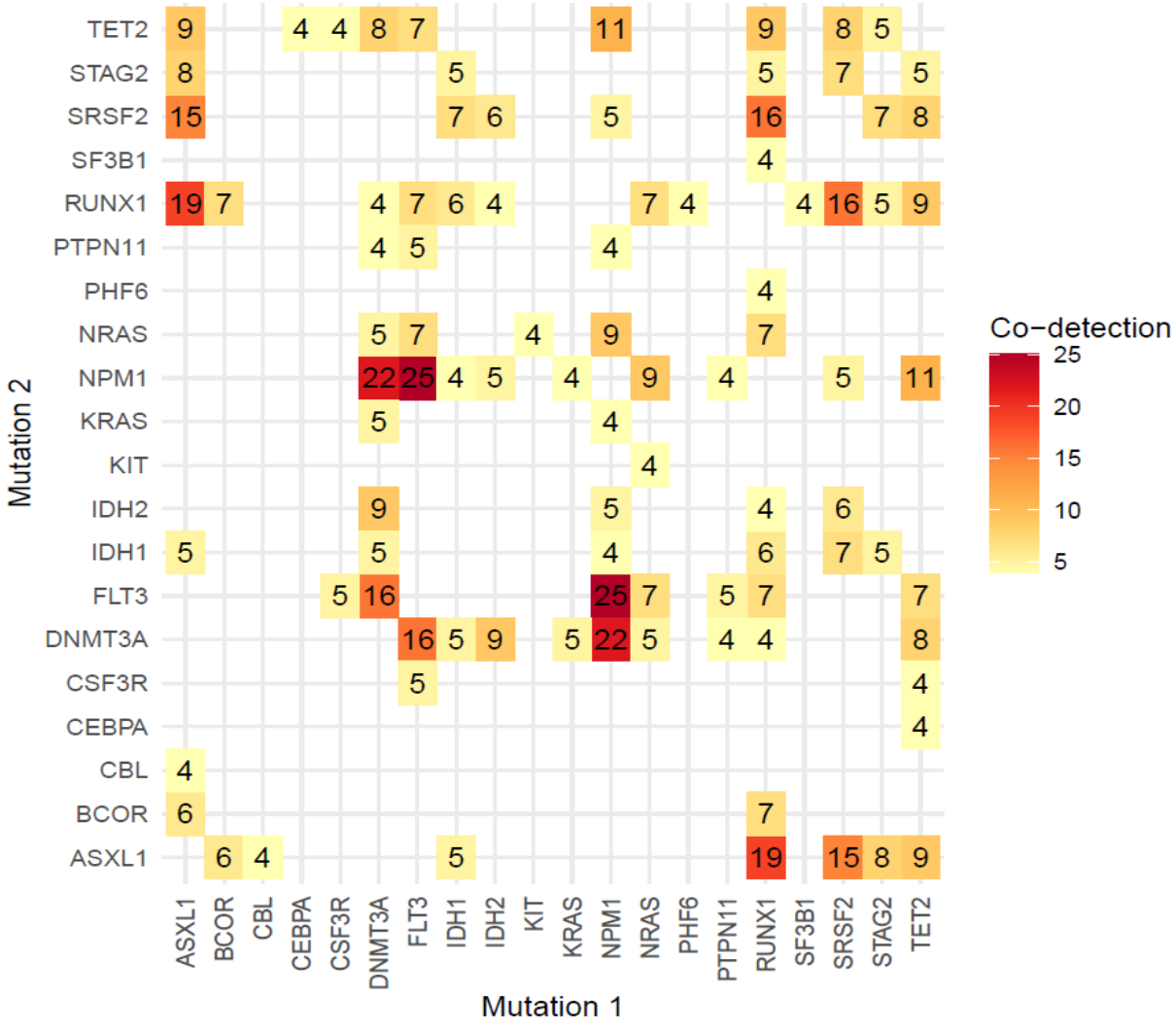


Figure 4 – Co-occurence of mutations

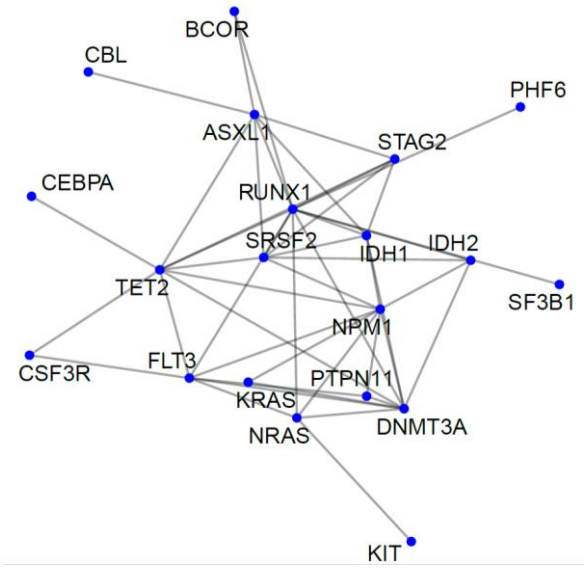
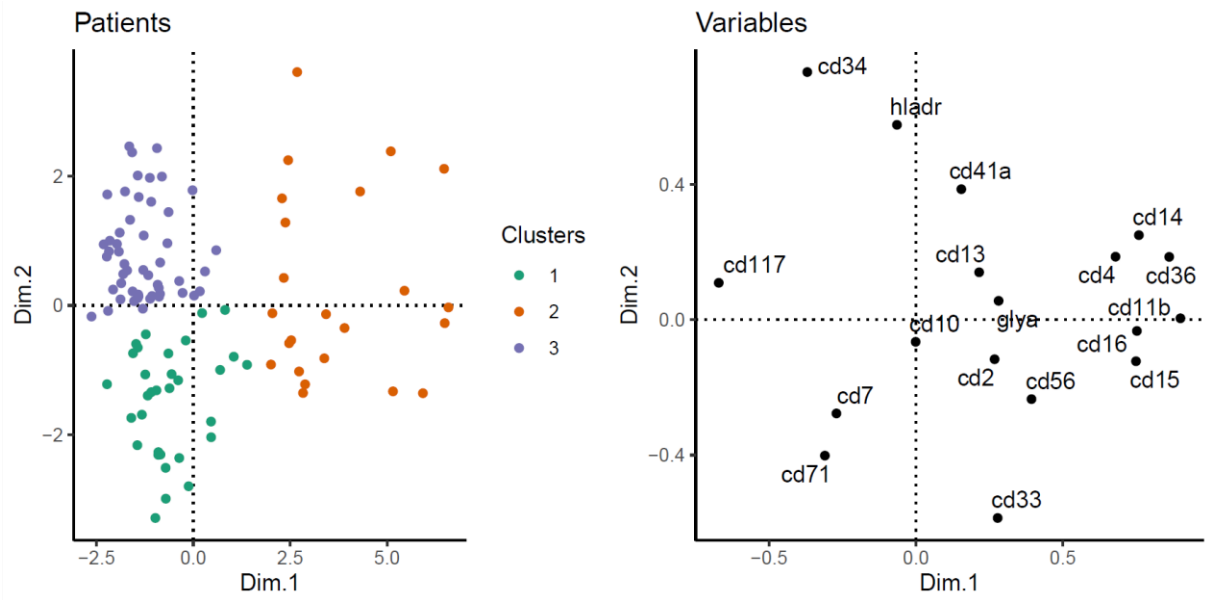


Figure 5 – Combinations of co-occurrence of mutations

Associations between phenotypical profile, NGS and karyotype

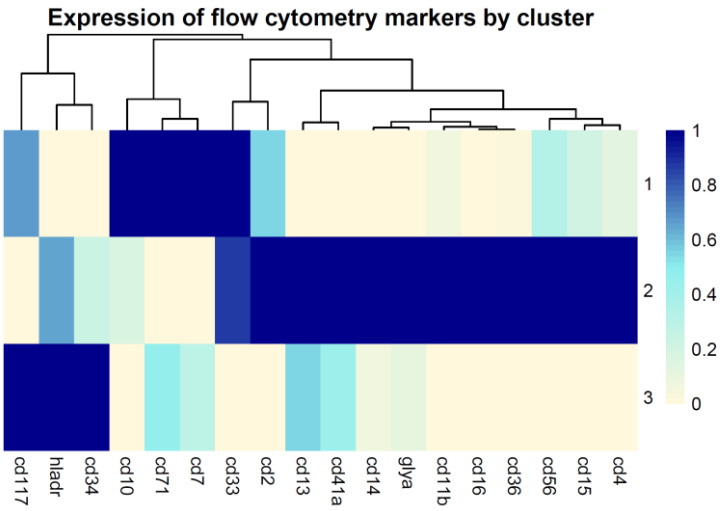
We performed a principal component analysis with the percent of antigen expression by blasts cells on the 106 patients that had an extended MFC analysis **(Figure 6)**. Three clusters were identified. The first cluster consisted of 32 patients expressing CD7, CD10, CD33 and CD71. The second one comprises 23 patients expressing multiple markers such as myeloid markers CD13, CD14, CD11b, CD36, and CD15, NK markers CD16 and CD56 and T cell markers CD2 and CD4. Lastly, the third cluster included 51 patients and expressed CD34, CD117 and HLADR suggesting less differentiated cells **(Figure 7).**



Patients are represented as colored points on the left graph. Each color is a phenotypic cluster. The right graph represents the phenotypic characteristics in the same dimensions.

Figure 6 – Principal component analysis of phenotypic characteristics

Association of these phenotypic clusters with mutational and cytogenetic profiles was screened using heatmaps presented in **Figure 8**. In the first cluster, we observed a higher incidence of NPM1 FLT3ITD DNMT3A, as well as WT1, KDM6A, NOTCH1, CEBPA and IDH2, and a higher incidence of normal karyotype or t(9;11) (intermediate risk cytogenetics). Cluster 2 was more often associated with IDH1, NRAS, RAD21, PTPN11 , CBL , JAK2 and CALR mutations, FLT3 TKD and TET2 mutations as well as 5 monosomy, del3q, add7q. Cluster 3 was associated with numerous mutations like RUNX1 , ASXL1, CSF3R, EZH2, SRSF2, BCOR, STAG2, U2AF1, TP53, SETBP1, ZRSR2. Multiple cytogenetic anomalies and more notably complex karyotype , monosomy 7 , monosomy 17 but also t(16;16) and t(8;21).



Lines represent clusters from Figure 2.

Figure 7 – Cluster-categorized phenotypic expression of blasts in flow cytometry (MFC)

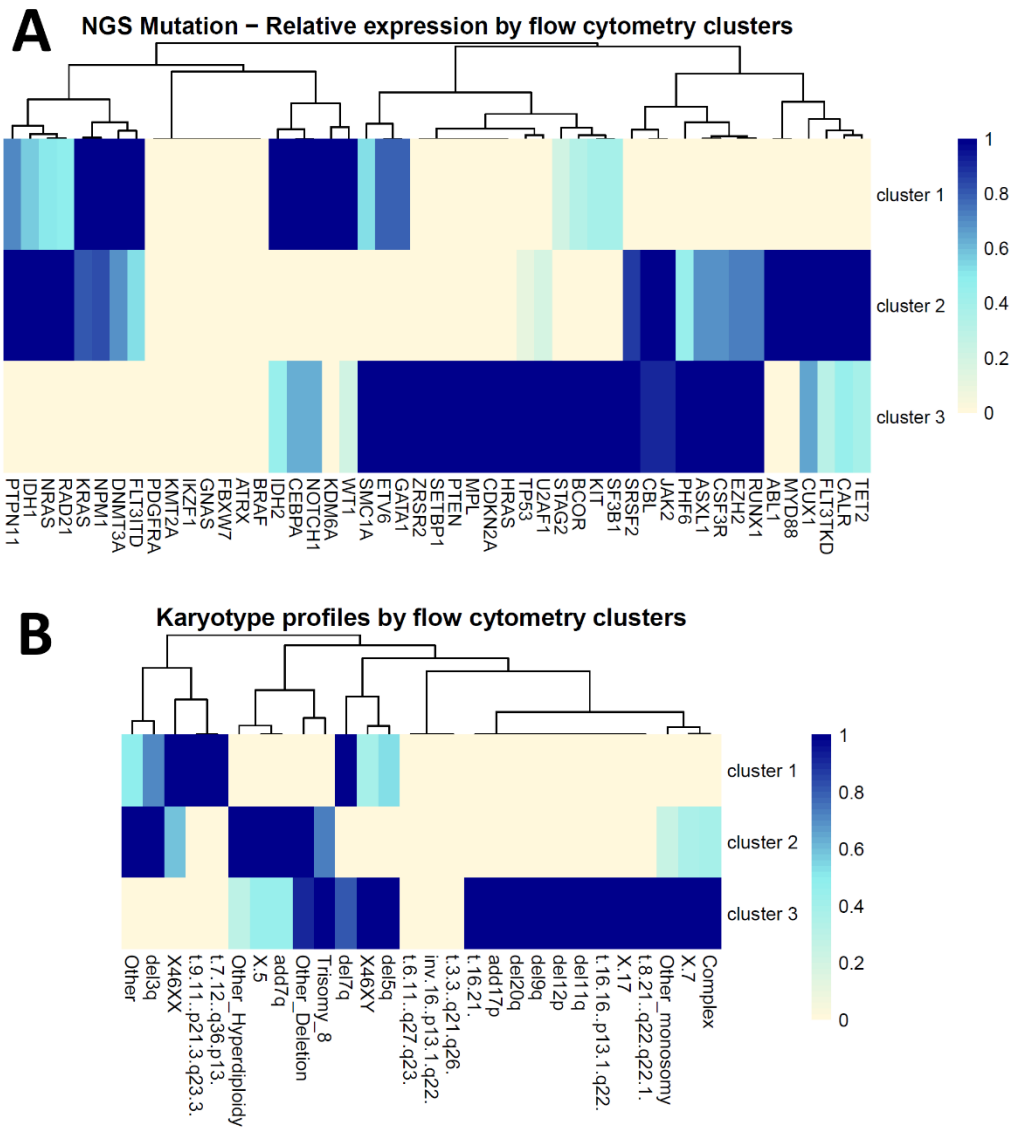


Figure 8 – Mutational and cytogenetic profiles of patients by MFC cluster

Outcomes of MFC clusters

We then compared outcomes between MFC clusters. The percentage of blasts at day 15 was significantly different across clusters 1, 2 and 3 (87% vs 80% vs 36%, respectively, p = 0.012). There was no significant difference for complete response , relapse or overall mortality **(Table 6)**.

A Kaplan Meier analysis of survival found no significant difference in survival after clusters (p=0.78) **(Figure 9)**.

	Cluster 1	Cluster 2	Cluster 3	p value*
Complete response	21/28 (75%)	17/22 (77%)	29/49 (59%)	0.199
No blasts at day 15	13/15 (87%)	8/10 (80%)	6/16 (36%)	0.012
Relapse	4/17 (24%)	6/14 (43%)	7/19 (37%)	0.563
Overall mortality	17/31 (55%)	12/22 (55%)	31/50 (62%)	0.755
A Chi-squared test was used when the sample size was >5, and a Fisher's test was used in other cases.				

Table 6 – Comparison of outcomes between MFC clusters

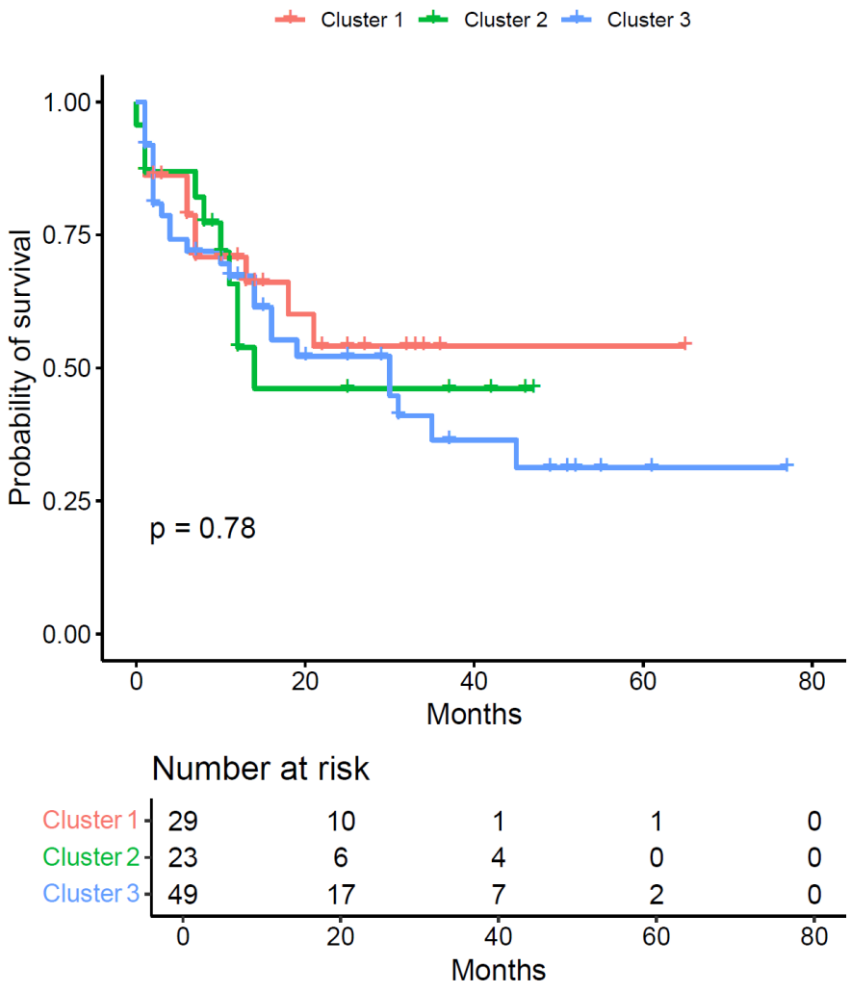


Figure 9 – Kaplan Meier survival analysis across CMF clusters

DISCUSSION

The main results of this study are a median age at diagnosis of 64 years [IQR 53;71], a prevalence of 77% for an ECOG score equal or inferior to 1 at diagnosis, a prevalence of 39% for a cardiovascular comorbidity such as hypertension, arrhythmias, or coronary artery disease. The median circulating blast percentage at diagnosis was 30%. Notably, 12.5% of patients had no circulating blasts. Secondary AML was observed in 47% of patients, and 51% were classified as having an adverse risk according to the 2022 ELN classification. A favorable risk was identified in 14% of patients, of whom 63% had an NPM1 mutation and 32% had a mutation involving the CBF. Cytogenetic analysis identified a complex karyotype in 13% of cases. Mutational analysis revealed frequent alterations in genes involved in leukemogenesis, notably NPM1 (28%), DNMT3A (26%), RUNX1 (24%), FLT3-ITD (22%), and TET2 (20%). Other frequently mutated genes included SRSF2 (16%), ASXL1 (16%), NRAS (16%), IDH1 (12%), IDH2 (12%), and TP53 (10%). The most common co-mutations were FLT3-ITD and NPM1 in 23 patients (15%), DNMT3A and NPM1 in 22 patients (14%), and ASXL1 and RUNX1 in 18 patients (12%).

Patient characteristics

Main characteristics like age and gender were comparable to literature (1). 77% of patients had an ECOG score of 0 or 1, so the performance status of patients was well preserved.

In our study, the prevalence of AML-MR was 47%. This finding is surprising comparing to epidemiological studies in AML patients. In an analysis of the PETHEMA registry with patients, Martinez-Cuadron *et al.* found that AML-MRC represents 27% of AML (29). We were not able to find an explanation for this particularly high rate. Moreover, AML-MRC are supposed to affect elderly patients, and our population is not old compared to epidemiological data.

Cardiovascular comorbidities were frequent in our population. We assumed that this could be linked to the high prevalence of AML-MRC via a high occurrence of clonal hematopoiesis of indeterminate potential (CHIP). CHIP is a newly defined entity revealed by genetic analyses of large populations. It refers to the acquisition of somatic mutations that drive clonal expansion without causing dysplastic hematopoiesis or cytopenia. Patients with these mutations are at higher risk of developing AML and other hematological malignancies, such as Myelodysplastic Neoplasm (MDN) (30). A study analyzing blood cells from 17,182

individuals found that among those aged 70 to 79, 9.5% had clonal mutations, with the incidence increasing with age. The most common mutations were found in *DNMT3A*, *TET2*, and *ASXL1*, and their presence was associated with an increased risk of hematologic cancer, all-cause mortality, coronary heart disease, and ischemic stroke. The rate of transformation of CHIP into overt hematologic disease is approximately 0.5-1% per year (31). We have no proof of a high amount of CHIP in our population, but this specific mutational profile is frequent.

Regarding risk stratification using ELN2017 , our population had a more pejorative risk than literature (32). This can be linked to the high prevalence of secondary AML in our population. Few published epidemiologic studies describe the prevalence of risk categories according to ELN2022. Our data suggests that patients are less likely to be ranked as favorable (14% vs 21%) and more likely to be ranked as adverse (51% vs 43%).

Evolution and treatment

Response to treatment

In our study the response rate after induction with intensive chemotherapy was 70% and the allogenic transplantation rate after first remission in those patients was 42%. These results seem closely coherent with the data from a large French trial in patients with AML comparing different regimen of intensive chemotherapy, in which the response rate after induction with intensive chemotherapy was 79%, and the allogenic transplantation rate was 42.3% at first remission (33).

In patients that received another treatment than intensive chemotherapy, the response rate of 42% was lower than expected. In a randomized trial comparing Azacitidine versus Azaciditine and Venetoclax, DiNardo et al. observed a response rate of 66,4% in the Venetoclax group and 28.3% in the control group (34). This data can be explained by the fact that in our subgroup of patients receiving non-intensive treatment, 60% had a combination of Azacitidine and Venetoclax and the remaining 40% had either Azacitidine monotherapy, either other combinations of treatments, with a lower expected response rate. Moreover, a subsequent number of patients receiving Azacitidine or Azacitidine and Venetoclax received suboptimal therapy due to intercurrents events such as intolerance, infections, or impaired physical state.

Survival

It is uneasy to compare our results of survival with literature, as the follow-up in this retrospective study could be too short, because some patients were diagnosed in 2023, sometimes only a few months before data were collected. It is known that median survival rates at 5 years for AML are approximately 31.9% (1,35). In our study, the 45% survival rate is described for a median time of follow-up of 2.9 [1.6 - 4.5] years.

Karyotype

One of the largest cohorts of cytogenetic data published is found in the studies of Grimwade *et al.* In 2001, they studied 1,065 AML patients with a median age of 66 years, reporting that 41-48% had no detectable cytogenetic abnormalities, while 14% had a complex karyotype, 10% had trisomy 8, 8% had monosomy 7 or del(5q), and 5% had monosomy 5 or del(7q) (16). Our findings are coherent to these results.

Grimwalde identified three prognostic groups, with favorable outcomes associated with t(15;17), t(8;21), or inv(16), as these abnormalities correlated with higher complete remission rates and lower relapse risk. Conversely, patients with a complex karyotype had a poor prognosis. Later, Grimwade *et al.* expanded on these findings by identifying additional cytogenetic abnormalities linked to poor outcomes, including abn(3q), inv(3)(q21q26), t(3;3)(q21;q26), add(5q)/del(5q), monosomy 5, monosomy 7, add(7q)/del(7q), and various 11q23 translocations. Additionally, t(9;22)(q34;q11), monosomy 17, and abn(17p) were also associated with poor prognosis. A complex karyotype was further refined to include cases with four or more unrelated cytogenetic abnormalities, reinforcing its association with significantly worse outcomes (16).

Phenotypical analysis

Identification of phenotypic patterns

In our principal component analysis, the markers found in cluster no.3 (CD34, CD117 and HLADR) are related to indifferenciated blasts, mimicking hematopoietic progenitor cells (HPC). They and are usually found in FAB 0 or 1 AML (36). In a data-driven classification study using flow cytometry, Simoes *et. al* identified 6 profiles of flow cytometry cells. They called one of these groups “HPC-like AML”, regarding to the expression of cd34, cd117 and hladr (36). It is interesting to notice that the NGS study in patients from HPC-like AML group

revealed that *RUNX1* was more frequent in this group. We also found a higher incidence of *RUNX1* mutations in this particular cluster.

The cluster no.1 was composed of patients with higher expression of CD7, CD10, CD33 and CD71. CD7, CD10 and CD33 can be seen in myeloid cells. Though CD71 is the receptor of transferrin, therefore an erythroblastic marker, it can also be found in non-erythroblastic bone marrow blast cells, as shown in the work of Qian Liu (37). This cluster seemed to be associated with a more important relative frequency of *NPM1*, *DNMT3a* and *FLT3-ITD* mutations and specific to *KMD6A* mutation, a gene involved in demethylation processes.

When comparing response to treatment between MFC clusters, response and relapse rates were similar but we observed significantly less patients having no blasts at day-15 bone marrow aspirate in cluster 3. Cluster 3 was also associated with complex karyotype and RUNX1, TP53, ASXL1, BCOR, EZH2 and other mutations known to have an adverse effect on prognosis (10). There was no significant difference in survival between MFC clusters. However, the Kaplan Meier curve seems to show a tendency for a greater overall mortality in cluster 3 patients, that would be expected regarding the mutational profile of these patients. Further analysis will be realized based on our data to evaluate more precisely the impact of these profiles on prognosis.

Mutational profiles

AML-MRC associated genes

The molecular profile of our population was likely impacted by the high prevalence of AML-MRC. AML-MRC is known to be linked to mutations of *TP53*, *RUNX1*, Splicing factors (*SRSF2*, *SF3B1*) and Epigenetic regulators (*ASXL1*, *IDH2*, *KMT2A*, *EZH2*) (38).

In our population, *RUNX1* was mutated in 24% of patients. In a large cohort of 2439 patients with newly diagnosed AML, it was only found in 10% patients. The same study found that it was associated to secondary AML (15.6% vs 5.5%, p <0.0001) a co-occurrence of mutation was frequent with epigenetic modifiers (*ASXL1*, *IDH2*, *KMT2A*, *EZH2*), spliceosome (*SRSF2*, *SF3B1*) and *STAG2*, *PHF6*, *BCOR*. Associated with older age, male gender, more immature morphology and secondary AML (15.6% vs 5.5%, p <0.0001) (39).

TET2 mutations were found in 26% of our patients. Epidemiologic data suggest that it usually occurs in approximately 13% of AML cases. (Chou, 2011) It plays a role in DNA demethylation. While their overall prognostic impact remains unclear, *TET2* mutations are

associated with older age, high white blood cell and blast counts, lower platelet counts, normal karyotype, and intermediate-risk cytogenetics. They frequently co-occur with trisomy 8, *NPM1*, and *ASXL1* mutations but are not typically associated with IDH mutations. Notably, *TET2* mutations have been linked to poor prognosis in AML patients with intermediate-risk cytogenetics, particularly when combined with other adverse molecular markers (40).

ASXL1 mutations were found in 15% of our patients. It occurs in approximately 5-11% of AML cases (41). These mutations are more common in older patients and are frequently associated with t(8;21), as well as cases lacking *NPM1* and *FLT3* mutations. They are also linked to *CEBPA* mutations. *ASXL1* mutations are generally associated with a poor prognosis, characterized by lower remission rates and worse overall outcomes (41).

The incidence of *TP53* mutations was not superior in our study (9%) than in epidemiologic data (8%) (42).

Other frequent genes

The most common gene mutation in our study was *NPM1* (28%). This finding was expected as mutations in *NPM1* represent the most common genetic anomaly in acute myeloid leukemia (AML), occurring in approximately 30% of cases (43,44). NPM1 encodes for Nucleophosphomin, a multifunctional protein localized in the nucleolus but capable of being aberrantly translocated to the cytoplasm in the presence of gene mutations. *NPM1* plays a crucial role in various cellular functions, including DNA repair and apoptosis regulation (45). Patients with *NPM1*-mutated AML typically present with high blood counts at diagnosis, a normal karyotype, and lack of CD34 expression on flow cytometry. This subtype is frequently associated with mutations in *FLT3*, *DNMT3A*, *TET2*, and *IDH*, *NRAS* and is generally linked to a favorable prognosis (12).

DNMT3A mutations were found in 25% of patients. They usually occur in approximately 18-22% of AML cases, most commonly affecting the R882 position, which results in a protein that inhibits the methyltransferase activity of the wild-type *DNMT3A*. While the exact mechanisms of leukemogenesis remain unclear, the prognostic impact of *DNMT3A* mutations remains controversial. Current data suggest that these mutations do not significantly affect overall survival; however, higher doses of anthracyclines may be more effective in *DNMT3A*-mutated AML (46–48).

FLT3-ITD and *FLT3-TKD* mutations occurred in 20% and 4% of our patients, respectively. The *FLT3* gene encodes a tyrosine kinase receptor that plays a key role in cell survival and proliferation. In acute myeloid leukemia (AML), two types of *FLT3* mutations have been identified: internal tandem duplication (ITD) in the juxtamembrane region and point mutations in the second tyrosine kinase domain (TKD). *FLT3-ITD* mutations, found in approximately 25% of AML cases, have been shown to impact prognosis, with a high mutant ratio correlating with poor outcomes (49). In contrast, *FLT3-TKD* mutations, present in about 7% of AML cases, have a controversial prognostic significance (50). FLT3 mutations are frequently associated with de novo AML, normal karyotype, high white blood cell counts, increased percentages of peripheral blood and bone marrow blasts, and elevated serum lactate dehydrogenase levels (51).

VAF dispersion

The study of VAF dispersion as shown in Figure 3 revealed that the median VAF level per mutation was heterogeneous. Specific mutations occur early in leukemogenesis and may facilitate the clonal expansion of hematopoietic stem cells, eventually leading to the progression to acute myeloid leukemia (AML). For example, epigenetic mutations in *DNMT3A*, *TET2*, and *ASXL1* have been identified in pre-leukemic cells years before the development of AML, suggesting that these mutations are early drivers of events that precede leukemogenic transformation. Other mutations may cooperate with these initiating events to cause disease progression, even though they do not initiate leukemia on their own. Logically, those cooperating mutations are likely to have a less important VAF. Among these, the *FLT3* gene is the most frequently mutated in this context (52).

Limits of the study

Our study has several limits. First, it is a retrospective and monocentric study, so our results regarding treatments, techniques employed for cytogenetic, phenotypic and molecular analysis can have local specificities and be responsible for a center effect. Some data could not be found due to the retrospective nature of the study, such as karyotypes for 2 patients, and MFC for 21 patients. MFC analysis studied an heterogeneous amount of markers among patients, thus allowing us to compare them with cytogenetic and mutational profiles in only 106 patients which share at least some studied antigenes. Secondly, the period limit of our study, due to the absence of systematic NGS analysis in our center before 2018 allowed us to include less patients than expected. This relatively low population size was responsible for a

poor number of co-occurrences of some mutations, which limits the power of analytic tests. Third, the screening method was not perfectly appropriate for the description of the global epidemiology of AML because it did not include the patients that had no treatment at all (*ie,* most of palliative care patients). This potential bias can be responsible of an overestimation of the proportion of patients with a satisfying performance status, or with a favorable evolution in our center. Still, 5 patients in our study had a palliative treatment and were still included. This can be explained by the fact that they were previously treated by Azacitidine for MDS before the diagnosis of secondary AML, and thus they were screened as they received a treatment usually employed in AML.

CONCLUSION

Our study describes the population of AML patients in the university hospital of Tours. In this monocentric cohort, the prevalence of AML-MRC was higher than expected compared to the most recent epidemiological data. We also found a high rate of *RUNX1*, *TET2* and *AXL1* mutations, which is likely explained by the higher frequence of those mutations in secondary AML.

We found that the co-occurrences of *NPM1/FLT3*, *NPM1/DNMT3A*, *RUNX1/ASXL1*, *RUNX1/SRSF2* and *FLT3/DNMT3A* were frequent. We also identified phenotypical patterns that seem to be linked to mutational and cytogenetic profiles

Further analysis and research are needed to study the statistic association between co-mutational profiles and biological, cytogenetic and phenotypic profiles in AML.

REFERENCES

1. National Cancer Institute. Cancer stat facts: leukemia—acute myeloid leukemia (AML). 2024. https://seer.cancer.gov/statfacts/html/amyl.html (accessed Sept 29, 2024).

2. Defossez G, Le Guyader-Peyrou S, Uhry Z, Grosclaude P, Colonna M, Dantony E, et al. Estimations nationales de l'incidence et de la mortalité par cancer en France métropolitaine entre 1990 et 2018. Synthèse. Saint-Maurice : Santé publique France, 2019. 20 p. Disponible à partir des URL : http://www.santepubliquefrance.fr/ ; https://geodes.santepubliquefrance.fr/ #c=home ; http://lesdonnees.e-cancer.fr/ ; https://www.e-cancer.fr.

3. Shysh AC, Nguyen LT, Guo M, Vaska M, Naugler C, Rashid-Kolvear F. The incidence of acute myeloid leukemia in Calgary, Alberta, Canada: a retrospective cohort study. BMC Public Health 2017;18:94.

4. Gangatharan SA, Grove CS, P'ng S, O'Reilly J, Joske D, Leahy MF, et al. Acute myeloid leukaemia in Western Australia 1991-2005: a retrospective populationbased study of 898 patients regarding epidemiology, cytogenetics, treatment and outcome. Intern Med J 2013;43:903–11.

5. Ostgard LS, Norgaard JM, Severinsen MT, Sengelov H, Friis L, Jensen MK, et al. Data quality in the Danish National Acute Leukemia Registry: a hematological data resource. Clin Epidemiol 2013;5:335–44.

6. Shallis RM, Wang R, Davidoff A, Ma X, Zeidan AM. Epidemiology of acute myeloid leukemia: Recent progress and enduring challenges. Blood Rev. juill 2019;36:70-87.

7. Shallis RM, Weiss JJ, Deziel NC, Gore SD. A clandestine culprit with critical consequences: Benzene and acute myeloid leukemia. Blood Rev. mai 2021;47:100736.

8. Khoury JD, Solary E, Abla O, Akkari Y, Alaggio R, Apperley JF, et al. The 5th edition of the World Health Organization Classification of Haematolymphoid Tumours: Myeloid and Histiocytic/Dendritic Neoplasms. Leukemia. juill 2022;36(7):1703-19.

9. Heuser M, Freeman SD, Ossenkoppele GJ, Buccisano F, Hourigan CS, Ngai LL, et al. 2021 Update on MRD in acute myeloid leukemia: a consensus document from the European LeukemiaNet MRD Working Party. Blood. 30 déc 2021;138(26):2753-67.

10. Döhner H, Wei AH, Appelbaum FR, Craddock C, DiNardo CD, Dombret H, et al. Diagnosis and management of AML in adults: 2022 recommendations from an international expert panel on behalf of the ELN. Blood. 22 sept 2022;140(12):1345-77.

11. Tettero JM, Freeman S, Buecklein V, Venditti A, Maurillo L, Kern W, et al. Technical Aspects of Flow Cytometry-based Measurable Residual Disease Quantification in Acute Myeloid Leukemia: Experience of the European LeukemiaNet MRD Working Party. HemaSphere. 22 déc 2021;6(1):e676.

12. Falini B, Martelli MP. Comparison of the International Consensus and 5th WHO edition classifications of adult myelodysplastic syndromes and acute myeloid leukemia. Am J Hematol. mars 2023;98(3):481-92.

13. Arber DA, Orazi A, Hasserjian RP, Borowitz MJ, Calvo KR, Kvasnicka HM, et al. International Consensus Classification of Myeloid Neoplasms and Acute Leukemias: integrating morphologic, clinical, and genomic data. Blood. 15 sept 2022;140(11):1200-28.

14. Attardi E, Savi A, Borsellino B, Piciocchi A, Cipriani M, Ottone T, et al. Applicability of 2022 classifications of acute myeloid leukemia in the real-world setting. Blood Adv. 12 sept 2023;7(17):5122-31.

15. Mrózek K, Heerema NA, Bloomfield CD. Cytogenetics in acute leukemia. Blood Rev. juin 2004;18(2):115-36.

16. Grimwade D, Hills RK, Moorman AV, Walker H, Chatters S, Goldstone AH, et al. Refinement of cytogenetic classification in acute myeloid leukemia: determination of prognostic significance of rare recurring chromosomal abnormalities among 5876 younger adult patients treated in the United Kingdom Medical Research Council trials. Blood. 22 juill 2010;116(3):354-65.

17. Yokota S, Kiyoi H, Nakao M, Iwai T, Misawa S, Okuda T, et al. Internal tandem duplication of the FLT3 gene is preferentially seen in acute myeloid leukemia and myelodysplastic syndrome among various hematological malignancies. A study on a large series of patients and cell lines. Leukemia. oct 1997;11(10):1605-9.

18. Papaemmanuil E, Gerstung M, Bullinger L, Gaidzik VI, Paschka P, Roberts ND, et al. Genomic Classification and Prognosis in Acute Myeloid Leukemia. N Engl J Med. 9 juin 2016;374(23):2209-21.

19. Pratcorona M, Brunet S, Nomdedéu J, Ribera JM, Tormo M, Duarte R, et al. Favorable outcome of patients with acute myeloid leukemia harboring a low-allelic burden FLT3-ITD mutation and concomitant NPM1 mutation: relevance to post-remission therapy. Blood. 4 avr 2013;121(14):2734-8.

20. Döhner H, Estey E, Grimwade D, Amadori S, Appelbaum FR, Büchner T, et al. Diagnosis and management of AML in adults: 2017 ELN recommendations from an international expert panel. Blood. 26 janv 2017;129(4):424-47.

21. Lachowiez CA, Long N, Saultz J, Gandhi A, Newell LF, Hayes-Lattin B, et al. Comparison and validation of the 2022 European LeukemiaNet guidelines in acute myeloid leukemia. Blood Adv. 9 mai 2023;7(9):1899-909.

22. Tbakhi B, O'Dwyer K. The VIALE-C Trial: Venetoclax and Low-dose Cytarabine in Older Patients With AML. The Hematologist. 29 août 2020;17(5).

23. Stone RM, Mandrekar SJ, Sanford BL, Laumann K, Geyer S, Bloomfield CD, et al. Midostaurin plus Chemotherapy for Acute Myeloid Leukemia with a FLT3 Mutation. N Engl J Med. 3 août 2017;377(5):454-64.

24. Montesinos P, Recher C, Vives S, Zarzycka E, Wang J, Bertani G, et al. Ivosidenib and Azacitidine in IDH1-Mutated Acute Myeloid Leukemia. N Engl J Med. 20 avr 2022;386(16):1519-31.

25. Wang ES, Montesinos P, Minden MD, Lee JH, Heuser M, Naoe T, et al. Phase 3 trial of gilteritinib plus azacitidine vs azacitidine for newly diagnosed FLT3mut+ AML ineligible for intensive chemotherapy. Blood. 27 oct 2022;140(17):1845-57.

26. Perl AE, Martinelli G, Cortes JE, Neubauer A, Berman E, Paolini S, et al. Gilteritinib or Chemotherapy for Relapsed or Refractory FLT3-Mutated AML. N Engl J Med. 31 oct 2019;381(18):1728-40.

27. Wang ES, Issa GC, Erba HP, Altman JK, Montesinos P, DeBotton S, et al. Ziftomenib in relapsed or refractory acute myeloid leukaemia (KOMET-001): a multicentre, open-label, multi-cohort, phase 1 trial. Lancet Oncol. oct 2024;25(10):1310-24.

28. Kwon MC, Thuring JW, Querolle O, Dai X, Verhulst T, Pande V, et al. Preclinical efficacy of the potent, selective menin-KMT2A inhibitor JNJ-75276617 (bleximenib) in KMT2A- and NPM1-altered leukemias. Blood. 12 sept 2024;144(11):1206-20.

29. Martínez-Cuadrón D, Megías-Vericat JE, Serrano J, Martínez-Sánchez P, Rodríguez-Arbolí E, Gil C, et al. Treatment patterns and outcomes of 2310 patients with secondary acute myeloid leukemia: a PETHEMA registry study. Blood Adv. 22 févr 2022;6(4):1278-95.

30. Steensma DP, Bejar R, Jaiswal S, Lindsley RC, Sekeres MA, Hasserjian RP, et al. Clonal hematopoiesis of indeterminate potential and its distinction from myelodysplastic syndromes. Blood. 2 juill 2015;126(1):9-16.

31. Jaiswal S, Fontanillas P, Flannick J, Manning A, Grauman PV, Mar BG, et al. Age-Related Clonal Hematopoiesis Associated with Adverse Outcomes. N Engl J Med. 25 déc 2014;371(26):2488-98.

32. Bataller A, Garrido A, Guijarro F, Oñate G, Diaz-Beyá M, Arnan M, et al. European LeukemiaNet 2017 risk stratification for acute myeloid leukemia: validation in a risk-adapted protocol. Blood Adv. 15 févr 2022;6(4):1193-206.

33. Mathilde HB, Pautas C, Bertoli S, Dumas PY, Raffoux E, Marchand T, et al. Current Results of Intensive Therapy in Younger Adults with Acute Myeloid Leukemia (AML): The Large Randomized French Backbone Intergroup (BIG)-1 Study on Behalf of the Filo, ALFA, and SFGM-TC Study Groups. Blood. 2 nov 2023;142:967.

34. DiNardo CD, Jonas BA, Pullarkat V, Thirman MJ, Garcia JS, Wei AH, et al. Azacitidine and Venetoclax in Previously Untreated Acute Myeloid Leukemia. N Engl J Med. 12 août 2020;383(7):617-29.

35. Sasaki K, Ravandi F, Kadia TM, DiNardo CD, Short NJ, Borthakur G, et al. De novo acute myeloid leukemia: A population-based study of outcome in the United States based on the Surveillance, Epidemiology, and End Results (SEER) database, 1980 to 2017. Cancer. 2021;127(12):2049-61.

36. Simoes C, Gonzalez C, Vergez F, Sarry A, Bertoli S, Ariceta B, et al. Data-driven flow cytometry classification of blast differentiation in older patients with acute myeloid leukemia. Blood Neoplasia. 1 sept 2024;1(3):100027.

37. Liu Q, Wang M, Hu Y, Xing H, Chen X, Zhang Y, et al. The Usefulness of CD71 Expression by Flow Cytometry in the Diagnosis of Acute Leukemia. Blood. 16 nov 2012;120(21):2533.

38. Baer C, Walter W, Stengel A, Hutter S, Meggendorfer M, Kern W, et al. Molecular Classification of AML-MRC Reveals a Distinct Profile and Identifies MRC-like Patients with Poor Overall Survival. Blood. 13 nov 2019;134(Supplement_1):2735.

39. Gaidzik VI, Teleanu V, Papaemmanuil E, Weber D, Paschka P, Hahn J, et al. RUNX1 mutations in acute myeloid leukemia are associated with distinct clinico-pathologic and genetic features. Leukemia. nov 2016;30(11):2160-8.

40. Chou WC, Chou SC, Liu CY, Chen CY, Hou HA, Kuo YY, et al. TET2 mutation is an unfavorable prognostic factor in acute myeloid leukemia patients with intermediate-risk cytogenetics. Blood. 6 oct 2011;118(14):3803-10.

41. Metzeler KH, Becker H, Maharry K, Radmacher MD, Kohlschmidt J, Mrózek K, et al. ASXL1 mutations identify a high-risk subgroup of older patients with primary cytogenetically normal AML within the ELN Favorable genetic category. Blood. 22 déc 2011;118(26):6920-9.

42. Devillier R, Mansat-De Mas V, Gelsi-Boyer V, Demur C, Murati A, Corre J, et al. Role of ASXL1 and TP53 mutations in the molecular classification and prognosis of acute myeloid leukemias with myelodysplasia-related changes. Oncotarget. 10 avr 2015;6(10):8388-96.

43. Falini B, Mecucci C, Tiacci E, Alcalay M, Rosati R, Pasqualucci L, et al. Cytoplasmic nucleophosmin in acute myelogenous leukemia with a normal karyotype. N Engl J Med. 20 janv 2005;352(3):254-66.

44. Falini B, Brunetti L, Sportoletti P, Martelli MP. NPM1-mutated acute myeloid leukemia: from bench to bedside. Blood. 8 oct 2020;136(15):1707-21.

45. Marando L, Huntly BJP. Molecular Landscape of Acute Myeloid Leukemia: Prognostic and Therapeutic Implications. Curr Oncol Rep. juin 2020;22(6):61.

46. Ley TJ, Ding L, Walter MJ, McLellan MD, Lamprecht T, Larson DE, et al. DNMT3A Mutations in Acute Myeloid Leukemia. N Engl J Med. 16 déc 2010;363(25):2424-33.

47. Yang L, Rau R, Goodell MA. DNMT3A in haematological malignancies. Nat Rev Cancer. mars 2015;15(3):152-65.

48. Sehgal AR, Gimotty PA, Zhao J, Hsu JM, Daber R, Morrissette JD, et al. DNMT3A Mutational Status Affects the Results of Dose-Escalated Induction Therapy in Acute Myelogenous Leukemia. Clin Cancer Res Off J Am Assoc Cancer Res. 1 avr 2015;21(7):1614-20.

49. Thiede C, Steudel C, Mohr B, Schaich M, Schäkel U, Platzbecker U, et al. Analysis of FLT3-activating mutations in 979 patients with acute myelogenous leukemia: association with FAB subtypes and identification of subgroups with poor prognosis. Blood. 15 juin 2002;99(12):4326-35.

50. Kayser S, Schlenk RF, Londono MC, Breitenbuecher F, Wittke K, Du J, et al. Insertion of FLT3 internal tandem duplication in the tyrosine kinase domain-1 is associated with resistance to chemotherapy and inferior outcome. Blood. 17 sept 2009;114(12):2386-92.

51. Fröhling S, Schlenk RF, Breitruck J, Benner A, Kreitmeier S, Tobis K, et al. Prognostic significance of activating FLT3 mutations in younger adults (16 to 60 years) with acute myeloid leukemia and normal cytogenetics: a study of the AML Study Group Ulm. Blood. 15 déc 2002;100(13):4372-80.

52. Welch JS, Ley TJ, Link DC, Miller CA, Larson DE, Koboldt DC, et al. The Origin and Evolution of Mutations in Acute Myeloid Leukemia. Cell. juill 2012;150(2):264-78.

APPENDIX

M0: Minimally differentiated AML
M1: AML without maturation
M2: AML with maturation
M3: Acute promyelocytic leukemia (APL)
M4: Acute myelomonocytic leukemia
M4Eo: AML with eosinophilia
M5: Acute monocytic leukemia
M6: Acute erythroid leukemia
M7: Acute megakaryoblastic leukemia

Appendix 1 – Fab classification

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1) Acute myeloid leukaemia with defining genetic abnormalities

Acute promyelocytic leukaemia with *PML::RARA* fusion
Acute myeloid leukaemia with *RUNX1::RUNX1T1* fusion
Acute myeloid leukaemia with *CBFB::MYH11* fusion
Acute myeloid leukaemia with *DEK::NUP214* fusion
Acute myeloid leukaemia with *RBM15::MRTFA* fusion
Acute myeloid leukaemia with *BCR::ABL1* fusion
Acute myeloid leukaemia with *KMT2A* rearrangement
Acute myeloid leukaemia with *MECOM* rearrangement
Acute myeloid leukaemia with *NUP98* rearrangement
Acute myeloid leukaemia with *NPM1* mutation
Acute myeloid leukaemia with *CEBPA* mutation
Acute myeloid leukaemia, myelodysplasia-related
Acute myeloid leukaemia with other defined genetic alterations

A key change in the classification is the elimination of the 20% blast requirement for AML with defining genetic mutations as well as the introduction of AML with other defined genetic alterations such as rare emerging genetic fusions. AML with BCR::ABL1 and AML with CEBPA mutation are the only ones from the group AML with a defined genetic abnormality that still require a threshold of 20% blasts at diagnosis. The definition of AML with CEBPA mutation has also changed and now includes single bZIP mutations as well as biallelic biCEBPA mutations.

2) Acute myeloid leukaemia with myelodysplasia related changes (AML-MRC)

The group of AML with myelodysplasia-related changes now called AML-MR is defined as > ou = 20% blasts with a myeloid immunophenotype and with specific cytogenetic and/or molecular anomalies

associated with MDS. This can occur de novo or after a known history of MDS. Morphology alone is not sufficient to make the diagnosis of AML-MR. The different abnormalities are listed below:

Defining cytogenetic abnormalities
Complex karyotype (≥3 abnormalities)
5q deletion or loss of 5q due to unbalanced translocation
Monosomy 7, 7q deletion, or loss of 7q due to unbalanced translocation
11q deletion
12p deletion or loss of 12p due to unbalanced translocation
Monosomy 13 or 13q deletion
17p deletion or loss of 17p due to unbalanced translocation
Isochromosome 17q
idic(X)(q13)
Defining somatic mutations
ASXL1
BCOR
EZH2
SF3B1
SRSF2
STAG2
U2AF1
ZRSR2

3) Therapy related AML : *The diagnosis of myeloid neoplasms post cytotoxic therapy (MN-pCT)is based on meeting the criteria for a myeloid neoplasm as well as a history of chemotherapy or large field radiation therapy for an unrelated neoplasm. This includes exposure to PARP1 inhibitors, methotrexate is excluded. a large proportion of this neoplasms is associated with TP53 mutations and therefore with a worse prognosis.*

4) AML with germline predisposition : *Myeloid Neoplasms with germline disposition is defined as AML MDS MPN and MDS/MPN in patients having genetic conditions known for an increased risk of this type of malignancies.*

Germline *CEBPA* P/LP variant (CEBPA-associated familial AML)
Germline *DDX41* P/LP variant
Germline *TP53* P/LP variant (Li-Fraumeni syndrome)
Germline *RUNX1* P/LP variant (familial platelet disorder with associated myeloid malignancy, FPD-MM)
Germline *ANKRD26* P/LP variant (Thrombocytopenia 2)
Germline *ETV6* P/LP variant (Thrombocytopenia 5)
Germline *GATA2* P/LP variant (GATA2-deficiency)
Severe congenital neutropenia (SCN)

Shwachman-Diamond syndrome (SDS)
Fanconi anaemia (FA)
Telomere biology disorders
RASopathies (Neurofibromatosis type 1, CBL syndrome, Noonan syndrome or Noonan syndrome-like disorders)
Down syndrome
Germline *SAMD9* P/LP variant (MIRAGE Syndrome)
Germline *SAMD9L* P/LP variant (SAMD9L-related Ataxia Pancytopenia Syndrome)
Biallelic germline *BLM* P/LP variant (Bloom syndrome)

5) AML defined by differentiation. *With the advances in the molecular and cytogenetic findings, the number of this cases in rapidly diminishing.*

Appendix 2 – WHO 2022 classification of AML

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 • bZIP in-frame mutated CEBPA
Intermediate	• Mutated NPM1 with FLT3-ITD • Wild-type NPM1 with FLT3-ITD (without adverse-risk genetic lesions) • t(9;11)(p21.3;q23.3)/MLLT3::KMT2A • Cytogenetic and/or molecular abnormalities not classified as favorable or adverse
Adverse	• t(6;9)(p23.3;q34.1)/DEK::NUP214 • t(v;11q23.3)/KMT2A-rearranged • t(9;22)(q34.1;q11.2)/BCR::ABL1 • t(8;16)(p11.2;p13.3)/KAT6A::CREBBP • inv(3)(q21.3q26.2) or t(3;3)(q21.3;q26.2)/GATA2, MECOM(EVI1) • t(3q26.2;v)/MECOM(EVI1)-rearranged • −5 or del(5q); −7; −17/abn(17p) • Complex karyotype, monosomal karyotype • Mutated ASXL1, BCOR, EZH2, RUNX1, SF3B1, SRSF2, STAG2, U2AF1, and/or ZRSR2 • Mutated TP53

Appendix 4 – 2022 ELN Risk Classification by Genetics at Initial Diagnosis

GROUPE ETHIQUE D'AIDE A LA RECHERCHE CLINIQUE POUR LES PROTOCOLES DE RECHERCHE NON SOUMIS AU COMITE DE PROTECTION DES PERSONNES
ETHICS COMMITTEE IN HUMAN RESEARCH

AVIS

Responsable de la recherche : Dr Nicolas VALLET / Areti CHANTZI
Titre du projet de recherche : Etude du profil mutationnel en association avec le profil clinique, biologique et phénotypique dans la leucémie aiguë myéloïde / COMUTAML

N° du projet : 2024 051

Le groupe éthique d'aide à la recherche clinique donne un avis

X FAVORABLE

☐ DÉFAVORABLE

☐ SURSIS A STATUER

☐ DÉCLARATION D'INCOMPÉTENCE

au projet de recherche n° 2024 051

A Tours, le 10/07/2024

Dr Thomas Léonard
Président du Groupe Ethique Clinique

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Appendix 5 – Ethics Committee in Human Research Approval

Vu, le Directeur de Thèse

Vu, le Doyen
De la Faculté de Médecine de Tours
Tours, le

CHANTZI Areti Effrosyni

52 pages – 6 tableaux – 9 figures

Résumé :
Les leucémies aiguës myéloïdes (LAM) sont des hémopathies malignes caractérisées par une prolifération clonale de progéniteurs myéloïdes immatures. Le diagnostic de LAM est confirmé par l'examen cytologique. Cet examen est complété par une analyse du phénotype des blastes afin de caractériser l'origine de la cellule tumorale. Les analyses cytogénétique et la recherche de mutation acquise par *Next Generation Sequencing* (NGS) permettent d'identifier les patients à haut risque de rechute. Si plusieurs études ont décrit les mutations présentes au diagnostic de LAM, peu décrivent les associations entre gènes mutés et les caractéristiques cliniques, phénotypiques, cytogénétiques. L'objectif de cette étude est de décrire les caractéristiques cliniques, cytogénétiques, phénotypiques et moléculaires des patients ayant été pris en charge au CHU de Tours. L'objectif secondaire est d'étudier les associations entre ces caractéristiques afin de déterminer si les profils mutationnels peuvent être associés à une présentation clinique, un profil cytogénétique ou phénotypique spécifiques.
Nous avons conduit une étude monocentrique rétrospective chez des patients diagnostiqués d'une LAM au CHU de Tours et ayant débuté un traitement entre 2018 et 2024 ayant eu une analyse génétique par la technique NGS au diagnostic retrouvant des mutations génétiques (étude COMUTAML CNIL #2024-099, IRB #2024-051).
Nous avons inclus 152 patients. L'âge médian était de 64 [IQR 53 ;71] ans au diagnostic. 45% étaient des femmes. 22% des patients avaient une hyperleucocytose > 50 G/L. Le taux médian de blastes circulants était de 30% au diagnostic. 12,5% de patients n'avaient pas de blastes circulants. 47% des patients avaient une LAM secondaire et 51% étaient de risque défavorable selon la classification ELN 2022. 14% des patients présentaient un risque favorable dont 63% avait une mutation NPM1 et 32% avaient une mutation impliquant le CBF. L'analyse cytogénétique a retrouvé un caryotype complexe dans 13% des cas.
L'analyse mutationnelle a révélé des altérations fréquentes dans des gènes impliqués dans la leucémogenèse, notamment NPM1 (28 %), DNMT3A (26 %), RUNX1 (24 %), FLT3-ITD (22 %) et TET2 (20 %). D'autres mutations fréquentes concernaient SRSF2 (16 %), ASXL1 (16 %), NRAS (16 %), IDH1 (12%), IDH2 (12%) et TP53 (10 %).
Les co-mutations les plus souvent associées étaient FLT3-ITD et NPM1 pour 23 patients (15%), DNMT3A et NPM 1 pour 22 patients (14%) et ASLX1 et RUNX1 pour 18 patients (12%).
Les caractéristiques cliniques, cytogénétiques, phénotypiques et moléculaires au diagnostic de LAM au CHU de Tours sont comparables avec d'autres études épidémiologiques retrouvées dans la littérature. On observe cependant une prévalence importante de la mutation RUNX1 dans notre population.
Des analyses complémentaires permettront de décrire les associations entre anomalies chromosomiques, phénotypiques et moléculaires.

Mots clés : Leucémie aiguë myéloïde, biologie moléculaire, cytométrie en flux, analyse cytogénétique, co-mutations

Jury :

Président du Jury : Professeur Emmanuel GYAN

Directeur de thèse : Docteur Nicolas VALLET
Membres du Jury : Docteur Martin ELOIT
Docteur Amelie FOUCAULT
Docteur Sébastien LACHOT

Date de soutenance : 30/04/2025