UNIVERSITÀ DEGLI STUDI DI GENOVA

SCUOLA DI SCIENZE MEDICHE E FARMACEUTICHE

CORSO DI LAUREA IN MEDICINA E CHIRURGIA

**Tesi di Laurea**

# CD-123 targeting as a salvage therapy for relapsed/refractory acute myeloid leukemia

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*A Jacopo,*
*in tempesta e bonaccia,*
*compagno di*
*vita.*

# ABSTRACT

**INTRODUCTION**: Relapsed/refractory acute myeloid leukemia (R/R AML) represents a major clinical challenge, given the scarcity of effective treatment options and the resulting dismal prognosis. The present multicenter phase II study, AML2020, the first of its kind in Europe and conducted within the GIMEMA (Gruppo Italiano Malattie Ematologiche) network, explores the efficacy and safety of the anti-CD123 immunotoxin Tagraxofusp in the R/R CD123+ AML setting. Tagraxofusp is made of recombinant Interleukin-3 fused with truncated diphtheria alpha-toxin (a protein synthesis inhibitor). Here, I analyze data from the 9 patients enrolled in the U.O. Clinic of Hematology, IRCCS Ospedale Policlinico San Martino, Genova.

**MATERIALS AND METHODS**: After the enrollment screening, Tagraxofusp was administered at 12 μg/kg on days 1 to 3 of the first cycle and then for 5 days in subsequent cycles. Each cycle had a duration of 21 days. Four patients had relapsed pathology, and five patients had refractory one. Three patients had an adverse ELN risk class.

**RESULTS**: T The median number of administered cycles was 2 (range 1 – 10). Of the 9 patients treated, 4 (44.5%) achieved partial response, 3 (33.3%) had no response, and 2 (22.2%) died early before hematologic evaluations. Median survival was 63 days (C.I. 95% 0 – 174.028 days), with overall survival at 2 months of 55.56%. Capillary leak syndrome appeared at any grade in 4 patients. Other most frequent side effects observed were: tumor differentiation and lysis syndrome, weight gain, and hypoalbuminemia.

**DISCUSSION**: Tagraxofusp, as a single agent, showed some antileukemic activity in R/R AML. The study cohort showed limited response and survival rates. However, some patients achieved stable disease for a few months. The toxicity profile was complex and required close monitoring. This study paved the way to investigate the activity of Tagraxofusp in combination with other drugs.

# ABSTRACT

**INTRODUZIONE**: La leucemia mieloide acuta recidivata/refrattaria (R/R AML) rappresenta un'importante sfida clinica, date le scarse opzioni terapeutiche e la prognosi infausta. Il presente studio multicentrico di fase II, AML2020, il primo del suo genere in Europa e condotto all'interno della rete GIMEMA, esplora l'efficacia e la sicurezza dell'immunotossina anti-CD123 Tagraxofusp nella AML R/R CD123+. Tagraxofusp è formato da Interleuchina-3 ricombinante fusa con alfa-tossina difterica tronca (inibente la sintesi proteica). Qui analizzo i dati dei 9 pazienti arruolati nell'U.O. Clinica Ematologica dell'IRCCS Ospedale Policlinico San Martino di Genova.

**MATERIALI E METODI**: Dopo l'arruolamento, Tagraxofusp è stato somministrato a 12 μg/kg nei giorni 1–3 del primo ciclo e poi per 5 giorni i cicli successivi. Ogni ciclo è durato di 21 giorni. Quattro pazienti presentavano una patologia recidivata e cinque una refrattaria. Tre pazienti avevano una classe di rischio ELN avversa.

**RISULTATI**: il numero mediano di cicli somministrati è stato di 2 (range 1 – 10). 4 (44,5%) hanno ottenuto una risposta parziale, 3 (33,3%) non hanno avuto risposta e 2 (22,2%) sono deceduti prima delle valutazioni ematologiche. La sopravvivenza mediana è stata di 63 giorni (C.I. 95% 0 - 174,028 giorni), con una sopravvivenza globale a 2 mesi del 55,56%. La sindrome da stravaso capillare è comparsa a qualsiasi grado in 4 pazienti. Altri effetti collaterali osservati sono stati: sindrome da differenziazione e lisi tumorale, aumento di peso e ipoalbuminemia.

**DISCUSSIONE**: Tagraxofusp in monoterapia ha mostrato una certa attività antileucemica nella AML R/R. La coorte di studio ha mostrato tassi di risposta e di sopravvivenza limitati. Tuttavia, alcuni pazienti hanno raggiunto una malattia stabile per alcuni mesi. Il profilo di tossicità ha richiesto un attento monitoraggio. Questo studio ha aperto la strada all'analisi dell'attività di Tagraxofusp in combinazione con altri farmaci.

# INDEX

# 1. Introduction

## 1.1. Definition

Acute Myeloid Leukemia (AML) is a neoplasm of Hematopoietic Stem Cells (HSCs) characterized by a block of differentiation of the involved cell, an aggressive monoclonal expansion of leukemic blasts, and an accumulation of neoplastic cells in the bone marrow (resulting in tri-linear pancytopenia), blood, and peripheral tissues.

## 1.2. Epidemiology

With an estimated incidence rate of 3.5 cases per 100,000 population/year in Italy[1], AML represents the leading acute leukemia of adulthood[2].

Although the age of onset is highly variable, the incidence of AML increases dramatically with age. If the incidence in the under-65 age group turns out to be 2.0 cases per 100,000 population/year, in individuals aged 65 years or older it rises to 20.1 cases per 100,000 population/year, with a median age at diagnosis of 68 years [3] (**Figure 1**).

According to data from the Italian Cancer Registry Association, it is estimated that 2,000 new cases of AML are diagnosed each year in Italy, divided into 1,200 male and 900 female individuals[4]. The incidence in males is slightly higher than in females, with an M/F ratio of 1.2-1.63[3].

Although the prevalence of this neoplasm is less than 5 cases per 10,000 population (which, by definition, makes it a rare disease), 19,389 people live in Italy after a diagnosis in 2020[5].

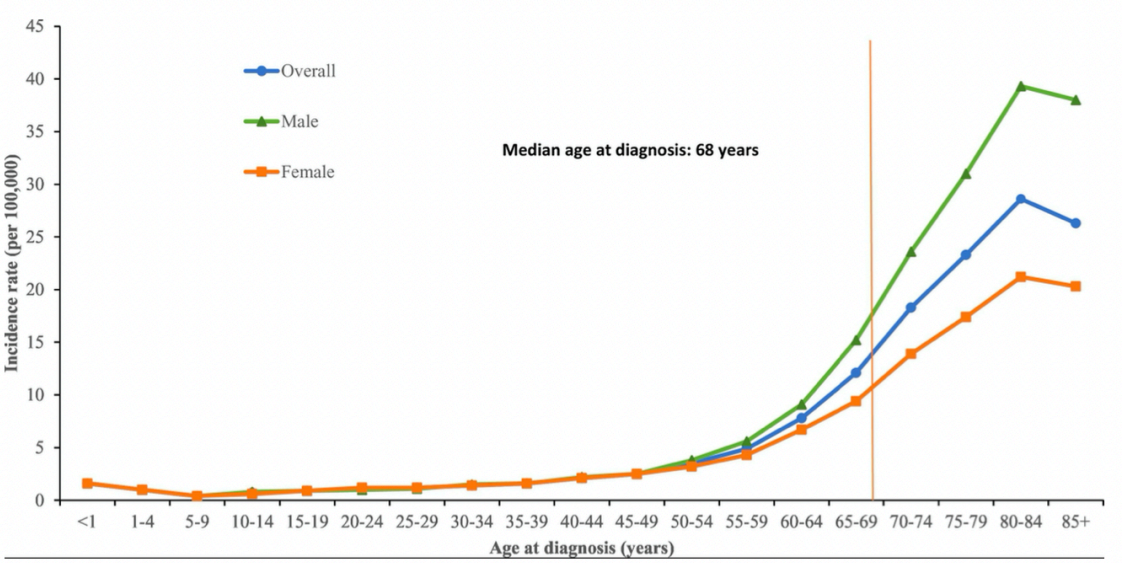


**Figure 1. Incidence of AML by age and sex in the U.S. population from 2011-2016**. Source: "Epidemiology of acute myeloid leukemia: Recent progress and enduring challenges"- Shallis RM, Wang R, Davidoff A, Ma X, Zeidan AM.[3]

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By 2023, 20,380 new AML diagnoses are expected in both sexes in the United States, and 11,310 deaths are expected for the same disease.[6]

## 1.3. Mortality

According to statistical analyses conducted on European patients, 5-years-overall-survival (OS5y) for all age groups and indifferent to classification subtype is estimated to be 17%[7].

Looking at the different neoplastic types, we see that survival varies greatly: 67% OS5y for Acute Promyelocytic Leukemia (APL, M3*), 23.6% for acute myelomonocytic leukemia (AMML, M4*), 18.6% for Acute Monocytic Leukemia (AMoL, M5*)[7].

Stratifying further by age group, we note essential differences: ranging from 47.4% OS5Y in the 15-49 age group, to 15.4% for individuals in the 50-69 age group, to 2.7% in patients aged 70 years or older[7].

*The acronyms given here refer to the French-American-British classification (FAB) of acute myeloid leukemias. See Chapter 1.6. of this manuscript for a more detailed description.

## 1.4. Etiology and risk factors

As can be seen from the epidemiology of AML (Chapter 1.2.), age plays an essential role in the onset of this condition[3], so much so that it can be considered one of its main risk factors[8]. With aging, the number of clonal mutations in hematopoietic progenitors increases with the risk of establishing monoclonal hematopoiesis, a significant risk factor for both AML and myelodysplasias (MDS)[9].

Although the vast majority of cases of acute myeloid leukemia occur without an apparent cause and can therefore be considered idiopathic, multiple etiologic agents have been described over the years that account for the occurrence of this disease in an increasing percentage of individuals.

Among these, it is crucial to mention cigarette smoking (considered the main among the known risk factors and strongly correlated with AML-associated deaths), a high Body Mass Index (BMI), occupational exposure to benzene and formaldehyde, and a low Socio-Demographic Index (SDI)[10].

In addition, it is essential to cite that some congenital disorders predispose to the onset of AML; the main ones include Li-Fraumeni syndrome (given by mutation of the TP53 gene resulting in alteration of the oncosuppressor p53), Kostmann syndrome, Fanconi anemia, familial platelet syndrome (also called gray platelet syndrome), and some aneuploidies such as Down syndrome[3].

Important risk factors in the development of AML are also previous treatment with chemotherapeutic drugs (especially topoisomerase inhibitors[11], such as etoposide and alkylating agents, including, most notably, melphalan[12]), immunosuppressive therapy, or ionizing radiation[13]; the onset of disease following exposure to these factors identifies a specific nosographic entity of AML: the therapy-related AML (t-AML)[14].

Previous hematological diseases such as, among all, myelodysplasias (MDS) and myeloproliferative neoplasms (myeloproliferative neoplasms, MPN: chronic myeloid leukemia, essential thrombocythemia, polycythemia vera, and myelofibrosis with splenic-hepatic metaplasia) also play a role in the development of AML. In these cases, we speak of acute myeloid leukemia related to antecedent-hematological disease (AHD-AML)[15].

In general, when it is possible to identify a specific cause (whether an exogenous agent, a congenital disorder or a previous hematologic disease) underlying the chromosomal or molecular alterations that led to the development of AML, one speaks of secondary acute myeloid leukemia (sAML)[15].

Interestingly, several studies indicate greater clinical aggressiveness and less response to different lines of treatment for sAML than for "de novo" forms: individuals with secondary forms tended to achieve a complete pathologic response in a lower percentage of cases and had a lower 5-year survival rate than primary forms[16,17].

## 1.5. Pathogenesis

The cell involved in the genesis of acute myeloid leukemia is the hematopoietic stem cell (HSC). This cell is endowed with two major features[18]:

1) Self-renewal
2) Ability to give rise to multipotent progenitors that will differentiate into the figured elements of the blood (**Figure 2**).

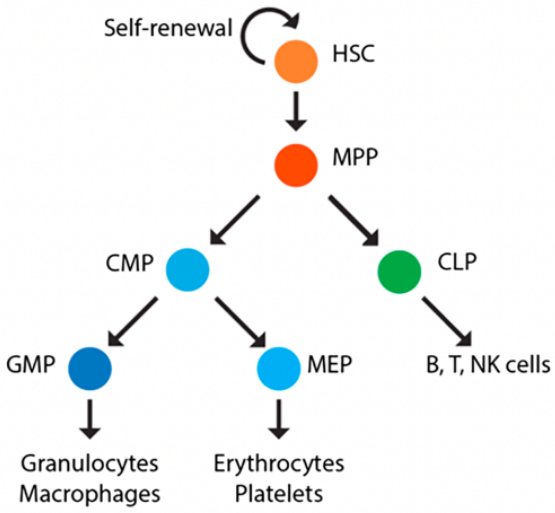


**Figure 2**: **Hemopoiesis tree**. As we see, it is a hierarchical structure with HSC at its apex. MPP = multipotent progenitor; CMP = common myeloid progenitor; CLP = common lymphoid progenitor; GMP = macrophage and granulocyte progenitor; MEP = megakaryocyte and erythrocyte progenitor. Source: "The cell of origin and the leukemia stem cell in acute myeloid leukemia" - Chopra M, Bohlander, SK Genes Chromosomes Cancer *2019;58(12):850-858. doi:10.1002/gcc.22805*.[19]

As a result of the causative factors seen in the previous chapter (as well as other mechanisms that have not yet been elucidated), it goes into the differentiative blockade and uncontrolled proliferation.

The pathogenesis and stages of this mutation are highly complex and involve numerous mechanisms, both at the level of individual genes and entire chromosomes.

Specifically, AML is characterized by the presence of a population of cells called cancer stem cells (Leukemia Stem Cells, LSCs), which, mirroring HSCs, represent the subset of cancer cells capable of self-renewal and, at the same time, generating all other cancer cells in the disease[19].

There are two modes of LSC genesis (**Figure 3**)[19]:

1. An HSC gains proliferative potential [Mut1a]
2. A multipotent progenitor gains self-regenerative capacity [Mut1b]

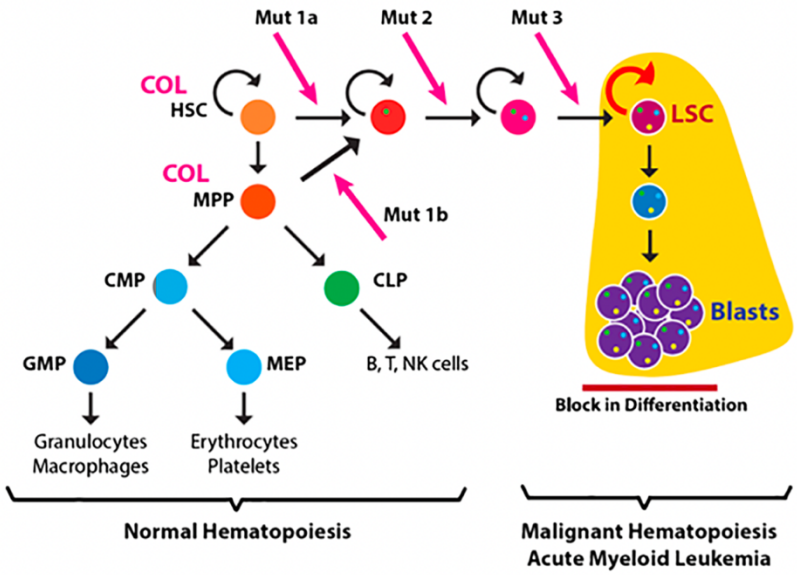


**Figure 3**: **Comparison of physiological and malignant hematopoiesis**. Source: "The cell of origin and the leukemic stem cell in acute myeloid leukemia" - Chopra M, Bohlander, SK Genes Chromosomes Cancer 2019;58(12):850-858. doi:10.102/gcc.2280515[19].

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Thus, mutations that can lead to LSC are basically of two types: mutations that confer increased proliferative potential and/or differentiation blockade (**Mut1a**, **Figure 3**) or mutations that allow for (re)gaining self-renewal capabilities (**Mut1b**, **Figure 3**)[19].

Interestingly, mutations can add up to each other (**Mut2**, **Mut3**, **Figure 3**), making the final LSC phenotypically even very distant from the HSC of origin[19]. To date, more than 200 mutated genes have been described in AML, and even on studies of hundreds of leukemic samples using next-generation sequencing (NGS) methods, no two cells with the same combination of mutations have been found, indicating the incredible heterogeneity of alterations that can lead to the development of LSC[20].

## 1.6. Classification

### 1.6.1. FAB classification

Until the second half of the 1970s, there were no unambiguous and globally accepted classifications of AML. In 1976, a joint effort of British, French, and U.S. authors gave rise to the first system of subdivision of the various types of acute myeloid leukemia: the FAB (French American British) classification system[21]. This classification refers to leukemic cells' morphological and cytochemical characteristics and identifies eight classes, numbered M0 to M7. This classification system has been revised and enriched multiple times over the years in order to increase its stadiative capacity.

**Table 1** shows the classes with their morphological and immunophenotypic characteristics[22].

**Table 1**: FAB classification.

| Class | Name | Morphologic features | Immunophenotype |
|---|---|---|---|
| **M0** | **Undifferentiated AML** | Undifferentiated morphology, Sudan black negativity. | CD13, CD33, CD117, MPO |
| **M1** | **AML without maturation** | Little evidence of maturation; cells show round nuclei with modest amounts of cytoplasm, which may sometimes appear granulated. | CD13, CD33, CD34, MPO |
| **M2** | **AML with maturation** | Evidence of maturation of the myeloid line; granulation is more evident. | CD13, CD33, CD117, MPO |
| **M3** | **Promyelocytic AML** | Hypergranular morphological appearance with the presence of Auer rods, round and bilobed nuclei with prominent nucleoli. | CD13, CD15, CD33, MPO |

| | | | |
|---|---|---|---|
| **M4** | **Myelomonocytic AML** | Morphology is similar to the monocytic lines with slightly granulated and grayish cytoplasm and folded nuclei; >20% of cells must derive from the monocytic line. | CD14, CD33 |
| **M5** | **Monocytic AML** | Morphology is similar to monocytic lines with the presence of folded nuclei and abundant modestly granulated cytoplasm. >80% of cells must derive from the monocytic line. | CD11, CD14, CD15 |
| **M6** | **Erythroblastic AML** | Presence of bizarre, megaloblastic, and often multinucleated erythroid precursors. | CD36, CD71, CD238 |
| **M7** | **Megakaryoblastic AML** | Megakaryoblast-like morphology with multinucleated cells and cytoplasmic budding. | CD36, CD41, CD42, CD61 |

With the advent of molecular biology and genome sequencing technologies, multiple genetic markers have been identified, leading to the creation of new classifications, such as the World Health Organization (WHO) classification, which was updated and supplemented in 2022 with the birth of the International Consensus Classification of Myeloid Neoplasms and Acute Leukemias[23].

The FAB system has been shown to have limitations in predicting prognosis and response to treatment, especially when compared with the new classification systems [24]. To date, the FAB system represents an essential first step in the initial diagnosis of AML (especially in settings with limited economic possibilities) but is being supplemented by the new cytogenetic and molecular classifications[24].

### 1.6.2. International Consensus Classification

The new classification of myeloid neoplasms and acute leukemias[23] (**Table 2**) retains many of the types of AML previously defined in the 2016 revision of the WHO[25] but includes other genetically related entities in order to make the classification more genetically defined[23,26]. Additional novelties are the identification of prior therapy (t-AML) and the presence of antecedent myeloid neoplasms (AHD-AML) or germline

disorders as qualifiers at diagnosis rather than as specific disease categories (**Table 3**). This is done to reduce confusion caused by the overlap of the previous AML categories. Two new classifications are also introduced: AML with TP53 mutation and AML with MDS-related gene mutations (AML with MDS), while the previous generic category of AML with MDS-related alterations is eliminated.

Finally, the classification adds to the previous three categories of AML that could be diagnosed with less than 20% blasts in the bone marrow or peripheral blood in the presence of specific genetic defects other types of neoplasia: in fact, if the WHO classification included in this category only AML defining abnormalities namely translocation of 8-21 chromosomes, chromosome 16 inversion, translocation 16-16 and translocation 15-17[27,28], now forms characterized by other mutations are also included.

**Table 2**: ICC 2022 classification of AMLs[23].

| Class | Percentage of blasts required for diagnosis |
|---|---|
| Acute promyelocytic leukemia (APL) with t(15;17)(q24.1;q21.2)/ PML:: RARα | ≥10% |
| APL with other RARA rearrangements | ≥10% |
| AML with t(8;21)(q22;q22.1)/RUNX1::RUNX1T1 | ≥10% |
| AML with inv(16)(p13.1q22) or t(16;16)(p13.1;q22)/CBFB::MYH11 | ≥10% |
| AML with t(9;11)(p21.3;q23.3)/MLLT3::KMT2A | ≥10% |
| AML with other KMT2A rearrangements | ≥10% |
| AML with t(6;9)(p22.3;q34.1)/DEK::NUP214 | ≥10% |
| AML with inv(3)(q21.3q26.2) or t(3;3)(q21.3;q26.2)/GATA2; MECOM(EVI1) | ≥10% |
| AML with other MECOM rearrangements | ≥10% |

| | |
|---|---|
| AML with other rare recurring translocations | ≥10% |
| AML with t(9;22)(q34.1;q11.2)/BCR::ABL1 | ≥20% |
| AML with mutated NPM1 | ≥10% |
| AML with in-frame bZIP CEBPA mutations | ≥10% |
| AML and MDS/AML with mutated TP53 | ≥20% |
| AML and MDS/AML with myelodysplasia-related gene mutations* | 10-19% (MDS-AML)<br>≥20% (AML) |
| AML with myelodysplasia-related cytogenetic abnormalities ° | 10-19% (MDS-AML)<br>≥20% (AML) |
| AML not otherwise specified (NOS) | 10-19% (MDS-AML)<br>≥20% (AML) |
| Myeloid sarcoma | / |

* Defined by mutations in ASXL1, BCOR, EZH2, RUNX1, SF3B1, SRSF2, STAG2, U2AF1, or ZRSR2
° Defined by complex karyotype, del(5q)/t(5q)/add(5q), 27/del(7q), 18, del(12p)/t(12p)/add(12p), i(17q), 217/add(17p) or del(17p), del(20q), and/or idic(X)(q13) clonal abnormalities

**Table 3**: AML diagnostic specifiers according to the new ICC 2022 classification[23].

| **Qualifiers** | **Features** |
|---|---|
| **Therapy-related** | Previous chemotherapy, radiotherapy, immune system-altering therapy |
| **Progressing from previous hematological diseases (MDS/MPN)** | MDS/MPN should be diagnosed through diagnostic standards |
| **Germline predisposition** | The main ones include CEBPA mutations, RUNX1, ETV6, Down syndrome, Fanconi anemia, Li-Fraumeni syndrome, Schwachman-Diamond syndrome, Diamond-Blackfan syndrome, and neurofibromatosis. |

### 1.6.3. Risk classification

As shown in the previous chapter, cytological and molecular complexity is one of the defining features of AML.

At the practical level, it becomes necessary to understand whether and how a given genetic alteration of the leukemic blast is aggressive and to correlate this finding with the clinical features of the neoplastic patient in order to achieve global risk stratification at the time of diagnosis. Indeed, it is well-established knowledge that specific genetic alterations are more impactful than others on the clinical course, response to therapy, and overall patient prognosis[29,30].

Over the decades, there have been several classifications to correlate the cytomolecular profile with a specific risk band.

At the European level, the most recent is the European LeukemiaNet genetic risk classification (ELN) of 2022[3131,32].

According to this classification's most recent and updated version, three risk classes are distinguished based on the genetic-molecular characteristics of the neoplasm: favorable, intermediate, and adverse (**Table 4**).

**Table 4**: 2022 ELN risk classification by genetics at initial diagnosis[32].

| Risk Category | Genetic abnormality |
|---|---|
| **Favorable** | • t(8;21)(q22;q22.1); RUNX1::RUNX1T1<br>• inv(16)(p13.1q22) or t(16;16)(p13.1;q22); CBFB-MYH11<br>• Mutated NPM1 without FLT3-ITD<br>• bZIP in-frame mutated CEBPA |
| **Intermediate** | • Mutated *NPM1* and *FLT3*-ITD<br>• Wild-type *NPM1* with *FLT3*-ITD (without adverse-risk genetic lesions).<br>• t(9;11)(p21.3;q23.3); *MLLT3::KMT2A*<br>• Cytogenetic and/or molecular abnormalities not classified as favorable or adverse. |
| **Adverse** | • t(6;9)(p23;q34.1); *DEK::NUP214*<br>• t(v;11q23.3)/*KMT2A* rearranged<br>• t(9;22)(q34.1;q11.2)/*BCR::ABL1*<br>• t(8;16)(p11;p13)/*KAT6A::CREBBP*<br>• inv(3)(q21.3q26.2) or t(3;3)(q21.3;q26.2); *GATA2, MECOM*(*EVI1*)<br>• t(3q26.2;v)/*MECOM(EVI1*)-rearranged<br>• -5 or del(5q); -7; -17/abn(17p)<br>• Complex karyotype, monosomal karyotype |

- Mutated *ASXL1, BCOR, EZH2, RUNX1, SF3B1, SRSF2, STAG2, U2AF1, ZRSR2*
- Mutated *TP53*

## 1.7. Clinical presentation

The clinical presentation of AML basically depends on two essential factors: marrow substitution by leukemic blasts resulting in pancytopenia and the presence of neoplastic infiltration of extramedullary tissues[33]. As a consequence of the first phenomenon, there will be (a) anemia and associated symptoms such as heart palpitation, pallor, hypotension, fatigue, and dyspnea; (b) thrombocytopenia with related manifestations: petechiae, ecchymoses, mucosal bleeding, metrorrhagia, purpura; (c) leukopenia: this will lead to immunodepression with increased susceptibility to infections (especially bacterial and fungal) with pneumonia, skin and mucosal infections (but, potentially, any type of opportunistic infection may manifest in the course of acute leukemia).

As a consequence of the extramedullary invasion of leukemic blasts[34] we will instead have hepatosplenomegaly, lymphadenomegaly, skin infiltration, and meningeal infiltration (with neurological symptoms and signs such as headache, vomiting, visual, hearing, and motility changes[35]).

The classic onset of acute leukemia symptoms is rapid, with a manifestation of symptomatology within a few weeks. In other cases, the presentation may be more insidious, with prolonged symptoms related to chronically compensated cytopathies over long periods. In both cases, the most typical presentation is that of symptoms related to bone marrow failure[33].

AML can also cause emergency medical conditions, including essentially hyperleukocytosis with leukostasis and disseminated intravascular coagulation (DIC, typically associated with APL)[33]. In the first case, we have a leukocyte count greater than >100,000 with accumulation (leukostasis) of these and consequent occlusion of the microcirculation of different districts such as lungs, brain, kidneys, heart with symptoms of failure of the affected organ[33,36]. The treatment involves adequate hydration, rapid chemotherapy treatment of underlying leukemia, and possibly leukapheresis[36].

DIC is characterized by the massive and simultaneous activation of the coagulation cascade with disseminated intravascular thrombosis and a resulting consummatory

coagulopathy and diffuse hemorrhagic manifestations. It is a dreaded complication of AML, particularly the APL form, which, by producing multiple pro-coagulant substances, leads to activation of the process[37]. The appropriate management provides for prompt recognition of the cause and its subsequent resolution. In cases of particularly low (<50,000/ml) or rapidly declining platelet counts, transfusion of platelet concentrates is necessary; in the course of APL, it is essential to start treatment with all-trans retinoic acid (ATRA) as soon as possible[38].

Finally, it is worth mentioning that AML can associate with paraneoplastic syndromes, including, most importantly, Sweet syndrome (SS), an acute febrile neutrophilic dermatosis characterized by recurrent fevers and the presence of pain and stiff, painful purplish papulo-nodular lesions that can occur all over the skin[39]. Sweet's syndrome is estimated to occur in about 1% of individuals diagnosed with AML[40].

## 1.8. Diagnosis

When it comes to AML, the diagnostic procedures to correctly confirm and classify this condition are multiple. Specifically, whenever we suspect AML, we should always do[32]:

1. A detailed history of the patient
2. Complete physical evaluation
3. Comprehensive biochemical and coagulative evaluation
4. Complete blood count (CBC) and differential count
5. Blood smear with cell count and morphological analysis
6. Bone marrow biopsy
7. Immunophenotype by flow cytometry
8. Cytogenetic and biomolecular analysis
9. Clinical evaluations for a complete diagnosis framing: performance status (ECOG/WHO), serology for HAV, HBV, HCV, HIV, CMV, EBV, HSV, VZV.
10. If specific indications exist, the following are required: chest CT scan, lumbar puncture, abdominal ultrasound, chest X-ray, and echocardiogram.

The CBC and blood smear show pancytopenia with a variable percentage and different morphology of blasts: at least two hundred nucleated cells must be counted on the blood

smear to make the diagnosis, while at least five hundred are needed in a bone marrow smear[32].

### 1.8.1. Immunophenotype

An essential step in the diagnosis and characterization of any hematologic malignancy is the immunophenotype. It consists of the evaluation of intra- and extracellular markers in order to understand the origin, differentiation status, and functional status of leukemic cells. The evaluation is done by flow cytometry. Immunophenotype is also vital to identify leukemia-associated immunophenotypes (LAIP), an essential step in monitoring minimal residual disease post-treatment[32]. **Table 5** summarizes the main markers of AML.

**Table 5**: Main immunophenotypic markers of AML[41].

| Cellular lineage | **Markers** |
|---|---|
| Precursors Granulocytic markers | CD34, CD117, CD33, CD13, HLA-D |
| Granulocytic markers | CD65, cytoplasmatic MPO |
| Monocytic markers | CD14, CD36, CD64 |
| Megakaryocytic markers | CD41 (glycoprotein IIb/IIIa), CD61 (glycoprotein IIIa) |
| Erythroid markers | CD235a (glycophorin A), CD36 |

Interestingly, the evaluation of some markers, while not diagnostic, gives essential information regarding potential therapeutic targets: these include CD44, CD87, CD116, CD123[41].

In addition, specific markers such as CD38, CD123 and CD133 can identify leukemic stem cells and thus offer targeted targets to eliminate this group of cells underlying the persistence of the disease[41].

In particular, CD123 (interleukin-3 receptor) has been shown to be mainly expressed in a subset of AML cells, making it a specific marker of leukemic stem cells and, therefore, an attractive pharmacological target[41,42].

CD123 is precisely the marker that was the focus of the study reported in this thesis (consult chapter 1.10. for a more detailed description).

### 1.8.2. Cytogenetic and biomolecular analysis

As described in Chapter 1.6.3. (Risk classification), cytogenetic and biomolecular assessments are essential not only for correct diagnosis but also for prognostic stratification of leukemia.

Specifically need to be researched and evaluated[32]:

1. Karyotype: to determine whether a karyotype is normal or abnormal, the evaluation must be performed on at least 20 metaphase bone marrow cells[31]; Complex karyotype is defined by at least 3 chromosome aberrations, regardless of their type and the individual chromosomes involved[43].
2. Mutation of specific genes such as FLT3, IDH1, IDH2, NPM1, CEBPA, DDX41, TP53, ASXL1, BCOR, EZH2, RUNX1, SF3B1, SRSF2, STAG2, U2AF1, ZRSR2
3. Gene rearrangements: PML::RARα, CBFB::MYH11, RUNX1::RUNX1T1, BCR::ABL1

## 1.9. Therapy

The goal of treatment in AML is to eradicate or control the disease. The ideal approach involves inducing a complete response with initial therapy, followed by consolidation and maintenance therapies to prolong remission and increase the duration of response. In selected patients, hematopoietic cell transplantation (HCT) could be indicated.

Suppose a patient is unable to tolerate intensive or non-intensive treatment options. In that case, the goal of therapy is to improve quality of life and reduce the incidence of disease-associated complications, involving palliative care services early if necessary[32].

Immediately after the diagnosis and right before the beginning of the treatment, it is to figure out whether the patient is fit or unfit for eradicant therapy (which is intended to lead to the disappearance of detectable disease hopefully). Treatment patterns, in fact, differ between the two groups of patients: the former will receive intensive polychemotherapy, the latter a less demanding one.

The stratification of patient fitness is classically done based on (a) age (the cut-off of 65 years is the one most commonly used to subdivide patients, although in some cases this limit is raised to 75 years for particularly fit subjects), (b) performance status (assessable

by ECOG and Karnofsky scores), (c) co-morbidities (such as lung disease, liver disease, previous solid tumors)[44]. In recent years, other criteria (which are becoming increasingly important in daily practice) have been added to the classic assessment of the patient who is a candidate for intensive chemotherapy: cognition, polypharmacotherapy, symptoms, and geriatric assessment[45].

### 1.9.1. Fit patients

In patients considered fit for intensive polychemotherapy, the treatment involves cycles of inductions followed by cycles of consolidation and maintenance.

Induction is classically done with the backbone combination of cytarabine and anthracyclines via the typical "3+7" scheme: three days of an anthracycline (idarubicin o daunorubicin) and seven days of cytarabine[31,32]. Other options include a combination of fludarabine, high doses of cytarabine, and granulocyte colony-stimulating factor (G-CSF; FLAG) and a combination of fludarabine, high-dose cytarabine and idarubicin (FLAI).

It is important to note that induction using the FLAI scheme resulted in a significant improvement in the clinical outcome of patients with mutated NMP1 and FLT3-ITD (which represent the most frequent genetic alterations in patients with acute myeloid leukemia[46]), regardless of the allelic load of FLT3-ITD thus being able to be a first-line treatment for patients with AML who have mutations in both of these genes[47].

To conclude, it is essential to mention one more option: the CPX-351 scheme; it consists of a liposomal formulation of cytarabine and daunorubicin in a fixed 5:1 molar ratio[48]. This drug has been shown to induce good-quality remission with acceptable toxicity in most patients, enhancing HSCT outcomes by reducing transplant-related mortality and post-transplant relapse rate[49]. It is important to note that this treatment option has received approval and is therefore indicated for the treatment of secondary or therapy-related AML[50].

After induction, a response to therapy is assessed by evaluating residual disease (RD, **Table 6**) via cytofluorimetry.

**Table 6**: **response criteria in AML**. Reproduced from Döhner H. et al, "*Diagnosis and management of AML in adults:2022 recommendations from an international expert panel on behalf of the ELN*"[32]

| Category | Definition |
|---|---|
| Complete remission (CR) | Bone marrow blasts <5%; absence of circulating blasts; absence of extramedullary disease; absolute neutrophil counts (ANC) ≥ 1 x $10^9$/L; platelet count ≥ 100 x $10^9$/L. |
| CR with partial hematologic recovery (CRh) | ANC ≥ 0,5 x $10^9$/L; platelet count ≥ 50 x $10^9$/L |
| CR with incomplete hematologic recovery (CRi) | All CR criteria except for residual neutropenia < 1.0 x $10^9$/L or thrombocytopenia < 100 x $10^9$/L; |
| Morphologic leukemia-free state (MLFS) | Bone marrow blasts <5%; absence of circulating blasts; absence of extramedullary disease; no hematologic recovery required |
| Partial remission (PR) | All CR hematologic criteria; 5% to 25% decrease in the percentage of bone marrow blasts and at least 50% decrease in the percentage of pre-treatment bone marrow blasts. |
| No response (NR) | Patients evaluable for a response but who do not meet the criteria for CR, CRh, CRi, MLFS or PR are classified as having no response before the response landmark. Patients who do not achieve a response by the designated reference point are defined as having a refractory disease. |
| Refractory disease | No CR, CRh, or CRi at the response landmark, after two cycles of intensive induction treatment or at a defined landmark, 180 days after initiating less intensive therapy. |
| Relapsed disease | Bone marrow blasts ≥ 5%; o reappearance of blasts in blood in at least two peripheral blood samples at least one week apart; or development of extramedullary disease |

If the patient achieves a CR (or CRh/CRi), we move on to consolidation, which has different approaches depending on the patient's genetic risk[31]:

1. Favorable-risk genetics: 2-4 cycles of IDAC

2. Intermediate-risk genetics: allogeneic HCT from matched or mismatched donor OR 2-4 cycles of cytarabine
3. Adverse risk genetics: Allogeneic HCT from a matched or unrelated donor

A third eventual step in treating AML might be maintenance: it involves a prolonged but time-limited course of treatment, usually less toxic, administered after RC is achieved with the goal of reducing the risk of recurrence[32]. It usually involves oral azacytidine and is used in patients not eligible for transplantation[51].

### 1.9.2. Unfit patients

Unfit patients, unable to sustain the toxicity of intensive polychemotherapy, are treated with treatment schemes aimed at disease control.

Multiple schemes can be used in this class of patients. Among the main ones, we indeed find the combination of azacitidine or decitabine and venetoclax[52]. Venetoclax is a highly selective oral inhibitor of BCL-2, a family of anti-apoptotic proteins; specifically, BCL-2 prevents the activity of the pro-apoptotic proteins BAX and BAK that result in increased mitochondrial permeability, the release of cytochrome C from mitochondria, activation of caspases, and cell death. By inhibiting BCL-2, venetoclax indirectly activates the described cascade[53].

The VEN/AZA scheme involves the administration of azacitidine on days 1-7 and the simultaneous and subsequent administration of venetoclax on days 3-28[32].

Two essential precautions should be taken during the use of venetoclax: (a) prevention of tumor lysis syndrome (TLS), for which prophylactic administration of uric acid-lowering drugs and careful monitoring of electrolytes are recommended; (b) dose adjustment when co-administering with CYP3A4 inhibitors, such as posaconazole (a drug frequently used during AML for prophylaxis of fungal infections)[32,54].

For patients who cannot tolerate antileukemic therapy or do not want any therapy, the treatment should be the best supportive care (BSC).

### 1.9.3. Treatment of acute promyelocytic leukemia

APL is an exception to the therapies seen in previous chapters. In fact, in recent decades, its treatment has been completely revolutionized, leading APL to be one of the leukemias with the best prognosis.

Currently, this condition's standard of care (SoC) is induction with the combination of all-trans retinoic acid (ATRA, a vitamin A derivative) and arsenic trioxide. These two substances bind the PML/RARα fusion protein leading to differentiation and apoptosis of leukemic cells[55].

Several studies have confirmed the non-inferiority of this approach compared to traditional chemotherapy but with markedly less hematologic toxicity[56].

### 1.9.4. New targets, new therapies

Biomolecular research has led, in recent years, to the discovery of multiple new therapeutic targets; this has made possible the development of new lines of therapy, broadening the therapeutic possibilities, especially of those forms that are not responsive to classical lines of treatment and leading to the emergence of precision medicine in AML[57].

Among the most significant new drugs (considering both those still in trials and those that have received approval only from few state drug agencies and are therefore not yet available in everyday clinical practice), it is essential to mention the following:

1. Gemtuzumab-ozogamicin (GO): a monoclonal antibody directed against CD33, expressed by some AML subtypes. The addition of GO to standard chemotherapy decreases the risk of recurrence and, in some studies, improves overall survival, especially in subjects with favorable risk rather than intermediate-risk disease[58].
2. Midostaurin, an FLT-3 tyrosine kinase inhibitor currently used as first-line (along with chemotherapy) in the induction, consolidation, and maintenance of FLT3-mutated forms of AML. Notably, midostaurin improved survival by slightly more than 7 percent when combined with the classic daunorubicin-cytarabine scheme[59].
3. Other mutated tyrosine kinase inhibitors, including gilteritinib, quizartinib, crenolanib, lestaurtinib, tandutinib[60]. While the latter are still under evaluation, the first two have become part of the therapeutic armamentarium; gilteritinib, the

most widely used, is routinely used as monotherapy for relapsed or refractory (R/R) FLT3-mutated AML patients[32].

4. Ivosidenib and enasidenib, inhibitors of mutated forms of isocitrate dehydrogenase (IDH, types 1 and 2). While enasidenib is used only as salvage therapy in subjects with refractory/recurrent mutated IDH2 AML, ivosidenib is also used (alone or in combination with azacitidine) in the treatment of forms of AML with isocitrate dehydrogenase type 1 mutation in subjects not candidates for intensive induction chemotherapy[32].
5. Magrolimab, a monoclonal antibody directed against CD47. It is a very recent product that induces phagocytosis of cancer cells, especially when combined with a drug that synergizes its effect, such as azacitidine[61].
6. SNDX-5613, a very recent drug still in phase 1 clinical trial. It inhibits menin, a nuclear protein involved in the cell cycle that often results dysregulated in forms of AML with KMT2A rearrangements or NPM1 mutations; so far, it has been exclusively evaluated in R/R forms[62].
7. Chimeric Antigen Receptor T cell (CAR-T cell). In recent years, CAR-Ts have become a prolific area of therapeutic research of hematologic malignancies, including AML. Although the results are auspicious, there are multiple difficulties in the AML setting that limit the full therapeutic potential of CAR-T cells: there are no truly leukemia-specific cell surface antigens that can be used as targets; normal HSCs often share leukemia cell antigens with a significant risk of toxicity; AML is a heterogeneous and complex disease that can evade the immune system through various immunosuppressive mechanisms with the risk of losing the efficacy of CAR-Ts[63]. Interestingly, many clinical trials to date had CD123 as the target of these engineered cells[64].

### 1.9.5. Refractory/Relapsed disease

The definition of refractory or relapsed disease (R/R) is shown in **Table 6**.

It is estimated that up to 57% of patients go on to primary refractory AML or relapse after relapse within 12 months of diagnosis[65].

At the time of clinical progression, it is crucial to keep in mind the possibility of clonal evolution and the emergence of relevant targets undetected at diagnosis; Therefore, it is essential to molecularly reexamine the disease upon relapse to identify patients who might be eligible for targeted salvage options (such as mutated FLT-3 or IDH1/2 inhibitors)[32].
To date, many therapeutic options can be employed in subjects with R/R forms; **Figure 4** shows the main ones (some of which are described in the previous chapter).

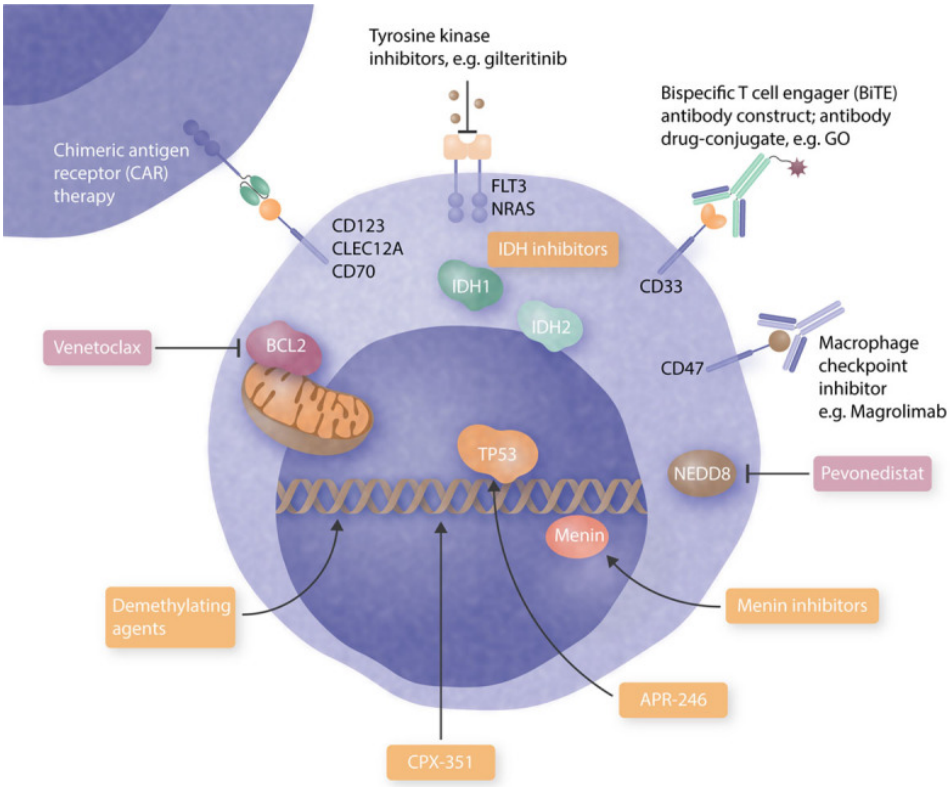



**Figure 4**: **Targets in relapsed/refractory AML**. BCL2 = B-cell leukemia/lymphoma-2; BiTE = bispecific T-cell engager; CAR = chimeric antigen receptor; CLEC12A = C-type lectin domain family 12 member A; FLT3 = fms-related tyrosine kinase 3; GO = gemtuzumab ozogamicin; IDH = isocitrate dehydrogenase; NEDD8 = neural-precursor-cell-expressed developmentally down-regulated 8. Source: Thol F, Heuser M - "Treatment for Relapsed/Refractory Acute Myeloid Leukemia", HemaSphere (2021) 5(6) E572[66].



It is important to emphasize that patient participation in clinical trials should be considered whenever possible in the case of new experimental treatment options.
The most common treatment regimens for patients with AML R/R include[32]:

1. Gilteritinib (AML with FLT- 3 mutation)

2. Ivosidenib or enasidenib (AML with IDH1/2 mutation)
3. Intermediate-dose cytarabine (with or without anthracycline)
4. FLAG-IDA
5. MEC (Mitoxantrone, Etoposide, Cytarabine)
6. CLAG-M (Cladribine, Cytarabine, Mitoxantrone)
7. Allogeneic HCT
8. Under certain conditions, consider a second HCT.
9. If HCT is not an option, HMA with or without venetoclax may be indicated.

Although treatment options appear numerous, patients with AML R/R have a markedly poor prognosis, and their treatment remains challenging and represents a significant unmet medical need[66].

In a cohort study, patients' median unadjusted overall survival was 5.3 months from the time of diagnosis of AML R/R, with a median OS of only 2 months for R/R patients receiving the best supportive care (BSC)[67].

An additional retrospective study by Zeichner et al. reported median overall survival (OS) in patients with relapsed and/or refractory acute myeloid leukemia to be just 4 months[68].

Therefore, further research is needed on the molecular mechanisms underlying refractoriness/recurrence and new therapeutic targets.

As has emerged several times in this manuscript, CD123 could be one of such targets.

## 1.10. CD123

CD123 constitutes the alpha subunit of the Interleukin-3 Receptor (IL-3R), which in turn belongs to the family of beta common (βc) receptors, which also includes Interleukin-5 Receptor (IL-5R) and granulocyte-monocyte colony-stimulating factor receptor (GM-CSF)[69].

This family of receptors plays numerous roles, including growth, survival, proliferation, and differentiation of hematopoietic cells, as well as immunity and inflammatory response[70]; also, working in unison with other structures, alters levels of the chemical messenger known as stromal cell-derived factor-1 (SDF-1) and its corresponding receptor CXCR4, which are involved in the movement of blood-forming cells in and out of the bone marrow[71].

**Figure 5** shows the structure of this receptor: as can be seen, it consists of two alpha chains (CD123) and two beta chains (CD131)[69].

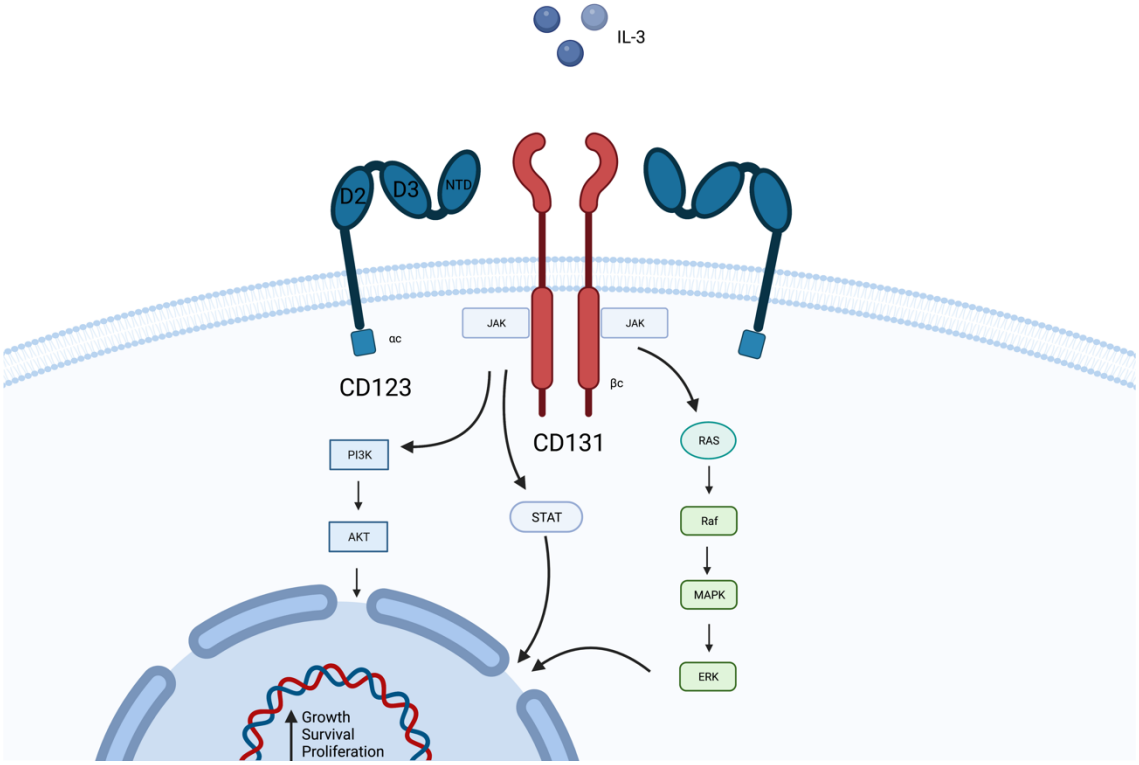



**Figure 5**: **IL-3R Pathway.**
Created with BioRender.com

The alpha chain (CD123) has a complex structure consisting of three extracellular domains, a transmembrane domain, and a short intracellular region[72]. The extracellular region of CD123 consists of two fibronectin III (FnIII)-like domains that bind cytokines (D2 and D3) and a third N-terminal domain (NTD)[73].

When IL-3 arrives and binds to the complex, dimerization of the receptor and activation of the intracellular cascade mediated by the JAK/STAT, Ras-MAPK, and phosphatidylinositol 3-kinase pathways occurs, leading to the aforementioned cellular effects[74].

CD-123 is physiologically expressed on basophils and a peculiar cell population: plasmacytoid dendritic cells[75]. Pathologically speaking, CD123 has been demonstrated on neoplastic cells in blastic plasmacytoid dendritic cell neoplasm (BPDCN), hairy cell

leukemia (HCL), acute myeloid leukemia (AML), and acute lymphoblastic leukemia/lymphoblastic lymphoma (ALL)[69,76].

Its upregulation in leukemia has brought it to prominence as a target that can be selectively inhibited in neoplastic cells[69].

The primary approach to assess CD123 expression by leukemic blasts is immunophenotyping by flow cytometry. The crucial aspect of this technique is its ability to identify antigens over a wide range and to assign a specific expression to particular groups of cells. By detecting fluorescence intensity, this method can indirectly determine the number of CD123 molecules expressed in different cell populations; the data are normalized against control cell populations (such as nucleated red blood cells, NRBC) or background fluorescence[69].

The percentage of myeloid neoplastic cells showing expression of this marker is variable. It is estimated that about 45% of AML cells express this receptor in high amounts[77,78].

Significantly, quantitative expression (measured as intensity change on cytometry) varies in the same cell population: in one study, it was estimated that about 80% of leukemic cells are CD123 positive in about 55% of AML patients[79].

Interestingly, the percentage of leukemic stem cells (LSCs) positive for this receptor is higher than that of other neoplastic populations, with an estimated 66%[80].

Moreover, CD123 overexpression on AML blast cells was associated with unfavorable outlooks, including reduced overall survival (OS) rates and clinical remission (CR)[78].

### 1.10.1. Blastic Plasmacytoid Dendritic Cells Neoplasm

When talking about CD123, one cannot fail to mention the hematologic neoplasm for which it is best known, not only because of the peculiarity of this form but also because of the particular overlap with some AML subgroups: plasmacytoid dendritic cell neoplasm (BPCDN).

BPCDN derives from plasmacytoid dendritic cell precursors (pDCs)[81]. The 2016 WHO classification of myeloproliferative neoplasms lists it as a separate classificatory entity immediately after acute myeloid leukemias to emphasize its proximity to this type of neoplasm rather than its lymphoid counterpart[25].

It is an extremely rare condition (its incidence is 0.000045%) with an ominous prognosis.

Clinically, this neoplasm involves multiple districts, including, most importantly, the skin (involved in 60-100% of cases), bone marrow, and lymph nodes[81].

From a diagnostic point of view, diagnosis is difficult, given the rarity and insidiousness of the neoplasm. Immunophenotypically, the blasts mainly show CD4, CD43, CD45, CD56, CD303, TCL1A, CD2AP, and, of course, CD123 (considered the pDC-associated antigen)[81].

Currently, one of the most promising therapies for this condition is Tagraxofusp (once known as SL-401), a drug directed explicitly against CD123, which is overexpressed at the level of BPDCN cells[82]. Tagraxofusp was approved for the treatment of BPDCN in 2018 by the Food and Drugs Administration (FDA) in the U.S. and in 2021 by the European Medicine Agency (EMA) in the European Union.

BPDCN interests us not only because of the many points of contact with myeloid neoplasms but also because there are forms of AML with a similar phenotype to BPDCN: BPDCN-like phenotype AML.

### 1.10.2. BPDCN-like phenotype AML

The BPDCN-like AML phenotype is a particular subcategory of AML expressing CD4, CD56, CD123[83]. An interesting work of Guolo F et Al. showed that this phenotype subgroup was associated with a significantly worse outcome in a subgroup of AML with isolated mutated NMP-1, a classically favorable subcategory of leukemia. Notably, the patients with a BPDCN-like phenotype showed a high level of minimal residual disease after induction (probably a symptom of chemoresistance) and had a three years overall survival of 25% (compared to the 77% of non-BPDCN-like phenotype)[83].

These data suggest that BPDCN-like AML may sub-stratify a distinct subtype among NPM1-mutated patients in order to improve prognostic yield[83,84].

In light of this, NPM1-mutated AML patients with BPDCN-like phenotype represent a population of subjects potentially to be treated with drugs used in the treatment of BPDCN, including, in particular, anti-CD123 drugs such as Tagraxofusp.

## 1.11. Tagraxofusp

Tagraxofusp (formerly known as SL-401) is one of many possible therapeutic options targeting CD123. It is a cytotoxin directed explicitly against CD123, consisting of recombinant human IL-3 fused with truncated diphtheria alpha-toxin (catalytic and translocation domains), a potent inhibitor of protein synthesis[85].

As illustrated in **Figure 6**, the IL-3 domain binds the specific receptor, which is then internalized by the process of endocytosis; within the cell, translocation of the diphtheria fragment into the cytosol occurs, followed by ADP ribosylation of elongation factor 2 (EF2) by the catalytic site of the toxin; EF2 normally displaces the tRNA-peptidyl complex from the A site to the P site: its inhibition irreversibly leads to inactivation of protein synthesis and cell death[86].

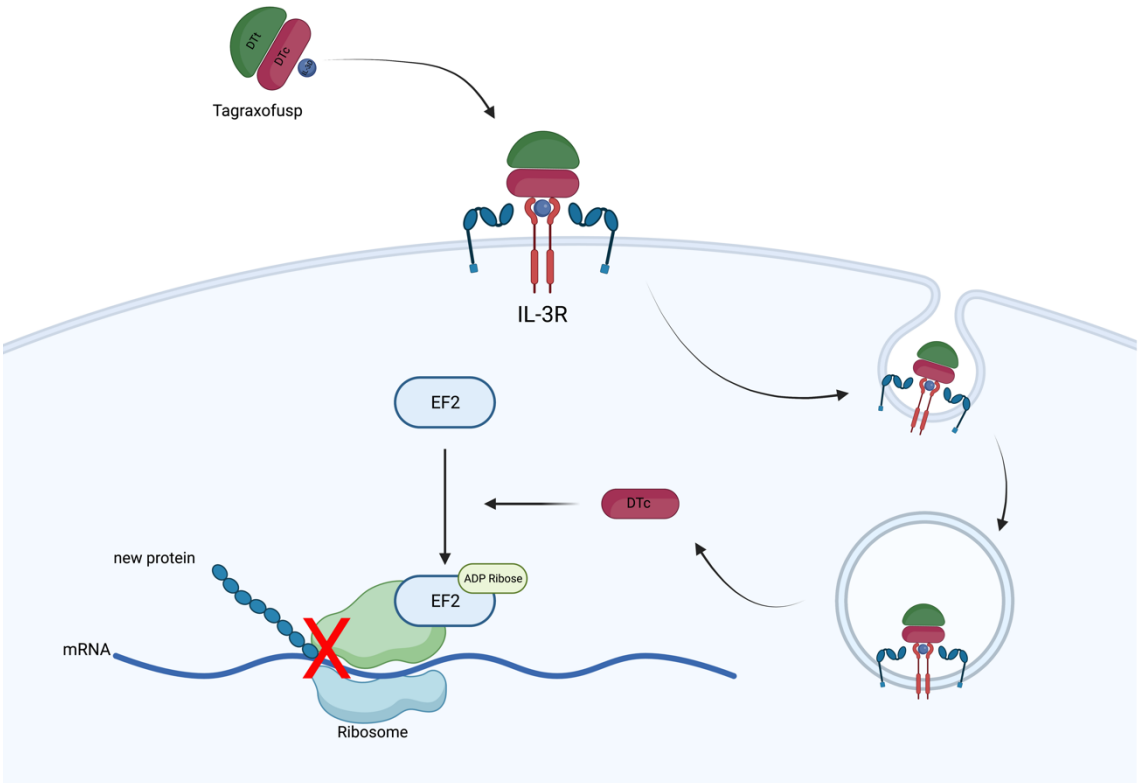



**Figure 6**: **Tagraxofusp mechanism of action**. DTt = diphtheria toxin translocation domain; DTc = diphtheria toxin catalytic domain; EF2 = elongation factor 2.
Created with BioRender.com

How this molecule induces cell death is different from that of other currently available therapies: in fact, it is able to kill cells even in the G0 phase because it hinders the process

of protein synthesis (which is a constitutive activity of the cell and not dependent on the cell cycle)[87].

Given its cell cycle-independent mechanism, it could be argued that this kind of drug also affects healthy bone marrow progenitors. However, toxicity on HSCs has been shown to be negligible in light of potent activity on leukemic cells[88].

It is also interesting to note that the cytotoxic component of Tagraxofusp is not subject to chemoresistance systems such as efflux pumps which make the drug particularly resistant to the defense mechanisms of tumor cells[89].

### 1.11.1. Drug safety

Although the effect on hematopoietic stem cells seems to be limited ~~and offensive~~, Tagraxofusp possesses some critical side effects that must be known for proper patient management.

In safety studies, the most common adverse reactions to Tagraxofusp (occurring in ≥15% of treated patients) were: hypoalbuminemia, increased hepatic cytolysis enzymes, thrombocytopenia, thrombocytosis, fever, nausea, fatigue, chills, hypotension, weight gain and capillary leak syndrome (which occurred in 17% of cases); approximately 23% of patients were found to develop at least one serious adverse effect and 6% had to stop treatment due to any grade of toxicity[90].

Capillary leak syndrome (CLS) is perhaps the most feared adverse reaction during treatment with Tagraxofusp; it is a rare, potentially fatal disorder characterized by the development of capillary hyper-permeability resulting in plasma extravasation, hypotension, and distributive shock; clinically, we see the classic triad of hypoalbuminemia, hemoconcentration, and hypotension along with other more nonspecific symptoms such as dyspnea, oliguria, edema, effusions[91].

In meta-analysis studies on cancer patients, it was seen that 52% of the forms were related to antineoplastic therapy, 43% to the underlying neoplasm, and about 5% were described as idiopathic; of the neoplasm-associated forms, more than 61% of the cases were hematologic neoplasms. The mortality rate of patients directly attributable to CLS was 6.5 percent[91].

Although the mechanisms of pathogenesis are not yet clear, several mechanisms have been proposed: increased levels of circulating T cells, endothelial injury and apoptosis, elevated levels of serum cytokines such as CXCL10, CCL2, IL-1β, IL-6, TNF-α, elevated plasma levels of vascular endothelial growth factor (VEGF)[91].

Given the small number of patients with capillary leak syndrome in the literature, there is a lack of sufficient evidence and international guidelines to guide the choice of therapy; the three therapeutic pillars with the most evidence to date are intravenous fluid infusion, glucocorticoids, and intravenous immunoglobulin (IVIG)[92].

It is important to note that after the condition has stabilized, pulmonary edema may verify due to the return of interstitial fluid to the intravascular space. Therefore, in this stage, fluid restriction and usage of diuretics may be indicated[92].

For a more accurate description of the management of capillary leak syndrome in the context of the current study, see Chapter 2.4.1.

### 1.11.2. Drug uses

As mentioned in chapter 1.10.1., Tagraxofusp has received FDA and EMA clearance for its use in BPDCN.

However, several studies are currently underway evaluating its use in other hematologic malignancies, including (a) relapsed/refractory chronic myelomonocytic leukemia[93], (b) relapsed/refractory myelofibrosis[94], (c) multiple myeloma[95], (d) in combination with azacitidine with or without venetoclax in CD123-positive AML, MDS, and BPDCN[96], and (e) consolidation therapy in MRD-positive AML[97].

The following chapters will report the methods, materials, and results of our study, the first European study using Tagraxofusp alone in the treatment of AML.

## 1.12. The study

The **GIMEMA AML2020 trial** represents the first European study to employ Tagraxofusp as salvage therapy for patients with refractory or relapsed acute myeloid leukemia presenting positivity to CD123 or with Blastic Plasmacytoid Dendritic Cell Neoplasm Immunophenotype-like.

It is a nonrandomized, open-label, ongoing multicenter phase II study and, in its initial design, it planned to enroll 50 patients divided into two cohorts:

- 25 patients with Relapsed/Refractory BPDCN-IF AML (CD123+, CD4+, CD56+)
- 25 patients with Relapsed/Refractory AML CD123+ (CD4-, CD56-)

The rationale for the clinical development of Tagraxofusp for AML patients with BPDCN-IF or CD123+ is based on several factors:

1. Cells of these specific AML subtypes have high expression of IL-3R;
2. The efficacy demonstrated by Tagraxofusp against cells with these types of characteristics (especially in BPCDNs).
3. The unmet medical need for these indications.

As of May 2023 (the data collection deadline for this thesis), approximately three years after the start of the study, there were 9 patients enrolled in the Genoa Center, all belonging to cohort B (R/R AML CD123+).

Therefore, the data and analyses reported in the following pages of this manuscript refer to the local enrolled population.

### 1.12.1. Aims of the study

The trial involves the evaluation of several primary and secondary endpoints:

- **Primary outcome**:
    - Evaluate the activity of Tagraxofusp in terms of Objective Response Rate (ORR), which includes Partial Response (PR) Complete Response (CR), and Complete Response with incomplete hematologic recovery (CRi).
- **Secondary outcomes**:
    - Safety;
    - Overall Survival (OS);
    - Response rate and survival outcomes;

# 2. Materials and Methods

## 2.1. Patient selection criteria

Patient enrollment within the study has strict eligibility or exclusion criteria:

1. **Inclusion criteria**:
    a. The patient has evidence of AML in peripheral blood and/or bone marrow with BPDCN-IF or with CD123+/CD4-/CD56- AML;
    b. The patient is ≥18 years of age;
    c. The patient must be refractory to conventional therapy or has relapsed after receiving conventional therapy (a maximum of two previous lines of therapy is allowed);
    d. The patient has an ECOG (Eastern Cooperative Oncology Group) score between 0 and 2;
    e. The patient has adequate organ function at baseline, including cardiac, renal, and liver function:
        - Left ventricular ejection fraction (LVEF) ≥ at the institutional lower limit of normal, as measured by two-dimensional echocardiography within 21 days before the start of therapy;
        - No clinically significant abnormalities on a 12-lead electrocardiogram (ECG);
        - Serum creatinine ≤1.5 mg/dL;
        - Serum albumin ≥3.2 g/dL;
        - Bilirubin ≤1.5 mg/dL (26 μmol/L);
        - Aspartate aminotransferase (AST) and alanine aminotransferase (ALT) ≤2.5 times the upper limit of normal (ULN);
    f. If the patient is a woman in the childbearing period, she must have a negative serum or urine pregnancy test at screening within 1 week before treatment;
    g. The patient has signed informed consent before the start of any study-specific procedure or treatment;

h. The patient is able to comply with the study visit schedule and other protocol requirements, including follow-up for survival assessment;
i. The patient (male and female) agrees to use acceptable contraceptive methods for the duration of the study and to continue using acceptable contraceptive methods for 1 week after the last infusion of Tagraxofusp.

2. **Exclusion criteria**:
   a. The patient has a diagnosis of acute promyelocytic leukemia (APL);
   b. The patient has clinically significant persistent grade ≥2[*98] toxicities from previous chemotherapy;
   c. The patient received treatment with chemotherapy, radiotherapy, or biologic therapy within 14 days of study entry;
   d. The patient received treatment with an investigational agent within 14 days of study entry;
   e. The patient has previously received treatment with Tagraxofusp;
   f. The patient has active disease and/or a history of cancer (excluding prior MDS) that could confound the assessment of study endpoints;
   g. Patients with a previous cancer diagnosis (within 2 years of entry) have been evaluated on a case-by-case basis. Patients with the following neoplastic diagnoses are eligible: nonmelanoma skin cancer, carcinoma in situ, intraepithelial neoplasia of the cervix, organ-confined prostate cancer without evidence of progressive disease;
   h. The patient presents with clinically significant or uncontrolled cardiovascular disease;
   i. The patient has an uncontrolled, clinically significant pulmonary disease;
   j. The patient has known or suspected central nervous system (CNS) leukemia. If suspected, CNS leukemia should be ruled out by imaging and/or cerebrospinal fluid examination;
   k. The patient is receiving immunosuppressive therapy-except low-dose prednisone (≤10 mg/day)-for the treatment or prophylaxis of graft-versus-

[*]The grading system given here and in the following paragraphs refers to the Common Terminology Criteria for Adverse Events (CTCAE) v. 5.0.

host disease (GVHD). If the patient has been receiving immunosuppressive treatment or prophylaxis for GVHD, the treatment(s) must have been discontinued at least 14 days prior to study treatment and there must be no evidence of grade ≥2 GVHD;

l. The patient has intercurrent uncontrolled illnesses, including but not limited to uncontrolled infections, disseminated intravascular coagulation, or psychiatric illnesses/social conditions that would limit compliance with study requirements;
m. The patient is pregnant or breastfeeding;
n. The patient is known to be positive for human immunodeficiency virus or has active or chronic hepatis B or C;
o. The patient is dependent on oxygen;
p. The patient has a medical condition that, in the opinion of the investigator, places the patient at an unacceptably high risk of toxicity;
q. The patient has AML and requires more than 1 g/day of hydroxyurea.
r. (Hydroxyurea is allowed at doses of ≤1 g/day);
s. The patient has a history of clinically significant allergic react ion or hypersensitivity to any drug or component of the study drug formulations.

## 2.2. Informed Consent

All participants received adequate information about the research objectives, potential adverse effects, procedures and possible risks, and treatment assignment details. They were also informed of the confidentiality of their medical data (accessed only by authorized personnel during the trial). The informed consent form (approved by the ethics committee before the start of the study) carries a date and version number to ensure its accuracy and completeness. Participants have been informed that their participation is entirely voluntary and that they can withdraw their consent and leave the study at any time without any negative impact on their subsequent medical care.

## 2.3. Therapeutic regimen and drug administration

Tagraxofusp was administered as an intravenous injectable drug in a 15-minute intravenous infusion.

Patients in the two cohorts were treated at each cycle with 12 μg/kg of the drug daily for 5 consecutive days, except for the first cycle in which administration (at the identical dosage) occurred for only 3 consecutive days. Each cycle of therapy lasted 21 days.

The initial schedule called for at least 4 cycles of administration, but not all patients reached this threshold.

According to the study design, the first cycle of Tagraxofusp was administered in an inpatient setting (starting from the day of the first infusion of the drug until 24 hours after the last infusion).

For subsequent courses of treatment, Tagraxofusp was administered either in an inpatient or outpatient setting as long as the environment had the necessary facilities and equipment to monitor patients with hematopoietic malignancies being treated closely. The decision to administer treatment inpatient or outpatient was made on a case-by-case basis at the investigator's discretion and according to the institution's capabilities and guidelines.

It is noteworthy that during the first cycle of treatment, the duration of Tagraxofusp dosing varied based on the patient's order of enrollment, with the first three patients receiving a 2-day dosing period, patients 4-6 receiving a 2-day dosing period with an optional third day if they met the criteria for continued dosing, and patients 7 and above receiving a 3-day dosing period. For all subsequent cycles, all patients received a 5-day dosing period (days 1-5).

It is also important to stress that in all cycles and schedules, there was the flexibility to delay the dose administration until day 10 of each cycle to allow for the resolution of any toxicity.

Patients who, for any reason, were unable to start a new course of treatment within 10 days of the previous course were excused from continuation.

## 2.4. Toxicity

As described in section 1.11.1 (drug safety), there are many possible side effects of Tagraxofusp. To give a complete picture, **Table 7** shows the principal expected adverse effects using the System Organ Classes (SOC) of the MedDRA hierarchy[99].

**Table 7** Expected adverse effects during administration of Tagraxofusp.

| MedDRA class | Side effects |
|---|---|
| **General disorders** | Asthenia<br>Chills<br>Fatigue<br>Pyrexia |
| **Gastrointestinal disorders** | Diarrhea<br>Nausea<br>Vomiting |
| **Hepatobiliary disorders** | Hepatic failure |
| **Nervous system and psychiatric disorders** | Dizziness<br>Headache<br>Metabolic encephalopathy<br>Anxiety<br>Confusional state<br>Insomnia |
| **Respiratory disorders** | Cough<br>Dyspnea<br>Epistaxis<br>Oropharyngeal pain<br>Pleural effusion<br>Pulmonary oedema |
| **Cardiac disorders** | Pericardial effusion<br>Tachycardia |
| **Skin and subcutaneous tissue disorders** | Angioedema<br>Petechiae<br>Pruritus<br>Rash |
| **Vascular disorders** | Capillary leak syndrome<br>Hypotension<br>Hypertension |

### 2.4.1. Capillary Leak Syndrome: presentation and management

Since capillary leak syndrome (CLS) is the most feared Tagraxofusp treatment adverse reaction, strict protocols were followed to suspect and treat it promptly in case of onset.

Before starting therapy, it was ensured that the patient had an adequate cardiac function, and that serum albumin was strictly ≥3.2 g/dL.

In addition, during treatment with Tagraxofusp, serum albumin levels were also monitored before the start of each dose and thereafter as clinically indicated. Patients were cyclically evaluated for any of the signs or symptoms of CLS described below.

CLS was defined by the presence of at least two of the following symptoms occurring within 7 days:

- Low blood albumin levels (including albumin <3.0 g/dL);
- Edema in various parts of the body (including weight gain ≥5 kg);
- Low blood pressure (including systolic blood pressure <90 mmHg);
- Cytokine release syndrome;
- Infusion-related reactions;
- Cardiac arrest;
- Failure of the heart and lungs;
- Failure of multiple organs to function;
- Dysfunction of multiple organs.

In case of the onset of edema, fluid overload, or hypotension, the management protocol includes administration of 25 g of intravenous albumin (every 12 h, or more frequently if possible) until serum albumin returns to ≥3.5 g/dL; in combination, 1 mg/kg of methylprednisolone (or equivalent) is administered daily until resolution of the sign/symptom of CLS or as clinically indicated.

If necessary, aggressive management of fluid status and hypotension is mandated, which could include intravenous fluids and/or diuretics or other methods of blood pressure management until the resolution of symptoms.

Note how it is possible to restart the administration of Tagraxofusp within the same treatment cycle if the patient's symptoms of CLS have disappeared and any measures were necessary to address hemodynamic instability. However, if the patient still has

symptoms or has required measures to correct hemodynamic instability, discontinuing the drug for the current cycle is mandatory.

If all signs and symptoms of CLS have resolved and the patient is hemodynamically stable, administration of Tagraxofusp can resume in the following cycle.

## 2.5. Clinical and laboratory evaluation

### 2.5.1. Pre-treatment screening

Selected patients underwent a battery of screening examinations in the 7 days directly preceding the start of treatment.

This screening included clinical, instrumental, and laboratory parameters in order to obtain a detailed overview of the patient's status at baseline:

I. **Clinical evaluations**:

1. Inclusion/exclusion criteria evaluation
2. Patient's personal and medical history
3. Complete physical Examination
4. Height and weight
5. Vital Signs
6. ECOG performance status

II. **Instrumental assays**:

1. Chest X-ray
2. ECG
3. Echocardiogram (with quantification of LVEF)

III. **Laboratory parameters**:

1. Complete blood count
2. Chemistry: albumin, alkaline phosphatase, ALT, AST, amylase, lipase, glucose, lactate dehydrogenase (LDH), major ions, creatinine, creatine kinase, bilirubin (direct and total), lipid profile, BUN, uric acid, amylase
3. Coagulation set-up: INR, aPTT, fibrinogen
4. Urinalysis
5. HIV, HBV, HCV serology

6. Manual differential counting of peripheral leukocytes
7. pregnancy testing
8. Bone marrow aspirate (if no previous complete bone marrow aspirate with cytogenetic and molecular stratification is available within 20 days of screening or if cytogenetic/molecular risk stratification has not been confirmed on any previous samples).

Several of these examinations were repeated at regular intervals during the treatment cycles. Specifically:

- Complete objective examination, ECOG, vital signs, assessments of complete remission (CR), if any, and residual disease (MRD) (both performed through bone marrow aspiration) were conducted at day 1 beginning of each cycle;
- CBC, chemistry, coagulation balance, and weight were evaluated every 7 days of treatment (starting from day 1 of each cycle).

### 2.5.2. End of treatment evaluation and follow-up

At the end of treatment, the following tests were repeated for each patient: objective examination, vital signs, ECOG, CBC, chemistry, coagulation asset, urinalysis, and bone marrow aspirate. The same examinations were repeated 30 days after the end of treatment and then every 3 months as indicated by the clinical follow-up according to local policy. It is important to mention that reasons were reported if a patient ceased treatment before the end of the schedule.

## 2.6. Treatment response assessment

Standard AML response criteria based on the 2017 ELN international consensus were used to assess treatment response[100].

The effectiveness of the treatment was measured by various parameters, including partial response (PR), complete response (CR), complete response with incomplete hematologic recovery (CRi), duration of response, disease-free survival (DFS), event-free survival (EFS), and overall survival (OS). **Table 6** provides detailed explanations of these parameters (see **Chapter 1.9.1.**).

The presence of minimal residual disease (MRD) was evaluated using either fresh bone marrow samples analyzed by multicolor flow cytometry or real-time PCR analysis if a molecular marker for MRD was identified at the time of diagnosis and could be evaluated. Samples are obtained at the time points established in the previous chapter.

It is essential to state that the cytofluorometric positivity cut-off for the definition of minimal residual disease (MRD) is established by the presence of 0.025% residual leukemic cells.

## 2.7. Statistical analysis

Treatment efficacy was analyzed based on data from all patients who received any dose of Tagraxofusp (intention to treat, ITT).

Patient characteristics were summarized using cross-tabulations for categorical variables and quantiles for continuous variables.

Adverse events were recorded and graded according to CTCAE v 5.0[98].

Dichotomous variables were compared using Fisher's exact test. Continuous variables were compared using the Wilcoxon rank test. Survival curves were built using the Kaplan-Meier method. All two-sided p-value lower than 0.05 were considered significant. All analyses were performed with IBM SPSS® v22.

# 3. Results

## 3.1. Patient's characteristics

From February 2021 to January 2023, 10 patients met the hematological criteria to proceed to the clinical screening phase for enrollment.

Of these, 9 successfully passed all the required examinations and were enrolled.

One patient was excluded from the trial because his left ventricular ejection fraction (LVEF) was below the required limit.

The median age at the start of treatment was 66 years, with a minimum of 52 and a maximum of 72.

Four out of 9 patients (44.4%) had acute relapsed leukemia, while the remaining 5 (55.6%) had leukemia refractory to treatment lines.

**Tables 8** and **9** show the demographic information of the enrolled patients.

**Tables 10** and **11** show, respectively, the hematologic status and previous lines of therapy the patients underwent before enrollment.

**Table 8**. Frequency and percentage of patients' sex categories.

| SEX | | |
|---|---|---|
| | **Frequency** | **Percentage** |
| **Male** | 3 | 33,3% |
| **Female** | 6 | 66,7% |

**Table 9**. The minimum, maximum, and median age of enrolled patients.

| AGE | | | |
|---|---|---|---|
| **Patients** | **Minimum** | **Median** | **Maximum** |
| 9 | 52 | 66 | 72 |

**Table 10**. AML status frequency and percentage at registration.

| AML status | Frequency | Percentage |
|---|---|---|
| **Relapsed** | 4 | 44,4% |
| **Refractory** | 5 | 55,6% |

**Table 11**. Frequency and percentage of previous lines of therapy

| N° of lines | Frequency | Percentage |
|---|---|---|
| **1** | 4 | 44,44% |
| **2** | 5 | 55,55% |

Although numerically small, the population examined had significant heterogeneity in its hematological status and previous treatments. Five of 9 patients (55,6%) arrived at enrollment with 2 prior lines of therapy. The types of treatment patients underwent before being included in the protocol were very heterogeneous, ranging from the classic CPX-351 protocol to experimental protocols such as FLAI-5-Venetoclax (AML1718). All of the enrolled patients received at least one line of intensive treatment.

**Table 12** summarizes the first-line protocols and their frequencies while **Table 13** summarizes the second-line protocols.

**Table 12**. Pre-enrollment 1st lines protocols and related frequencies and percentages.

| Protocol | Frequency | Percentage |
|---|---|---|
| **CPX-351** | 4 | 44,44% |
| **FLAI – 5 – Venetoclax (AML1718)*** | 2 | 22,22% |
| **FLAI – 5 – ARA/C** | 2 | 22,22% |
| **PETHEMA protocol** | 1 | 11,11% |

*GIMEMA **AML1718** is an ongoing trial designed to evaluate the hematologic response following the combination of the FLAI-5 regimen and the pro-apoptotic agent Venetoclax[101].

To further emphasize the critical compromised state of the patients at the time of enrollment, it is essential to point out that after the first treatment protocol, 3 patients underwent allogeneic bone marrow transplantation (Allo-BMT), resulting in relapse/refractoriness. Of these patients, one had performed the CPX-351 protocol, one the FLAI - 5 ARA/C scheme, and one the FLAI - 5 - Venetoclax scheme. The former two patients showed persistently refractory disease, while the latter relapsed after transplantation.

**Table 13**. Pre-enrollment 2nd lines protocols and related frequencies and percentage.

| Protocol | Frequency | Percentage |
|---|---|---|
| Azacitidine – Venetoclax | 4 | 80,00% |
| Decitabine – Venetoclax | 1 | 20,00% |

As indicated in the materials and methods chapter, white blood cells in peripheral blood and the percentage of bone marrow blasts were assessed before administration of the first dose of Tagraxofusp (Cycle 1/Day 1). **Charts 1** and **2** show the peripheral white blood cell count (median 4930/µl) and the percentage of bone marrow blasts (median 50%), respectively. As seen from the graphs, there is again heterogeneity in clinical presentation at the start of treatment.

**Chart 1**. Peripheral White Blood Cell (WBC) at Cycle 1/Day 1 and corresponding median.

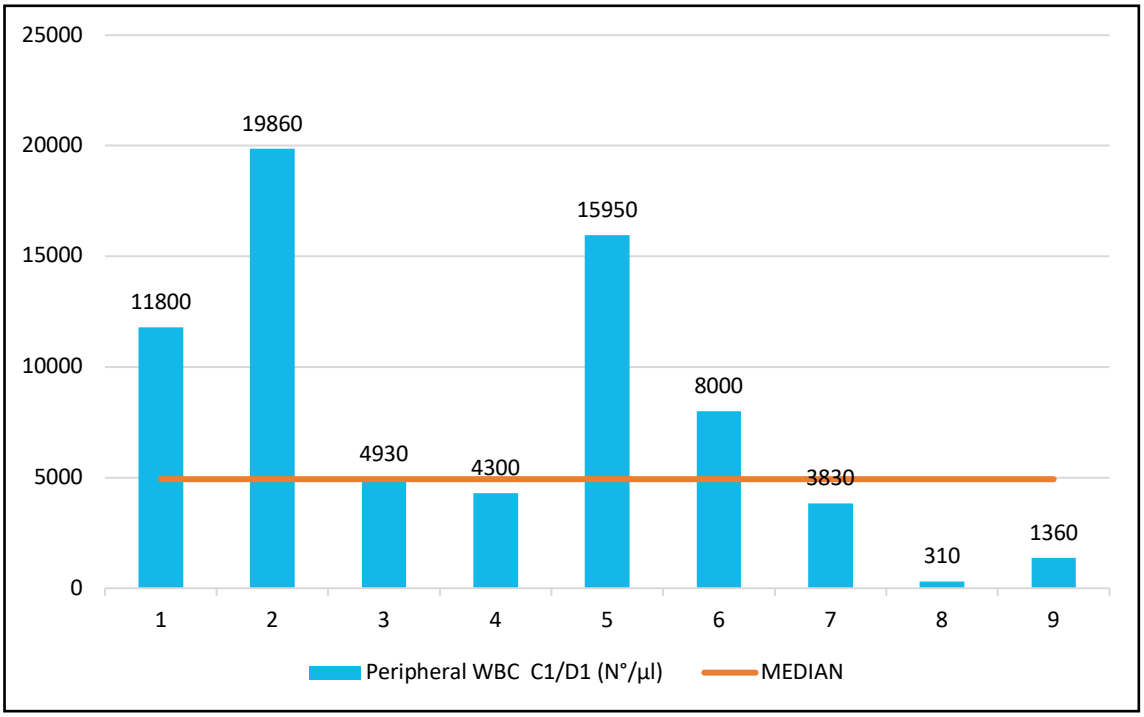
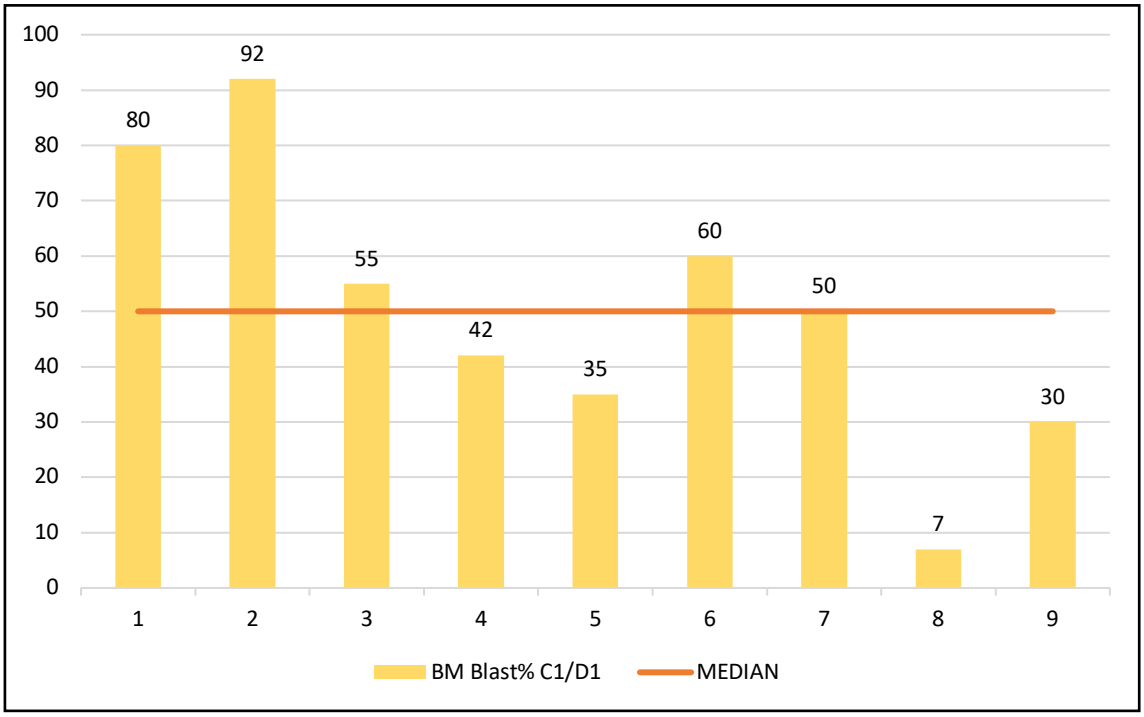



**Chart 2**. Bone Marrow Blast percentage at Cycle 1/Day 1.



It is also important to note the karyotypic heterogeneity of patients at the time of induction. Although the majority (6) of patients had a normal karyotype (46, XY/XX), 3 patients (33,3% of the total) had a prognostically unfavorable karyotype. **Table 14** summarizes the detected karyotypes and the corresponding ELN risk classes (see **Chapter 1.6.3**). As can be seen, no patient had a favorable risk class.

**Table 14**. Leukemic blasts karyotypes at the onset.

| Patient | Karyotype | ELN risk class |
|---|---|---|
| **N°1** | 44, XX, del(4), del(5)(q13q33), del(7)(q22), add(11p), add(12p), del(13)(q14), -14, -16, ad(17p) | Adverse |
| **N°2** | 46, XX | Intermediate |
| **N°3** | Complex hyperdiploid | Adverse |
| **N°4** | 46, XX | Intermediate |
| **N°5** | 46, XY | Intermediate |
| **N°6** | 46, XY | Intermediate |
| **N°7** | 46, XX | Intermediate |
| **N°8** | 46, XX | Intermediate |
| **N°9** | 43, XX, t(1;1;12)(q11;p13;p13), -4, -5, -7, -12, der(16)add(q24), der(17)add(q21, der(18)add(q23), +mar(15) | Adverse |

Regarding CD123 expression, the patients showed another substantial heterogeneity. Evaluation, performed by flow cytofluorimetry, demonstrated (as already known in the context of acute myeloid leukemia clones) significant heterogeneity in marker expression, identifying bright populations (with a substantial homogeneity in the expression of the marker) and dim forms (with varying percentages of clones for CD123).
In two cases, the populations identified were more than one with mutual differences in CD123 expression, indicating further variability in the picture.

**Table 15** provides a summary of the populations and their expression rates.

**Table 15.** CD123 expression in patients blast populations. "Bright" indicates high Mean Fluorescence Intensity (MFI) in the marker's expression by the blasts. In contrast, "dim" indicates a lower MFI in which not all blasts evaluated by the instrument express CD123 (in this case, expression rates are reported). "+" indicates a brighter (hence denser) expression of the marker. The percentage indicates the proportion of leukemic blasts expressing the marker CD123 with an MFI over the threshold to define positivity.

| Patient | BRIGHT | DIM | Population | Percentage |
|---|---|---|---|---|
| **N°1** | | x | | 96% |
| **N°2** | x | | Population A<br>Population B | 100% (++)<br>100% (+) |
| **N°3** | | x | | 91% |
| **N°4** | | x | | 65% |
| **N°5** | | x | | 28% |
| **N°6** | | x | | 69% |
| **N°7** | | x | Population A<br>Population B | 55%<br>0% |
| **N°8** | | x | | 30% |
| **N°9** | | x | | 100% |

## 3.2. Cycles and Overall Response Rate (ORR)

As shown in **Table 16**, all patients received at least one dose of Tagraxofusp. The median number of cycles administered was 2, with a maximum of 10 (1 patient) and a minimum of 1 (2 patients).

**Table 17** shows the number of patients for each cycle.

**Table 16**. Overview of administered Tagraxofusp cycles.

| Number of cycles | | | |
|---|---|---|---|
| **N°** | **Minimum** | **Median** | **Maximum** |
| **9** | 1 | 2 | 10 |

**Table 17.** Number of patients that could reach each cycle.

| | Cycle | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | **C1** | **C2** | **C3** | **C4** | **C5** | **C6** | **C7** | **C8** | **C9** | **C10** |
| **N°** | 9 | 7 | 3 | 3 | 2 | 2 | 1 | 1 | 1 | 1 |

Only 7 out of 9 patients were evaluable after the first cycle of Tagraxofusp because two patients died before evaluation could occur (early death; refer to **Chapter 3.4.** for data related to toxicity and causes of death of patients).

The best hematological response was a partial response (PR), obtained in 4 out of 9 patients (54.6%) considering any cycle.

Of those who achieved a partial response, 2 out of 4 patients achieved it only after the second course of treatment.

3 out of 9 patients (33.3%) never achieved any response, reaching a maximum of two cycles of treatment before death.

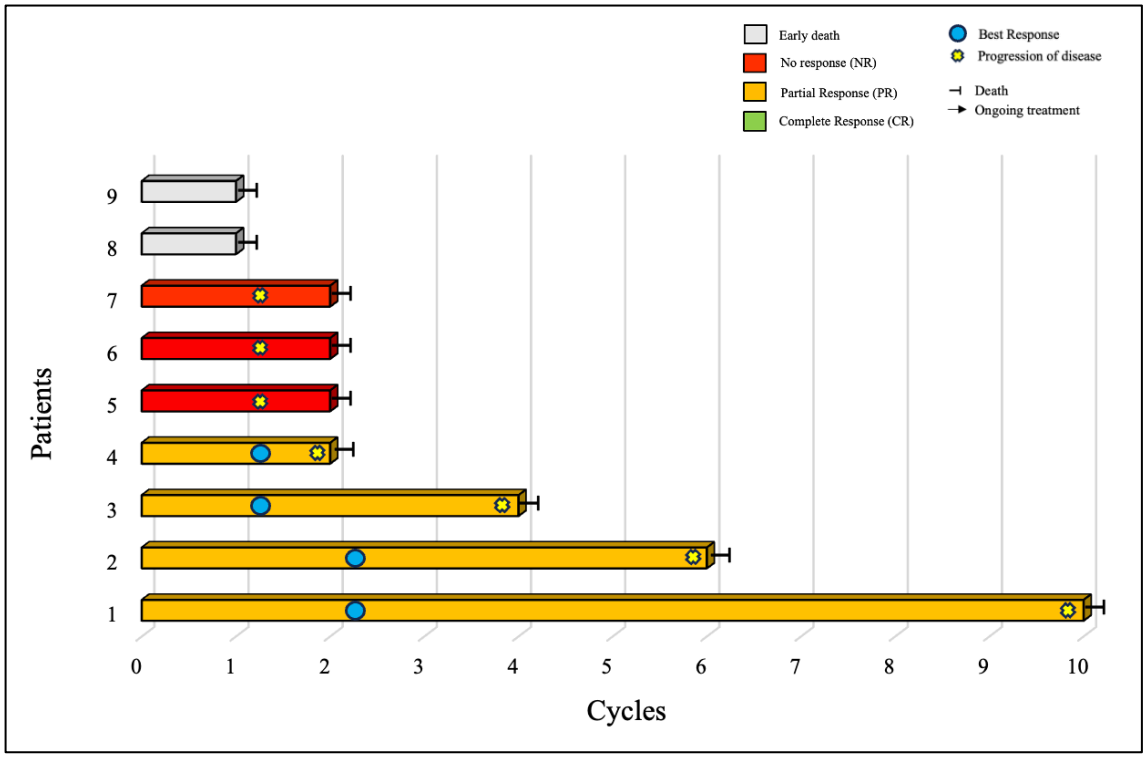
**Table 18** summarizes the ORR at any treatment cycle with Tagraxofusp and related percentages.

**Table 18**. Best Objective Response Rate (ORR) at any cycle.

| | **Frequency** | **Percentage** |
|---|---|---|
| **Complete Response (CR)** | 0 | 0,00% |
| **Partial Response (PR)** | 4 | 44,5% |
| **No response (NR)** | 3 | 33,3% |
| **Early death** | 2 | 22,2% |

It is important to note that few patients have achieved their best hematological response (PR) in the first cycle, but as can be seen from **Chart 3**, 2 out of the 4 patients who achieved PR did so after the second cycle. In contrast, 2 patients managed to get a response after the first administration.

**Chart 3**. Administered Tagraxofusp cycles and corresponding ORR for each patient. As can be seen, two patients could not be evaluated due to an early death. No patients have achieved a complete response. A green dot and a yellow cross were used to indicate two essential time points: the cycle at which the best response was achieved and the cycle at which disease progression started, respectively.

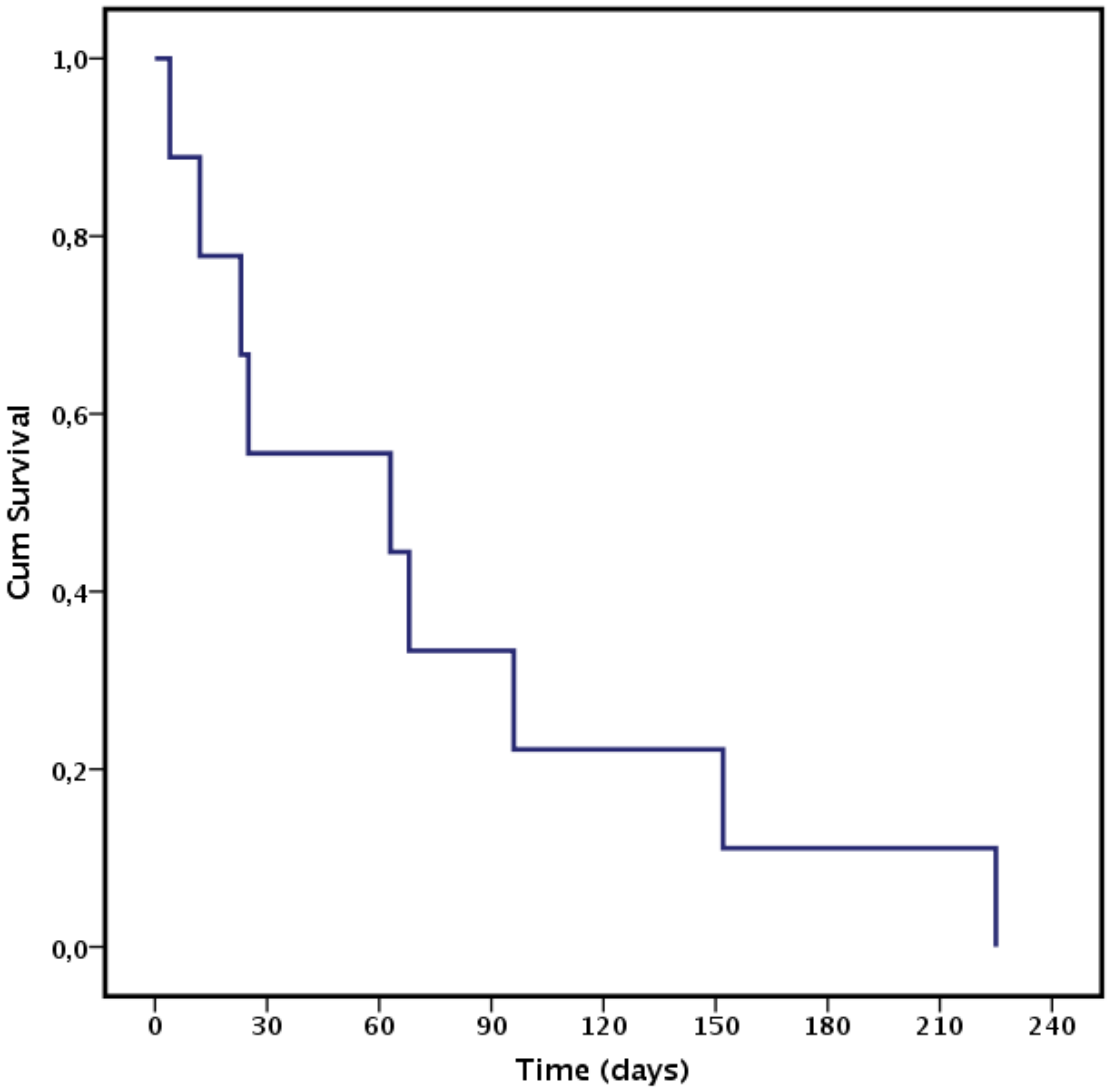



## 3.3. Overall Survival (OS)

When writing this manuscript, all patients reached the death event. The outcome occurred heterogeneously from patient to patient, with a minimum of 4 days from the first day of the first Tagraxofusp cycle (which, in this specific case, was more likely due to acute disease progression than drug ineffectiveness) to a maximum of 225 days. Median survival was 63 days (C.I. 95% 0 – 174.028 days), and overall survival (OS) at 2 months was 55.56%.

**Chart 4** shows the Kaplan-Meier-type survival curve.

**Chart 4.** Kaplan-Meier cumulative survival curve. Median survival: 63 days (C.I. 95% 0 – 174.028 days). 2 months OS 55,56%; 3 months OS 33,3%; 6 months OS 11.1%




## 3.4. Safety and related toxicities

As pointed out several times in various chapters of this manuscript, Tagraxofusp is a drug with a complex toxicity profile.

**Chart 5** shows which and how many side effects occurred during the drug administration. Adverse events with a CTCAE grade (v. 5.0) greater than or equal to 3 are shown in red, while adverse effects with grades 1-2 are shown in green.

The most feared adverse event was Capillary Leak Syndrome (CLS) which occurred in 4 patients (44.5% of treated patients), and half of the cases presented with grade ≥3. More specifically, CLS occurred in the first cycle (C1) in 3 out of 4 patients and the second cycle (C2) in the fourth patient.

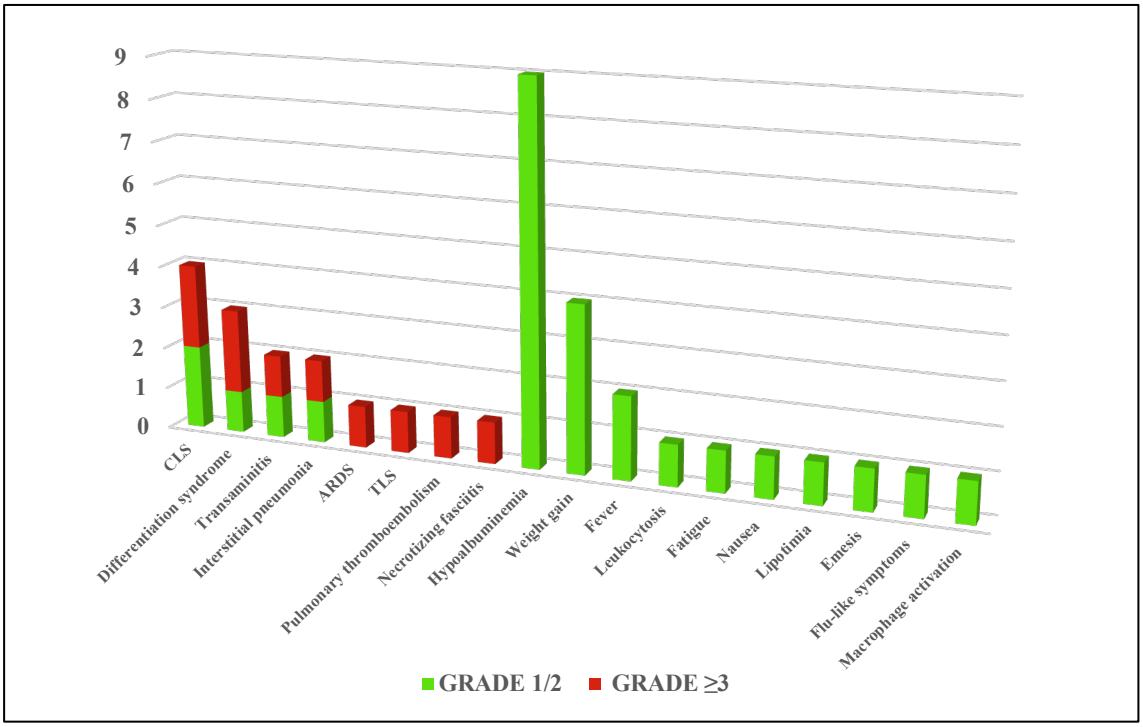
Another frequently encountered effect was differentiation syndrome (a clinical syndrome characterized by dyspnea, fever, weight gain, hypotension, and pulmonary infiltrates), which occurred in 3 patients (33.3% of the total), and one patient was fatal in the second cycle (C2, grade 5).

Regarding additional side effects with grade ≥3, we found: pneumonia (2 patients), ARDS (1 patient), tumor lysis syndrome (TLS, 1 patient), pulmonary embolism (PE, 1 patient), and necrotizing fasciitis (1 patient).

In the patient who presented PE, the event proved fatal at cycle 2 (C2, grade 5).

Among grade 1-2 side effects, the most frequent event was, as expected, hypoalbuminemia (which was treated as per the datasheet; see chapter 2.4.1), which occurred in 100% of patients, accumulation gain (44.5% of patients), fever (33.3% of patients), leukocytosis, fatigue, nausea, lipemia and dizziness, emesis, flu-like symptoms, and macrophage activation (each of which was experienced by a single patient).

**Chart 5**. Adverse events during the administration of Tagraxofusp at any cycle. CTCAE grades v 5.0 ≥3 in red, grades 1-2 in green. The proportion of patients who experienced each adverse event is shown.




Regarding the causes of death, 44.5% of patients (4 out of 9) died from primary disease progression. Of these, two patients completed only one course of therapy and died before the complete evaluation of the hematologic status (early death, see **chapter 3.2.**); specifically, one died from differentiation syndrome and the other from septic shock (highly likely from cytomegalovirus reactivation after bone marrow transplant).

Regarding other causes of death, the following were recorded: another septic shock event that resulted in multi-organ failure (MOF, cycle 6), acute central nervous system bleeding (cycle 4), and pulmonary thromboembolism (cycle 2).

As mentioned in the previous paragraph, two out of 9 patients (22.2%) died from complications due to systemic response due to uncontrolled disease (one due to pulmonary embolism and the other due to differentiation syndrome, at cycle 2 and 1, respectively).

# 4. Discussion

AML2020, as repeatedly pointed out in this paper, was the first European experience of using Tagraxofusp (a smart molecule directed against CD123) outside of its approved context: the plasma dendritic cell neoplasm (BPCDN).

The rationale for using this drug within the scope of acute myeloid leukemias was mainly related to CD123 having expressions within the blastic populations of certain types of myeloid leukemia. Therefore, its targeting could lead to promising hematological responses.

It should be noted that the biological target of Tagraxofusp has a highly heterogeneous course in populations of acute myeloid leukemia compared to BPDCN cells. Most patients treated had only partial (dim) positivity to the CD123, with very different values between one patient and the other. Since there are tumor cells that are not positive for the marker, the drug may not be ideal as exclusive monotherapy except in highly selected cases. However, the extensive use of cytofluorometry and the detailed molecular analysis of neoplastic populations, although few in number, allowed us to understand the cellular markers of AML better.

The response rates of the cohort presented in this thesis are meager and statistically insignificant due to the small number of patients analyzed. For at least two patients (22,2% of the total), it was impossible to carry out adequate assessments as the death occurred before the hematological evaluation of the first time-point, making the results even more limited. However, it is essential to note that a significant number of treatment cycles have been achieved in at least two cases (reaching 6 and 10 cycles, specifically), achieving a good stabilization of a highly proliferating and otherwise lethal disease within a few months to indicate heterogeneity of response to Tagraxofusp.

Overall survival at 2 months (55.56%) was slightly higher than the median survival reported in the literature of subjects with AML R/R receiving the best supportive care (50%)[67]. Certainly, in obtaining this data, the significant clinical complexity of treated patients and the fact that 2 of them (22.2% of the total) died early in the trial before hematologic evaluations weighed heavily.

In any case, as indicated in Chapter 3.2. no patient in our cohort has achieved complete remission; the best response has been partial remission (PR) of the disease (which occurred in 4 patients out of 9, 44,5% of the total).

Interestingly, in other centers, some patients have obtained complete remission with an effective bridge therapy to transplant, further indicating the extreme response heterogeneity to this drug. Concerning this condition, it is necessary to carry out further studies and refine the management schemes for clinical complications that may occur during drug administration.

Regarding the toxicity profile, Tagraxofusp has proven as a complex drug. However, it is essential to note that this drug has received fast approval from BPDCN treatment agencies and that fatal toxicity cases have already occurred during the approval studies.

Our cases showed a complex profile despite the precautions for managing side effects. The administration of the drug must be done following the indications and, in parallel, it is essential to carefully choose the appropriate patient and fit to receive it (especially at the stages when the drug is administered as single agent without any other therapy that can help to counteract or limit its toxicity). For this reason, recruitment requirements have been extraordinarily stringent, and in some cases, it has become necessary to combine supportive cytoreductive therapies.

Even with the necessary precautions, we have had cases of substantial toxicity that.

The worst more frequent side effect was, as expected, capillary leak syndrome (CLS). This condition was observed in 4 patients (44.5% of the total), and two presented a ≥3 grade condition requiring immediate intervention.

The high frequency of presentation of CLS in our small study population requires us to carry out further studies and refine the management schemes for clinical complications that may occur during drug administration.

In conclusion, Tagraxofusp has proved an interesting addition to the therapeutic paraphernalia against a very unfavorable prognostic condition such as acute refractory/relapsing leukemia: it showed effects of cytoreduction and tumor lysis with moderate disease containment in some patients. However, given the critical acute toxicity and the not excellent therapeutic response (at least in our small sample), further studies are needed to refine the therapeutic regimen, improve the toxicity profile and, where

appropriate, insert Tagraxofusp into polypharmacological schemes in order to increase its clinical capacity and reduce its side effects, especially in clinically compromised patients and in high-risk settings.

# References


1. Associazione Italiana Oncologia Medica et al. *I Numeri Del Cancro in Italia 2022*.

2. Tallman MS, Wang ES, Altman JK, et al. Acute Myeloid Leukemia, Version 3.2019, NCCN Clinical Practice Guidelines in Oncology. *Journal of the National Comprehensive Cancer Network*. 2019;17(6):721-749. doi:10.6004/jnccn.2019.0028

3. Shallis RM, Wang R, Davidoff A, Ma X, Zeidan AM. Epidemiology of acute myeloid leukemia: Recent progress and enduring challenges. *Blood Rev*. 2019;36:70-87. doi:10.1016/J.BLRE.2019.04.005

4. Fondazione AIRC per la Ricerca sul Cancro. Leucemia mieloide acuta. https://www.airc.it/cancro/informazioni-tumori/guida-ai-tumori/leucemia-mieloide-acuta

5. Associazione Italiana Oncologia Medica et al. I Numeri Del Cancro in Italia 2021.

6. Siegel RL, Miller KD, Wagle NS, Jemal A. Cancer statistics, 2023. *CA Cancer J Clin*. 2023;73(1):17-48. doi:10.3322/caac.21763

7. Maynadie´ M, de Angelis R, Marcos-Gragera R, et al. Survival of European patients diagnosed with myeloid malignancies: A HAEMACARE study. *Haematologica*. 2013;98(2):230-238. doi:10.3324/haematol.2012.064014

8. Zjablovskaja P, Florian MC. Acute myeloid leukemia: Aging and epigenetics. *Cancers (Basel)*. 2020;12(1). doi:10.3390/cancers12010103

9. Jaiswal S, Ebert BL. Clonal hematopoiesis in human aging and disease. *Science (1979)*. 2019;366(6465). doi:10.1126/science.aan4673

10. Yi M, Li A, Zhou L, et al. The global burden and attributable risk factor analysis of acute myeloid leukemia in 195 countries and territories from 1990 to 2017: Estimates based on the global burden of disease study 2017. *J Hematol Oncol*. 2020;13(1). doi:10.1186/s13045-020-00908-z

11. Cowell IG, Austin CA. Mechanism of generation of therapy related leukemia in response to anti-topoisomerase II agents. *Int J Environ Res Public Health*. 2012;9(6):2075-2091. doi:10.3390/ijerph9062075

12. Greene MH, Harris EL, Gershenson DM, et al. *Melphalan May Be a More Potent Leukemogen than Cyclophosphamide*.; 1986. http://annals.org/pdfaccess.ashx?url=/data/journals/aim/19637/

13. Patel AA, Rojek AE, Drazer MW, et al. Therapy-related myeloid neoplasms in 109 patients after radiation monotherapy. In: *Blood Advances*. Vol 5. American Society of Hematology; 2021:4140-4148. doi:10.1182/bloodadvances.2021004964

14. Strickland SA, Vey N. Diagnosis and treatment of therapy-related acute myeloid leukemia. *Crit Rev Oncol Hematol*. 2022;171:103607. doi:10.1016/J.CRITREVONC.2022.103607

15. Lalayanni C, Gavriilaki E, Athanasiadou A, et al. Secondary Acute Myeloid Leukemia (sAML): Similarly Dismal Outcomes of AML After an Antecedent Hematologic Disorder and Therapy Related AML. *Clin Lymphoma Myeloma Leuk*. 2022;22(4):e233-e240. doi:10.1016/J.CLML.2021.09.019

16. Kim S, Yoon SS, Hong J, et al. Characterization and prognosis of secondary acute myeloid leukemia in an asian population: AML with antecedent hematological disease confers worst outcomes, irrespective of cytogenetic risk. In: *Anticancer Research*. Vol 40. International Institute of Anticancer Research; 2020:2917-2924. doi:10.21873/anticanres.14269

17. Neugut AI, Robinson E, Nieves J, Murray T, Tsai WY. *Poor Survival of Treatment-Related Acute Nonlymphocytic Leukemia*. http://jama.jamanetwork.com/

18. Mosaad YM. Hematopoietic stem cells: An overview. *Transfusion and Apheresis Science*. 2014;51(3):68-82. doi:10.1016/J.TRANSCI.2014.10.016

19. Chopra M, Bohlander SK. The cell of origin and the leukemia stem cell in acute myeloid leukemia. *Genes Chromosomes Cancer*. 2019;58(12):850-858. doi:10.1002/gcc.22805

20. Genomic and Epigenomic Landscapes of Adult De Novo Acute Myeloid Leukemia. *New England Journal of Medicine*. 2013;368(22):2059-2074. doi:10.1056/NEJMoa1301689

21. Bennett JM, Catovsky D, Daniel M therbse, Flandrin G, G Galton DA, Gralnick HR. *Proposals for the Classification of the Acute Leukaemias*.; 1976.

22. Schiffer CA, Stone RM. Morphologic Classification and Clinical and Laboratory Correlates. In: Kufe DW, Pollock RE, Weichselbaum RR, et al., editors. Holland-Frei Cancer Medicine. 6th edition. Hamilton (ON): BC Decker; 2003. Available from: https://www.ncbi.nlm.nih.gov/books/NBK13452/.

23. Daniel A. Arber et al. International Consensus Classification of Myeloid Neoplasms and Acute Leukemias: integrating morphologic, clinical, and genomic data.

24. Segeren CM, van 't Veer MB. *The Netherlands JOURNAL OF MEDICINE ELSEVIER The FAB Classification for Acute Myeloid Leukaemia-Is It Outdated?* Vol 49.; 1996.

25. Arber DA, Orazi A, Hasserjian R, et al. The 2016 revision to the World Health Organization classification of myeloid neoplasms and acute leukemia. *Blood*. 2016;127(20):2391-2405. doi:10.1182/blood-2016-03-643544

26. Arber DA, Hasserjian RP, Orazi A, et al. Classification of myeloid neoplasms/acute leukemia: Global perspectives and the international consensus classification approach. *Am J Hematol*. 2022;97(5):514-518. doi:10.1002/ajh.26503

27. Vardiman JW, Thiele J, Arber DA, et al. The 2008 revision of the World Health Organization (WHO) classification of myeloid neoplasms and acute leukemia: Rationale and important changes. *Blood*. 2009;114(5):937-951. doi:10.1182/blood-2009-03-209262

28. Yang JJ, Park TS, Wan TSK. Recurrent cytogenetic abnormalities in acute myeloid leukemia. *Methods in Molecular Biology*. 2017;1541:223-245. doi:10.1007/978-1-4939-6703-2_19

29. Papaemmanuil E, Gerstung M, Bullinger L, et al. Genomic Classification and Prognosis in Acute Myeloid Leukemia. *New England Journal of Medicine*. 2016;374(23):2209-2221. doi:10.1056/nejmoa1516192

30. Metzeler KH, Herold T, Rothenberg-Thurley M, et al. Spectrum and prognostic relevance of driver gene mutations in acute myeloid leukemia. Published online 2016. doi:10.1182/blood-2016-01

31. Döhner H, Estey E, Grimwade D, et al. Diagnosis and management of AML in adults: 2017 ELN recommendations from an international expert panel. *Blood*. 2017;129(4):424. doi:10.1182/BLOOD-2016-08-733196

32. Hartmut Döhner et al. Diagnosis and management of AML in adults: 2022 recommendations from an international expert panel on behalf of the ELN. Blood 2022; 140 (12): 1345–1377. doi: https://doi.org/10.1182/blood.2022016867.

33. Juskevicius R, Thompson MA, Shaver A, Head D. Clinical Presentation, Diagnosis, and Classification of Acute Myeloid Leukemia. In: *Hematologic Malignancies*. Springer Science and Business Media Deutschland GmbH; 2021:11-55. doi:10.1007/978-3-030-53633-6_2

34. Wilson CS, Medeiros LJ. Extramedullary Manifestations of Myeloid Neoplasms. *Am J Clin Pathol*. 2015;144(2):219-239. doi:10.1309/AJCPO58YWIBUBESX

35. Siegal T, Benouaich-Amiel A, Bairey O. Neurologic complications of acute myeloid leukemia. Diagnostic approach and therapeutic modalities. *Blood Rev*. 2022;53:100910. doi:10.1016/J.BLRE.2021.100910

36. Röllig C, Ehninger G. How I treat hyperleukocytosis in acute myeloid leukemia. *Blood*. 2015;125(21):3246-3252. doi:10.1182/BLOOD-2014-10-551507

37. Ikezoe T. Pathogenesis of disseminated intravascular coagulation in patients with acute promyelocytic leukemia, and its treatment using recombinant human soluble thrombomodulin. *Int J Hematol*. 2014;100(1):27-37. doi:10.1007/S12185-013-1463-0/FIGURES/2

38. ten Cate H, Leader A. Management of Disseminated Intravascular Coagulation in Acute Leukemias. *Hamostaseologie*. 2021;41(2):120-126. doi:10.1055/A-1393-8302/ID/JR200090-36

39. Guarneri C, Wollina U, Lotti T, et al. Sweet's Syndrome (SS) in the Course of Acute Myeloid Leukaemia (AML). *Open Access Maced J Med Sci*. 2018;6(1):105. doi:10.3889/OAMJMS.2018.006

40. Kazmi SM, Pemmaraju N, Patel KP, et al. Characteristics of Sweet's syndrome in patients with acute myeloid leukemia. *Clin Lymphoma Myeloma Leuk*. 2015;15(6):358. doi:10.1016/J.CLML.2014.12.009

41. Béné MC, Nebe T, Bettelheim P, et al. Immunophenotyping of acute leukemia and lymphoproliferative disorders: a consensus proposal of the European LeukemiaNet Work Package 10. *Leukemia*. 2011;25(4):567-574. doi:10.1038/LEU.2010.312

42. Jin L, Lee EM, Ramshaw HS, et al. Monoclonal Antibody-Mediated Targeting of CD123, IL-3 Receptor α Chain, Eliminates Human Acute Myeloid Leukemic Stem Cells. *Cell Stem Cell*. 2009;5(1):31-42. doi:10.1016/J.STEM.2009.04.018

43. Mrózek K. Acute Myeloid Leukemia with a Complex Karyotype. *Semin Oncol*. 2008;35(4):365. doi:10.1053/J.SEMINONCOL.2008.04.007

44. Cortes JE, Mehta P. Determination of fitness and therapeutic options in older patients with acute myeloid leukemia. *Am J Hematol*. 2021;96(4):493. doi:10.1002/AJH.26079

45. Klepin HD. Definition of Unfit for Standard Acute Myeloid Leukemia Therapy. *Curr Hematol Malig Rep*. 2016;11(6):537-544. doi:10.1007/S11899-016-0348-8/FIGURES/1

46. Chauhan PS, Ihsan R, Singh LC, Gupta DK, Mittal V, Kapur S. Mutation of NPM1 and FLT3 Genes in Acute Myeloid Leukemia and Their Association with Clinical and Immunophenotypic Features. *Dis Markers*. 2013;35(5):581. doi:10.1155/2013/582569

47. Minetto P, Candoni A, Guolo F, et al. Fludarabine, High-Dose Cytarabine and Idarubicin-Based Induction May Overcome the Negative Prognostic Impact of FLT3-ITD in NPM1 Mutated AML, Irrespectively of FLT3-ITD Allelic Burden. *Cancers (Basel)*. 2021;13(1):1-13. doi:10.3390/CANCERS13010034

48. Lim WS, Tardi PG, Dos Santos N, et al. Leukemia-selective uptake and cytotoxicity of CPX-351, a synergistic fixed-ratio cytarabine:daunorubicin formulation, in bone marrow xenografts. *Leuk Res*. 2010;34(9):1214-1223. doi:10.1016/J.LEUKRES.2010.01.015

49. Guolo F, Fianchi L, Minetto P, et al. CPX-351 treatment in secondary acute myeloblastic leukemia is effective and improves the feasibility of allogeneic stem cell transplantation: results of the Italian compassionate use program. *Blood Cancer J*. 2020;10(10):96. doi:10.1038/S41408-020-00361-8

50. Lancet JE, Cortes JE, Hogge DE, et al. Phase 2 trial of CPX-351, a fixed 5:1 molar ratio of cytarabine/daunorubicin, vs cytarabine/daunorubicin in older adults with untreated AML. *Blood*. 2014;123(21):3239. doi:10.1182/BLOOD-2013-12-540971

51. Wei AH, Döhner H, Pocock C, et al. Oral Azacitidine Maintenance Therapy for Acute Myeloid Leukemia in First Remission. *N Engl J Med*. 2020;383(26):2526-2537. doi:10.1056/NEJMOA2004444

52. DiNardo CD, Pratz K, Pullarkat V, et al. Venetoclax combined with decitabine or azacitidine in treatment-naive, elderly patients with acute myeloid leukemia. *Blood*. 2019;133(1):7-17. doi:10.1182/BLOOD-2018-08-868752

53. Guerra VA, DiNardo C, Konopleva M. Venetoclax-based therapies for acute myeloid leukemia. *Best Pract Res Clin Haematol*. 2019;32(2):145-153. doi:10.1016/J.BEHA.2019.05.008

54. Agarwal SK, DiNardo CD, Potluri J, et al. Management of Venetoclax-Posaconazole Interaction in Acute Myeloid Leukemia Patients: Evaluation of Dose Adjustments. *Clin Ther*. 2017;39(2):359-367. doi:10.1016/J.CLINTHERA.2017.01.003

55. Lallemand-Breitenbach V, Guillemin MC, Janin A, et al. Retinoic Acid and Arsenic Synergize to Eradicate Leukemic Cells in a Mouse Model of Acute Promyelocytic Leukemia. *J Exp Med*. 1999;189(7):1043. doi:10.1084/JEM.189.7.1043

56. Lo-Coco F, Avvisati G, Vignetti M, et al. Retinoic Acid and Arsenic Trioxide for Acute Promyelocytic Leukemia. *New England Journal of Medicine*. 2013;369(2):111-121. doi:10.1056/NEJMOA1300874/SUPPL_FILE/NEJMOA1300874_DISCLOSURES.PDF

57. Döhner H, Wei AH, Löwenberg B. Towards precision medicine for AML. *Nature Reviews Clinical Oncology 2021 18:9*. 2021;18(9):577-590. doi:10.1038/s41571-021-00509-w

58. Castaigne S, Pautas C, Terré C, et al. Effect of gemtuzumab ozogamicin on survival of adult patients with de-novo acute myeloid leukaemia (ALFA-0701): a randomised, open-label, phase 3 study. *The Lancet*. 2012;379(9825):1508-1516. doi:10.1016/S0140-6736(12)60485-1

59. Stone RM, Mandrekar SJ, Sanford BL, et al. Midostaurin plus Chemotherapy for Acute Myeloid Leukemia with a FLT3 Mutation . *New England Journal of Medicine*. 2017;377(5):454-464. doi:10.1056/NEJMOA1614359/SUPPL_FILE/NEJMOA1614359_DISCLOSURES.PDF

60. Zhao JC, Agarwal S, Ahmad H, Amin K, Bewersdorf JP, Zeidan AM. A review of FLT3 inhibitors in acute myeloid leukemia. *Blood Rev*. 2022;52:100905. doi:10.1016/J.BLRE.2021.100905

61. Sallman D, Asch A, Kambhampati S, et al. AML-196: The First-in-Class Anti-CD47 Antibody Magrolimab in Combination with Azacitidine Is Well Tolerated

and Effective in AML Patients: Phase 1b Results. *Clin Lymphoma Myeloma Leuk*. 2021;21:S290. doi:10.1016/S2152-2650(21)01694-3

62. Stein EM, Aldoss I, DiPersio JF, et al. Safety and Efficacy of Menin Inhibition in Patients (Pts) with MLL-Rearranged and NPM1 Mutant Acute Leukemia: A Phase (Ph) 1, First-in-Human Study of SNDX-5613 (AUGMENT 101). *Blood*. 2021;138(Supplement 1):699. doi:10.1182/BLOOD-2021-146944

63. Mardiana S, Gill S. CAR T Cells for Acute Myeloid Leukemia: State of the Art and Future Directions. *Front Oncol*. 2020;10:697. doi:10.3389/FONC.2020.00697

64. Koedam J, Wermke M, Ehninger A, Cartellieri M, Ehninger G. Chimeric antigen receptor T-cell therapy in acute myeloid leukemia. *Curr Opin Hematol*. 2022;29(2):74. doi:10.1097/MOH.0000000000000703

65. Walter RB, Othus M, Burnett AK, et al. Resistance prediction in AML: analysis of 4601 patients from MRC/NCRI, HOVON/SAKK, SWOG and MD Anderson Cancer Center. *Leukemia*. 2015;29(2):312-320. doi:10.1038/LEU.2014.242

66. Thol F, Heuser M. Treatment for Relapsed/Refractory Acute Myeloid Leukemia. *Hemasphere*. 2021;5(6):E572. doi:10.1097/HS9.0000000000000572

67. Brandwein JM, Saini L, Geddes MN, et al. Outcomes of patients with relapsed or refractory acute myeloid leukemia: a population-based real-world study. *Am J Blood Res*. 2020;10(4):124. doi:10.1097/01.hs9.0000562468.89369.69

68. Zeichner SB, Gleason S, Antun AG, et al. Survival of Patients Diagnosed with Primary Refractory and Relapsed Acute Myeloid Leukemia from 2008-2012: A Single Institution Experience. *Blood*. 2015;126(23):4955-4955. doi:10.1182/BLOOD.V126.23.4955.4955

69. El Achi H, Dupont E, Paul S, Khoury JD. CD123 as a Biomarker in Hematolymphoid Malignancies: Principles of Detection and Targeted Therapies. *Cancers 2020, Vol 12, Page 3087*. 2020;12(11):3087. doi:10.3390/CANCERS12113087

70. Broughton SE, Dhagat U, Hercus TR, et al. The GM–CSF/IL-3/IL-5 cytokine receptor family: from ligand recognition to initiation of signaling. *Immunol Rev*. 2012;250(1):277-302. doi:10.1111/J.1600-065X.2012.01164.X

71. Wittwer NL, Brumatti G, Marchant C, et al. High CD123 levels enhance proliferation in response to IL-3, but reduce chemotaxis by downregulating CXCR4 expression. *Blood Adv*. 2017;1(15):1067-1079. doi:10.1182/BLOODADVANCES.2016002931

72. Barry SC, Korpelainen E, Sun Q, et al. Roles of the N and C Terminal Domains of the Interleukin-3 Receptor α Chain in Receptor Function. *Blood*. 1997;89(3):842-852. doi:10.1182/BLOOD.V89.3.842

73. Broughton SE, Hercus TR, Nero TL, et al. A dual role for the N-terminal domain of the IL-3 receptor in cell signalling. *Nat Commun*. 2018;9(1). doi:10.1038/S41467-017-02633-7

74. Hercus TR, Dhagat U, Kan WLT, et al. Signalling by the βc family of cytokines. *Cytokine Growth Factor Rev*. 2013;24(3):189-201. doi:10.1016/J.CYTOGFR.2013.03.002

75. Jegalian AG, Facchetti F, Jaffe ES. Plasmacytoid Dendritic Cells: Physiologic Roles and Pathologic States. *Adv Anat Pathol*. 2009;16(6):392. doi:10.1097/PAP.0B013E3181BB6BC2

76. Interleukin-3 receptor alpha chain (CD123) is widely expressed in hematologic malignancies - PubMed. Accessed March 9, 2023. https://pubmed.ncbi.nlm.nih.gov/11726317/

77. Testa U, Pelosi E, Frankel A. CD 123 is a membrane biomarker and a therapeutic target in hematologic malignancies. *Biomark Res*. 2014;2(1):4. doi:10.1186/2050-7771-2-4

78. Testa U, Riccioni R, Militi S, et al. Elevated expression of IL-3Rα in acute myelogenous leukemia is associated with enhanced blast proliferation, increased cellularity, and poor prognosis. *Blood*. 2002;100(8):2980-2988. doi:10.1182/BLOOD-2002-03-0852

79. Bras AE, de Haas V, van Stigt A, et al. CD123 expression levels in 846 acute leukemia patients based on standardized immunophenotyping. *Cytometry B Clin Cytom*. 2019;96(2):134. doi:10.1002/CYTO.B.21745

80. Hwang K, Park CJ, Jang S, et al. Flow cytometric quantification and immunophenotyping of leukemic stem cells in acute myeloid leukemia. *Ann Hematol*. 2012;91(10):1541-1546. doi:10.1007/S00277-012-1501-7/TABLES/4

81. Sapienza MR, Pileri A, Derenzini E, et al. Blastic Plasmacytoid Dendritic Cell Neoplasm: State of the Art and Prospects. *Cancers (Basel)*. 2019;11(5). doi:10.3390/CANCERS11050595

82. Pemmaraju N, Lane AA, Sweet KL, et al. Tagraxofusp in Blastic Plasmacytoid Dendritic-Cell Neoplasm. *New England Journal of Medicine*. 2019;380(17):1628-1637. doi:10.1056/NEJMOA1815105/SUPPL_FILE/NEJMOA1815105_DATA-SHARING.PDF

83. Guolo F, Minetto P, Kunkl A, et al. A Blastic Plasmacytoid Dendritic Cell Neoplasm-like Phenotype Identifies a Subgroup of NPM1-Mutated AML Patients

with Worse Prognosis While Has Not Predictive Value in NPM1-Wt AML. *Blood*. 2018;132(Supplement 1):5243. doi:10.1182/BLOOD-2018-99-118984

84. Guolo F, Minetto P, Clavio M, et al. Prognostic relevance of a blastic plasmacytoid dendritic cell neoplasm-like immunophenotype in cytogenetically normal acute myeloid leukemia patients. *Leuk Lymphoma*. 2020;61(7):1695-1701. doi:10.1080/10428194.2020.1737685

85. Frankel AE, Ramage J, Kiser M, Alexander R, Kucera G, Miller MS. Characterization of diphtheria fusion proteins targeted to the human interleukin-3 receptor. *Protein Eng*. 2000;13(8):575-581. doi:10.1093/PROTEIN/13.8.575

86. Frankel A, Rossi P, Kuzel T, Foss F. Diphtheria Fusion Protein Therapy of Chemoresistant Malignancies. *Curr Cancer Drug Targets*. 2005;2(1):19-36. doi:10.2174/1568009023333944

87. Jedema I, Barge RMY, Frankel AE, Willemze R, Falkenburg JHF. Acute myeloid leukemia cells in G0 phase of the cell cycle that are unresponsive to conventional chemotherapy are sensitive to treatment with granulocyte-macrophage colony-stimulating factor/diphtheria toxin fusion proteins. *Exp Hematol*. 2004;32(2):188-194. doi:10.1016/j.exphem.2003.11.005

88. Kovtun Y, Jones GE, Adams S, et al. A CD123-targeting antibody-drug conjugate, IMGN632, designed to eradicate AML while sparing normal bone marrow cells. *Blood Adv*. 2018;2(8):848-858. doi:10.1182/BLOODADVANCES.2018017517

89. Frankel AE, Hall PD, McLain C, Safa AR, Tagge EP, Kreitman RJ. Cell-specific modulation of drug resistance in acute myeloid leukemic blasts by diphtheria fusion toxin, DT388-GMCSF. *Bioconjug Chem*. 1998;9(4):490-496. doi:10.1021/BC980015A

90. Pemmaraju N, Kantarjian H, Sweet K, et al. Integrated Safety Analysis of Tagraxofusp, a CD123-Directed Targeted Therapy, in Patients with Hematologic Malignancies. *Blood*. 2021;138(Supplement 1):2318-2318. doi:10.1182/BLOOD-2021-145344

91. Shin J Il, Lee KH, Lee IR, et al. Systemic Capillary Leak Syndrome (Clarkson Syndrome) in Cancer Patients: A Systematic Review. *J Clin Med*. 2018;7(11). doi:10.3390/JCM7110418

92. Siddall E, Khatri M, Radhakrishnan J. Capillary leak syndrome: etiologies, pathophysiology, and management. *Kidney Int*. 2017;92(1):37-46. doi:10.1016/J.KINT.2016.11.029

93. Patnaik MM, Ali H, Gupta V, et al. Results from Ongoing Phase 1/2 Clinical Trial of Tagraxofusp (SL-401) in Patients with Relapsed/Refractory Chronic Myelomonocytic Leukemia (CMML). *Blood*. 2018;132(Supplement 1):1821. doi:10.1182/BLOOD-2018-99-119302

94. Yacoub A, Patnaik MM, Ali H, et al. A Phase 1/2 Study of Single Agent Tagraxofusp, a First-in-Class CD123-Targeted Therapy, in Patients with Myelofibrosis That Is Relapsed/Refractory Following JAK Inhibitor Therapy. *Blood*. 2021;138(Supplement 1):140. doi:10.1182/BLOOD-2021-145276

95. Ray A, Mo CC, DU T, et al. Novel Cytokine-Mediated Mechanism of Action Identified By Quantitative Seroproteomics in Multiple Myeloma Patients Treated with Tagraxofusp, a Novel CD123-Directed Targeted Therapy. *Blood*. 2021;138(Supplement 1):1620. doi:10.1182/BLOOD-2021-153458

96. Lane AA, Stein AS, Garcia JS, et al. Safety and Efficacy of Combining Tagraxofusp (SL-401) with Azacitidine or Azacitidine and Venetoclax in a Phase 1b Study for CD123 Positive AML, MDS, or BPDCN. *Blood*. 2021;138(Supplement 1):2346-2346. doi:10.1182/BLOOD-2021-147486

97. Results from Ongoing Phase 1/2 Trial of SL-401 As Consolidation Therapy in Patients with Acute Myeloid Leukemia (AML) in Remission with Minimal Residual Disease (MRD). *Blood*. 2017;130:2583. doi:10.1182/BLOOD.V130.SUPPL_1.2583.2583

98. Common Terminology Criteria for Adverse Events (CTCAE) v5.0. Accessed April 13, 2023. https://ctep.cancer.gov/protocoldevelopment/electronic_applications/ctc.htm#ctc_50

99. MedDRA Hierarchy. Accessed April 13, 2023. https://www.meddra.org/how-to-use/basics/hierarchy

100. Döhner H, Estey E, Grimwade D, et al. Diagnosis and management of AML in adults: 2017 ELN recommendations from an international expert panel. *Blood*. 2017;129(4):424. doi:10.1182/BLOOD-2016-08-733196

101. Marconi G, Piciocchi A, Audisio E, et al. Gimema AML1718 Part 1: Planned Interim Analysis of a Safety Run-in and Phase 2 Open-Label Study of Venetoclax, Fludarabine, Idarubicin and Cytarabine (V-FLAI) in the Induction Therapy of Non Low-Risk Acute Myeloid Leukemia. *Blood*. 2022;140(Supplement 1):1705-1707. doi:10.1182/BLOOD-2022-157393

# RINGRAZIAMENTI