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Therapeutic strategies in high-risk acute myeloid leukaemia eligible for intensive chemotherapy

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Abstract
High-risk acute myeloid leukaemia is still a therapeutic challenge. Recently, classification has been revised and identified more patients who could be integrated into this poor-risk disease. Venetoclax acts synergistically with hypomethylating agents but also with intensive chemotherapy and showed promising results specifically in the adverse risk AML population. A new formulation of intensive chemotherapy, CPX-351, is approved for this specific population, therapy-related AML and myelodysplasia-related change AML. This review focuses on the place of intensive chemotherapy in high-risk AML, including a focus on *TP53*-mutated AML.

Introduction
Standard of care for acute myeloid leukaemia is still intensive chemotherapy (IC) followed by allogeneic stem cell transplantation depending on the cytogenetic and molecular profiles of the disease. Nevertheless, a hypomethylating agent in combination with venetoclax (VEN) could be an option in patients not eligible for intensive chemotherapy and/or in patients with adverse risk based on the ELN classification. The definition of high-risk leukaemia is not well standardised but usually, we define this subgroup of patients as adverse risk based on the ELN classification. Recently, ELN classification evolved to include new molecular abnormalities such as *TP53* mutation, myelodysplasia-related gene mutations, and myelodysplasia-related cytogenetic abnormalities [1]. Complete remission (CR) rate obtained after induction chemotherapy was 67.5% with DAC (daunorubicin, fludarabine, cladribine) [2], 74% with MDACC FIA (fludarabine, cytarabine, idarubicin) [3], 82% with FLAI + GO (fludarabine, cytarabine, idarubicin, gemtuzumab ozogamycin) [4] and 84% with FLAG-IDA (fludarabine, cytarabine, idarubicin, G-CSF) [5]. Overall survival (OS) reported with DAC was 45% at 3 years, with MDACC FIA was 57% at 2 years, with FLAI + GO was 63% at 2 years and with FLAG-IDA was 44% at 8 years. The goals of improving IC in AML are efficacy in improving CR rates and irradicating measurable residual disease (MRD); safety in reducing early mortality and durability in preventing relapse and transitioning to alloHSCT if indicated. The primary goal is to decrease relapse and increase cure rates.

Venetoclax in combination with intensive chemotherapy
Some studies combined VEN with intensive chemotherapy (IC). Twenty-nine patients were treated with FLAG-IDA + VEN [6] showing 90% of composite CR (cCR), 96% of negative MRD and 1-year OS at 94%. Fifty patients were treated with CLIA + VEN [7] showing 94% cCR, 82% negative MRD and 1-year OS at 85%. Fifty-one patients were treated with « 5+2 » + VEN [8] showing 72% cCR, and a median OS at 11.2 months. A retrospective study which compared IC vs IC + VEN in 279 patients was performed [9]. No significant difference was observed in terms of overall response rate (ORR) 86% vs 95% or in terms of cCR 86% vs 91%. Nevertheless, we observed significantly more CR MRD- in IC + VEN 86% vs 61% (p=0.0028). Moreover, CR MRD- was significantly higher in ELN adverse 87% vs 48% (p=0.0059). More patients could undergo alloSCT after IC + VEN 72% vs 58% (p=0.012), and 82% vs 46% (p<0.0001) in ELN adverse AML. We observed a significant improvement with median event free survival (EFS) not reached vs 12 months (p=0.002) and a median OS not reached vs 21 months (p=0.03) in IC + VEN and IC subgroups, respectively.

CPX-351
CPX-351 is a dual-drug liposomal encapsulation of cytarabine and daunorubicin that delivers a synergistic 5:1 drug ratio into leukaemia cells to a greater extent than normal bone marrow cells. CPX-351 is approved in newly diagnosed AML-MRC (myelodysplasia-related changes) and therapy-related AML patients. In the phase 3 clinical trial, CPX-351 was associated with a higher ORR (CR/CRi) (47.7% vs 33.3%) and a higher rate of subsequent allo-SCT (34% vs 25%) vs « 3+7 [10]. We observed an increase of median OS in the global cohort 9.33 months vs 5.95 months, more pronounced in allotransplanted patients not reached vs 10.25 months in CPX-351 vs « 3+7 », respectively [11]. These results were confirmed in the real-life experience from several countries. In France, 103 patients were reported from 12 centres. ORR was 59% and 57% of patients obtained MRD <10-3. Median OS was 16.1 months and was not reached in allotransplanted patients [12]. In Italy, 71 patients were reported from 31 centres. ORR was 65% and 62.5% of patients obtained MRD <10-3. Median OS was not reached in the global cohort and in allotransplanted patients [13].

Extension of CPX-351 indication in all high-risk AML difined by ELN 2022

We designed a new study to extend CPX-351 indication to treatment-naive patients ≥50 years of age with de novo AML except t-AML or secondary AML eligible for intensive therapy. We stratified by genomic the population based on the presence of *SRSF2, SF3B1, U2AF1, ZRSR2, ASXL1, EZH2, BCOR, STAG2* or *RUNX1* mutation. Patients are randomized 1:1 between CPX-351 vs "3+7". We estimated that 48% of patients achieve MRD using leukaemia-associated immunophenotype (LAIP) <10-3 with one cycle of conventional 7+3 and the primary objective is to demonstrate a 20% increase in MRD LAIP<10-3 with CPX-351 to 68% after the first course. We plan to include 210 patients to answer to this question. This clinical trial is currently enrolling (NCT05260528).

Focus on *TP53*-mutated AML eligible for IC

TP53-mutated AML are very poor disease with a low rate of CR/CRi and a short median duration of response and OS. Any treatment compared to AZA alone or usual IC as "3+7" showed an improvement in this setting. For patients eligible for IC, we observed 29% of CR using CPX-351 and 40% using « 3+7 », with a median OS at 4.5 months using CPX-351 and 5.1 months using « 3+7 [14]. For patients not eligible for IC, even if we observed a higher rate of CR using VEN in combination with hypomethylaling agents (CR/CRi rate: 17%, 41% and 57% for AZA alone, AZA + VEN or decitabine + VEN, respectively), we didn't find an improvement in term of OS (median OS: 4.9, 5.2 and 5.2 months for AZA alone, AZA + VEN or decitabine + VEN, respectively) [15-17]. Magrolimab, an anti-CD47 antibody targeting the « don't eat me » signal interacting with macrophages showed promising results in combination with AZA alone with a CR/CRi rate at 42% and a median OS at 10.8 months [18]; and in combination with AZA + VEN showing CR/CRi rate at 64% and a median OS at 10.4 months [19]. Two randomized phase clinical trials evaluating AZA + magrolimab (ENHANCE-2, NCT04778397) and AZA + VEN + magrolimab (ENHANCE-3, NCT04435691) were designed and enrolled some patients but FDA halted them following negative results of ENHANCE (NCT04313881) clinical trial in myelodysplastic syndromes and about safety concerns.

Conclusions

To summarize, IC needs to be improved in high-risk AML. Venetocax seems to be a good partner to IC. CPX-351 showed a better response, OS and better safety and should be a backbone for the development of new combinations in this setting. Molecular high-risk AML defined by ELN 2022 could be considered in future clinical trials as ALFA 2101. MRD could guide therapy including allogeneic stem cell transplantation as part of the strategy for intensively treated patients. Unfortunately, treatment of *TP53*-mutated AML remains a challenge.

Conflict of interest

The author declares a conflict of interest with Jazz Pharma, Abbvie, BMS/Celgene and Gilead.

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Acute myeloid leukaemia: First debate on leukaemia biology

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Abstract
The contribution of the leukaemic microenvironment (LME) for acute myeloid leukaemia (AML) development and maintenance is gaining much and increasing interest. A better understanding of the cellular interactions operating in the LME is likely to improve the effectiveness of immunotherapy approaches and strategies, whose clinical applications have been globally unsatisfactory so far. During EUROLEUK 2023, the first debate was focused on the immunobiology of AML. An outstanding panel of speakers addressed the crucial questions regarding the interactive network operating in the AML microenvironment and pointed out some of the most relevant biological mechanisms underlying the induction of immune tolerance through the generation of highly suppressive T regulatory cells along with the expansion of exhausted and senescent effector T cells. A special focus was dedicated to the lesson from allogeneic hematopoietic stem cells (alloSCT), where a large body of evidence indicates the compelling role of immune activation as part of the successful outcome of alloSCT, but also as a critical mechanism leading to leukaemia evasion from immunological pressure.

Acute myeloid leukaemia (AML) is the most common leukaemia in adults representing about 3% of all cancer cases and 25% of all leukemias. The average age at diagnosis is about 68 years, with an estimated 5-year survival rate of around 7% in patients over 65, compared to 45% in younger patients [1]. AML is a genetically heterogeneous clonal disease deriving from a rare bone marrow (BM) leukaemia stem cell (LSC) population with identifiable somatic mutations in 97.3% of all cases [2]. Regarding its onset, AML may be subdivided into de novo (primary) AML, therapy-related AML (t-AML) and secondary AML (sAML), the latter emerging alongside previous hematologic disorders, such as myeloproliferative neoplasms or myelodysplastic syndromes (MDS) and representing a model for understanding the transition from normal hematopoiesis to AML development [3]. Besides age and comorbidities, the genetic profile of leukaemia cells has played and still plays an essential role as a prognostic factor and a key predictive parameter for selecting the type and intensity of induction and post-induction therapy [4]. However, the molecular characterization of leukaemia cells does not always translate into genetic-targeting clinical actions. Consequently, most patients receive standard-of-care treatments, accounting for most cases of chemotherapy combinations, unchanged since 1973 [5]. In this scenario, despite some crucial advances in the understanding of the biology of AML and the recent approval of novel strategies, such as venetoclax and hypomethylating agent combinations in chemotherapy-ineligible patients [6], the overall prognosis of AML patients is still poor due to the high rate of relapse, which translates into a small number of long-term survivors. Cell-intrinsic mechanisms of resistance to therapy result in the persistence of LSCs, hampering complete remission and leading to relapse. However, heterogeneous clinical response is observed even within the same molecular subgroup [7], suggesting that additional factors beyond genetic background are causative of patient outcomes. In that, the contribution of the leukaemic microenvironment (LME) for AML development and maintenance is gaining much and increasing interest [8].

The LME comprises a complex network of immune and non-immune cells. The immune compartment of LME is mainly composed of T effector (Teff) and regulatory (Treg) cells, NK cells, dendritic cells (DCs), innate lymphoid cells (ILCs),

myeloid-derived suppressor cells (MDSCs), macrophages, all potentially contributing to leukaemia cell development, proliferation, and survival [9]. In AML, aberrant cytokine production and a profound dysregulation of the frequency and function of immune cell subsets have been described. In particular, increased immune-suppressive cell populations, e.g. Tregs and MDSCs, induce a specific milieu that interferes with the anti-leukaemia immune response, favouring immune escape and limiting the response to therapy [10]. In turn, leukaemia cells are known to shape and remodel the immune microenvironment by modulating the expression of immune checkpoint inhibitors on T cells, secreting immune-inhibitory soluble factors and increasing the local and systemic metabolite composition [11].

Non-immune cellular elements, i.e., endothelial, stromal, and osteoprogenitor cells, also contribute to the dysregulation of the BM microenvironment and malignancy progression, concomitantly with the accumulation of driver mutations in hematopoietic stem cells (HSCs) [12]. Among non-immune BM microenvironment components, mesenchymal stromal cells (MSCs) are well-known regulators of HSC differentiation and BM structural architecture. MSC dysregulation has been considered a crucial step in leukaemogenesis and BM dysfunction [13]. MSCs support leukaemia cell survival and metabolic requirements and tune the anti-tumour immune response and responsiveness to treatments through different mechanisms, including cell-to-cell contact, exosome production, and secreted factors [14]. In turn, leukemia cells remodel the transcriptome, proteome, and function of MSCs contributing to an immunosuppressive AML cell-protective phenotype [15]. BM vascular architecture and function are also altered in AML with increased permeability, altered perfusion, and release of normal HSCs to the periphery.

Collectively, a compelling body of evidence has provided an extensive characterization of the biological processes occurring in the AML microenvironment. However, a comprehensive understanding of the cellular and functional interactome within LME and between leukaemia cells and LME is far from settled. This knowledge gap may explain why, despite their potential to circumvent some of the cell-intrinsic resistance mechanisms to conventional therapies, immune system-centered therapeutic interventions aimed at harnessing the immune system against AML have led to unsatisfactory and disappointing clinical results.

During EUROLEUK 2023, the first debate was focused on the immunobiology of AML. An outstanding panel of speakers addressed the crucial questions regarding the interactive network operating in the AML microenvironment and pointed out some of the most relevant biological mechanisms underlying the induction of immune tolerance through the generation of highly suppressive T regulatory cells along with the expansion of exhausted and senescent effector T cells. The latter may represent a hallmark of some subtypes of AML, especially those showing adverse molecular and cytogenetic features at diagnosis, tightly interconnected with a wide spectrum of inflammatory modifications that have been reported in the bone marrow of AML patients. In that, a better understanding of the transition from myelodysplastic syndrome to AML from the perspective of an immune tolerogenic microenvironment was discussed as a potential paradigm to be considered for a wider and comprehensive characterisation of the immune landscape of myeloid malignancies. The contribution of somatic leukaemia cell-intrinsic driver mutations in the creation of an immunosuppressed and inflamed leukaemia microenvironment is also emerging as a crucial point, which indicates the need to integrate the genomic profile of clonal cells with the characterisation of the immune microenvironment as part of a novel approach to AML classification and risk-adapted stratification systems. Finally, the lesson from allogeneic hematopoietic stem cells (alloSCT) was part of the debate. A large body of evidence indicates the compelling role of immune activation as part of the successful outcome of alloSCT, but also as a critical mechanism leading to leukaemia evasion from immunological pressure, ultimately leading to relapse. An integrated approach, which combines omics technologies and a functional approach, is revealing the interdependence of clonal and immune cells in transplanted AML patients.

Conflict of interest

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Drug-based maintenance strategies post-allogeneic stem cell transplantation – are we there yet (and will we be)?

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Abstract
Maintenance therapy following allogeneic stem cell transplantation is increasingly being seen as a potential integral part of the treatment of hematologic malignancies such as acute myeloid leukaemia and acute lymphoblastic leukaemia. There is considerable interest in maintenance therapy using targeted drugs where applicable targets are available, such as *FLT3*- and *IHD*-mutations, as well as non-specific therapies aiming to either exert a direct anti-leukaemic effect or enhance the graft-versus-leukaemia effect. This review will discuss the background for these strategies and highlight the most promising targets of treatment.

Introduction
Allogeneic stem cell transplantation (ASCT) is the mainstay of consolidation therapy for a variety of malignant hematologic diseases, including acute myeloid leukaemia (AML) myelodysplastic syndromes and acute lymphoblastic leukaemia (ALL). During the last decades, ASCT has become safer with the advent of reduced intensity conditioning regimens and improved supportive care. With decreasing transplant related mortality, relapse is the most common cause of treatment failure in AML after ASCT. About 20–30% relapses occur within in the first 1–2 years following ASCT and in relapsed AML the 1-year survival is less than 20% with limited treatment options [1,2].

We have seen promising developments with novel opportunities to manipulate the anti-leukaemic effect of the graft after transplant and ASCT is no longer seen as a static mode of treatment, but rather as part of the treatment continuum making therapy safer and reducing the risk of relapse. This can be done using the conditioning regimen, post-transplant immunosuppressive strategies and both drug-based and immunotherapeutic strategies in order to induce a direct anti-leukaemic effect through the elimination of any residual leukaemia as well as stimulate the graft-versus-leukaemia (GvL) effect without increasing the risk of GvHD.

Drug based maintenance - What's on the shelf?
During the last years several drugs have become available, mainly in clinical trials for post-transplant maintenance aiming to reduce the risk of relapse after ASCT. The most widely studied class of drugs are the epigenetic modifying agents, such as azacytidine, decitabine and panobinostat. With the availability of novel targeted agents, such as FLT3-inhibitors, IDH-inhibitors, Bcl-2 inhibitors and menin-inhibitors, these have been incorporated into clinical trials (NTC04027309, NCT03839771, NCT04065399, NTC02400255) [3]. The FLT3-inhibitor sorafenib has been widely used [4,5] and results from novel agents in clinical trials are awaited.
The goal of post-transplant drug-based maintenance is to enhance the anti-leukaemic activity with the capability of eradicating emerging resistant clones, manipulate the kinetics of relapse and delay the requirement for DLI as well as augment the alloreactive effect with an acceptable safety profile, especially with regard to cytopenias, infections, GVHD, and NRM.

Targeting the epigenetic pathways – azacytidine, decitabine and panobinostat
Azacytidine has known anti-leukaemic activity *in vitro* and *in vivo*. Early preclinical animal models with azacytidine

demonstrated both direct anti-leukaemic activity as well as activation of CD8+ tumour-specific T cells through upregulation of aberrant methylated leukemic antigens on leukemic cells and augments regulatory T cell activity potentially reducing the risk of GvHD in murine models [6].

With the well-known and acceptable safety profile, azacytidine and decitabine are the most widely studied epigenetic modifying drugs post-allotransplant [7]. The trials have often had limited numbers of patients, making the results difficult to interpret with confidence. Maintenance with oral azacitidine (Onureg) in the QUAZAR-AML-001 trial [8]. showed an improved overall survival and relapse-free survival after induction chemotherapy in older patients with AML in CR. This has prompted larger trials using oral azacytidine after ASCT for AML and MDS and the randomized placebo-controlled AMADEUS phase III trial (NCT04173533) is fully recruited and results will be expected soon.

Histone deacetylase inhibitors, such as panobinostat, have been shown to have both direct and immunomodulatory activity. Promising results were reported in the phase I/II PANOBEST trial in 42 patients with high-risk AML or MDS in CR after ASCT with 2-year overall survival (OS) and relapse-free survival are 81% and 75%, respectively. The cumulative incidence of relapse and non-relapse mortality across all dose levels was 20% and 5% [9]. The confirmatory phase III ETAL-4/HOVON-145 trial (NCT04326764) was prematurely stopped.

Bcl2-inhibitors - venetoclax

Venetoclax has provided a novel backbone for the treatment of AML in a significant proportion of patients with promising CR rates and an excellent safety profile. Following this development, we are now seeing several studies investigating the safety and feasibility of venetoclax with or without the combination with hypomethylating agents after allotransplantation. The main side effect causing some concern in this setting is the marrow suppression with cytopenias complicating dosing.

A trial by Kent et al looking at Venetoclax post-transplant reported 6-month OS and progression-free survival (PFS) of 87% with Venetoclax 400 mg daily for 1 year. However, 11% of patients discontinued due to adverse effects or transplant complications and half required dose interruption and adjustments due to side effects including cytopenias and GI-related side effects [10[. Wei and colleagues reported a 2-year OS of 85.2% and 2-year EFS of 84.7% with venetoclax 200 mg/day and low-dose decitabine in a phase II study in 20 patients [11]. The randomized phase 3 VIALE-T trial is currently recruiting (NCT04161885).

IDH inhibitors

The IDH1-inhibitor has received FDA approval in combination with azacytidine for patients >75 years old or unfit for intensive chemotherapy. Enasidenib is approved for *IDH2*-mutated relapsed or refractory AML. Both drugs have shown a favourable safety profile in relapsed AML and several post-transplant studies are ongoing (NCT03564821, NCT03515512, NCT03728335, NCT04522895).

Fathi et al reported results from a phase I trial of ivosidenib maintenance following ASCT for *IDH1*-mutated AML. Treatment was initiated between days 30 and 90 and given for up to 12 28-day cycles at a recommended phase 2 dose of 500 mg daily in 16 patients. The 2-year cumulative incidences of relapse and NRM were 19% and 0%, respectively. The 2-year PFS was 81%, and the 2-year OS was 88%, showing safety and tolerability as well as promising PFS and OS in this setting [12].

Fathi et al also reported results with the IDH2-inhibitor enasidenib post-transplant for 19 patients with *IDH2*-mutated myeloid malignancies. Two-year PFS and OS were 69% (95% CI: 39-86%) and 74% (95% CI, 44-90%), respectively [13].

FLT3 inhibitors

FLT3 mutations (*FLT3-ITD* and *FLT3-TKD*) are among the most common genetic molecular abnormalities in patients with AML, resulting in uncontrolled proliferation of leukemic blasts [14,15]. There is increasing evidence that measurable residual disease (MRD) predicts an increased risk of relapse after allogeneic stem cell transplantation16 and there is great interest in approaches to eradicate residual disease post-transplantation with drugs to enhance the GvL effect or with tyrosine kinase inhibitors targeting the *FLT3* mutation.

The SORMAIN trial with post-transplant sorafenib maintenance was pivotal in showing a reduced risk of relapse. In this trial, 83 patients were randomized to sorafenib or placebo starting 60 to 100 days post-transplant for 24 months duration or until disease progression or intolerability. The 2-year RFS of 85 % vs 53,3% in the sorafenib and placebo groups, respectively. There was a higher rate of acute and chronic GVHD in the sorafenib group (76.8%) versus the control group (59.8%) [4]. More recent data seem to reproduce these findings in an open-label, randomized phase III trial [5]. This trial administered sorafenib for a shorter duration than recommended by current consensus guidelines and included exclusively patients aged 18–60 years and myeloablative conditioning regimens.

Midostaurin is approved for first-line treatment of FLT3+ AML and maintenance after chemotherapy. The AML-SG 16–10 and Radius trials looked at the impact of midostaurin as maintenance therapy post-transplant. The RADIUS trial showed that midostaurin could be safely added to standard-of-care after ASCT and improved RFS at 18 months after ASCT. The trial was not powered to detect treatment difference, but showed a trend toward benefit with midostaurin [17]. In the AML-SG 16–10 trial, midostaurin was given during induction chemotherapy and as a 1-year maintenance. Results were compared with a historical cohort of 415 patients treated on 5 prior AMLSG trials and with patients treated on the placebo arm of the Cancer and Leukemia Group B (CALGB) 10603/RATIFY trial. In comparison with historical controls, the addition of midostaurin to intensive therapy led to a significant improvement in outcomes in younger and older patients with AML and FLT3-ITD [18].

Gilteritinib is a novel FLT3-inhibitor and has been approved for the treatment of relapsed and refractory FLT3+ AML. Ongoing trials include maintenance post-induction (NCT04027309). The MORPHO trial studied gilteritinib as post-transplant maintenance for *FLT3-ITD* AML [3]. Although the trial did not achieve its primary study endpoint of relapse-free survival at the primary analysis, the data prospectively demonstrated a correlation between MRD and survival in post-HCT therapy in *FLT3-ITD* AML, thus providing important data for the rationale of MRD-guided therapy for these patients. In MRD+ patients pre- or post-transplant 43 out of 91 patients relapsed in the placebo group versus 26 out of 89 patients in the gilteritinib group.

Menin-inhibitors

One of the currently most promising classes of drugs are the menin-inhibitors. These drugs stop the genes affected by altered *KMT2A* or *NPM1* from being expressed and have significant anti-leukaemic activity in these subgroups of AML [19]. In the AUGMENT-101 trial for R/R AML with *KMT2A* rearrangement, 9 patients resumed revumenib. CRc was maintained in 6 of 9 patients on maintenance. One patient with reported MRD after HSCT converted to MRD-negative status following initiation of revumenib maintenance therapy. Long-term responses, including conversion to MRD-negative status, were seen in heavily pre-treated patients with AML with a safety profile consistent with that previously reported for the AUGMENT-101 study [20].

Conclusion

There is increasing evidence that transplant outcomes may be improved with the use of post-transplant interventions, including drugs with anti-leukaemic activity or with the ability to enhance the GvL effect. The choice of treatment must be tailored to each patient's disease and transplant characteristics, including the risk of toxicity of drug-drug interactions, active GvHD and/or active infections. Some high-risk mutations with a historically poor prognosis, like *FLT3-ITD*, have targeted therapy options that may prove valuable in reducing the risk of relapse. This may be true also for *KMT2A*- and *MLL*-mutated AML with novel menin-inhibitor.

It seems important to start early, enabling the eradication of remaining leukaemic cells and/or accelerating GVL reconstitution. The duration of maintenance is yet to be determined and the question of monotherapy vs combination therapy is also not clear. Also, the financial burden of adding high-cost treatment after ASCT can be an issue. Patients need to be enrolled in clinical trials whenever possible.

Conflict of interest

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Definitions of acute myeloid leukaemia and their clinical significance according to the WHO 2022 and ICC classification

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Abstract

In 2022, two concurrent classification systems for acute myeloid leukaemia (AML) were introduced, replacing the 2016 version of the World Health Organization (WHO). While the WHO 2022 and the International Consensus Classification (ICC) exhibit significant overlap, distinctions arise in certain key aspects of AML definition and specific subgroups. Notably, the blast threshold defining AML differs between the two systems, with the new entity MDS/AML replacing the 2016 WHO myelodysplastic syndrome with elevated blasts-2 according to the ICC, but not in the WHO 2022 classification. Moreover, in the WHO 2022 system, AML-defining genetic aberrations still permit the diagnosis of AML regardless of the blast count in bone marrow or blood. In contrast, the ICC introduces a 10% threshold for this category. Consequently, in a small number of cases, the diagnosed myeloid neoplasm may differ between the two classifications. The most notable distinctions in subgroup definitions involve AML with myelodysplasia-related aberrations, including *TP53*, as well as AML with *CEBPA* mutations. This review examines the significant changes from the 2016 to the 2022 classification systems, highlighting their similarities and differences and discussing the ensuing implications for clinical practice.

Introduction

For many years, acute myeloid leukaemia (AML) has been classified according to the World Health Organization (WHO) classification of tumours, which integrates clinical features, morphology, immunophenotyping, and genetics [1–5]. Our understanding of the biology of hematologic neoplasms continuously evolves, leading to revisions of the classification every 5 to 10 years [6].

To ensure widespread use and acceptance of the third edition, released in 2001, the WHO collaborated with the Society for Hematopathology, the European Association for Haematopathology and a Clinical Advisory Committee(CAC)comprising leading pathologists, oncologists, haematologists, and geneticists. This collaboration extended for the fourth (2008) and revised fourth (2016) edition [7]. In an effort to streamline the selection of editors and authors for the fifth edition, released in 2022, the WHO opted for a process called informed bibliometrics instead of the CAC[6]. However, some contributors to earlier editions deemed the CAC still necessary and organised one in September 2020. The findings of this CAC were not included in the fifth WHO edition but were published separately in the International Consensus Classification of Myeloid and Lymphoid Neoplasms (ICC) [7,8]. Consequently, there are now two competing classification systems for myeloid neoplasms, which complicates accurate patient diagnoses [2,8].

In this context, we discuss the similarities and differences between the two 2022 classifications for AML, and provide a summary of previously published data assessing both classifications in real-world cohorts. Finally, we address their implications for the European LeukemiaNet (ELN) 2022 risk classification, the most commonly used risk stratification system in AML.

2022 Classifications of AML – Similarities and differences

Boundary between MDS and AML

According to the WHO 2016 classification, AML was defined if the blast percentage in peripheral blood (PB) or bone marrow (BM) was equal to or exceeded 20%. Exceptions were defined for genetic abnormalities like core-binding factor AML or acute promyelocytic leukaemia (APL), which have been previously considered as AML regardless of the blast count [9]. If the blast proportion exceeded 5% in PB or 10% in BM, but did not reach the 20% cut-off, an MDS with excess blasts 2 (MDS-EB2) was diagnosed, especially when BM dysplasia or PB cytopenias were present [1].

Because of the increasing recognition of shared common pathogenic mechanisms between MDS and AML, and the high observer-dependency of blast enumeration [2], the blast cut-off that defines the boundary between both diseases was re-assessed in the WHO 2022 classification and the ICC. In the end, both classifications retained 20% blasts to define AML, but highlighted the biologic continuum between MDS and AML, and the need to offer patients a broader range of therapeutic approaches [2,7]. Therefore, the ICC introduced the new category of MDS/AML (defined by a blast proportion of 10-19% in PB or BM), replacing the former MDS-EB [2,7]. This group is further divided into MDS/AML with mutated *TP53*, MDS/AML with myelodysplasia-related (MR) gene mutation, MDS/AML with MR cytogenetic abnormalities and MDS/AML not

otherwise specified (NOS). In contrast, the WHO 2022 classification rejected to lower the blast cut-off to define AML with the argument of the risk of overtreatment and the mere replacement of one arbitrary cut-off by another [2]. Subsequently, the WHO 2022 retained the previous blast thresholds of 5-19% in PB and 10-19% in BM for MDS-EB2, but renamed it to myelodysplastic neoplasm with increased blasts 2 (MDS-IB2) to acknowledge the neoplastic behaviour of the disease [2].

With the newly defined ICC entity MDS/AML, the question arose whether these patients should be risk-stratified according to the MDS (i.e. Molecular International Prognostic Scoring System [IPSS-M]) or AML risk classification (i.e. ELN 2022), which was assessed by Huber et al. in a cohort of 137 MDS/AML patients [10]. For both classifications, there was a clear tendency towards higher risk groups with 10% of patients having moderate high, 29% high, and 45% very high IPSS-M risk, and 9% of patients having intermediate, and 91% adverse ELN 2022 risk, driven by the high incidence of MR gene mutations defining ELN 2022 adverse risk. According to the IPSS-M, there was a clear outcome separation, and overall survival (OS) was comparable to that of a published MDS cohort. In contrast, while OS still differed according to the ELN 2022, outcomes were significantly better than in a published AML cohort. The authors concluded that in MDS/AML patients, the IPSS-M should remain the preferred risk stratification system [10].

Table 1. Comparison of AML with defining genetic abnormalities according to the WHO 2022 classification and the ICC, as defined in the published recommendations [2,7]

WHO 2022	ICC
AML with defining genetic abnormalities*	
APL with *PML::RARA* fusion	APL with t(15;17)(q24.1;q21.2)/*PML::RARA* APL with other *RARA* rearrangements**
AML with *RUNX1::RUNX1T1* fusion	AML with t(8;21)(q22;q22.1)/*RUNX1::RUNX1T1*
AML with *CBFB::MYH11* fusion	AML with inv(16)(p13.1q22) or (16;16)(p13.1;q22)/ *CBFB::MYH11*
AML with *DEK::NUP214* fusion	AML with t(6;9)(p22.3;q34.1)/*DEK::NUP214*
AML with *BCR::ABL1* fusion*	AML with t(9;22)(q34.1;q11.2)/*BCR::ABL1**
AML with *KMT2A* rearrangement	AML with t(9;11)(p21.3;q23.3)/*MLLT3::KMT2A* AML with other *KMT2A* rearrangements**
AML with *MECOM* rearrangement	AML with inv(3)(q21.3q26.2) or t(3;3)(q21.3;q26.2)/*GATA2; MECOM(EVI1)* AML with other *MECOM* rearrangements**
AML with *NPM1* mutation	AML with mutated *NPM1*
AML with *RBM15::MRTFA* fusion	-
AML with *NUP98* rearrangement	-
AML with other defined genetic alterations	AML with other rare recurring translocations
AML with *CEBPA* mutation*	AML with in-frame bZIP *CEBPA* mutations
AML, MR*	**AML (≥ 20%) and MDS/AML (10-19%) with mutated *TP53*** **AML (≥ 20%) and MDS/AML (10-19%) with MR gene mutations** **AML (≥ 20%) and MDS/AML (10-19%) with MR cytogenetic abnormalities**
AML, defined by differentiation	***AML, not otherwise specified***
AML with minimal differentiation	-
AML without maturation	-
AML with maturation	-
Acute basophilic leukaemia	-
Acute myelomonocytic leukaemia	-
Acute monocytic leukaemia	-
Acute erythroid leukaemia	-
Acute megakaryoblastic leukaemia	-

Relevant differences are highlighted in bold.

* In patients with defining genetic abnormalities, no blast cut-off (WHO 2022) or a 10% cut-off (ICC) is sufficient to diagnose AML. Exceptions remain the marked cases, i.e. AML with *BCR::ABL1* fusion and AML with *CEBPA* mutation (according to WHO 2022) as well as AML with t(9;22)(q34.1;q11.2)/*BCR::ABL1* (according to ICC), in which at least 20% blasts have to be present to diagnose AML.

** In these subgroups the ICC define specific rearrangements:

For APL with other *RARA* rearrangements: t(1;17)(q42.3;q21.2)/*IRF2BP2::RARA*; t(5;17)(q35.1;q21.2) /*NPM1::RARA*; t(11;17)(q23.2;q21.2)/*ZBTB16::RARA*; cryptic inv(17q) or del(17)(q21.2q21.2) / S*TAT5B::RARA,STAT3::RARA*; Other genes rarely rearranged with *RARA*:*TBL1XR1* (3q26.3), *FIP1L1* (4q12), *BCOR* (Xp11.4).

For AML with other *KMT2A* rearrangements: t(4;11)(q21.3;q23.3)/*AFF1::KMT2A*; t(6;11)(q27;q23.3)/ *AFDN::KMT2A*; t(10;11)(p12.3;q23.3)/*MLLT10::KMT2A*; t(10;11)(q21.3;q23.3) / *TET1::KMT2A*; t(11;19)(q23.3;p13.1)/*KMT2A::ELL*; t(11;19)(q23.3;p13.3)/*KMT2A::MLLT1*.

For AML with other *MECOM* rearrangements: t(2;3)(p11~23;q26.2)/*MECOM*::?; t(3;8)(q26.2;q24.2)/ *MYC,MECOM*; t(3;12)(q26.2;p13.2)/ETV6::*MECOM*; t(3;21)(q26.2;q22.1)/*MECOM::RUNX1*

Abbreviations: AML, Acute myeloid leukaemia; ICC, International Consensus Classification, MR, Myelodysplasia-related; WHO, World Health Organization.

AML defined by genetics

With the growing understanding of the pathogenesis and biology of AML the WHO 2022 classification as well as the ICC extended the list of AML-defining genetic abnormalities (Table 1) [2,7]. In comparison to the defined sub-classification of AML in the WHO 2016, the WHO 2022 classification broadened existing groups with defined gene fusions to also incorporate rare fusion partners. This affects APL with t(15;17)(q24.1;q21.2)/PML::RARA (now APL with *PML::RARA* fusion), as well as AML with t(9;11)(p21.3;q23.3); *MLLT3-KMT2A* (now AML with *KMT2A* rearrangement) and AML with inv(3)(q21.3q26.2) or t(3;3)(q21.3;q26.2); *GATA2, MECOM* (now AML with *MECOM* rearrangement). All may have a variety of fusion partners and can be cryptic on conventional karyotyping.2 Additionally, the WHO 2022 classification now recognizes AML with *RBM15::MRTFA* fusion (formerly *RBM15::MKL1*) and AML with *NUP98* rearrangement as independent subgroups [2]. In contrast, the ICC still retained the cytogenetically defined WHO 2016 subgroups, but added subgroups of APL with other defined *RARA*, and AML with other *KMT2A*, or *MECOM* rearrangements [7]. Furthermore, both classifications introduced a new subgroup of "AML with other defined genetic alterations" (WHO 2022) [2,7], or "AML with other rare recurring translocations" (ICC) [7], providing a place for previously unknown AML entities.

With regard to molecular abnormalities, both classifications acknowledge AML with *NPM1* mutation and AML with *CEBPA* mutation as distinct entities. However, the latter was changed from AML with bi-allelic *CEBPA* mutations in the WHO 2016 classification [1], to AML with *CEBPA* mutations in the WHO 2022 classification [2], which comprises bi-allelic mutations as well as single mutations in the basic leucine zipper (bZIP) region of *CEBPA*. Within the ICC, this group only includes AML with in-frame *bZIP* mutations and is named accordingly [7].

As mentioned before, both classifications re-assessed the blast threshold to define AML, which was also done for the group of AML with defining genetic abnormalities. Within the WHO 2022, no blast count is necessary to diagnose AML with defining genetic abnormalities, with two exceptions: AML with *BCR::ABL1* (to avoid an overlap with chronic myeloid leukaemia in myeloid blast phase), and AML with *CEBPA* mutation (due to insufficient data)[2]. According to the ICC, a blast count of at least 10% was kept for AML with defining genetic abnormalities - again except for AML with t(9;22)(q34.1;q11.2)/*BCR::ABL1*, which still requires 20% for AML diagnosis [7].

AML-MR

The WHO 2016 entity of AML with myelodysplasia-related changes (MRC), whose diagnosis was made by detection of at least 50% dysplastic cells in more than one cell line, a history of antecedent MDS or the presence of MR cytogenetic abnormalities, was also refined [1]. Both WHO 2022 and ICC removed morphology as a diagnostic criterion, but added a panel of MR gene mutations to define AML-MR [2,7]. In addition, the WHO 2022 retained the history of MDS or myelodysplastic/myeloproliferative neoplasm overlap (MDS/MPN) as a diagnostic criterion for this group, which the ICC did not.

While in the WHO 2022 classification AML-MR represents a single category summarizing cytogenetic and molecular abnormalities [2], the ICC further subcategorizes into three groups – AML with mutated *TP53*, AML with MR gene mutations, and AML with MR cytogenetic abnormalities [7]. Because of its aggressive behaviour and dismal prognosis, AML with mutated *TP53* is recognized as a separate entity by the ICC, for which a mutation with a variant allele frequency (VAF) ≥10% must be present [7]. In contrast, the WHO 2022 does not include *TP53* mutations as a diagnostic criterion for AML-MR.

Several other gene mutations, i.e. in *ASXL1, BCOR, EZH2, SF3B1, SRSF2, STAG2, U2AF1* and *ZRSR2* are now acknowledged to define AML-MR and included in both classifications [2,7]. Additionally, the ICC incorporated *RUNX1* mutations into AML-MR, replacing the provisional WHO 2016 entity AML with mutated *RUNX1* [7]. The WHO 2022 completely omitted this entity because of the lack of sufficient unifying characteristics [2]. However, due to the frequent co-occurrence of other MR gene mutations, the majority of *RUNX1* mutated cases remain in the category AML-MR [11].

With regard to cytogenetic abnormalities, both classifications updated their definition criteria, which are summarized in Table 2 [2,7]. Of note, only the WHO 2022 recognized monosomy 13 or del(13q), as well as del(11q) as aberrations justifying the diagnosis of AML-MR [2], whereas only the ICC acknowledged trisomy 8 and del(20q) [7].

While MR gene mutations were included in both 2022 classifications based on their ability to more precisely define this specific subgroup than morphology alone, they also seem to have a prognostic significance in AML. In general, a shorter event-free survival and OS for individuals with these mutations was described [12], especially within the ELN 2017 intermediate risk group [12]. Further analyses suggested that also the number and VAF of gene mutations are relevant, as patients with one MR gene mutation or lower VAFs had longer OS compared to patients with more than one MR mutation or higher VAFs [13–15]. However, the adverse prognostic impact of MR gene mutations might be abrogated by an allogeneic hematopoietic stem cell transplantation (HSCT). In this context, a longer OS and relapse-free survival (RFS) was observed in de novo AML patients with at least one MR gene mutation who underwent HSCT, compared to patients after chemotherapy consolidation [16]. Other studies suggested a benefit for HSCT in a time-dependent Cox regression analysis [15], or a very favourable 2-year OS of 77% in *SRSF2*-mutated AML patients after HSCT [17]. In addition, in patients that underwent an allogeneic HSCT, those classified as adverse ELN 2022 risk due to the presence of an MR gene mutation had the most favourable outcome within the adverse risk group, which rather resembled that of patients classified as intermediate risk [18].

While overall, *TP53* mutations were recognized to provide adverse risk in myeloid neoplasm, recent data in MDS suggested especially dismal outcomes in the presence of a *TP53* multi-hit status (defined as ≥2 *TP53* mutations, a single *TP53* mutation together with a cytogenetic deletion of the *TP53* locus, a VAF ≥ 50%, or a copy-neutral loss of heterozygosity at the TP53 locus) [19]. Therefore, the WHO 2022 and ICC only included multi-hit *TP53* mutations in the group of MDS with bi-allelic *TP53* inactivation or MDS with mutated *TP53*, respectively [2,7]. In contrast, in AML, also the presence of monoallelic TP53 alterations showed poor outcomes [20,21], which was only worsened by a co-occurring complex karyotype [20,21]. Finally, patients with mutant *TP53* seem to have dismal outcomes, irrespective of whether MDS-IB or AML was the underlying myeloid neoplasm, which again underlines the biologic continuity between both diseases [20,21].

AML not defined by genetics

Due to the increasing knowledge regarding the genetic abnormalities in AML, this subgroup gradually decreased over time. However, there are still some AML cases without a defined genetic driver. According to the WHO 2016, these cases were regarded as AML-NOS and subcategorized based on the degree and type of differentiation [1]. In the WHO 2022 classification this was retained, and only the term AML-NOS was replaced by AML, defined by differentiation (Table 1) [2]. In contrast, in the ICC the term AML-NOS was kept, but the subcategorization was omitted due to its limited prognostic significance [7].

Table 2. Comparison of AML-MR defining cytogenetic abnormalities according to the WHO 2022 classifcation and the ICC, as defined in the published recommendations [2,7]

WHO 2022	ICC 2022
Complex karyotype (≥ 3 abnormalities)	
del(5q) or t(5q)	del(5q), t(5q) or **add(5q)**
-7, del(7q) or **t(7q)**	-7, del(7q)
-	**+8**
del(11q)	-
del(12p) or t(12p)	del(12p), t(12p) or **add(12p)**
-13 or del(13q)	-
del(17p) or **t(17p)**	**-17**, del(17p) or **add(17p)**
i(17q)	
-	**del(20q)**
idic(X)(q13)	

Relevant differences are highlighted in bold.

Abbreviations: AML, acute myeloid leukaemia; ICC, International Consensus Classification, MR, Myelodysplasia-related; WHO, World Health Organization.

Of note, acute erythroid leukaemia (AEL), previously named pure erythroid leukaemia [1], remains an entity in the WHO 2022 classification, because of its aggressive phenotype with a high prevalence of bi-allelic *TP53* mutations. In contrast, in the ICC, AELs are usually included in the newly introduced subcategory of AML-MR (with mutated *TP53*) [7].

Hierarchy

In the WHO 2022 classification, AML with defining genetic abnormalities supersedes AML defined by differentiation with the exception of AEL, which is prioritised over AML-MR due to its distinctive morphologic feature, regardless of de novo or secondary origin [2]. Similarly, in the ICC, AML with defining genetic abnormalities take precedence over AML-MR and AML-NOS. In addition, the group of AML-MR is further divided and prioritised as follows: AML with mutated *TP53*, AML with MR gene mutations, and AML with MR cytogenetic abnormalities (Figure 1) [7].

Diagnostic qualifiers

In the WHO 2016 classification, myeloid neoplasm (i.e. AML, MDS, MDS/MPN) occurring therapy-related or with germline predisposition were regarded as separate entities [1]. In the WHO 2022 classification, these categories are re-named into "post cytotoxic therapy" and "associated with germline variant", respectively [2], and now re-considered as disease qualifiers that should be added to the disease type and classification. In this way, the substantial overlap to genetically defined AML subgroups, which better reflect the disease biology and individual risk, becomes more emphasized. Furthermore, exposure to PARP inhibitors was introduced as a qualifying criterion for AML post-cytotoxic therapy due to its link to complex karyotypes and mutations in DNA damage repair genes like *TP53* or *PPM1D* [22].

Also, the ICC eliminated the prior standalone entities therapy-related myeloid neoplasm and myeloid neoplasm with germline predisposition, but recognizes them as a qualifier to the diagnosis, naming them "therapy-related" and "germline predisposition" [7]. In contrast to the WHO 2022, also the history of MDS or MDS/MPN is highlighted as a qualifier and named "progressing from MDS" and "progressing from MDS/MPN" [7].

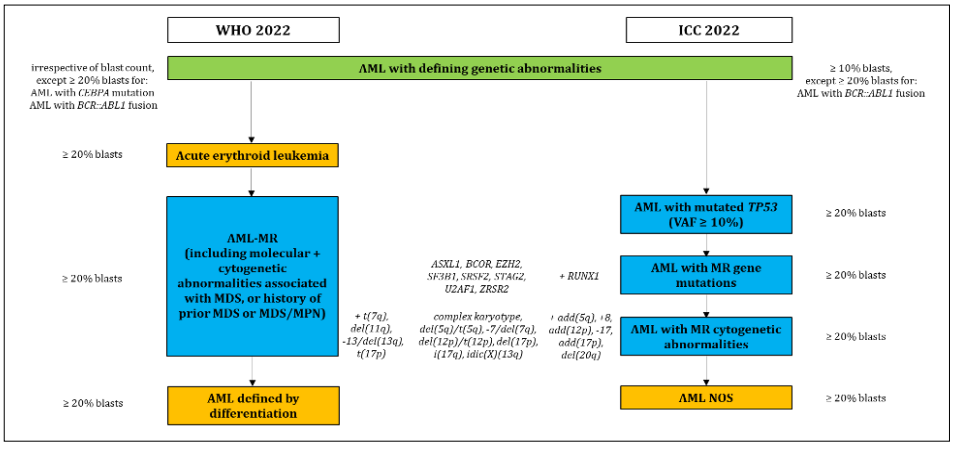
To further underline the importance of this more genetically based classification, OS of chemo-consolidated patients with secondary and therapy-related AML was generally shorter compared to de novo AML patients [23], which was attributed to a higher incidence of adverse cytogenetics. However, when outcomes were analyzed within distinct cytogenetic or ELN risk groups, this impact was less present, especially in patients with intermediate and adverse risk [23,24], and patients consolidated by HSCT [24].

Real world assessments of WHO 2022 and ICC

Changes between the WHO 2016 and the 2022 classifications

There are already two published studies comparing patient allocation and outcomes according to both 2022 classifications with the WHO 2016 and with one another [14,25]. Huber et al. reclassified 1451 patients (717 with MDS, 734 with AML) [25], while Attardi et al. reclassified 1001 patients with AML [14]. Here, a significant shift between the WHO 2016 and WHO 2022 (in 23% of cases) or ICC (in 24% of cases) was shown [14]. As expected, the number of patients classified as NOS according to WHO 2016 (39% and 13%) decreased to 24% and 5% (WHO 2022, now 'defined by differentiation') and 27% and 5% (ICC), respectively [14,25]. Matching this, both studies observed an increase in patients classified as AML-MR in the 2022 classifications due to the inclusion of MR gene mutations [2,7]: according to WHO 2016, 18% and 22% of patients were classified as AML-MRC, which increased to 35% and 28% according to WHO 2022 and 31% and 26% according to ICC, respectively [14,25].

Figure 1: Hierachical classification of AML according to WHO 2022 and ICC.



Abbreviations: AML, acute myeloid leukaemia; MR, myelodysplasia-related; NOS, not otherwise specified; VAF, variant allele frequency.

With the elimination of the WHO 2016 provisional entity AML with mutated *RUNX1* in both 2022 classifications, 93% and 96% of these patients were reclassified as AML with MR gene mutations according to the ICC (which kept *RUNX1* as MR-defining). Still, due to the high co-incidence of *RUNX1* and *ASXL1* or spliceosome mutations, also 74% and 77% of patients were classified as AML-MR according to the WHO 2022 [14,25].

Discrepancies in patient allocation according to WHO 2022 and ICC

The classification of most patients was congruent according to WHO 2022 or ICC, with only 14% and 13% of patients classified differently [14,25]. The main reasons for discrepant classifications were differences in the definition of AML with mutated *CEBPA, KMT2A*- and *MECOM* rearranged AML, and AML-MR between WHO 2022 and ICC [25]. While in general, differences regarding the main diagnosis (MDS or AML) between WHO 2022 and the ICC were very rare (<1%), only in 4/16 patients upstaging was concordant in both classifications [25]. These rare but relevant events epitomize the problem of having two classifications and show how the choice of classification may critically influence available treatment options.

Lastly, the inclusion of AML with mutated *TP53* in the ICC, but not WHO 2022, seems to have the most relevant real-world implications. Attardi et al. demonstrated that 91% of AML with mutated *TP53* according to the ICC were reclassified as AML-MR according to the WHO 2022 [14]. Huber et al. showed the other way around, that 23% of AML-MR patients according to the WHO 2022 harboured a *TP53*-mutation [25].

Prognostic relevance of the WHO 2022 and ICC classification

Although the unique biological – and not prognostic - features of each entity drove disease categorisation in all three classifications, the inclusion of certain genetic features also are of prognostic relevance [25]. Huber et al. demonstrated that OS for AML-MR(C) was shorter in the WHO 2016 (median 0.4 years) and the WHO 2022 (median 0.5 years) compared to the ICC (median 1.0 years). This was likely mediated by the exclusion of *TP53*-mutated patients, whose short OS (median OS 0.1 years) was best shown in the respective category of ICC [25]. With the exclusion of patients with MR gene mutations from AML defined by differentiation, long-term OS improved from approximately 10% after 10 years for AML-NOS according to WHO 2016 to approximately 20% after 10 years for AML defined by differentiation according to WHO 2022 and AML-NOS according to ICC [25].

In both 2022 classifications, only minor changes within the genetically defined AML groups were made [2,7], which did not translate into a significant change of OS for AML with defined fusion genes, or mutated *NPM1* [25]. Only for AML with mutated *CEBPA* non-aligning definitions between the three classifications lead to a noticeable difference in OS (median OS 5.0 years in WHO 2016, 4.1 years in WHO 2022, and not reached in ICC) [25].

ELN 2022

Adjustment of recommendations

The first ELN recommendations for diagnosis and management of AML were published in 2010 [26], and later updated - following the WHO 2016 classification - in 2017 [1,27], and - following the ICC – in 2022 [28]. Compared to the ELN 2017 classification, the distribution into three genetic risk groups – favourable, intermediate, adverse – was kept, but some adjustments made following new insights into AML disease biology and prognosis. First, only *bZIP* in frame *CEBPA* mutations, now define favourable risk, irrespective of whether they occur mono- or biallelic [28]. The former emphasized varied risk depending on a high or low *FLT3-ITD* allelic ratio (AR) has been omitted, and now the presence of a *FLT3-ITD* defines intermediate risk irrespective of the AR or co-occurring *NPM1* mutations. *NPM1* mutations remain to identify favourable risk AML, however, in cases of co-occurring adverse risk cytogenetics, patients should be classified to have adverse ELN 2022 risk. In addition to mutations in *ASXL1, RUNX1*, or *TP53*, which define adverse risk AML since the ELN 2017 classifications, the MR gene mutations (affecting the genes *BCOR, EZH2, SF3B1, SRSF2, STAG2, U2AF1,* and *ZRSR2*) now additionally define adverse ELN 2022 risk. Congruent to the ICC, only mutated *TP53* with a VAF >10% is considered to define adverse ELN 2022 risk.

Prognostic relevance

Several studies analyzed changes in the risk distribution and prognosis of the ELN 2022 compared to the ELN 2017 classification. Overall, approximately 85% of analysed patients remained in their respective risk group, while 5% were reclassified to have more favorable, and 10 % to have more adverse risk [14,18,29,30]. Reclassification of ELN from 2017 to 2022 occurred most often in patients with low *FLT3-ITD* AR from favourable to intermediate risk and in patients with MR gene mutations from intermediate to adverse risk [29]. Several authors independently showed the prognostic relevance of the ELN 2022 classification regarding the achievement of complete remission, OS, RFS, and relapse incidence, especially in patients treated with intensive chemotherapy and consolidated by HSCT [10,14,18,29–31].

However, in most studies, the ELN 2022 classification did not allow for a more precise risk prediction than the ELN 2017. Some analyses also suggested that older age as an independent prognostic factor might allow for a better risk classification than the ELN 2022 [29,31]. In addition, especially for patients treated with the non-intensive combination hypomethylating agents and venetoclax, the ELN 2022 seems to have limited prognostic potential [32]. In this context, a molecular prognostic signature including mutated *FLT3-ITD, NRAS, KRAS*, and *TP53*, was suggested as a useful alternative [33].

Conclusion

With the advent of new technologies and our growing understanding of AML biology, the assessment of molecular data has become increasingly relevant for precisely diagnosing and stratifying the risk of AML patients. While this reflects the complexity of AML, these analyses are not universally

available in all countries and clinics due to their significant cost, the requirement for high technical expertise, and the current lack of adequate standardization. Consequently, a substantial proportion of AML patients face hindrances in achieving proper risk stratification.

Despite existing discrepancies, there is a considerable degree of agreement between the WHO 2022 and ICC, with congruent allocation in more than 80% of AML patients in real-life cohorts [14,25]. The variations primarily revolve around bone marrow blast cut-offs, biological aspects, or slight differences in the inclusion criteria for corresponding subgroups. Notably, these differences between both classifications highlight areas that may require further discussion and adjustment in the near future.

Nevertheless, the coexistence of two parallel classification systems poses challenges for treating physicians and health authorities, creates confusion among patients, and may complicate the inclusion criteria for future clinical trials. Consequently, we believe it is crucial to once again strive towards a unified cancer classification system, fostering a common language that can be shared by the international scientific community.

Conflict of interest

The authors declare no conflict of interest.

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Therapeutic strategies in *TP53* AML not eligible to intensive chemotherapy

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Abstract

Despite improvements in the last years, acute myeloid leukaemia (AML) remains very challenging to treat in high-risk elderly patients, especially *TP53* AML patients, unfit for intensive chemotherapy. With 3 case reports, we illustrated the therapeutic opportunities in these patients. If in some *TP53* AML patients complete response can be obtained, long responses are rare and the disease is considered as not curable in unfit patients. New therapeutic strategies may emerge in the future. We strongly believe that the recent identification of clonal hematopoiesis as a pre-leukemic event could offer new preventive strategies, especially in the *TP53* setting.

Introduction

The understanding of acute myeloid leukaemia (AML) has evolved drastically over the past decade. With the lens being shifted to a molecular level, it has unveiled a large heterogenous spectrum of molecular identities that define the disease, its evolution and prognosis, and guide the treatment strategies [1]. However, AML remains very challenging to treat in high-risk elderly patients, especially *TP53* AML patients, unfit for intensive chemotherapy.

Ten years ago, the AZA-AML-001 trial compared 5'-Azacitidine to the conventional care regimens (CCR; including best supportive care, subcutaneous cytarabine or intensive chemotherapy) in treating older patients (65 years or older) newly diagnosed AML with more than 30% of bone marrow (BM) blasts [2]. Patients eligible for hematopoietic stem cell transplantation were not included in this trial. The trial failed to show a significant improvement in the overall survival (OS) in the global population but was considered safe and manageable in this difficult-to-treat AML population. Moreover, in a subgroup analysis of the trial, 5'-Azacitidine was shown to be more beneficial in AML patients whose cytogenetics inflicted a poor prognosis (such as chromosome 5 or 7 abnormalities or complex karyotypes) or whose molecular profiles harboured an unfavourable mutation. *TP53*-mutated AML patients had an improved median OS compared to their counterparts in the CCR arm with a median OS of 7.2 vs 2.4 months [3]. The potential increased sensitivity of *TP53*-mutated AML to hypomethylating agent was confirmed by the high rate of complete response (CR) in *TP53*-mutated AML patients treated with Decitabine even if these responses were not long-lasting [4], suggesting a potential interest in hypomethylating agent-based combination therapies.

The addition of Venetoclax to 5'-Azacitidine led to improved survival compared with 5'-Azacitidine alone in patients with newly diagnosed AML unfit for intensive treatment (VIALE-A trial) [5]. However, these findings were not the same in all the population subgroups. Individuals with unfavourable molecular profiles and more specifically, those carrying the *TP53* mutation, had a lower benefit from the combination of 5'-Azacitidine-Venetoclax than individuals with more favourable profiles [6], when compared to 5'-Azacitidine alone Moreover, in pooled data from the phase 1b trial (NCT02203773) and the phase 3 trial (VIALE-A), the median OS for *TP53*-mutated patients, *TP53*-WT with *FLT3-ITD*-mutated or *K-NRAS*-mutated patients and *TP53*-WT with *FLT3-ITD*-WT or *K-NRAS*-WT were 5.52 months (95%CI 2.19-7.59), 12.12 months (95%CI 7.26-15.15), and 26.51 months (95%CI 20.24-32.69) respectively. These data were confirmed by real-life studies showing a very poor OS for *TP53*-mutated AML patients [7].

Other combinations failed to improve survival compared to 5'-Azacitidine alone or CCR; for instance, the ENHANCE-2 trial failed to prove that the monoclonal antibody (anti-CD47) Magrolimab would have a survival benefit when added to 5'-Azacitidine, similarly to Eprenetapopt (TP53-reactivating compound) which did not have an added benefit when given with 5'-Azacitidine in treating *TP53*-mutated AML patients.

Despite the poor results, responses to treatment have been achieved in a few cases. The following three case reports may not be representative of the whole unfit *TP53* AML population but will shed light on the molecular complexity of this subgroup of *TP53*-mutated AML and its potential therapeutic response.

CASE 1

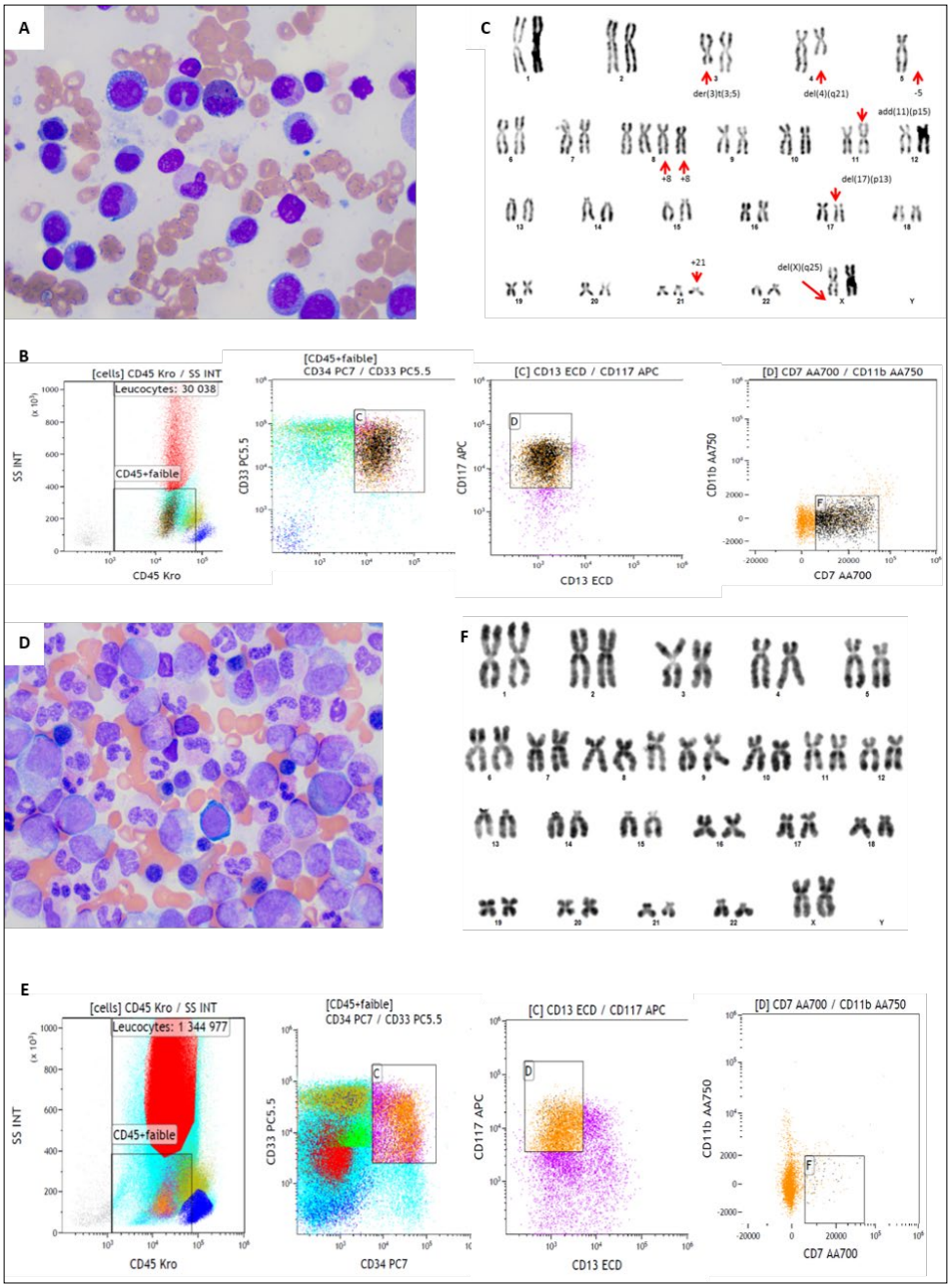
A 75-year-old female patient, with an ECOG performance status of 2 to 3, known to have Chronic Obstructive Pulmonary Disease and Aortic insufficiency, was diagnosed with AML with complex karyotype and *TP53* mutation.

The diagnosis was based on a complete blood count at presentation that revealed a white blood cell count (WBC) of 3.5 G/L with 5% blasts, haemoglobin of 9.9 g/dl and platelets count of 95 G/L. A bone marrow aspirate was performed revealing AML with myelodysplasia-related changes (22% of blasts) (fig 1.A) with a Leukemia-associated immunophenotyping (LAIP) on flow cytometry with positive CD34/33/117/7 (fig 1.B). Cytogenetic analysis revealed a complex karyotype including a deletion of chromosome 17 (fig

1.C). An NGS revealed a *TP53 H179N* mutation with a 65% variant allele frequency (VAF). The multidisciplinary tumour board was held and treatment with 5'-Azacitidine + Venetoclax was decided. Evaluation after three cycles of 5'-Azacitidine -Venetoclax was in favour of a complete cytologic remission (fig 1.D), a negative minimal residual disease (MRD) on flow cytometry (fig 1.E), and a cytogenetic remission (fig 1.F). However, no NGS was done.

This is one of the double-hit *TP53*-mutated AML patients who presented the best response to 5'-Azacitidine-Venetoclax in our centre. We picked this example as this patient reached a complete cytological, immunophenotypic, and cytogenetic response with 5'-Azacitidine-Venetoclax, however, this patient had a relapse of his AML after the 10th cycle and died shortly after. This case translates the idea that a complete remission (CR) does not necessarily dictate a better overall survival. A study has shown the efficacy of a 10-day treatment with Decitabine in high-risk *TP53*-mutated AML patients, in achieving complete molecular remission. However, this high degree of decitabine sensitivity allowed outgrowth of a preexisting subclone in all cases, implicating an early relapse [4]. This was also shown in the analysis of the phase III study VIALE-A trial and a preceding phase IB study that showed a high CR rate in *TP53*-mutated AML when treated with the combination of 5'-Azacitidine-Venetoclax without improving the overall survival or affecting the duration of remission [8].

Fig 1. Bone marrow evaluation of a *TP53*-mutated AML patient at diagnosis (A-C) and after three cycles of 5'-Azacitidine-Venetoclax (D-F). Myeloid blasts shown on the bone marrow aspiration evaluation (A), confirmed on the flow cytometry with positive CD34/33/117/7 (B) and associated with a complex karyotype including a deletion of chromosome 17 (C), at diagnosis. After 3 treatment cycles with 5'-Azacitidine-Venetoclax, evaluation revealed a complete remission with no or minimal blasts on the bone marrow analysis (D), confirmed by the flow cytometry with a low CD34/33/117/7 signalling (E) and a karyotype no longer expressing the chromosome 17 deletion (F).



CASE 2

An 86-year-old female patient, with an ECOG performance status of 1, with a known medical history of breast cancer treated with partial mastectomy and axillary lymph node dissection and radiotherapy, in remission since 2016. She was diagnosed with t-AML in June 2020 based on a bone marrow aspiration revealing 28% blasts with LAIP on flow cytometry, a normal karyotype, and with an NGS revealing a *DNMT3A, IDH2, TET2, SH2B3,* and *TP53* mutations *[DNMT3A R730H* & *A639V* (VAF 27% and 14%), *IDH2 R140Q* (VAF 12%), *TET2 R123C & N275Ifs* (VAF 3% and 3%), *SH2B3 splice exon2* (VAF 3%), *TP53 R248Q & C124F* (VAF 2% and 2%)]. As expected, the choice of treatment which the multidisciplinary tumour board agreed on was 5'-Azacitidine-Venetoclax and an evaluation after the second cycle was in favour of complete cytologic remission with an *NGS* panel expressing the *DNMT3A, IDH2, TET2,* and *SH2B3* mutations without the *TP53* mutation. In October 2023, after 43 cycles of 5'-Azacitidine-Venetoclax, the patient is still in complete response.

This case highlights the importance of the risk-stratification of the AML based on VAF of the *TP53* mutation and not by the sole presence or absence of this culprit mutation. For instance, it has been suggested that

the International Consensus Classification (ICC) may have under-estimated the toll that has a 'multi-hit' *TP53* mutation with a VAF of <10% on the prognosis, in the same way that the World Health Organization (WHO) may under-estimated the poor prognosis inflicted by the presence of a monoallelic *TP53* MDS with 10-19% blasts as well as *TP53*^{mut} VAF ≤49% in the presence of CK [4,8]. These two classifications wouldn't classify patients with *TP53* mutation of VAF <10% as high-risk AML which seems to be the case for our patient in Case 2.

CASE 3

A 66-year-old female, with an ECOG performance status of 1, was known to have breast cancer diagnosed in 2011 with metastases to the bones despite five lines of treatment. In September 2020, she initially presented with a myelodysplastic syndrome with an excess of blasts; MDS EB-1 with a very high R-IPSSM risk score, a complex karyotype, and an NGS panel revealing a *TP53* splice exon 4 (11%) & R273H (7%). This patient was transfusion-dependent with an initial hemoglobin level of 7.6 g/dl.

The multidisciplinary tumour board was held and decided to treat with 5'-Azacitidine. An evaluation after a third cycle was in favour of a progression into AML with 35% blasts, with worsening of the transfusion dependence (haemoglobin of 6.6g/dl and platelets of 12 G/L). The NGS panel revealed then mutations of *TP53* R273H (21%) and *IDH1* R132C (23%). A treatment with Ivosidenib monotherapy was therefore initiated and 3 months into therapy, the patient became transfusion-independent with a partial response (fig 2A and 2B) and neutrophil recovery. Unfortunately, the patient died 9 months after initiation of Ivosidenib treatment from a progression of breast cancer with a stable AML disease.

This case highlights the potential benefit of targeting mutations or pathways other than the *TP53*, such as the above-mentioned *IDH1* mutation, in a relapsed or refractory AML. It also suggests that in case of a relapsed or refractory disease, a new NGS evaluation could be of clinical significance as some subclones could emerge and proliferate while targeting the initial mutation. This key concept was highlighted in the IDHENTIFY trial which showed an improved response to treatment with enasidenib compared to CCR in late-stage *IDH2*-mutated relapsed or refractory acute myeloid leukaemia where around 14% of the patients had an associated *TP53* mutation [11,12]. Moreover, a recent abstract described, through single-cell DNA profiling, the acquisition of an *IDH1* mutation in a *TP53* AML patient treated with CD47 antibody, 5'-Azacitidine and Venetoclax, thus confirming that this phenomenon may not be incidental [13].

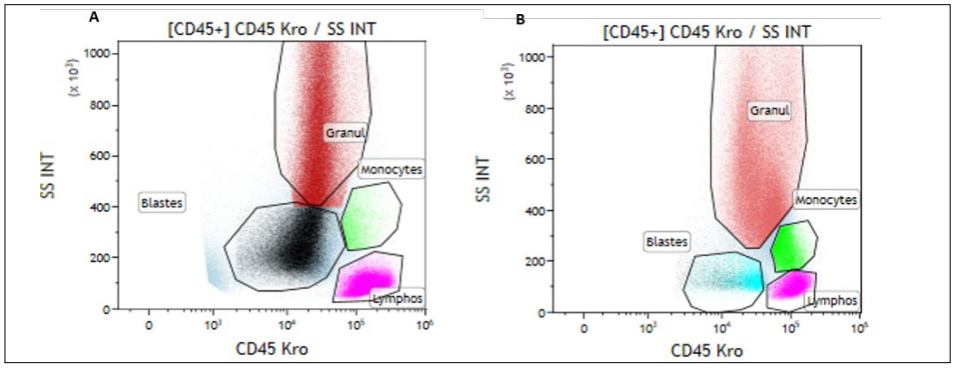
Conclusions

Here, we deliberately chose unfit *TP53* AML patients who had the best response to the treatment in our centre. Unfortunately, especially in the case of multi-hit *TP53* mutations, the disease is considered as not curable whatever the intensity of the treatment. When possible it is recommended to enroll these patients into clinical trials for alternative strategies. So far "promising drugs" have not translated into "efficient drugs" but the substantial number of abstracts with new drugs tested in *TP53* AML this year at the ASH congress is encouraging and highlights that emerging therapies seek to address these unmet needs.

New therapeutic strategies may emerge in the future. Indeed, the availability of NGS technologies represents a breakthrough in the field of AML, especially for a better understanding of the disease pathogenesis. *TP53* mutations are known to be enriched in therapy-related myeloid neoplasm14 and the recent identification of clonal hematopoiesis (CH) as a preleukemic state and its high incidence in cancer survivors may open up new perspectives. Incidental detection of CH through liquid profiling of cell-free DNA, germline testing or exploration of cytopenia became a custom in clinical practice. It helps to identify patients at high risk of developing AML or MDS, especially in the case of *TP53* CH, and contributes to the development of prevention strategies.

More and more centres created CH clinic or specific molecular tumour boards to improve clinical management of patients [15] and some clinical trials [16] are now opening for early therapeutic interventions which could lead the way to new perspectives in the management of patients at risk of developing *TP53* AML.

Fig 2. Immunophenotypic evaluation by flow cytometry of the bone marrow of the patient before (A) and 3 months after treatment with Ivosidenib (B), showing the shift in signalling and the net decrease in the blasts' expression and differentiation syndrome.



Conflict of interest

Lina El Murr has no conflict of interest to declare.

Jean Baptiste Micol: Honoraria - Jazz Pharmaceuticals (less than $10,000 in a single calendar year), AstraZeneca (less than $10,000 in a single calendar year), Astellas Pharma (less than $10,000 in a single calendar year); Consulting or Advisory Role - AbbVie (less than $10,000 in a single calendar year), Gilead Sciences (less than $10,000 in a single calendar year); Travel, Accommodations, Expenses - AbbVie (less than $10,000 in a single calendar year).

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Advances and remaining challenges in adult acute lymphoblastic leukaemia

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Abstract

In recent years treatment of acute lymphoblastic leukaemia (ALL) has seen marked improvements, facilitated largely by the introduction of targeted therapies and the use of measurable residual disease (MRD) to guide therapy. Tyrosine kinase inhibitors for Philadelphia positive ALL and antibody-based therapeutics including the bispecific T cell engager (BiTE) blinatumomab have not only demonstrated increased efficacy but have enabled de-escalation of conventional induction chemotherapy with a resultant reduction in toxicity. Combining these various treatment modalities in first-line therapy has led to a reassessment of the role of allogeneic hematopoietic cell transplantation (HCT) but is also substantially benefitting elderly and unfit patients not considered to be transplant candidates. Despite these advances, leukemic relapse remains a profound challenge, mandating close attention to MRD with prompt intervention in case of molecular relapse or persistence to prevent overt disease recurrence. Newer modalities such as chimeric antigen receptor (CAR) T cells and several small molecules hold promise to further improve patient outcomes.

Introduction

The past few years have seen advances in the therapeutic management of B-lineage acute lymphoblastic leukaemia (ALL) that border on the revolutionary, with paradigm shifts in relation to the use of chemotherapy, treatment intensity, hemopoietic cell transplantation (HCT) and utilization of targeted therapies. Concerning the latter, it is convenient to distinguish time periods before and after the introduction of immunotherapies, specifically monoclonal antibodies targeting cell surface antigens (inotuzumab ozogamicin), bispecific antibodies (blinatumomab) and more recently CAR T-cell therapy. For Philadelphia chromosome-positive (Ph+)/*BCR::ABL1*-positive ALL, we can additionally delineate a pre- and post-tyrosine kinase inhibitor (TKI) era.

Lessons and questions from the TKI era

ABL1-directed TKI were the first class of targeted agents that proved to be game changers; successive generations of TKI were able to induce – as single agents or in combination with corticosteroids - a complete remission in nearly all patients diagnosed with Ph+ ALL. This, plus a substantially lower toxicity and early death rate with improved outcomes facilitated

reduced-intensity induction treatment as a new therapeutic principle [1-3]. Moreover, the use of TKI in combination with established post-remission regimens (chemotherapy and HCT) resulted in survival outcomes equivalent to those of patients with Philadelphia-negative ALL [4].

As a caveat, the concept of using TKI alone in combination with substantially reduced intensity chemotherapy applies to induction therapy for Ph+ ALL, but not to consolidation cycles, at least in the absence of immunotherapy. This was demonstrated by an increased relapse rate in patients in whom high-dose cytarabine was omitted from consolidation therapy in a recent randomised, nilotinib-based trial (Graaph-2014) by the GRAALL group [5]. Decreasing chemotherapy intensity likewise does not apply to CNS-directed prophylaxis, which may benefit from an increase in the number of intrathecal chemotherapy cycles, although an optimal regimen remains to be defined [6].

The question of which TKI is the best has been the subject of intense debate and is not quite obsolete despite the results of the recent randomised PhALLCON trial, which showed superior molecular responses of the to-date most potent TKI ponatinib compared with the first-generation TKI imatinib, although overall survival was not significantly different [7]. In many countries, ponatinib or even the second-generation TKI dasatinib and nilotinib are not available as first-line therapy, making equitable access to these drugs an important global endeavour. This is underscored by recent real-world evidence from a middle-income country, illustrating the need for further improvement outside of the clinical trial setting [8]. Additionally, the differential side effect profiles of the various TKI´s may determine the initial choice of TKI: in case of ponatinib and nilotinib, their cardiovascular risk profile requires particular consideration in patients at risk of myocardial infarction or peripheral or arterial vascular events. This risk has been reported to be alleviated by reducing the TKI dose, but systematic long-term observations are still limited [9].

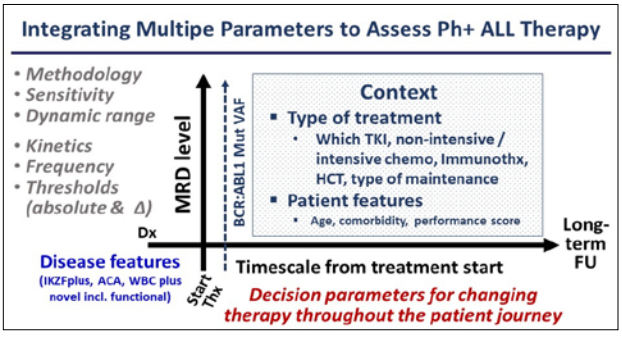
A still unresolved issue is the optimal use of TKI as maintenance after HCT. Most experience has been gathered with imatinib, but all approved TKI can be safely administered. Second- and third-generation TKI are preferable in patients with more advanced and high-risk disease; in the absence of randomised data, recommendations on the duration after HCT range from 1 to 5 years of TKI following HCT [10-12]. In reality, prolonged administration may prove challenging in the face of low-grade but long-term toxicities. If tolerability is poor, serial assessment of measurable residual disease and the initial relapse risk can help motivate the patient and guide post-transplant maintenance therapy. If supervised closely, pre-emptive, MRD-triggered use of TKI has been shown to be as effective as its prophylactic use in a randomised trial [13].

Another emerging question concerns the potential for safely discontinuing TKI in patients who have not undergone HCT [14]. As parameters predictive of the likely success of such an attempt are speculative, attempts to emulate the experience with treatment-free remissions in chronic myeloid leukaemia (CML) should be done exclusively in the setting of a clinical trial outside of a post-allogeneic transplant setting.

The improvements achieved with TKI plus chemotherapy combinations have spawned a vigorous debate about the continued role HCT, which traditionally was an almost mandatory component of post-remission therapy for Ph+ ALL. The majority of clinical trials showed superiority of strategies based on allogeneic HCT in the context of TKI plus chemotherapy regimens, and the combination of ponatinib with pediatric-inspired chemotherapy followed by alloHCT has yielded excellent outcomes [15], experience that will be relevant when the newer immunotherapeutic approaches discussed below are not available. Nevertheless, a growing body of data has identified patient subsets, defined by good molecular responses, who did not appear to benefit from transplant [16,17]. In this context, the importance of assay sensitivity as a determinant of predictive power of MRD has become increasingly apparent [18,19]. The major limitation of available evidence is the lack of comparability of trials in terms of molecular response, due to variability of methods for assessing MRD, their sensitivity, thresholds used for clinical decision making, time points of the analysis and therapeutic context including transplant modalities. In addition, few studies have considered additional clinical and genetic risk factors in their analysis of outcomes with or without HCT. Notably, the presence of additional recurring gene deletions, e.g. of *IKZF1, CDKN2, PAX5* and others has been shown to have a profound impact on survival of Ph+ ALL patients, even among good molecular responders [20,21]. Whether the negative prognostic impact of these factors will be mitigated by the newer immunotherapy approaches remains to be determined.

Based on this experience it would be desirable to develop more comprehensive algorithms for risk assessment rather than relying on rather simplistic approaches to MRD assessment (Figure). This should be facilitated by more sensitive and standardisable MRD quantitation by next-generation sequencing NGS and increasing availability of whole exome or whole genome sequencing and RNA-Seq. Ideally, these molecular studies would be implemented prospectively and across large cooperative study groups, encompassing both adult and paediatric patient cohorts to improve our understanding of the different

Figure



disease biology between these groups. Clearly, MRD analysis and molecular stratification are central to the management not only of Ph+ ALL but all subtypes of B- or T-cell precursor ALL.

Immunotherapy for B-cell precursor ALL: blinatumomab and Inotuzumab

The addition of antibody constructs targeting cell surface antigens to our therapeutic armamentarium have had an even greater impact on our treatment strategies for ALL than the TKI as they are not restricted to a specific molecular subtype. Their major promise is the potential for enabling chemotherapy-free (except for intrathecal CNS prophylaxis) regimens by combining targeted agents of different classes, building on their high efficacy in the face of low to moderate toxicity. By extension, it is hoped that HCT can be avoided in a majority of patients. The paradigm-changing D-ALBA trial demonstrated the feasibility and efficacy of a chemotherapy-free regimen which combined dasatinib with blinatumomab as front-line therapy for Ph+ ALL [22]. In a recent update of this trial with a median follow-up of 53 months, overall and event-free survival were 80.7% and 74.1%, respectively [23]. It appeared that this combination was effective in eliminating clones with kinase domain mutations that were resistant to dasatinib. While the regimen was chemotherapy-free, it is noteworthy that a substantial proportion of patients, mostly those with persistent MRD, still underwent alloHCT, which was however associated with a quite low rate of transplant-associated mortality. The ponatinib-based successor trial will determine whether results can be further improved by using ponatinib as a TKI.

Evidence that the chemotherapy-free combination of blinatumomab and ponatinib may not require a subsequent HCT comes from a trial conducted by the MDACC, in which only 2% of patients underwent alloHCT and 2-year overall survival was 89% [24]. Follow-up is still short, and it is worth noting that half of the relapses occurred in the CNS, again emphasising the need to improve CNS prophylaxis in the era of `exclusively´ targeted therapies.

In Philadelphia-negative B-lineage ALL omission of chemotherapy is less straightforward since the TKI class of agents cannot be employed, with the exception of some cases of *BCR::ABL1*-like or early T-progenitor ALL. Accordingly, the focus in Ph negative ALL is on combining immune-oncology agents with standard or reduced-intensity chemotherapy regimens, given in various sequences. The addition of 4 cycles of blinatumomab to standard consolidation chemotherapy was superior to chemotherapy consolidation alone in a randomised phase 3 trial of newly diagnosed patients with *BCR::ABL1*-negative ALL [25]. providing the first evidence that blinatumomab significantly improved survival for both MRD negative and MRD positive patients in CR1. More recent trials are evaluating strategies incorporating both inotuzumab and blinatumomab in established front-line chemotherapy regimens such as the HyperCVAD. Initial reports suggest excellent hematologic and molecular response rates and low early mortality, but do not conclusively address the role of HCT as a definite postremission therapy. This important question needs to be addressed in a prospective randomised fashion, with patient stratification by standardised MRD and molecular analyses. One of the challenges of such a trial is the constantly evolving therapeutic landscape, in which new drugs and cellular therapies will continue to enter the clinical testing stage and if effective will prove to be significant confounding factors. At present, younger and fit patients without a good molecular response and certain patient subsets including Ph-like/*BCR::ABL1*-like ALL, *KMT2A* rearranged ALL and a hypodiploid karyotype should be considered for alloHCT.

Minimising toxicity by reducing chemotherapy intensity is particularly relevant in elderly and frail patients and is being explored by several centres and cooperative study groups. Inotuzumab alone or with low-dose chemotherapy, followed by chemotherapy, blinatumomab or both has been effective at inducing CR rates of 80-90%, mostly MRD negative, with a low induction mortality of less than 5%. Again, the importance of intrathecal chemotherapy as CNS prophylaxis cannot be overemphasised. Whether systemic chemotherapy can be completely omitted by administering inotuzumab plus blinatumumab-based regimens is unknown and is being explored primarily in elderly patients deemed ineligible for chemotherapy. Encouraging results in patients 70 years and older were obtained in an MDACC trial combining inotuzumab, blinatumomab and rituximab, although follow-up is still too short to assess long-term outcomes. Similarly, the Alliance A 041703 trial is evaluating an inotuzumab induction followed by inotuzumab and blinatumomab maintenance in patients ≥60 years [26]. At the time of reporting, 9 of 33 patients had relapsed, highlighting not only the substantial challenges in treating elderly ALL patients but also the ability of ALL to evade multi-pronged targeted therapies. Accordingly, alloHCT with reduced intensity conditioning may be an option even for elderly patients who are deemed fit enough for the procedure or become fit enough after achieving a CR with low-toxicity salvage therapy.

Relapsed and refractory ALL

Treatment of patients with recurrent or resistant (r/r) disease remains the probably greatest challenge in the management of ALL. Combinations of inotuzumab, blinatumomab and chemotherapy have improved outcomes compared with historical experience, reaching approximately 40% at three years. A caveat is that as immunotherapy increasingly takes the role of standard front-line therapy, relapses will no longer respond as well to these same agents, an observation already made with sequential generations of TKI in r/r Ph+ ALL. Introduction of newer agents such as BH3 mimetics (venetoclax), menin inhibitors for KMT2A rearranged ALL, and the allosteric BCR::ABL1 inhibitor asciminib for Ph+ ALL show promise in the salvage setting, their main utility is likely to be as early front-line therapy. Transplantation is generally

regarded as the definite treatment option for patients with r/r disease who achieve complete remission with salvage therapy, despite some immunotherapy-based studies showing little additional impact of HCT.

CAR T-cells targeting CD19 have emerged as a new promising therapeutic modality for advanced ALL, and two products have been approved for B-ALL in second relapse or in first relapse after HSCT in patients younger than 26 years (Tisegenlecleucel) and for r/r B-ALL in general (Brexucabtagene autoleucel). MRD-negative responses have been observed in approximately 60-90% of pediatric, AYA and adult patients with r/r ALL [27-31], but the median duration of response has been just over one year [30,31]. This raises the question of what the optimal sequencing of available salvage therapies for patients with R/R B-ALL is and whether HSCT following CAR T-cell therapy provides long-term benefits [32]. The type of prior therapies impacts on outcomes, patients who had previously received blinatumomab, inotuzumab or HCT experienced inferior survival. Disease biology as evidenced by time from HCT to relapse (< 6 months vs. ≥6 months) was also highly predictive of outcome after CAR T-cell therapy [33], as was tumour burden, disease kinetics, CAR T-cell persistence and fitness [25,31]. These factors have implications for the positioning of CART within the overall treatment strategy and raise the issue of whether HCT should be used to consolidate a CR achieved by CAR T-cells. While it was shown that CD19.28z CAR T-cells followed by a consolidative alloHSCT can provide long-term durable disease control in children and young adults with r/r B-ALL, there is no conclusive evidence for a benefit of HCT after CAR T-cell therapy. In view of these challenges, current strategies to improve CAR T-cell efficacy focus on multispecific CAR T-cells to overcome immune escape and new CAR designs [35-37]. These exciting developments should not however obscure the fact that the cost of HCT and CART therapy and the manufacturing process of the latter remain major challenges in providing equitable access to these therapies.

Conclusions

An increasing number of patients with ALL are now being cured by combining different types of targeted agents and positioning them in the early first-line setting. The overall reliance on cytotoxic drugs is decreasing and minimization of toxicity caused by chemotherapy has become a key concept, particularly during induction. Allogeneic HCT retains its central role in the treatment of r/r ALL but immunotherapy-based regimens seem to offer little or no benefit to an increasing number of patients, especially those with a very good molecular response. While the critical importance of MRD in informing treatment decisions is universally accepted, a lack of standardization and consensus on exactly how it should be conducted remain obstacles to its optimal use. CAR T-cells are emerging as the next major, highly effective components of ALL therapy but substantial toxicity issues need to be overcome before routinely integrating them into first-line therapy. How to best position these cellular therapies within a therapeutic regimen, including in relation to HCT, remains investigational. Treatment of older patients continues to be problematic, a better understanding of the different biology of ALL in young and older patients should be a focus of translational research. The main goal of treatment has to be the prevention of overt relapse at all costs, facilitated by optimal front-line therapy, risk-orientated stratification and meticulous implementation of state-of-the-art MRD monitoring. Even with the dramatically improved tools now at our disposal, optimal patient management requires enormous attention to detail and recognition of patient-specific parameters such as response depth and dynamics, tolerability of treatment and quality of life, psychological factors and social support structures, among others. All of these are best delivered in the context of a clinical trial.

Conflict of interest

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Is allogeneic transplantation necessary in acute myeloid leukaemia of intermediate risk in the first complete remission?

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Abstract

The choice of post-induction treatment in patients with acute myeloid leukaemia (AML) at intermediate risk poses a significant challenge, particularly due to the lack of clear evidence regarding the benefits of allogeneic hematopoietic stem cell transplantation (allo-HSCT) in this population. The complexity is heightened by genetic diversity and modifications in diagnostic and cytogenetic risk classifications. Although allo-HSCT is considered the preferred option, especially for high-risk relapse cases, its benefit in those in complete remission (CR) with negative measurable residual disease (MRD) remains uncertain. In fact, several studies support the re-stratification of intermediate-risk patients, considering both MRD and mutational profile. The optimization of conditioning regimen intensity is also a subject of debate, as myeloablative regimens (MAC) have shown advantages over reduced-intensity regimens (RIC) in patients with positive MRD but not in those with negative MRD. Finally, the use of allo-HSCT in AML with intermediate-risk *FLT3-ITD* mutations in the era of FLT3 inhibitors is also in question due to the outcomes observed with these drugs. Therefore, the development of comparative clinical trials among various therapeutic options emerges as the most effective approach to advance in stratification and provide precise clinical guidance in the management of intermediate-risk AML.

Introduction

Acute myeloid leukaemia (AML) is a heterogeneous clonal disease caused by the accumulation of acquired somatic genetic alterations leading to uncontrolled proliferation of immature hematopoietic precursors. This genetic diversity becomes particularly relevant in patients with AML classified as intermediate risk according to the European LeukemiaNet (ELN) guidelines. The emergence of new markers (genetic, functional, post-remission, etc.) and targeted therapies (FLT3 inhibitors) has defined more precisely the prognosis, leading to the reclassification of these patients into favourable or adverse risk groups. Currently, post-remission treatment options for the intermediate-risk patient group are controversial due to the uncertainty regarding the benefits of allogeneic hematopoietic stem cell transplantation (allo-HSCT), recommended for patients in the adverse risk group.

Current indications and outcomes of allo-HSCT in AML

AML represents the most common indication for allo-HSCT, serving as a cornerstone in its therapeutic approach. In recent decades, there has been a sustained increase in the number of transplants performed in AML patients, attributed to advances in conditioning regimens (such as the implementation of non-myeloablative and reduced-intensity regimens) and the emergence of alternative sources of progenitors (expanding the availability of donors).

Typical indications for allo-HSCT include: (1) patients in first complete remission (CR1) if the risk of relapse exceeds 35-40%; (2) patients in second or subsequent complete remissions; and (3) patients refractory to the first-line treatment regardless of risk [1].

The decision to undergo allo-HSCT in CR1 is made by weighing the risk-benefit ratio, influenced by the reduction in relapse risk (CIR) and the increase in non-relapse mortality (NRM) [2]. Although the evaluation should be personalised considering factors such as age, functional status, and comorbidities, it has been observed that patients who derive greater benefits are those with intermediate or unfavourable risk according to the ELN 2017 criteria, or favourable risk with measurable residual disease (MRD) persistence after consolidation/intensification treatment.

This indication is based on the findings of several studies comparing outcomes in patients with available versus unavailable donors, demonstrating better overall survival (OS) in high-risk patients defined by cytogenetic characteristics. In this regard, the HOVON-SAKK group study analysed AML patients in CR1 eligible for allogeneic transplantation based on the availability of an HLA-identical family donor (32%) or unavailable donor (58%). Although treatment-related mortality (TRM) was significantly higher in the donor group (21% vs. 4%), both CIR and disease-free survival (DFS) were lower in the donor-available group. Subgroup analysis revealed significantly better DFS and OS in patients with a donor belonging to the intermediate or adverse cytogenetic risk group. From this, it was concluded that AML patients with intermediate or adverse cytogenetic risk significantly benefited from allo-HSCT [3].

Changes in the definition of intermediate risk: Differences between ELN 2017 and 2022

The continuous progress in genetic knowledge, resulting from gene expression

analyses and next-generation sequencing (NGS), has enhanced the identification of biomarkers related to acute leukaemia leading to enhanced diagnostic and prognostic precision. The genetic, cytogenetic, and molecular advancements justified the update of the previously unified fourth edition of the World Health Organization Hematolymphoid Tumor Classification [4], resulting in the current fragmentation into two distinct classifications: the fifth edition of the World Health Organization Hematolymphoid Tumor Classification [5] and the International Consensus Classification [6].

These changes have brought about a reclassification of intermediate-risk patients, as highlighted by the study of Huber et al, emphasizing the following findings: (1) a reduction in entities defined by morphology, decreasing from 13% in the WHO 2016 Classification to 5% in the WHO 2022 Classification and ICC; (2) an increase in the incidence of MDS-related AML, rising from 22% in the WHO 2016 Classification to 28% in the WHO 2022 Classification and 26% in the ICC, after the inclusion of myelodysplasia-related gene mutations in this group and (3) the reclassification of AML-*RUNX1*; (4) the existence of different inclusion criteria for *CEBPA* mutant AML in each classification, as well as those related to myelodysplasia (the ICC excludes *TP53*-mutated AMLs from this group) [7].

Similarly, the European LeukemiaNet 2022 guidelines were updated following the criteria proposed by the ICC. The intermediate-risk group was mainly affected by two changes: (1) all patients with *FLT3-ITD* mutations were moved to the intermediate-risk group regardless of the coexistence of *NPM1* mutations and *FLT3-ITD* allelic ratio; and (2) AMLs with molecular alterations not classified as adverse or favourable have decreased in account of the inclusion of AMLs with myelodysplasia-related gene mutations in the adverse-risk group [8].

The consequences of this update have been analysed in other studies where ELN 2022 was not a better prognostic tool compared to ELN 2017 in the intermediate-risk group [9,10].

In order to validate ELN 2022, the PETHEMA group conducted an analysis of the effects of the classification change in a cohort of 546 patients eligible for intensive treatment. A decrease in the representation of favourable and intermediate-risk groups, accompanied by an increase in the adverse group was observed compared to the ELN 2017 classification. This phenomenon was attributed to a change in risk group in 14.5% of patients, mainly due to the lack of recognition of *FLT3* allelic ratio prognosis and the inclusion of AML with myelodysplasia-related gene mutations in the adverse risk group. To improve the prognostic capacity of ELN 2022, subgroup analysis revealed that patients included in the adverse risk group but with a single mutation related to myelodysplasia-associated genes had higher survival than those with ≥2 mutations. These patients had a similar survival to the intermediate-risk group, suggesting the appropriateness of placing this subgroup of patients in the intermediate-risk category rather than the adverse-risk group [11].

Results of allo-HSCT vs auto-HSCT vs standard consolidation chemotherapy in intermediate-risk AML

Controversy surrounds the optimal post-induction treatment for intermediate-risk patients, as, unlike their adverse-risk counterparts, there is no clearly demonstrated benefit in employing allo-HSCT as a post-remission strategy.

Traditionally, post-remission intensification through allo-HSCT has been associated with increased OS and a reduced risk of relapse. However, TRM has led to the consideration of alternative therapeutic approaches, among which auto-HSCT stands out. Auto-HSCT results in fewer complications due to the absence of the graft-versus-leukaemia effect. To understand the effect of these therapeutic options, the PETHEMA group retrospectively analysed the outcomes of a cohort of intermediate-risk cytogenetic patients who underwent auto-HSCT or allo-HSCT in CR1 after intensive chemotherapy. The results indicated that allo-HSCT was significantly associated with better OS, leukaemia-free survival (LFS), CIR and NRM compared to auto-HSCT. This study suggests that within the intermediate cytogenetic risk group in CR1, auto-HSCT may be a valid option for patients with favourable molecular risk, while allo-HSCT should be the preferred post-remission strategy for patients with intermediate or adverse molecular risk [12].

On the other hand, the ETAL-1 clinical trial allowed the comparison of outcomes between intermediate-risk cytogenetic AML patients randomly assigned to receive allo-HSCT or conventional consolidation chemotherapy with the option of rescue allo-HSCT in case of relapse. Despite premature closure due to slow patient enrolment, notable results included allo-HSCT being associated with significantly longer DFS but similar OS compared to the chemotherapy group. Although the classification scheme used was appropriate at the initiation of the trial in 2012, it does not reflect current guidelines for classifying AML patients. Therefore, a substantial portion of the patients included in this study would now be classified in the adverse or favourable-risk groups according to ELN 2017 and 2022 criteria [13].

In another study, the outcomes of haploidentical transplantation were explored compared to intensive chemotherapy as post-induction therapy in intermediate-risk cytogenetic AML patients in CR1 without an HLA-identical family donor. The 3-year LFS and OS were significantly better in the group receiving haploidentical transplantation than in the chemotherapy group. In multivariate analysis, the type of post-remission treatment (haplo-HSCT vs chemotherapy) was an independent risk factor in both the overall cohort and when stratified by minimal residual disease after the second consolidation. The authors concluded that, in the absence of an HLA-identical family donor, haploidentical transplantation could be a superior post-remission therapy to chemotherapy as a first-line post-remission treatment for intermediate-risk cytogenetic AML [14].

Importance of MRD and post-remission treatment in intermediate-risk AML

Measurable residual disease (MRD) is one of the most important markers for predicting the risk of post-remission relapse, as its positivity implies the presence of leukaemia cells not detectable below the sensitivity limit of optical microscopy. Supporting this assertion, Araki et al analysed the survival difference based on MRD in a cohort of 359 patients undergoing allo-HSCT in CR. MRD was assessed using 10-color multiparametric flow cytometry (MFC), and any level of positivity was considered MRD positive. The results concluded that patients with MRD-negative CR had significantly higher OS and PFS compared to MRD-positive or actively diseased patients, with similar outcomes between the last two groups [15].

Due to its predictive capability, various studies have explored incorporating MRD (via MFC, PCR, or NGS) into algorithms that integrate it alongside the genetic risk of the patients, aiming to select the most suitable therapeutic strategy based on their post-remission relapse risk. This approach becomes relevant in the absence of data supporting the benefits of allo-HSCT in intermediate-risk patients.

The GIMEMA AML 310 clinical trial (NCT01452646) was designed to determine the post-remission treatment strategy considering cytogenetic/genetic factors and post-consolidation MRD levels using flow cytometry in young patients with de novo AML. In this context, intermediate-risk AML patients with MRD-positive CR1 received allo-HSCT, while those with MRD-negative CR1 received auto-HSCT. The comparison between both cohorts revealed similar rates of OS and PFS. Thus, the antileukemic effect exerted by allogeneic transplantation in intermediate-risk patients with MRD positivity was demonstrated and implied that intermediate-risk patients achieving MRD negativity may not necessarily require allo-HSCT, contributing to reducing potential toxicity [16].

MRD could also play a crucial role in determining the type of conditioning regimen used in allo-HSCT. This hypothesis is based on a retrospective analysis of MRD using NGS by Hourigan et al from the BMT CTN 0901 clinical trial (NCT01339910), where AML patients in CR were randomly assigned to receive myeloablative conditioning (MAC) or reduced-intensity conditioning (RIC) allo-HSCT. The analysis revealed MRD negativity in 32% of patients treated with MAC and 37% treated with RIC, showing similar OS. However, OS and CIR at 3 years were significantly worse in MRD-positive patients receiving RIC conditioning compared to those receiving MAC conditioning. This finding suggests that, in AML patients with genomic MRD detected before allo-HSCT, MAC regimens might result in better survival compared to RIC regimens [17].

In response to the growing evidence on the impact of MRD on prognosis and therapeutic decisions, Jentzsch et al. refined ELN 2022 based on post-remission MRD to improve its prognostic capacity. In this way, the intermediate group of ELN 2022 was reclassified as favourable if they achieved MRD negativity and adverse if they presented MRD positivity. This change resulted in 50% of patients initially classified as intermediate risk in ELN 2022 now being categorized as favourable risk [18].

Perhaps the only way to answer the question of the best post-remission treatment in intermediate-risk AML patients would be to conduct randomized clinical trials using a more up-to-date risk strategy, where MRD-negative AML patients are randomly assigned between allo-HSCT, auto-HSCT, and consolidation chemotherapy [19].

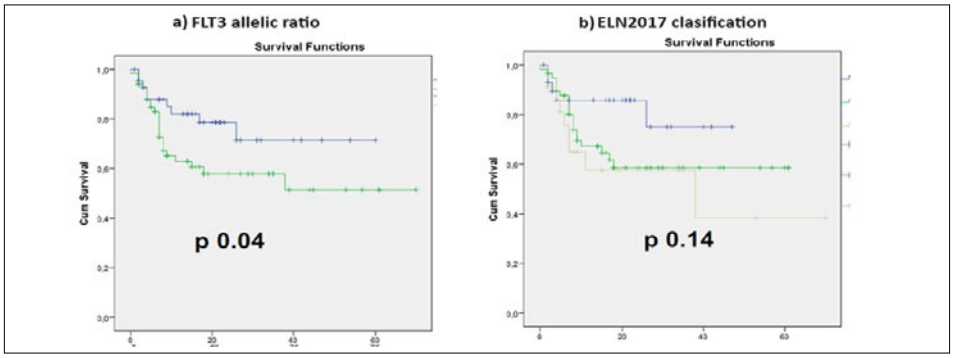
Post-remission treatment in AML patients with *FLT3-ITD* mutation

One of the significant changes introduced in the ELN 2022 update is the inclusion of AML with *FLT3-ITD* mutation in the intermediate-risk group, regardless of allelic ratio or coexistence with *NPM1* mutation. These factors previously determined favourable, intermediate, or adverse risk with different post-consolidation therapeutic approaches. The addition of FLT3 inhibitors along with standard intensive chemotherapy has improved OS and PFS, sparking a debate about the need for allo-HSCT in all *FLT3-ITD* mutated patients.

Döhner et al conducted a subanalysis of the RATIFY clinical trial to evaluate the prognostic impact of *NPM1/FLT3-ITD* genotypes according to ELN 2017 classification. They found that the addition of midostaurin provided significant benefits in all three risk groups in terms of overall survival and suggested that only *NPM1*^{wt}/*FLT3-ITD*^{mut} patients benefit from allo-HSCT [20].

A retrospective analysis of 27 Spanish centres with 175 AML patients with *FLT3* mutations examined the "real-world" impact of adding midostaurin to intensive chemotherapy. When comparing ELN 2017 intermediate-risk group patients who received allo-HSCT with those who continued treatment with midostaurin, no significant differences in OS were observed (Figure 1). Consequently, it can be concluded that the role of allogeneic transplantation in AML patients with *FLT3* mutations in the era of FLT3 inhibitors is not clearly defined [21].

Figure 1. Effect of the allelic ratio of FLT3 and the ELN 2017 classification on overall survival in patients with AML with *FLT3* mutation treated with midostaurin. In figure 1a, FLT3 ratio ≥ 0.5 (green) and FLT3 ratio < 0.5 (blue). In figure 1b: Favorable risk (blue), intermediate risk (green), and adverse risk (yellow).



Conclusions

- Post-first remission treatment in intermediate-risk AML patients remains controversial.
- Due to changes in risk stratification, post-remission treatment options for intermediate-risk AML patients should be continuously updated.
- Allogeneic transplantation remains the preferred option, especially if the risk of relapse is high.
- However, it is unclear whether allogeneic transplantation is preferable in patients who achieve complete remission with negative measurable residual disease.
- MAC regimens are clearly advantageous over RIC in patients undergoing Allo-HSCT with detectable MRD.
- The role of allogeneic transplantation in *FLT3-ITD* mutated patients belonging to the ELN 2022 intermediate-risk group is controversial in the era of FLT3 inhibitors.

Conflict of interest

The authors declare no competing financial interests.

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Immunotherapeutic strategies after allogeneic stem cell transplantation

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Abstract
In this article, we describe current immunotherapeutic strategies to reduce transplant-related morbidity and mortality and to enhance disease control strategies after allogeneic hematopoietic cell transplantation.

Introduction
Both the success (cure) as well as the disaster (transplant-related death) that may occur after allogeneic hematopoietic cell transplantation (allo-HCT) are ascribed to the non-optimal immune-reconstitution in the immune-ablated recipient. Thus, harnessing the immune system after allo-HCT by applying immunotherapeutic (IT) strategies to reduce transplant-related morbidity and mortality (TRM) and to enhance disease control remains a challenge for the transplant physician [1].

IT strategies to enhance anti-viral immunity
IT strategies to enhance the anti-viral immunity and reduce TRM include the application of mono- or multi-virus-specific T cells manufactured from T cells of the original donor [2]. As such advanced IT strategies can be produced only in experienced and well-equipped centres with good manufacturing practice (GMP) facilities legislated by national authorities to produce point of care (POC) advanced therapeutic medicinal therapies (ATMPs), still only a minority of patients have access to them. "Off the shelf" ready-to-use T-cell therapies generated from third-party donor-derived T cells are currently tested in prospective clinical trials, with one of such products against EBV+ PTLD (tabelecleucel) having gained FDA and EMA approval.

IT strategies to combat Graft versus Host Disease
IT strategies to reduce Graft versus Host Disease (GvHD) are more challenging as these should also not harm the Graft versus Leukemia (GvL) effect. Emerging evidence suggests a central role of T-regulatory reconstitution in establishing the desired optimal GvHD and GvL balance after allo-HCT. T-cell depletion (TCD) strategies with ATG or alemtuzumab are effective in preventing GvHD not only by depleting naïve T cells but also by establishing a higher regulatory to naïve T cell ratio after allo-HCT [3]. Post-transplant cyclophosphamide (PTCY) prevents efficiently GvHD, especially chronic GvHD, mainly via a preferential recovery of Tregs and myeloid suppressors which conquer surviving alloreactive T cells [4]. As the current paradigm indicates that immune tolerance is determined by a balance of Tregs over T-effector cells, Tregs hold a great promise to combat GvHD and several approaches have been attempted to translate an adoptive Treg cell immunotherapy to the clinic. As far as manufacturing of Tregs is concerned, the most documented and common approach is to isolate natural Tregs (nTregs) through CD4+CD25+ selection, however, this approach was accompanied by co-infusion of a high proportion of alloreactive CD25+ T cells along with the intended nTreg population. To circumvent such purity and safety issues, a novel approach implementing a GMP-compliant closed-system flow cytometry (FACS) sorter to isolate the CD127low fraction of the CD4+CD25+ nTregs has been successfully employed in the first and only phase III randomized clinical trial of nTregs, with exceptional preliminary data [5]. Since nTregs constitute a rare population in the periphery challenging their clinical application, many groups, including ours, have sought to convert conventional T cells to induced Treg (iTregs). Our approach aspired to mimic the mechanism of successful physiological immunotolerance during pregnancy, the best example of semi-allogeneic tolerance in nature, in which a highly potent immunomodulatory molecule called human-leucocyte-antigen-G (HLA-G) is expressed in the placenta to protect the foetus from the maternal immune attack. HLA-G is epigenetically silenced after prenatal life and in normal healthy tissues but may putatively re-expressed in pathological conditions aiming to mitigate immune aggression by suppressing various immune effector cells. We were the first to show that in allo-HSCT recipients, HLA-G is de novo expressed in GvHD sites and in peripheral blood T cells which upon their isolation by flow cytometry (FACS)-sorting proved to have strong *in vitro* immune-suppressive properties (HLAG+ T-suppressors) [6]. Subsequently, we managed to robustly *ex vivo* generate on small and large-clinical scale a highly *in vitro* and *in vivo* immunosuppressive induced T-regulatory (iTreg) population enriched in HLA-G expressing cells, termed iG-Tregs [7,8]. We successfully translated our iG-Treg production methods into GMP-compatible manufacturing processes, and we initiated the first-in-human phase 1/2 clinical trial of *ex vivo* generated iG-Tregs in adult patients undergoing allo-HCT from an HLA-matched sibling donor to prevent GvHD or treat refractory chronic GvHD [9]. Our preliminary exploratory results hint towards long-term

persistence of infused iG-Treg clonotypes and the emergence of increased diversity of the Treg repertoire. Besides our *ex vivo* generated iG-Treg products, other iTregs have proceeded to phase 1/2 clinical studies showing the feasibility and safety of this approach with encouraging results [10,11]. All these approaches have in common the exposure of T cells to regulatory-inducing mediators e.g. decitabine, TGF-beta, or tolerogenic dendritic cells. Novel manufacturing approaches which are now tested in phase I/II clinical trials include the use of genetically engineered Tregs, like viral-based systems to induce chimeric antigen receptor CAR-Tregs or CRISPRCas9 genome editing to deplete or activate endogenous antigens [12]. The major challenge of the use of Tregs in the clinic remain in identifying the most effective Treg subsets with stable regulatory function and long-term persistence *in vivo*. Other challenges include production issues as an ATMP, as these are produced on an individual patient basis, in time-consuming, complex and still expensive manufacturing processes. A third-party and "off-the-shelf" Treg bank could overcome such limitations.

IT strategies to reduce relapse

Minimal residual disease (MRD) after allo-HCT may be used as a predictor of impending relapse and should be part of routine follow-up for transplanted patients, however, a clear recommendation on how to best implement MRD testing and MRD-directed therapy after allo-HCT is still lacking [13]. The MRD techniques continue to advance (eg, error-corrected NGS, MRD from circulating DNA) and are expected to improve the accuracy of assessment of clonal and/or immunological changes (e.g. HLA loss) in low-volume residual disease, thus enabling a more rational therapeutic intervention than is currently possible. Less progress has been made in monitoring the speed and quality of GvL reconstitution. Unlike chemotherapy, which induces an antileukemic effect of short duration, the GvL effect is prolonged, with unique and non-quantifiable dynamics in different individuals, and may require several months to eradicate any persisting tumour cells. Recent reports suggest that the increased frequency of regulatory T cells and exhausted leukaemia-specific T cells in bone marrow or the co-expression of inhibitory molecules on circulating T cells represents a dysfunctional GvL pattern that permits eventual relapse [14,15]. Understanding the interplay between GvL and MRD post-allo-HCT remains a major challenge.

Prophylactic or preemptive donor lymphocyte infusion (DLI) may improve outcomes, yet convincing evidence from randomized trials is lacking [16-18]. Open questions remain about the dose intensity and the total number of infusions that are necessary to achieve long-term remissions. The landscape of cellular and targeted immunotherapy is evolving rapidly and is increasingly used also as an IT strategy after allo-HCT. Hypomethylating agents (azacitidine and decitabine) may beneficially influence the balance between GvL and GVHD by enhancing the immunological visibility of leukaemia cells (eg, through the expression of silenced cancer/testis antigens and activation of interferon responses) while mitigating GvHD through expansion of regulatory T cells [19]. Better results were found when azacitidine was given together with DLIs [20]. Extended azacitidine dosing using the oral formulation of the drug and panobinostat (deacetylase inhibitor) have shown promising results in prophylactic phase 1/2 studies [21]. Case series reported the efficacy of immune checkpoint inhibitors (anti-CTLA-4 and anti-PD-1/PD-L1) in relapsed disease after allo-HCT, but their use is associated with high rates of severe, often life-threatening GvHD and thus the administration of these drugs in the preemptive MRD setting is not justified outside a clinical trial [22,23]. FLT3 inhibitors enhance the GvL effect and have been shown efficacious in preventing relapse or in treating *FLT3-ITD*–mutant acute myeloid leukaemia (AML) relapse, especially when combined with DLI. While the phase 3 BMT-CTN 1506/MORPHO trial of gilteritinib did not demonstrate statistically significant improvement of relapse-free survival (RFS), there was a clinical improvement of RFS among patients with detectable MRD before and after allo-HCT [24, 25]. The isocitrate dehydrogenase (IDH) inhibitors (ivosidenib and enasidenib) are currently tested in *IDH*–mutated AML as maintenance and salvage therapy after allo-HCT. Interferon-a and interleukin-2 alone or together with DLIs have also been tested as immunomodulators in the MRD preemptive setting, but with doubtful effects and safety concerns.

Conclusion

Much remains unknown regarding the dynamic evolution of the immune system in the allo-HCT recipient and how we can dictate it. How much immunosuppression after allo-HCT do we need? How can we establish immune tolerance long-term? How can we detect and correct a dysfunctional GvL pattern? IT strategies have been developed and have shown promising results in small patient series. A significant challenge will be to perform well-designed prospective clinical trials of IT in these relatively small patient populations [26].

Conflict of interest

None related to this work.

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Why should we opt for continuous therapy in chronic lymphocytic leukaemia?

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Abstract

The treatment paradigm for Chronic Lymphocytic Leukemia (CLL) has undergone a remarkable revolution, transitioning from traditional therapies to targeted agents and immunotherapy. This evolution has been characterised by the emergence of Bruton tyrosine kinase (BTK) inhibitors and Bcl-2 inhibitors which have reshaped CLL management. The ongoing debate surrounding continuous versus fixed-duration (FD) therapy underscores the nuanced considerations involved in optimising treatment efficacy while minimising toxicity and preserving patient quality of life.

Continuous therapy with BTK inhibitors, notably exemplified by second-generation agents like acalabrutinib and zanubrutinib, has emerged as a cornerstone of CLL treatment, offering sustained disease control and durable responses across diverse patient populations. However, challenges persist, including the occurrence of adverse events, in particular cardiovascular events, the development of resistance mutations, and economic implications.

Efforts to mitigate these challenges are underway, with research focusing on dose optimization, alternative therapies for patients intolerant to standard agents, and the development of novel agents targeting resistant mutations. Individualised treatment approaches, informed by patient-specific factors such as comorbidities, biological markers and treatment preferences, are crucial in navigating the complexities of CLL management.

Despite challenges, the outlook for CLL patients is increasingly optimistic, with ongoing advancements poised to further enhance treatment outcomes and quality of life. With a continued focus on refining treatment strategies and addressing unmet needs, the CLL treatment landscape is poised for continued evolution, promising improved efficacy, tolerability, and overall patient care.

Introduction

The chronic lymphocytic leukaemia (CLL) treatment landscape has seen remarkable evolution since 2010. Traditional therapies like chemotherapy and monoclonal antibodies were once mainstays, but targeted therapies and immunotherapies have transformed management. Bruton tyrosine kinase (BTK) inhibitors such as ibrutinib and acalabrutinib have demonstrated efficacy in inhibiting CLL progression by targeting B-cell receptor signalling pathways. Similarly, Bcl-2 inhibitors, like venetoclax, disrupt anti-apoptotic mechanisms in CLL cells. Furthermore, CAR T-cell therapy and bispecific antibodies are promising avenues for engineering patients' T cells to target CLL cells. With personalised medicine approaches and ongoing clinical trials, the CLL treatment landscape continues to evolve, offering improved outcomes and quality of life for patients [1].

The debate between continuous therapy and fixed-duration (FD) therapy in CLL reflects the complexity of balancing treatment efficacy with toxicity and patient quality of life. Continuous therapy, often involving BTK inhibitors or Bcl-2 inhibitors, aims to sustain disease control over the long term, potentially preventing relapse but necessitating ongoing drug exposure and monitoring. Conversely, FD therapy, typically utilising targeted therapy combinations of Bcl-2 inhibitor plus an anti-CD20 monoclonal antibody or a BTK inhibitor, aims for a finite treatment period, potentially allowing for treatment-free intervals but risking disease recurrence. Both approaches have demonstrated efficacy, and the choice depends on individual patient factors, including comorbidities, treatment tolerance, and preferences, emphasising the importance of shared decision-making in CLL management. Ongoing research aims to refine treatment strategies and optimise outcomes in this dynamic landscape.

When we talk about continuous therapy we usually refer to treatment with BTK inhibitors, while only few studies investigated the role of Bcl-2 inhibitor as a single agent in continuous therapy [2-4].

Ibrutinib, the first-in-class BTK inhibitor, revolutionising the treatment landscape of CLL and other B-cell malignancies, showed efficacy in both treatment-naive and relapsed/refractory CLL [5,6]. Its mechanism involves covalent irreversible inhibition of BTK, disrupting B-cell receptor signalling and promoting apoptosis in malignant B cells. Long-term results from the RESONATE trial demonstrated that ibrutinib significantly prolonged progression-free survival (PFS) and overall survival (OS) compared to ofatumumab in relapsed/refractory CLL patients [5]. Additionally, the RESONATE-2 trial showed superior PFS, OS, and overall response rate of ibrutinib compared to chlorambucil in treatment-naive CLL patients, particularly in those with high-risk genomic features [6]. Notably, ibrutinib showed sustained efficacy across patient subgroups, regardless of del(17p) or *TP53* mutation status, making it a cornerstone therapy for CLL management, offering durable responses and improved survival outcomes.

Acalabrutinib, a second-generation BTK inhibitor, offers a more selective inhibition

profile, potentially reducing off-target effects and improving tolerability. Results from the ELEVATE-TN trial demonstrated that acalabrutinib significantly improved PFS compared to standard chemoimmunotherapy in treatment-naive CLL patients [7]. Moreover, the ELEVATE-RR trial which compared acalabrutinib directly with ibrutinib in relapsed/refractory CLL patients, acalabrutinib demonstrated superiority in terms of PFS [8]. This trial showcased acalabrutinib's efficacy and safety profile compared to ibrutinib. Notably, acalabrutinib exhibited lower rates of adverse events such as atrial fibrillation, hypertension and bleeding events. These findings position acalabrutinib as an effective and well-tolerated treatment option for CLL patients, particularly those with comorbidities or a need for long-term therapy.

Zanubrutinib, another second-generation BTK inhibitor, exhibits potent BTK inhibition with minimal off-target activity, contributing to its favourable safety profile. In both the ALPINE and SEQUOIA trials, zanubrutinib demonstrated significant efficacy in treating CLL and small lymphocytic lymphoma (SLL). Zanubrutinib notably improved PFS compared to standard ibrutinib in the ALPINE trial for relapsed/refractory cases [9], and surpassed bendamustine plus rituximab in PFS and overall response rates in the SEQUOIA trial for treatment-naive patients [10]. Moreover, zanubrutinib exhibited a favourable safety profile, with low rates of adverse events such as atrial fibrillation and bleeding incidents, reinforcing its efficacy and tolerability as a frontline or salvage therapy option for CLL/SLL. While the better safety profile is likely related to the high selectivity of zanubrutinib for BTK, the improved outcome might be related to the better pharmacokinetic, while its plasma concentration always above the IC50, higher BTK occupancy within the lymph nodes, and less treatment discontinuation due adverse events [11].

The favourable safety profile of second-generation BTK inhibitors, coupled with their efficacy in CLL, position them as a compelling treatment option, potentially offering improved tolerability and quality of life for patients compared to ibrutinib [12].

Although we would like to treat all patients with FD therapy, several points favour the use of continuous therapy with BTK inhibitors in CLL. We herein addressed the most pros and cons of continuous therapy with BTKi in patients with CLL.

Pros of continuous therapy

There are several favourable aspects for the use of continuous BTKi such as the efficacy in high-risk or bulky disease and the easier schedule. CLL displays a wide spectrum of clinical and biological diversity, ranging from those who will never require treatment to patients with short-term disease control and who relapse several times. The former an enriched in patients with a mutated *IGHV* gene and 13q deletion at FISH (fluorescence in situ hybridization), while the latter are featured by an unmutated IGHV gene, *TP53* abnormalities (deletion and/or mutation) and complex karyotypes [13-15].

In Table 1 we summarised results of sub-analyses from clinical trials both in treatment-naive and relapse/refractory patients with *TP53* abnormalities or complex karyotype who received fixed duration therapy with venetoclax-obinutuzumab [16] or venetoclax-ibrutinib [17,18] or venetoclax-rituximab [19] versus ibrutinib [5,6], acalabrutinib or zanubrutinib. Among treatment-naive patients the 3 years PFS is about 15-20% higher with a continuous BTK inhibitor therapy (range 75%-84%) than an FD (range 57%-73%), while differences are smaller among relapse-refractory patients [19-21].

In addition, both the CLL14 and the Murano trials, using venetoclax-obinutuzumab and venetoclax-rituximab in treatment-naive and relapse patients, respectively, showed that patients with bulky nodes (defined

Table 1. Comparisons of fixed-duration and continuous therapies in patients with *TP53* abnormalities and complex karyotype.

	***TP53* abnormalities (including del17p by FISH and/or *TP53* mutation by sanger or NGS)**				
	Therapies	**Trials**	**2-y PFS**	**3-y PFS**	**median PFS**
TREATMENT NAIVE	VG	CLL14	~71%	~62%	~48m
	I+V	Captivate Glow Flair	~85% n.a. n.a.	~73% n.a. n.a.	n.r. n.a. n.a.
	Ibrutinib	Pooled analysis	~87%	79%	78m
	Acalabrutinib	Pooled analysis	~88%	78%	n.r.
	Zanubrutinib	Sequoia	~88%	~80%	n.r.
RELAPSED	VR	Murano	~80%	~51%	36m
	I+V	Vision Clarity	n.a. n.a.	n.a. n.a.	n.a. n.a.
	Ibrutinib	Resonate Alpine	~72% 55%	~52% ~40%	40.7m ~26m
	Acalabrutinib	Polled	~72%	~54%	~40m
	Zanubrutinib	Alpine	78%	~50%	38.6m
	Complex karyotype				
TREATMENT NAIVE	VG	CLL13 no CK / iCK / hCK CLL14 NO CK / CK	~95% / ~95% / ~82% ~95% / ~80%	~85% / ~85% / ~57% ~80% / ~79%	n.r. / n.r. / ~43m n.r. / n.r.
	I+V	Captivate CK	~85%	~70%	n.r.
	Ibrutinib	Pooled analysis no CK / CK	~84% / ~84%	~78% / ~75%	n.r.
	Acalabrutinib	Pooled analysis CK	~91%	84%	n.r.
	Zanubrutinib	Sequoia no CK / CK	~95% / ~85%	~85% / ~80%	n.r. / n.r.
RELAPSED	VR	Murano no CG / CG	~92% / ~80%	~78% / ~50%	60 / 42m
	Ibrutinib	OSU study no CK / iCK / hCK	~82% / ~75% / ~55%	~80% / ~68% / ~52%	n.r. / 60m / ~36m
	Acalabrutinib	Pooled analaysis CK	~75%	~56%	~39m

VG = venetoclax-obinutuzumab, I+V = ibrutinib plus venetoclax, n.a.= not available, n.r. = not reached, PFS = progression free survival, CK = complex karyotype (≥3 chromosomal abnormalities), iCK = intermediate CK (3-4 chromosomal abnormalities), hCK = high CK (≥ 5 chromosomal abnormalities); CG = genome complexity (≥ 3 abnormalities); OSU study =. study by the Ohio state university.

as patients whose lymph nodes were 5cm or larger) had almost 2-fold higher risk of relapse than patients with small lymph nodes [16,19]. Conversely, BTK inhibitors were highly active in the lymph nodes, regardless of their size, being able to cause their shrinkage already after a few days from the start of the drug [5,6].

One of the cornerstones of fixed duration therapy is the capability of combination therapies to reach undetectable measurable residual disease (uMRD, $<10^{-4}$) leading to long-term disease remissions. However, while the rates of uMRD are high for previously untreated patients [16-19], these rates tend to decrease in heavily treated patients [20,21] (Table 2). Despite uMRD being very rare with BTK inhibitors, continuous treatment allows for long-term disease remission. [5-7,10].

Furthermore, another point forward the use of continuous treatment with BTK inhibitor is the easier schedules and formulations [6,7,10]. Since they are available as a capsule or tablet, they can be easily taken by the patients at home once a day for ibrutinib, twice a day for acalabrutinib and zanubrutinib. Conversely, access to the outpatient clinic for intravenous drugs, such as obinutuzumab or rituximab, or the management of the venetoclax ramp-up phase might be an issue for some elderly patients with comorbidities and/or for their caregivers [16,18,19]. In fact, recent retrospective studies confirmed the efficacy and feasibility of BTK inhibitors in octogenarian patients [22,23].

Cons of continuous therapy

There are also several aspects against the use of continuous BTKi such as adverse events, emergence of resistance or mutations and costs.

It is well known that a relevant number of patients develop adverse events, in particular cardiovascular events like atrial fibrillation, hypertension, bleeding and diarrhoea, and some of them discontinue therapy due to adverse events [5-7,10,24-27]. However, we learn that most adverse are common during the first years of treatment but their incidence decreases in the next years [5,6]. This is the case for diarrhoea, fatigue, bleeding and infections while the rate of hypertension onset increases during ibrutinib treatment in the Resonate-2 trial [6]. In addition, some life-threatening ventricular arrhythmias events have been reported with ibrutinib and calabrutinib [28]. However, the rates of atrial fibrillation, hypertension and ventricular arrhythmias are much lower with acalabrutinib and Zanubrutinib [10,28]. Of note, a recent analysis demonstrated that patients who decrease ibrutinib dose after a cardiovascular adverse event experience a very low rate of adverse event recurrence and a trend for a longer PFS [29], suggesting that lower doses of ibrutinib might be safer.

Interestingly, second-generation BTK inhibitors have been explored in patients who discontinued ibrutinib due to intolerance. A phase 2 study by Shadman M. et al showed that 68% of ibrutinib-intolerant and 73% of acalabrutinib-intolerant patients did not have recurrence of the same adverse events during zanubrutinib treatment. Of those adverse events that recurred during zanubrutinib treatment, none were at a higher grade, and 75% among ibrutinib-intolerant and 40% among acalabrutinib-intolerant patients were at a lower grade [30].

One of the major concerns regarding continuous treatment is the emergence of mutations of BTK or downstream signalling pathways gene such as *PLCG2* [31-33]. Although these mutations can arise both in patients failing BTK inhibitor, up to 65% of cases, and in responding patients, almost 10% of cases [31], Woyack J. et al showed that *BTK* mutations are more rare in patients taking ibrutinib as first-line therapy (3%) compared with relapsed patients (30%) [32].

According to the literature, different kinds of *BTK* mutations exist. The most common mutation, i.e. *C481x*, involves the ATP binding site of BTK leading to the inability of ibrutinib, acalabrutinib, or zanubrutinib to covalently bind and inhibit the kinase [31,32,34]. Other, less common *BTK* mutations include *L474x*, also known as a gatekeeper, and *L528W*, also known as kinase-dead mutation [32,34]. Pirtobrutinib, a new non-covalent BTK inhibitor given as continuous therapy, showed activity in heavily pretreated patients who previously relapsed after covalent BTK inhibitor in the phase 3 BRUIN trial [35]. In addition, pirtrobrutinib was able to clear *C481x*-mutated clones [34]. Other new drugs are being developed, such as the chimeric degradation activation compound, which can bind the E3 ligase complex and BTK, leading to its polyubiquitination and proteasomal degradation. These agents, such as BGB-16673, NX-5948 and NX-2127, might be active in patients harbouring *L474x* and/or *L528W* mutations [36-38].

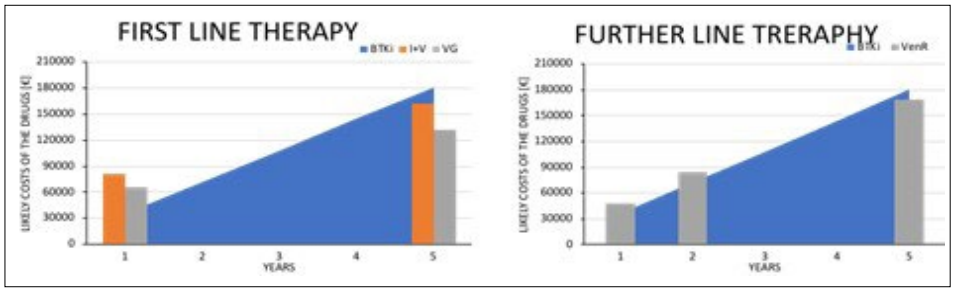
Finally, the cost of FD therapy is lower than that of continuous therapy. However, if we consider retreatments, the whole cost of treatment for two FD regimes is not much lower than that of continuous

Table 2. Comparisons of measurable residual disease (MRD) rates according to the line of therapy.

	Flow-cytometry PB MRD EoT				
	Therapies	Studies	uMRD4	uMRD2	dMRD2
TREATMENT NAIVE	VG	CLL13 CLL14 (AS-PCR)	86% 76%	9% 5%	0% 4%
	I+V	Captivate MRD Captivate FD Glow	75% 77% 61%	n.a.	n.a.
RELAPSED	VR	Murano (all) 2L 3L +3L	70% 69% 78% 55%	19% 24% 12% 9%	10% 7% 9% 36%
	I+V	Vision Clarity	30% ~57%	57% ~33%	57% ~33%

VG = venetoclax-obinutuzumab, I+V = ibrutinib plus venetoclax, VR = venetoclax-rituximab, uMRD4 = undetectable measurable residual disease $<10^{-4}$ events, uMRD2 undetectable measurable residual disease $<10^{-2}$ events, dMRD2 detectable measurable residual disease $>10^{-2}$ events, n.a.= not available.

Figure 1. Cost of treatments



We considered cost of BTKi and venetoclax as 3,000€/months, obinutuzumab all 8 doses 30,000€, Rituximab biosimilar all 6 cycles 12.000€

therapy either in the frontline or relapse setting. This is shown in Figure 1.

Conclusions

Although we would like to treat all patients with CLL with FD therapy, most of them will receive at least one continuous therapy in their lives. The debate between continuous therapy and FD therapy underscores the complexity of balancing treatment efficacy with toxicity and patient quality of life. Continuous therapy with BTK inhibitors has emerged as a cornerstone of CLL treatment, offering sustained disease control and durable responses across patient subgroups. The ease of administration and favourable safety profiles of second-generation BTK inhibitors such as acalabrutinib and zanubrutinib make them compelling treatment options, particularly for patients with comorbidities or long-term therapy needs. However, continuous therapy is not without its challenges. Adverse events, the emergence of resistance mutations, and higher costs are notable considerations. Yet, ongoing research is exploring strategies to mitigate these challenges, including dose adjustments, alternative therapies for intolerant patients, and novel agents targeting resistant mutations.

Ultimately, the choice between continuous therapy and FD therapy depends on individual patient factors, highlighting the importance of shared decision-making in CLL management. With continued advancements and refinement of treatment strategies, the outlook for CLL patients continues to improve, offering hope for better outcomes and quality of life. Accordingly, a recent pooled analysis of patients treated frontline with ibrutinib within three clinical trials, showed that initiating BTK inhibitor as first-line therapy improved overall survival to rates similar to an age-matched population.

Further insight will come from the CLL17 trial, a prospective randomised open-label multicenter phase 3 trial assessing ibrutinib versus venetoclax-obinutuzumab versus ibrutinib plus venetoclax for patients with previously untreated CLL.

BTK inhibitors have transformed CLL management, offering durable responses, prolonged progression-free survival, and improved quality of life for patients. Ongoing research aims to further elucidate their optimal sequencing, combination strategies, and long-term outcomes, highlighting the continuous evolution of CLL treatment. Up to me, contraindications to BTK inhibitors are a family history of sudden death, a history of ventricular tachycardia or fibrillation, uncontrolled or severe hypertension needing at least 3 drugs and the use of warfarin.

Conflict of interest

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