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Review

Precision Medicine Treatment in Acute Myeloid Leukemia Is Not a Dream

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Abstract: The development of molecular studies to define the somatic genetic alterations has revolutionized the diagnostic and therapeutic management of acute myeloid leukemia (AML). AML is a highly heterogenous disease that includes many molecular subtypes; each subtype is heterogeneous both for the presence of variable co-mutations and complex combinations of clones and subclones, changing during disease evolution and in response to treatment. The treatment of AML is changing from standardized schemes of induction and consolidation chemotherapy to tailored approaches according to molecular and genetic profiles and to targeted therapy. Several molecularly targeted therapies have been approved for the treatment of some AML patients, including mutation-specific targeted drugs such as FLT3, IDH1 and IDH2 inhibitors, mutation-independent targeted drugs such as the Bcl2 inhibitor venetoclax, the hedgehog inhibitor glasdegib and the CD33-targeted drug gemtuzumab ozogamicin. Furthermore, recent studies have shown the feasibility of a personalized medicine approach for the treatment of AML patients, where the therapy decisions are guided by the results of genomic studies.

Keywords: leukemia; acute myeloid leukemia; molecular classification; targeted therapy; personalized medicine; next generation sequencing; genomic study

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1. Introduction

The development of massive parallel sequencing techniques has revolutionized the study of human cancers, allowing to sequence the entire genome and to provide detailed information on the genetic alterations present in tumor cells. The techniques of next generation sequencing (NGS) allowed to define the most recurrent genetic alterations observed in cancer cells, including gene mutations, small insertions/deletions (indels), gene fusions, alternative splicing and copy number alterations. NGS can provide, in a few days, the profile of genetic alterations in the blood or bone marrow samples from a patient with leukemia.

These dramatic progresses in the study of genomic alterations have considerably contributed to improve the understanding of the genetic alterations occurring in a heterogeneous disease, such as acute myeloid leukemia (AML). The current diagnostics of AMLs implies cytomorphology analysis, multiparameter flow cytometry, cytogenetics and molecular genetics. NGS studies have allowed to define the genomic landscape of AMLs, in its complexity and heterogeneity; ≥90% of AMLs display at least one gene mutation [1–4]. Different patterns of genetic instability are observed in AML cells; in fact, about 20% of AML patients can be defined according to fusion genes, 31% by chromosomal aneuploidies and 46% by gene mutations only [5]. Frequently, AML patients share mutations observed in normal subjects with clonal hematopoiesis; however, the majority of these patients acquired ≥two mutations, with clonal distribution [5]. The molecular classification of AMLs identified some major molecular subtypes: (i) AMLs characterized by peculiar translocation events (balanced rearrangements) leading to the formation of fusion genes and correspondent fusion proteins, including inv(16) with *CBFB-MYH11*, t(15;17) with

PML-RARA, t(8;21) with *RUN1-RUNXT1*, inv(3) with GATA2-MECOM, *MLL* fusions and t(6;9) with *DEK-NUP214*; (ii) AMLs exhibiting chromatin-spliceosome gene abnormalities, including mutations of genes involved in RNA splicing (*SRSF2, SF3B1, U2AF1, ZRSR2*), chromatin (*ASXL1)* and transcription *RUNX1)*; (iii) AMLs characterized by *TP53* mutations, complex karyotype alterations and copy-number chromosome alterations; (iv) AMLs displaying mutations of the nucleophosmin 1 (*NPM1*) gene, mutually exclusive to other genomic rearrangements and with frequent co-mutations in hydroxymethylation genes (*DNMT3A, TET2, IDH1, IDH2*); (v) AMLs with chromosomal aneuploidies, characterized by double *CEBPA* mutation, with *GATA2 and NRAS* co-mutations found in about 30% of cases; (vi) AMLs with $IDH2^{R172}$ mutation, defined as a distinct subgroup for the mutual exclusivity with *NPM1* mutation and other class-defining lesions [3,4].

The application of NGS techniques has led to the identification of 40–50 genes recurrently mutated in AMLs [6–8]. Driver gene mutations play a key role in AML development with a clear pathogenetic and prognostic relevance [6–8]. The identification of these mutations has led to the individuation of molecular targets for mutation-based targeted therapy [9–13]. This progress has involved a careful definition of AML subtypes at the level of their main mutational events, of their genotypic and phenotypic heterogeneity, thus defining sensitive tools for risk stratification of these patients and for a sensitive and accurate evaluation of therapy response and for better planning the optimal treatment for each patient. Examples of treatment improvements are related to the development and to the clinical introduction of mutation-specific targeted small molecule inhibitors against mutant *FLT3* or mutant *IDH1/IDH2*. In parallel, the introduction in the therapeutic armamentarium of venetoclax, a Bcl2 inhibitor, in association with hypomethylating agents is providing a consistent improvement in the survival of a part of older AML patients [9–13]. Thus, recently some new drugs were approved for the treatment of AML patients (Table I). Unfortunately, a significant proportion of patients develop resistance to these novel therapies whose molecular mechanism has been identified, in part bypassed by rationally designed combination therapies [9–13]. The final demonstration that these targeted treatments result in a clear benefit in terms of overall survival requires time and the careful definition of the most responsive AML patients.

The aim of this review paper is to provide an outline of the major contributions of molecular studies on AMLs to the development of a targeted/personalized treatment.

2. The Fundamental Contribution of Precision Medicine to a More Rational and Predictive Risk Stratification of AML Patients

A correct risk stratification of AML patients is of fundamental importance for the adoption of the potentially optimal treatment strategy for each patient. Some clinical parameters and the integration of immunophenotypic characteristics, cytogenetic abnormalities and molecular mutations, co-occurring or in isolation, contributed to a more refined prognostic assessment.

The development of genomics has improved our understanding of AML development and resulted in novel modes of AML risk stratification that have been in part adopted in recently proposed classifications of AMLs. Prognostic risk of AMLs is defined at diagnosis according to the presence of specific cytogenetic and molecular aberrations [14–16]. Criteria for AML classification and risk stratification have been proposed by several organizations, including the European Leukemia NET (ELN) [14], National Comprehensive Cancer Network (NCCN) [15] and World Health Organization (WHO) [16]. The NCCN and ELN guidelines are the most adopted and stratify AML patients into three different risk groups: Favorable, intermediate and poor/adverse [14,15]. The most adopted risk classification is the 2017 ELN risk stratification; according to this classification, patients are classified into one of the three risk groups, including favorable, intermediate and adverse. Favorable prognosis group includes AMLs with acute promyelocytic leukemia (APL) t(15;17)(q22;q12), balanced translocations t(8;21)(q22;q22), biallelic mutated *CEBPA* and inv(16)(p13.1q22), mutated *NPM1* without *FLT3-ITD* or with $FLT3\text{-}ITD^{low}$. The intermediate group comprises mutated *NPM1* with $FLT3\text{-}ITD^{high}$, WT-*NPM1* without or with $FLT3\text{-}ITD^{low}$, t(9;11), *MLLT3-*

MLL and cytogenetic abnormalities neither favorable or adverse. The adverse AML group comprises AMLs with complex karyotype, inv(3)(q21q26)/t(3;3)(q21;q26), *DEK-NUP214* t(6;9)(p23;q34), *RPN1-EVI1*, t(6;11), −5 or del(5q), −7 or abnormal (17p) or monosomal karyotype, *TP53* mutations, *RUNX1* mutations, *ASXL1* mutations, *FLT3-ITD^{high}* isolated without *NPM1* mutations and with normal karyotype. The NCCN and ENL adopt a similar classification scheme for favorable-risk AMLs, although the criteria for favorable risk differ in some respects in these two evaluation systems [14,15]. It is important to point out that each of these risk groups is consistently heterogeneous, even considering the favorable-risk AML group. Thus, a consistent genotypic and clinical heterogeneity exists within the favorable risk AML, a variability observed also in single molecularly-defined AML subtypes.

A recent study by Herold and coworkers, on 1116 adult AML patients not selected by genetics, validated the ELN-2017 classification and showed that: (i) In 599 patients < 60 years, the overall survival (OS) was 64% for ELN-2017 favorable, 42% for intermediate-risk and 20% for adverse-risk AMLs; (ii) In 517 patients > 60 years, corresponding five-year overall survival (OS) was 37%, 16% and 6% [17]. The analysis of the mutational profile showed that the large majority of *RUNX1, ASXL1* and *TP53* mutations were observed in the adverse risk group; *SRSF2* and *BCOR* mutations were more frequent in the adverse group than in the two other groups; *MLL-PTD* mutations were more frequent in the adverse and intermediate groups, compared to the favorable group; *NRAS* and *DNMT3A* mutations were more frequent in the favorable-intermediate groups compared to the adverse group [17]. These authors proposed to refine the 2017 ELN classification by separating a very favorable subgroup (patients with inv(16)/t(16;16) or biallelic *CEBPA* mutations) from the favorable group, and a very adverse subgroup (patients with *TP53* mutations and a complex karyotype) from the adverse group [17].

The 2017 ELN stratification system has provided and continues to provide an essential support to the risk evaluation of AML patients both in clinical current practice and in clinical trials. However, it is evident that 2017 ELN cannot predict the real risk of a part of AML patients, resulting in either an underestimation or in an overestimation of the individual patient's risk. Furthermore, the 2017 ELN is based on scoring systems that are intrinsically limited by significant heterogeneity existing in AML subtypes. Several recent studies have provided evidence that the 2017 ELN classification can be implemented through a better evaluation of the impact of the individual AML mutational profile; only a technology like NGS makes it feasible to capture the genetic heterogeneity underlying AML heterogeneity, at individual level.

Risk stratification systems integrating mutational or gene expression data were found to add prognostic value to the current ELN risk classification [18,19]. Risk classification of AML based on a combination of molecular and clinical data may contribute to improve AML patient stratification. An example of this approach is given by the prognostic model for AML patients recently proposed by Ma and coworkers; in this model, several parameters including age, hematopoietic cell transplantation-comorbidity index, white blood cell count, hemoglobin, biallelic *CEBPA, DNMT3A* mutations, *FLT3-ITD/NPM1* status and ELN cytogenetic risk status were identified as independent prognostic factors for overall survival in multivariate analysis [20]. This model showed a good performance with a C-index of 0.74 and can be applied to both young and older AML patients, and allows also the distinguishing of eligible candidates for hematopoietic stem cell transplantation [20].

3. The Contribution of the Machine Learning Approach to Improve the Assessment of AML Diagnosis and Prognosis

Machine learning is a branch of computer science and statistics that represents a form of artificial intelligence, based on the development of predictive and descriptive models by learning from training data rather than being pre-programmed according to rigid schemes; the learning approach implies both supervised learning and an unsupervised learning [21,22]. Therefore, machine learning can be considered as a form of interpretation and analysis of a specific reality based on the accumulation and elaboration of thousands of

data, allowing the development of algorithms suitable to analyze the individual complexity and heterogeneity. Thus, it is not surprising that machine learning has rapidly found many applications in medicine from diagnosis, to prognosis and treatment [23]. The applications include also the management of hematological malignancies and particularly of AMLs, at the level of the analysis of genomic and gene expression data for diagnostic, prognostic and therapeutic purposes [21,22,24].

Various recent studies have shown the support of a machine learning approach to the analysis of genomic and transcriptomic data on AML samples.

Some studies were focused to explore a machine learning approach based on large dataset of mutational profiles and clinical data to perform diagnosis of several bone marrow myeloid neoplasms [25] and to predict the outcomes of myelodysplasias, myeloproliferative disorders and chronic myelomonocytic leukemia, particularly for that concerns the risk of AML transformation [26]. Radakovich et al. have used a machine learning approach to explore the genotype-phenotype correlations in patients with MDS and related myeloid malignancies using a large genomic database based on 2697 patients [27]. This analysis showed some associations between genotype and clinical phenotype: *SF3B1* mutations were associated with normal karyotype and some clinical features and *TP53* mutations were associated with complex karyotype; clinical characteristics were also associated with specific genomic alterations: Normal karyotype correlated with the presence of *SF3B1, ZRSR2* and *DNMT3A* mutations and absence of *TP53, ASXL1* and *KRAS* mutations, while age <65 years was associated with the presence of *NRAS* and *JAK2* mutations and the absence of *TET2, SF3B1* and *SRSF2* mutations [27]. These observations support the existence of a link between mutational data and clinical characteristics [27].

Many studies showed the consistent impact of machine learning approach in the discovery of algorithms to improve the AML classification, prognosis prediction and outcomes and screening of drug sensitivity.

Gerstung et al., through the analysis of 1540 AML patients with available matched genomic-clinical data (knowledge bank), developed multistage statistical models more accurately predicting likelihoods of remission, relapse and mortality [24]. This study was based on a modified regression-based method for estimating the likelihood of survival whether a patient received hematopoietic stem cell transplantation in first remission or after relapse [28]. Particularly, this study showed that: (i) Clinical and demographic factors, such as patient age, performance status and blood counts, exerted the most influence on early death rates, including death in remission (due to treatment-related mortality); (ii) genomic features, mostly influencing the dynamics of disease remission and relapse [24]. Using a knowledge bank to model patient outcomes, a substantial fraction (about 1/3) was reclassified and would have their treatment altered compared to current recommendations [28]. Furthermore, personal tailored management decisions could reduce the number of hematopoietic stem cell transplants by 20–25%, while maintaining overall survival rates [28]. Furthermore, about 15% of ELN favorable risk patients are predicted to potentially benefit from stem cell transplantation in first complete remission [28]. It is important to point out that the accuracy of the predictive potential of knowledge bank-based systems largely depend on continuously updated databases based on thousands of patients [28].

Recently, Fleming and coworkers proposed a machine learning (ML) approach to develop a hierarchical prognostic risk model that hierarchically categorizes cytogenetic and molecular factors into groupings that accurately predict survival [25]. This approach was used to explore two large cohorts of AML patients that were classified into four prognostic groups: good (30%), intermediate (26%), poor (26%) and very poor (18%); the ELN2017 classification evaluated these AMLs as: good (39%), intermediate (31%) and poor (30%) [29]. It is important to note that, in this system of AML prognostication, a large number of molecular parameters was taken in account: Complex karyotype, inv(16), $CEBPA^{dmut}$, inv(3)/t(3;3), *FLT3-ITD*, spliceosome mutations (*U2AF1, SRSF2* or *SF3B1*), $NPM1^{mut}$ (in the absence of *FLT3-ITD*), t(8;21), *MLL* translocations, $NRAS^{mut}$, $TP53^{mut}$, $ASXL1^{mut}$ [29]. This evaluation system allowed the prognostication of many AML subgroups: (i) In

the group characterized by complex karyotype, the presence of high-risk monosomies or chromosomal abnormalities or *TP53* mutations have a very poor prognosis, whereas complex karyotypes without these alterations have a better prognosis; (ii) $CEBPA^{dmut}$ AMLs have a good prognosis, particularly when associated with *NRAS* mutations; (iii) co-occurrence of *FLT3-ITD* and spliceosome mutations was associated with very negative outcome; (iv) *FLT3-ITD* high allelic ratio (>0.5) has a very poor prognosis when present in the absence of concomitant *NPM1* mutations; (v) triple mutant *NPM1/DNMT3A/FLT3-ITD* AMLs display a poor prognosis; (vi) AMLs with spliceosome mutations display a poor prognosis when associated with *ASXL1* mutations or *ASXL1* heterozygous deletion; (vii) among *NPM1*-mutant AMLs, *NRAS* co-mutations identified a subgroup associated with good prognosis, whereas those associated with *IDH1* mutations display an intermediate prognosis; (vii) the presence of *KIT* mutations in t(8;21) AMLs was associated with an intermediate prognosis [29].

Using a machine learning approach, Shreve et al. have developed a novel prognostic model of AMLs that incorporates clinical, cytogenetic and mutational data to predict personalized outcomes specific of individual patients [26]. This study was based on genomic data from 3421 patients; a machine learning algorithm capable of accounting for survival was used to generate the new model: In this model, clinical and molecular variables were randomly selected for inclusion in determining overall survival [30]. The analysis of the mutational impact in the various cytogenetic risk groups showed that the most frequently mutated genes in low risk were *NRAS, KIT, FLT3* and *KRAS*, in intermediate risk were *NPM1, FLT3, DNMT3A, IDH2, TET2* and in high risk were *TP53, DNMT3A, NRAS, RUNX1, PTPN11* [30] (Figure 1). Importantly, when assayed on individual data from four cohorts of patients, this evaluation system showed a C-index for overall survival ranging from 0.80 to 0.85 compared to 0.59 when using the 2017 ELN criteria [30]. This study reached also another important conclusion concerning the differential impact of genomic alterations on overall survival in each cytogenetic risk group, thus indicating all the complexities relative to the incorporation of mutational data into risk stratification [30].

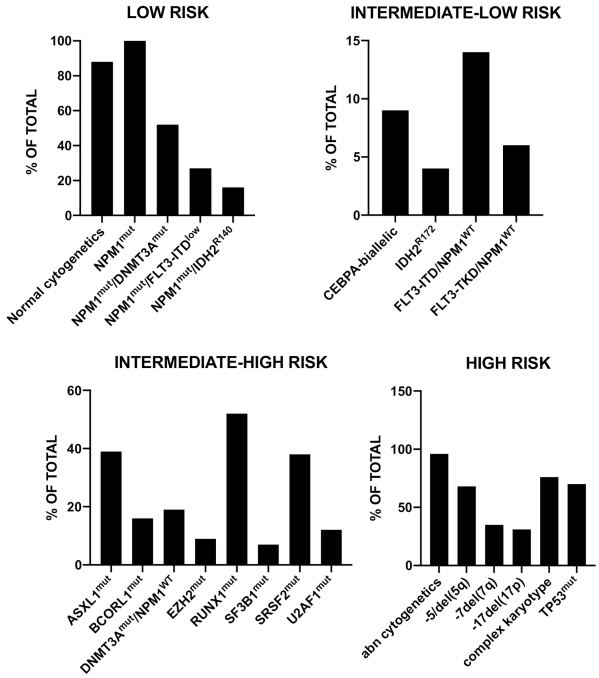

Figure 1. Recurrent gene mutations observed in a group of 3421 AML patients subdivided into three prognostic risk groups (low risk, intermediate risk and high risk), according to a machine learning algorithm accounting for survival. The data are reported in Shreve et al., 2019 [30]. Abbreviations: mut: mutant; del: deletion; abn: abnormal.

Awada et al. have used the machine learning approach to improve the subclassification and prognostication of AML through the collection and analysis of genomic data from a multicenter cohort of 6788 AML patients [31]. Using a logistic regression model some mutations resulted enriched in pAML (*CEBPA*^{mono}, *CEBPA*^{biallelic}, *DNMT3A*, *FLT3-ITD*, *FLT3-TKD*, *GATA2*, *IDH1*, *IDH2*^{R140}, *NRAS*, *NPM1*, *WT1*) and other mutations in sAML (*ASXL1*, *RUNX1*, *SF3B1*, *SRSF2*, *U2AF1*, *−5/del(5q)*, *−7/del(7q)*, *−17/del(17P)*, *del(20q)*, *+8* and complex karyotype) [31]. Using the Bayes latent class analysis, four unique genomic clusters of distinct prognoses were identified: low risk, intermediate-low risk, intermediate-high risk and high risk [27]. The generation of a random forest model allowed the extraction of invariant genomic features driving each group; the main genomic alterations observed in these four groups are reported in Figure 2 [31].

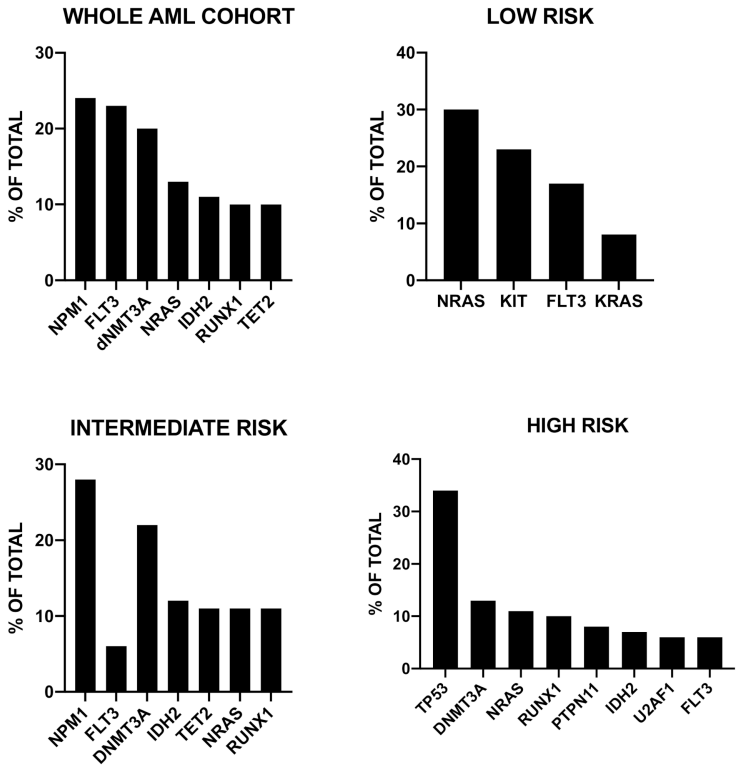

Figure 2. Recurrent genetic abnormalities observed in 6788 AML patients stratified into four prognostic risk groups (low risk, intermediate-low risk, intermediate-high risk and high risk), according to a machine learning algorithm. The data are reported in Awada et al., 2020 [31].

Siddiqui et al. have shown the potential of machine learning algorithms, trained using factors available at the time of admission for AML treatment, to predict death during the patient's hospitalization; this study was based on the acquisition of data relative to a total of 29,613 patients with AML [32].

The machine learning approach was shown to be very useful for a prognostic identification of AMLs with specific driver mutations. Supervised machine learning analysis of the profile of genetic alterations observed patients with *RUNX1-RUNXT1* AMLs identified the presence of concurrent *NRAS* mutations and the absence of mutations in *ASXL2*, *RAD21*, *KIT* and *FLT3* genes and low mutational burden as conditions of favorable genetic risk [33]. According to these data, the patients were stratified into a poor genetic risk group associated with lower overall survival and relapse-free survival, compared to the group of patients classified as good genetic risk [33]. In another study, Patkar and coworkers, using the machine learning approach, have developed a scoring model for the risk stratification of AML patients with *NPM1* mutations; in this model, the five top variables to predict the outcomes of these leukemias were *NPM1* VAF (variant allelic frequency), *FLT3-ITD*

VAF, presence of *IDH2* mutations, *DNMT3A R882* mutation, and type A *NPM1* mutation: The presence of type A *NPM1* and *IDH2* mutations and low levels of *FLT3-ITD* VAF and *NPM1* VAF and absence of *DNMT3A* mutations were favorable prognostic factors [34]. This scoring system allowed to stratify *NPM1*-mutant AMLs into three groups with favorable, intermediate and poor genetic risk, exhibiting a remarkably different outcome in terms of overall survival and relapse-free survival. Furthermore, a strong statistical correlation was observed between ML-derived genetic risk and post-induction flow cytometry minimal residual disease [34].

The analysis of gene expression profiling was essential to improve the molecular classification of AMLs and to identify new biomarkers suitable for clinical studies. Initial studies have led to the identification of gene expression signatures with a prognostic predictive value. Thus, Bullinger et al. reported the identification of a 133-gene clinical-outcome predictor, which accurately predicted overall survival, including also patients with AML with a normal karyotype [35]. Li et al., through the analysis of a very large set of data derived from different cohorts of AML patients, identified a robust prognostic signature composed of 24 genes, capable of predicting overall survival and event-free survival. Furthermore, this signature provides a significant improvement of the ELN risk classification of AML [36]. Marcucci et al., through an epigenetic analysis involving the analysis of genes with differently methylated regions in older AML patients, reported the identification of seven genes whose expression was associated with outcome: A low score was associated with a better complete remission rate and longer disease-free survival and overall survival [37]. Ng et al. used an approach aiming to develop predictive and/or prognostic biomarkers related to stemness, based on the identification of genes that are differentially expressed between leukemic stem cell-positive and leukemic stem cell-negative cell fractions derived from 78 AMLs. Using this approach, they identified 17 genes whose expression score was highly prognostic in five different cohorts of AML patients, comprising different AML subtypes [38]. More recently, some machine learning studies were based on the analysis of transcriptomic AML profiles. Warnat-Herresthal reported the use of LASSO (Least Absolute Shrinkage and Selection Operator) regression analysis, a machine learning method allowing to automatically select the most predictive characteristics, for the automatic diagnosis and classification of AMLs based on transcriptomic data [35]. This analysis utilizes data from 105 studies, involving 12,029 AML, ALL (Acute Lymphoblastic Leukemia), MDS patients and normal subjects; this system identified AMLs based on transcriptomic data, with >99% accuracy [39]. Interestingly, Wagner et al. used an artificial neural network (ANN)-based machine learning approach to a dataset of 593 AML and identified a three-gene expression signature comprising *CALCRL, CD109* and *LSP1,* predictive of both overall and relapse-free survival [40]. This three-gene prognostic index separated the adult AML patients in each 2017 ELN cytogenetic risk category into subgroups with different survival probabilities and allowed also the identification of patients with high-risk features [40]. The prognostic impact of this three-gene index was validated in different cohorts of AML patients, including childhood AML [40]. In another study, Rouphail and coworkers have used a machine learning approach, based on the analysis of transcription data relative to 242 AML patients, mainly *NPM1*-mutated, to define differences in transcription signatures of $NPM1^{mut}$/*FLT3-ITD* compared to $NPM1^{mut}$/*FLT3-WT* [41]. The algorithm that this study developed, identified 20 genes that are highly specific for $NPM1^{mut}$/*FLT3-ITD* AMLs; these genes affect key biochemical pathways involved in the regulation of cell differentiation, proliferation, mitochondrial oxidative phosphorylation, histone modification and lipid metabolism [41].

Other few recent studies have started to explore the possible contribution of machine learning approach to develop models to predict the outcome of allogeneic stem cell transplantation. Thus, Gandelman and coworkers provided preliminary evidence that machine learning computational studies may better reveal biomarkers and stratify risk of patients with hematological malignancies undergoing allogeneic SCT, than the current approach based on cumulative severity [42]. Choi and coworkers have shown the feasibility of the

machine learning-based approach, using random forest models, to predict survival after allogeneic stem cell transplantation in hematologic malignancies [43]. Finally, Nazha and coworkers have developed a personalized prediction model for outcomes after allogeneic SCT in patients with MDSs; the algorithm developed in this model identified the following variable prior to cell transplant impacting overall survival: Age, *TP53, RAS, JAK2, ZRS2* and *CUX1* mutations, cytogenetic profile, conditioning regimen, donor age, WBC count, hemoglobin and diagnosis of therapy-related MDS [44]. Importantly, this novel model is able to provide survival probability at different time points specifically for a given patient [44].

A machine learning-based approach was used also in drug discovery and development. Thus, Lee et al. have reported the development of a novel method for predicting AML drug sensitivities; this approach incorporates information learned from the Tumor Cancer Genome Atlas to help to support the link between genetic alterations and the pattern of drug sensitivity [45]. Using this approach, the authors identified *SMARCA4* as a marker and driver of sensitivity to the topoisomerase II inhibitors mitoxantrone and etoposide, showing that the increased sensitivity predicted by the model was confirmed by in vitro assays [41]. Other studies reported the use of machine learning in drug screening: Chen et al. adopted machine learning to evaluate potential STAT3 inhibitors in AML [46]; Cutler and Fridman developed a machine learning-based model to predict high sensitivity to the compound FLX925, a FLT3 inhibitor, in AML [47].

Machine learning algorithms have been also applied to the detection and analysis of minimal residual disease. Minimal (or measurable) residual disease refers to the detection of residual leukemic cells below the threshold for morphological recognition and represents an important tool to evaluate the response after anti-leukemia treatment and is an important prognostic indicator for AML. MRD (Minimal Residua Disease) can be detected either using molecular assays or multiparameter flow cytometry (MFC). MFC has been and is extensively used in the detection of MRD in various hematological malignancies, including AML and MDS. However, the current methodology of MFC is complex at the level of data interpretation for the problem of manual flow cytometer gating. To bypass these difficulties, Ko et al. have used two machine learning techniques to develop an MFC interpretation algorithm for MRD detection using two large cohorts of AML and MDS patients [48]. High clinical validity of the algorithm was demonstrated through appropriate outcome prediction in the post-induction chemotherapy setting [48].

4. An Integrated Approach Is Required for the Development of Personalized Medicine in AML

The diagnosis of AMLs requires a multidisciplinary, integrated diagnostic approach, based on cytomorphology, cytochemistry, immunophenotyping, cytometry and molecular genetics [49]. A multidisciplinary diagnostic approach is today fundamental for appropriate AML subtype identification, patient prognostication and to define optimal therapy and also for definition of markers to monitor response to therapy [49]. This approach is particularly important in view of the development of precision medicine.

Furthermore, functional approaches, such as ex vivo drug sensitivity and resistance profiling, may cooperate with genomic, epigenomic and transcriptomic data in the identification of new targeted therapies and thus increase the number of drugs that can be tailored to AML patients [50].

Tyner and coworkers performed an integrative analysis of clinical, cytogenetic, molecular genetic and transcriptional data, together with in vitro testing of primary samples, examining drug sensitivity against 122 different compounds [46]. This functional genomic analysis was performed on a large cohort of 562 AML patients based on whole exome sequencing, RNA-sequencing and ex vivo drug sensitivity analyses [51]. This approach showed several relevant findings: (i) Genetic subgroups, including *TP53* or *ASXL1* mutations, were associated with widespread drug sensitivity; (ii) a sensitivity of *FLT3-ITD* mutant AMLs to FLT3 inhibitors; (iii) *NRAS*-mutant AMLs resistant to most of the drugs, but sensitive to MAPK inhibitors; (iv) *IDH2*-mutant AMLs are sensitive to several drugs,

whereas the contrary is true for *IDH1*-mutant AMLs; (v) *RUNX1*-mutant AMLs are sensitive to PIK3C/MTOR inhibitors; (vi) AMLs with mutations of spliceosome genes display a peculiar pattern of drug sensitivity; (vii) triple mutant *NPM1/FLT3/DNMT3A* AMLs are sensitive to ibrutinib [51]. Co-occurrence of some genetic mutations and some gene expression clusters were associated with and predicted response to specific drugs [51].

Recent advancements in understanding of the molecular alterations of AMLs have determined the generation of a growing number of molecularly targeted drugs, such as FLT3 and IDH inhibitors. However, several limiting factors hinder the development of effective single-agent targeted therapies, including the more or less pronounced heterogeneity of AML subtypes, the emergence or amplification of pre-existing subclones leading to relapse, and protective signals mediated by the tumor microenvironment. The combination of drugs that target different pathways may represent a valuable strategy to improve the response and to reduce the resistance mechanisms by tumor cells. Thus Kurtz et al. have evaluated, on fresh AML blast cells, the sensitivities to combinations of molecularly targeted drugs acting on different cell-signaling responses, and have correlated these responses with the diagnostic clinical/genetic/cytogenetic and cellular features of the various patient samples [52]. These studies showed that, for AML cells, several combinations of targeted agents that include venetoclax (a Bcl2 inhibitor) and a kinase inhibitor are affective [52].

Various authors have reported automated systems for ex-vivo drug testing capable of predicting chemosensitivity in AML patients [53–55].

Other studies have evaluated the chemogenomic landscape of molecularly-defined AML subsets. Thus, Simon et al. have evaluated, in parallel, the mutational spectrum and gene expression profile of *RUNX1*-mutated AMLs and have correlated these results to drug sensitivity assayed in vitro [56]. Chemical screening showed that most *RUNX1*-mutated AML specimens are sensitive to glucocorticoids, resulting in an inhibitory effect on cell proliferation [56]. Moison et al. have reported a comprehensive genomic and transcriptomic analysis of a cohort of AML patients with complex karyotype and identified in these AMLs the frequent (about 80%) aberrant expression of the *HMGA2* oncogene, in a *TP53*-independent manner [46]. *HMGA2* mediated sensitization of complex karyotype AMLs to G2/M checkpoint, thus offering a potential therapeutic opportunity using drugs such as CHK1 and PLK1 inhibitors [57].

As above reported, venetoclax-based therapy (venetoclax in association with hypomethylating agents such as azacytidine or decitabine) can induce responses in about 70% of older previously untreated AML patients. However, upfront resistance, as well as acquired resistance determining relapse limit the effectiveness of this treatment. Zhang and coworkers have used an integrated genomic and functional screen data analysis to identify biomarkers predicting venetoclax sensitivity and resistance in AML and to identify venetoclax combination strategies to bypass resistance mechanisms [54,55]. By integrating the clinical data, exome and RNA sequencing, and inhibitor data from samples derived from approximately 200 samples of treated patients, several conclusions were reached: A myelomonocytic phenotype of leukemic cells (as supported by high CD14 expression), upregulation of *BCL2A1* and *CLEC7A* and mutations of *PTPN11* and *KRAS* were associated with resistance to venetoclax and multiple venetoclax combinations; venetoclax in combination with an inhibitor of the antiapoptotic protein MCL1 (AZD5991) induced synthetic lethality and bypassed venetoclax resistance [58,59].

Another study confirmed a link between *PTPN11* mutations and venetoclax resistance and showed also that mutant *PTPN11* induces, in leukemic cells, an increase of oxidative phosphorylation and glycolysis: This metabolic modification determines resistance to venetoclax that can be bypassed by a MCL1 inhibitor [60]. Recent studies have characterized AMLs bearing *PTPN11* mutations showing several peculiar findings: Frequent myelo-monocytic morphology; frequently co-mutated with *NPM1* and *FLT3-ITD* and less frequently with *IDH2* and complex karyotype; an adverse prognosis compared to *PTPN11-WT* AMLs (8.4 vs. 13.6 months of median overall survival) [61].

Another study confirmed the resistance to venetoclax of monocytic AMLs: These leukemic cells have a distinct transcriptomic profile, lose expression of BCL2 and rely on MCL1 to mediate oxidative phosphorylation and survival [62].

Spinner et al. used an ex-vivo drug screening to define novel drug sensitivity patterns for informing personalized therapy in a group of 21 MDS patients resistant to hypomethylating agents [63]. Ex-vivo drug screening was performed within a clinically actionable time frame (a median time of 15 days) and showed drug sensitivity patterns heterogeneous, defining distinct patient clusters with differential sensitivity to hypomethylating agents, anthracyclines, histone deacetylase inhibitors and kinase inhibitors. Furthermore, a synergy between hypomethylating agents and venetoclax was observed [63]. These results on drug sensitivity informed personalized therapy. In 21 patients with ex vivo and in vivo clinical response data, the ex-vivo drug sensitivity screening platform showed a positive predictive value of 0.92, negative predictive value of 0.82, and overall accuracy of 0.85 [63].

While the above reported studies supported a role of integrated chemogenomic approach to identify AML subsets associated with sensitivity/resistance to specific drugs or to identify new potential treatments in AML subtypes, other recent studies directly implied the chemogenomic approach into a clinical trial.

Snijder et al. evaluated, in a prospective study, the feasibility and efficacy of ex-vivo drug-response profiling to guide personalized treatment selection across large panels of possible treatments for patients affected by aggressive hematological malignancies, including AMLs [64]. This study was based on a new image-based, drug-response profiling technique called pharmacoscopy, which uses high-throughput, automated confocal microscopy, immunofluorescence and singe-cell image analysis [64]. Pharmacoscopy, retrospectively predicted the clinical response of 20 AML patients to induction therapy with 88% accuracy [64]. Seventeen patients received the pharmacoscopy-guided treatment, providing preliminary evidence that this treatment is feasible, safe and effective [64].

In another study, Collignon et al. have assessed the feasibility of a tailored treatment strategy guided by systematic ex vivo drug sensitivity/resistance profiling and targeted NGS for patients with refractory/relapsed AML [65]. A tailored treatment strategy could be achieved in 47/55 AML patients: Five based only on targeted NGS, six on drug sensitivity/resistance profiling and 36 on both techniques [65]. The tailored treatment strategy was available in <21 days for 28 patients participating to the study; three to four potentially active drugs were selected for each patient; five patients resulted resistant to the whole panel of drugs tested [65]. Seventeen patients received a tailored treatment strategy and resulted in 4 complete remissions, one partial remission and five decreased peripheral blast cell counts [65].

5. Challenges in Clinical Development of Targeted Therapies for AML

The fundamental aim of targeted therapy consists in improving overall survival compared to the best standard therapy. The results obtained using various agents targeting mutant FLT3 or IDH showed that while it is possible to obtain a consistent number of objective responses, it is much more difficult to obtain an improvement of overall survival compared to standard therapy.

The analysis of the clinical results observed using the various FLT3 inhibitors provides an example of this problem. Three FLT3 inhibitors, midostaurin, quizartinib and gilteritinib, have shown the capacity to induce significant therapeutic effects on FLT3-mutated AMLs in the context of randomized clinical studies.

The phase III randomized RATIFY study showed that midostaurin, in association with standard induction chemotherapy, administered during induction and consolidation phases, to de novo AML patients with *FLT3-TKD* or *FLT3-ITD* mutations, significantly improved overall survival compared to chemotherapy plus placebo: At four years, overall survival of 51.4% vs. 44.3%; median overall survival of 74.7 months vs. 25.6 months [66]. On the basis of these results, midostaurin was approved for the treatment of adult AML patients with newly diagnosed *FLT3*-mutated AMLs [66]. A recent update of the follow-up

at five years of this study confirmed, in part, the results previously reported. For patients treated with midostaurin plus chemotherapy versus placebo plus chemotherapy: Event-free survival was 45.2% vs. 30.1%; disease-free survival was 67.3% vs. 53.4%; however, the overall survival was similar in the two groups [67]. A sub-analysis of molecular AML subtypes showed that *NPM1*-mutated and *CBF*-mutated AMLs display a significantly improved overall survival in the group treated with midostaurin [68].

In AML patients with refractory/relapsed FLT3-mutated AMLs, two second-generation FLT3 inhibitors, quizartinib and giltertinib were evaluated at clinical level. Quizartinib showed clinical activity as single treatment in refractory/relapsed *FLT3*-mutated AML patients, with a 47% of marrow complete responses [69]. In a phase III randomized study (QUANTUM-R trial) on 367 AML patients with refractory/relapsed *FLT3*-mutated AML patients, quizartinib improved complete remission rates and overall survival compared to investigator choice salvage chemotherapy—48% vs. 27% and 6.2 months vs. 4.7 months, respectively [70]. Thus, the effect of quizartinib on overall survival was minimal for this category of patients. Similarly to quizartinib, gilteritinib, another second generation FLT3 inhibitor, displayed activity as single-agent in refractory/relapsed *FLT3*-mutated AMLs (CHRYSALIS trial), inducing 37% of marrow complete responses [71]. In a randomized phase III study (ADMIRAL trial), giltertinib administration was associated with higher complete remission rates and overall survival compared to investigator choice salvage chemotherapy—54% vs. 22% and 9.3 months vs. 5.6 months, respectively [72]. A secondary analysis of the ADMIRAL trial showed that treatment with gilteritinib compared with salvage chemotherapy induces more refractory/relapsed *FLT3*-mutant AMLs to achieve complete responses, to proceed to hematopoietic stem cell transplantation and to remain alive at one year [73]. An analysis carried out both in the CHRYSALIS and in the ADMIRAL trials provided evidence that patients with refractory/relapsed AMLs who received prior FLT3 inhibitors (midostaurin or sorafenib) were able to achieve remission with gilteritinib (about 50% of responding patients) [74].

A comparative analysis of the results obtained in QUANTUM-R and in ADMIRAL trials suggested comparable results in terms of complete responses with quizartinib or with gilteritinib; this comparison suggested also that remission is achieved faster with quizartinib, while response may be durable and survival potentially longer with gilteritinib [75].

These two FLT3 inhibitors have been tested also in combination therapies. Thus, quizartinib is under evaluation in combination with azacytidine or low-dose AraC in older *FLT3-ITD*-mutated AML patients, reporting a complete remission rate of 83% and a median overall survival of 18.6 months [76]. In a similar ongoing study (LACEWING trial), using gilteritinib in place of quizartinib, 67% of complete responses were observed [77]. However, at the end of December 2020, Astellas Company reported that in the phase III LACEWING trial gilteritinib failed to extend survival in newly diagnosed AML patients and thus failed to meet its primary overall survival endpoint.

Other ongoing clinical studies are exploring the FLT3 inhibitors gilteritinib or quizartinib in combination with the Bcl-2 inhibitor venetoclax. Preliminary interesting results were reported in a study based on the administration of gilteritinib in combination with the BCL2 inhibitor venetoclax to a cohort of heavily pretreated *FLT3*-mutated AML patients, showing 84% of molecular complete responses [78]. In line with this observation, Maiti et al. have recently reported the results on the treatment with venetoclax, FLT3 inhibitor and decitabine of 30 *FLT3*-mutated AML patients, 14 previously treated and 16 treatment-naïve. In previously treated AMLs, the complete remission rate was 64% and MRD negativity rate was 88%; in treatment-naïve AML patients, the complete remission rate was 88% and the MRD negativity was 100%, with an overall survival rate at two years of 90% [79]. These observations suggest that the giltertinib plus venetoclax may have great therapeutic impact. The combination of venetoclax with quizartinib is under evaluation in an ongoing study. Preliminary results on 11 *FLT3*-mutated AML patients, mostly refractory/relapsing, treated with quizartinib, venetoclax and decitabine showed a high response rate (90%) and a six months overall survival rate of 86% [80].

The results of induction chemotherapy trials in association with second generation FLT3 inhibitors such as quizartinib or giltertinib will be of fundamental importance, and will allow to perform a comparison with the results observed in the RATIFY trial with midostaurin. In this context, Pratz et al. recently reported a phase 1 study assessing the tolerability and antileukemic effects of gilteritinib plus induction chemotherapy and high-dose AraC consolidation chemotherapy, and as single-agent maintenance therapy in adults with newly diagnosed AML; patients achieving complete remission undergo HSCT and resume gilteritinib treatment post-HSCT [81]. Median overall survival for *FLT3*-mutant patients was not reached and the survival probability at 8, 12, 26, 52 and 104 weeks was 98%, 95%, 93%, 83% and 72%, respectively [81]. Based on these results, randomized clinical trials of induction and consolidation chemotherapy plus gilteritinib versus midostaurin have been initiated. Preliminary results in the phase I trial based on quizartinib administration in association with standard induction chemotherapy have shown 74% of complete responses [82].

Crenolanib is a potent type I multikinase inhibitor with activity against *PDGFR, FLT3-ITD* and *FLT3-TKD* mutations, including resistance-conferring point mutations [83]. Phase II clinical studies have shown the efficacy of crenolanib as single-agent in refractory/relapsing *FLT3*-mutated AML patients, reporting a rate of complete remission ranging from 23% to 39% in patients naïve to treatment with FLT3 inhibitors and 5% among patients previously exposed to FLT3 inhibitors [84,85]. Two phase II clinical studies have shown promising activity of crenolanib in association with chemotherapy. One of these two studies showed a high complete remission rate of 85% in patients with newly diagnosed *FLT3*-mutated AML undergoing treatment with crenolanib plus chemotherapy: At a median follow-up of 29.3 months, 70% of patients remained alive and disease-free [86]. The other study enrolled newly diagnosed *FLT3*-mutated adult AML patients with an age comprised between 61 and 75 years: the treatment with crenolanib plus chemotherapy induced a complete remission rate 86% [87]. An ongoing phase III randomized multi-center study (NCT 03258331) was designed to compare the efficacy of crenolanib with that of midostaurin when administered following induction chemotherapy, consolidation chemotherapy and bone marrow transplantation in newly diagnosed AML patients with *FLT3* mutation. Two recent reports on a limited number of patients treated on compassionate basis provided evidence of clinical benefit of crenolanib in *FLT3*-mutated adult AML patients relapsing after previous gilteritinib treatment [88] and in *FLT3*-mutated pediatric AML patients relapsing after multiple previous treatments and harboring resistant *FLT3-ITD* and *FLT3-TKD* mutations [89].

The clinical studies based on the use of FLT3 inhibitors for the treatment of FLT3-mutated AML patients clearly support the rationale of this therapeutic strategy but at the same time indicate the difficulties of improving patient's survival. Thus, these studies indicate in some instances the need of selecting subgroups of *FLT3*-mutated AML patients (see the final results of the midostaurine studies) or the need of selecting the appropriate FLT3 inhibitor (see the studies using quizartinib or gilteritinib in relapsed/refractory AML patients). The combination studies based on the use of quizartinib or gilteritinib have shown promising results, but phase III randomized studies are required to prove that these initial promising results will translate into an improved overall survival.

Oral small-molecule inhibitors of mutant *IDH1* (ivosidenib) and *IDH2* (enasidenib) have been tested in preclinical studies and then have been evaluated at clinical levels, showing efficacy in AML patients with *IDH1* and *IDH2* mutations, respectively. A phase I clinical study on relapsed/refractory AML patients with *IDH1*-mutated AMLs ivosidenib induced a complete remission rate of 21.6%, with an overall survival of 8.8 months [90]. Enasidenib, in a phase II study on relapsed/refractory *IDH2*-mutant AMLs, induced a 20.6% complete remission rate with an overall survival of 9.3 months, with an estimated one-year survival of 39%; in patients achieving a complete response, the median overall survival was 19.7 months [91]. An update of this study showed that response rates were similar for patients in relapse or with refractory disease and for patients with either *IDH2-R140* or

IDH2-R172 mutations [92]. On the basis of the clinical activity of these IDH inhibitors, the FDA approved ivosidenib and enasidenib for patients with refractory/relapsed *IDH1*- and *IDH2*-mutated AML, respectively, in 2018. Ivosidenib was approved also for the treatment of newly-diagnosed AML patients with *IDH1* mutations who are old (≥75 years of age) on the basis of the results of a phase I trial showing a complete remission rate of 42%, with median overall survival of 12.6 months; furthermore, *IDH1* mutation clearance was observed in 65% of patients achieving a complete response [93]. Similarly, Pollyea et al. reported the results of a phase I/II study showing in a group of 39 older *IDH2*-mutant AMLs treated with enasidenib a response rate of 30.8%, with 18% of complete responses and a median overall survival of 11.3 months; in these patients, the presence of *DNMT3A* mutations was associated with complete responses [94].

Paschka et al. have recently reported a comparison of the outcomes in a group of 105 *IDH1*-mutant AML patients treated with ivosidenib after at least two previous treatments with historical control, showing that: Ivosidenib-treated patients showed a significantly improved overall survival compared to historical control (8.1 months vs. 2.9 months, respectively) [95].

Unfortunately, a significant proportion of *IDH*-mutant AML patients show primary or secondary resistance to IDH inhibitors. Among the mechanisms of primary resistance, an important role is played by mutations in *NRAS/KRAS* genes or in other genes encoding MAPK effectors, such as *PTPN11, NF1* and *FLT3* mutations, enriched at baseline in patients with primary resistance to ivosidenib and enasidenib [96]. Mechanisms of secondary resistance englobe mutations at the level of *IDH* genes, involving isoform switching from *IDH1* to *IDH2* mutation or vice versa [97] or development of second-site *IDH2* missense mutations at the level of the nonmutant allele [98].

In order to improve the clinical response to IDH inhibitors, combination therapies that target both leukemic clones/subclones IDH-dependent and IDH-independent have been attempted. Notable examples are given by combination studies using IDH inhibitors with hypomethylating agents or chemotherapy or BCL2 inhibitors.

In a phase I study, DiNardo and coworkers have evaluated the safety and efficacy of ivosidenib in association with azacytidine in newly diagnosed *IDH1*-mutant AML patients ineligible for intensive chemotherapy, showing an overall response rate of 61% and 12-month survival of 82%; mutant IDH1 clearance was observed in 71% of patients achieving complete remission [99,100]. The results of this study have supported the development of the phase III randomized AGILE study, aiming to compare event-free survival in patients receiving azacytidine + ivosidenib compared to that observed in patient treated with azacytidine+placebo. A similar study was performed in *IDH2*-mutant AML patients involving the randomization of 101 patients to treatment with azacytidine alone or in association with enasidenib: the combination treatment was associated with significantly improved complete remission and overall response rates and significant mutant *IDH2* VAF reductions compared with treatment with azacytidine alone [99]. An update evaluation of this study showed that overall response rate, duration of remission and complete remission rates were all significantly improved in azacytidine+enasidenib-treated patients compared to azacytidine alone [101].

IDH mutations were detectable in about 40–50% of AML patients in remission and are associated with an increased risk of disease relapse, as compared to those with undetectable *IDH1/IDH2* mutations [102]. This finding strongly supports the rationale of associating chemotherapy with IDH inhibitors in an attempt to reduce/abrogate residual leukemic disease. In 2018, Stein and coworkers have reported the preliminary results of a phase I trial involving the administration of ivosidenib or enasidenib in association with induction chemotherapy in patients with newly diagnosed *IDH1*-mutant or *IDH2*-mutant AMLs [103]. Forty-one patients were treated with ivosidenib and induction chemotherapy—93% of patients with de novo AML achieved a complete response and 46% of those with sAML; 77 patients were treated with edasidenib and induction chemotherapy: 73% of patients with de novo AML achieved a complete response and 63% of those with sAML [104]. The

final report of this phase Ib study involved 60 patients with *IDH1*-mutated AMLs and 91 with *IDH2*-mutated AMLs; complete remission rates were 72% and 63%, respectively; in patients achieving complete response, 39% of those receiving ivosidenib had mutant *IDH1* clearance by digital PCR and 23% of those receiving enasidenib displayed mutant *IDH2* clearance [103]. However, although results have not yet published, the phase III IDHENTIFY study evaluating enasidenib plus best supportive therapy, versus conventional care regimens, failed to support the primary endpoint consisting to show an improved overall survival using the IDH2 inhibitor in older patients with refractory/relapsed AMLs with *IDH2* mutations.

Recent studies have shown the consistent efficacy of the BCL2 inhibitor venetoclax in combination with either hypomethylating agents (azacytidine or decitabine) or low-does AraC for the treatment of elderly AML patients [105,106]. The most responsive patients to the treatment with venetoclax plus low-dose AraC are those with *IDH1/IDH2* mutations (with a median overall survival of 19.4 months) and to the treatment with venetoclax in combination with hypomethylating agents (with a median overall survival of 24.4 months) [106]. The molecular characterization of older AML patients undergoing treatment with venetoclax plus low-dose AraC or azacytidine showed that high-response rates were associated with *NPM1* and *IDH2* mutations. Particularly, *IDH2* mutations were absent among patients resistant to these treatments [107]. In contrast, the frequency of *IDH1* mutations does not seem to be associated with the response to venetoclax [108].

DiNardo et al. have recently reported the results of a confirmatory, randomized clinical study (VIALE-A) involving 431 newly diagnosed AML patients treated with azacytidine+venetoclax or azacytidine plus placebo. At a median follow-up of 20.5 months, the median overall survival was 14.7 months in the azacytidine-venetoclax group and 9.6 months in the control group; the incidence of complete remissions was higher with azacytidine+venetoclax than in the control regimen (66.4% vs. 28.3%) [107]. Importantly, in patients with *IDH1* and *IDH2* mutations, the incidence of remission was 75.4% in the azacytidine+venetoclax group compared to 10.7% in the control group; in patients with *IDH1* and *IDH2* mutations, overall survival at 12 months was 66.8%, compared to 35.7% in the control group [107]. Very recently, Pollyea and coworkers reported the results of an ongoing phase III study involving the evaluation of venetoclax+azacytidine vs. azacytidine plus placebo in a group of 107 *IDH1/IDH2*-mutated treatment-naïve AML patients unfit for intensive treatment either due to comorbidities and/or age $\geq$ 75 years [109]. Venetoclax+azacytidine compared to azacytidine monotherapy resulted in higher response rates and median overall survival for both *IDH1*-mutated (complete remission—59% vs. 9%; median overall survival—17.5 months vs. 2.2 months) and *IDH2*-mutated (complete remission—80% vs. 6%; median overall survival—not-reached vs. 13.0 months) [109].

In parallel to the use of azacytidine as a hypomethylating agent, other studies have investigated another hypomethylating agent, decitabine. To this end, a phase II trial explored older AML patients (>60 years) not eligible for intensive chemotherapy, secondary AML and relapsed/refractory AML [110]. In this study, patients received decitabine and venetoclax in the induction and in the consolidation phases. A final report of this study was recently published [110]. The median overall survival was 18.1 months in newly diagnosed AMLs; 7.8 months in untreated secondary AMLs; 6.0 months in treated secondary AMLs; 7.8 months in refractory/relapsed AMLs [110]. Importantly, some molecularly-defined AML subsets showed a consistent sensitivity to this treatment. Particularly, *IDH1*-mutant and *IDH2*-mutant AMLs showed among newly diagnosed AML patients a complete remission rate of 84%, a median duration of response and of overall survival not reached; among previously treated, high-risk *IDH1*-mutant and *IDH2*-mutant AML patients showed a complete remission rate of 50%, with an MRD negativity in 25% of cases and a median overall survival of 7.8 months [111].

Few studies have explored patient outcomes after failure of frontline therapy with venetoclax and hypomethylating agents. Thus, Maiti et al. have investigated 41 patients relapsing after venetoclax plus hypomethylating agent treatment, and reported a very

short overall survival of 2.4 months for these patients. In this group of patients, all *IDH1* and *IDH2*-mutant AML patients had adverse-risk cytogenetics and co-occurring mutations in *TP53, NRAS, KRAS, FLT3* and *KIT* [110]. Hammond and coworkers have recently reported the analysis of response patterns of 65 *IDH*-mutant AML patients with newly diagnosed or refractory/relapsing disease treated with venetoclax and a hypomethylating agent [112]. A total of 79% of patients achieved an objective response, with 69% of complete responses; 90% of complete responders displayed a negative flow cytometry minimal residual disease; presence of a RAS pathway and/or *TP53* mutation was associated with a lower frequency of complete responses in refractory/relapsing patients [112]. The analysis of the results in different groups of patients showed that: The combination of venetoclax and hypomethylating agents induced a very high rate of complete responses, with a long duration of response, in *IDH1/IDH2*-mutated AMLs, in the frontline setting and in *NPM1* co-mutated cases; about 50% of the treated patients retain an *IDH* mutation detectable by next generation sequencing; good outcomes have been observed also in the relapsed setting, although the presence of *TP53/RAS* mutations confer resistance to the treatment; high rates of salvage responses were observed in relapsed patients switched from venetoclax plus hypomethylating agent to IDH inhibitor treatment [112].

Venetoclax was explored in association with low-dose and with intensive chemotherapy. Concerning the studies with low-dose chemotherapy, venetoclax was associated with low-dose cytarabine. In a first study, 82 older AML patients, not eligible for intensive chemotherapy (50% with sAML and 32% with poor-risk cytogenetics), were enrolled and treated with low-dose AraC, associated with venetoclax: de novo AMLs displayed 71% complete remission and median duration of response of 11.6 months; sAML showed 35% complete remission and a median duration of response of 8.1 months [106]. Patients with *NPM1* and *IDH1/IDH2* mutations had better outcomes, with complete remission rates of 89% and 72%, respectively [108]. The confirmatory VIALE-C trial randomized 211 older AML patients to the treatment with low-dose AraC with or without venetoclax: The median overall survival was 8.4 months in the double treatment and 4.1 months in the single treatment arm; the event-free survival was 4.7 months versus 2.0 months; the complete remission rates were 48% and 13% for the venetoclax combination and low-dose chemotherapy alone [113]. Concerning the patients with *IDH1/IDH2* mutations, the complete remission rate was 57% in the double treatment compared to 38% in the single treatment arm; the overall survival was 10.8 months compared to nine months, respectively [113].

The CAVEAT study involved the treatment of older AML patients with an initial treatment with seven-day administration of venetoclax, followed by venetoclax in combination with intensive chemotherapy; the overall response of complete remissions was 72–97% in de novo AMLs and 42% in secondary AMLs [114,115]. *NPM1*-mutant and *IDH1/IDH2*-mutant AMLs are the AMLs achieving greatest bone marrow blast reduction after seven days of pre-treatment with venetoclax; complete remissions were observed in 100% of *IDH2*-mutant AMLs and in 62% of *IDH1*-mutant AMLs [107,109]. *IDH2*-mutant AMLs displayed the longest overall survival; *IDH1*-mutant AMLs are less sensitive to this treatment [114,116].

The data related to the efficacy and to the favorable safety profiles of venetoclax and of IDH1/IDH2 inhibitors have strongly supported the evaluation of the drug association of venetoclax and IDH inhibitors, aiming to demonstrate a potential synergy between these two drug types. Preliminary results of the trial NCT03471260, a phase I/II trial of a combination of venetoclax with ivosidenib, with or without azacytidine in AML patients with mutated *IDH1*, were recently presented for the first 18 evaluated patients: The global complete remission rate was 89% (100% with ivosidenib+venetoclax 800 mg, 67% with ivosidenib+venetoclax 400 mg and 67% with ivosidenib+venetoclax 400 mg + azacytidine) [117]. After a median follow up of 3.5 months, median overall survival was not reached in treatment-naïve patients, and 9.7 months in refractory/relapsing patients; half of patients who achieved complete response also were MRD negative [115].

In conclusion the clinical studies carried out using IDH1/IDH2 inhibitors support the rationale of using these drugs for the treatment of *IDH*-mutant AMLs. However, the clinical results obtained using these drugs in monotherapy are limited and combination therapy approaches are required. Future studies will be required to determine the optimal treatment for *IDH1* or *IDH2*-mutant AML patients, younger or older, with de novo or refractory/relapsing disease. Particularly, randomized clinical trials will be required to confirm the therapeutic impact of BCL2 inhibitor with hypomethylating agents or with chemotherapy on *IDH*-mutant AMLs. In addition, to determine whether the addition of IDH inhibitors to these regimens will be compatible with an acceptable safety profile and will improve the therapeutic response.

6. Personalized Therapy for AML Patients Is Feasible

Given the consistent advances in the understanding of the molecular alterations occurring in AMLs, there was a great expectancy that data deriving from NGS analyses could be integrated into therapy decisions, to support the development of an individual, patient-adapted therapeutic approach, a condition formulated by the so-called precision or personalized medicine [117]. Surprisingly, concrete applications of personalized medicine, consisting in clinical trials where the therapy decisions for frontline therapy are guided by the results of genomic studies, remain very limited [118].

Thus, in spite the dramatic improvements in our understanding the molecular basis of AML, molecular data in AML have been used predominantly for prognostication and for second-line therapeutic choices after induction therapy and not for initial therapeutic options, with the exception of FLT3 inhibitors. The clinical application of precision medicine implies the definition of the right drug, for the right patient, at the right time. The practical application of this approach is hampered by the time required to obtain the data on the profile of molecular abnormalities observed in the leukemic blasts of each AML patient. Currently, AML treatment is started rapidly after diagnosis, thus precluding the opportunity to consider the individual mutational profile of the patient for treatment decisions. However, at variance with this common view, in a retrospective analysis on 599 newly diagnosed AML patients, Bertoli et al. explored the potential impact of time from diagnosis to treatment (TDT) on overall survival, early death and response rate [119]. The median TDT in this study was eight days; in multivariate analysis, TDT had no impact on overall survival and was not associated with response rate and early death [119]. Thus, this study supported that waiting seven to 10 days for laboratory tests to characterize leukemias at the molecular level and to design adapted, personalized treatment at diagnosis seemed possible [119].

A recent study, the Beat AML trial (this clinical study englobes 11 sub-studies), provided evidence supporting the feasibility of precision medicine for elderly AML patients [120,121]. The Beat trial aims to evaluate the feasibility of using NGS to assign treatment tailored to individual genomics of elderly patients with AML within seven days of diagnosis [120,121]. Enrollment criteria included age of $\geq$60 years at diagnosis of AML and absence of any previous treatment, with exception of hydroxyurea. In an initial phase, the investigators have shown the feasibility of completing the evaluation of the cytogenetic and mutational profiles and of assigning patients to a targeted therapy within seven days of samples arriving at the reference laboratory [120]. In line with this initial information, at the end of the study, on 395 eligible patients enrolled in the study, 94.7% of these patients had genetic and cytogenetic analysis completed within seven days [121]. Once NGS results are received, patients are assigned to therapy according to the best option for curability based on dominant clones of any of the following genetic abnormalities: Core Binding Factor; *NPM1* mutation/*FLT3* wild-type; *MLL* rearrangements; *IDH2* mutations; *IDH1* mutations; *TP53* mutations; complex karyotype with no *TP53* mutations; *FLT3-ITD* or *FLT3-TKD* mutations; *TET2/WT1* mutations; marker-negative AMLs [121]. Each of these molecular groups is admitted to a specific treatment option. Of the 374 AML patients with complete genetic analysis, 224 were enrolled in a Beat AML sub-study, whereas the

remaining 171 patients were treated with standard of care therapy, 28 with investigational therapy and 40 with palliative care; nine patients died before treatment assignment [121].

Importantly, thirty-day mortality was less frequent and overall survival was significantly longer for patients enrolled on the Beat AML sub-studies, versus those who were elected for standard-of-care treatment [121]. The results of this trial are important because they support the feasibility of personalized medicine for the large majority of AML patients, and indicate that a delay in therapy to perform molecular profiling is safe [121]. Acute events requiring more urgent therapy were observed in 26 patients and 32 patients began therapy before treatment assignment [121]. It is important to note that 30-day mortality was 3.7% for patients electing to enroll on Beat AML trial (244 patients), whereas it was 20.4% in patients electing standard of care (103 patients) [121]. Overall survival was significantly longer in the Beat AML group (12.8 months, with 54.7% of patients surviving at 12 months) compared to standard of care (3.9 months, with 27.6% of patients surviving at 12 months) or palliative care (0.6 months, with 11% of patients surviving at 12 months) groups, but not to the group of 28 AML patients treated with an alternative protocol with investigational therapy (not reached, with 57.4% of patients surviving at 12 months).

In conclusion, this prospective precision medicine trial raises several important observations: (i) For the majority of older AML patients, a delay in therapy to perform detailed molecular profiling is safe; (ii) a precision medicine approach requires a coordinated effort by investigators, patients and caregivers, genomic and cytogenetic laboratories; (iii) the majority of enrolled patients could be assigned to a specific molecular-adapted therapy based on the analysis of the dominant AML clone; (iv) patients elected to receive the treatment based on the molecular profiling display lower early death rate and superior overall survival, compared to patients elected to receive standard of care [121]. However, the last point must be considered with some caution, in that, the standard of care treatment did not involve the use of venetoclax plus azacytidine, a drug combination that seems to improve the overall survival in older AML patients.

While this study provides a strong support to the feasibility of a personalized treatment for AML patients using genetic information to match patients to targeted therapies, additional studies will be required to demonstrate that this approach leads to better survival rates than traditional one-size-fits all treatment approaches. The Leukemia and Lymphoma Society, using the Beat AML infrastructure, intends to launch the Stops MDS trial for patients with myelodysplastic syndrome and the LLS (Leukemia Lymphoma Society) PedAL (Pediatric Acute Leukemia) trial for children with acute leukemia.

7. Conclusions

Individualizing patient treatment is a main objective of whole oncology, and particularly of hematology, for the therapy of hematological neoplasia. Reaching this objective has been elusive for long-time for a number of limiting factors. However, the recent progresses in the study of genetic abnormalities of tumors and particularly of AMLs have led to decipher the heterogeneous genetic abnormalities underlying this disease and to define tailored treatments targeting AMLs bearing specific genetic abnormalities. In parallel, the development of integrated diagnostic and prognostic systems, supported by artificial intelligence platforms, have globally optimized the capacity to perform an accurate diagnosis, classification and risk stratification of individual AML patients. Finally, recent studies have supported the feasibility of a personalized treatment for AML patients, where the therapy decision was guided by the results of genomic studies, performed within a frame of time compatible with clinical activity.

However, the future of personalized treatments for AML patients remains difficult for various reasons: The complexity of these studies were limited to highly specialized medical centers; the requirement of an optimal organization, based on the interaction between different technological teams with the clinical unit; the necessity of demonstrating the clinical benefit of these treatments in terms of patient's survival with respect to the standard-of-care; and the cost of these treatments.

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