BBA - Reviews on Cancer 1872 (2019) 80–88

Contents lists available at ScienceDirect

BBA - Reviews on Cancer

journal homepage: www.elsevier.com/locate/bbacan

Review

FLT3 overexpression in acute leukaemias: New insights into the search for molecular mechanisms

Caroline Pires Poubel[a,b,1], Marcela B. Mansur[a,1], Mariana Boroni[b], Mariana Emerenciano[a,*]

[a] Division of Clinical Research, Research Centre, Instituto Nacional de Câncer (INCA), Rua André Cavalcanti 37, Rio de Janeiro, RJ 20231050, Brazil
[b] Bioinformatics and Computational Biology Lab, Research Centre, Instituto Nacional de Câncer (INCA), Rua André Cavalcanti 37, Rio de Janeiro, RJ 20231050, Brazil

ARTICLE INFO

Keywords:
Acute leukaemia
FLT3
Molecular mechanisms
Overexpression
Targeted therapy

ABSTRACT

FLT3 overexpression is a recurrent event in various acute leukaemia subtypes. This transcriptional deregulation is important to define the prognostic risk for many patients. Of note, the molecular mechanisms leading to this gene upregulation are unknown for a substantial number of cases. In this Mini-Review, we highlight the role of *FLT3* overexpression in acute leukaemia and discuss emerging mechanisms accounting for this upregulation. The benefits of using targeted therapy are also addressed in the overexpression context, posing other therapeutic possibilities based on state-of-the-art knowledge that could be considered for future research.

1. Introduction

Recent studies involving omics sciences have been successful into describing alterations in single genes or networks leading to benefits in diagnosis and treatment of patients with cancer. A noteworthy example is *FMS-like tyrosine kinase 3* (*FLT3*) gene overexpression that is a recurrent event in several acute leukaemia subtypes. Remarkably, the presence or absence of this transcriptional aberration is important to define the high prognostic risk as well as to provide the best choice of treatment strategy for many patients [1–4].

FLT3 gene is located on chromosome 13q12.2 and consists of 24 exons spanning 96 kb. This gene encodes a transmembrane ligand-activated receptor tyrosine kinase (RTK) of 993 amino acids that belongs to the class III RTK family which also includes platelet-derived growth factor receptor, macrophage colony-stimulating factor receptor and stem cell factor receptor (aka c-KIT). FLT3 plays a crucial role in early haematopoiesis and is expressed on CD34-positive haematopoietic stem cells, multipotential myeloid and B-lymphoid progenitor cells and monocytic cells. FLT3 receptor comprises five extracellular, a transmembrane (TMD), a juxtamembrane (JMD) and two cytoplasmic tyrosine kinase (TKD) domains. Binding of FLT3 ligand leads to the receptor homodimerization, resulting in downstream TKD phosphorylation and other mediators. Ultimately, this signalling cascade culminates in cell growth and inhibition of apoptosis [5].

FLT3 protein is commonly overexpressed in blast cells from patients diagnosed with acute myeloid leukaemia (AML) and B-cell precursor acute lymphoblastic leukaemia (B-ALL). Studies have reported that this event occurs due to the presence of *FLT3* gain-of-function (GOF) mutations, however, a significant number of leukaemic cases lacking *FLT3* GOF mutations have a higher *FLT3* expression level compared to normal bone marrow samples. It has been already demonstrated that *FLT3* overexpression is associated with autophosphorylation and an unfavourable prognosis in acute leukaemias [5,6]. For *FLT3*-related leukaemias, it is central to discuss the clonal architecture of the disease, once it has already been shown that *FLT3* GOF mutations are usually subclonal to putative founder mutations in AML, highlighting the presence of an underlying clonal heterogeneity in these leukaemias [7,8]. In case of *FLT3* overexpression, it is difficult to infer how the clonal variegation of this event would impact in the natural history of this disease.

Abbreviations: FLT3, FMS-like tyrosine kinase 3; RTK, receptor tyrosine kinase; TMD, transmembrane domain; JMD, juxtamembrane domain; TKD, tyrosine kinase domain; AML, acute myeloid leukaemia; B-ALL, B-cell precursor acute lymphoblastic leukaemia; GOF, gain-of-function; ITD, internal tandem duplication; TARGET, therapeutically applicable research to generate effective treatment; TCGA, the cancer genome atlas; WGS, whole genome sequencing; WES, whole exome sequencing; T-ALL, T-cell acute lymphoblastic leukaemia; ETP-ALL, early T-cell precursor acute lymphoblastic leukaemia; *KMT2A*-r, *KMT2A* rearrangements; OS, overall survival; PRC2, polycomb repressive complex 2; H3K27me3, trimethylation of H3 at lysine 27; miR, microRNA; RPKM, Reads Per Kilobase Million; FPKM, Fragments Per Kilobase Million

* Corresponding author at: Molecular Cancer Study Group, Division of Clinical Research, Research Centre, Instituto Nacional de Câncer (INCA), Rua André Cavalcanti 37, 2° floor, Rio de Janeiro, RJ 20231050, Brazil.
E-mail address: memerenciano@inca.gov.br (M. Emerenciano).
[1] These authors contributed equally to this work.

https://doi.org/10.1016/j.bbcan.2019.06.001
Received 3 May 2019; Received in revised form 31 May 2019
Available online 12 June 2019

In this Mini-Review, we will address the recent scenario of *FLT3* gene in the leukaemic context, highlighting known and emerging mechanisms of *FLT3* overexpression. Furthermore, we will discuss the current and potential therapeutic options and the results obtained with recent clinical trials.

2. *FLT3* recurrent alterations

2.1. Internal tandem duplication

In acute leukaemia, the most common type of *FLT3* GOF mutation is an internal tandem duplication (*FLT3*-ITD) of the intracellular JMD. This elongated FLT3 receptor loses the capacity of auto-inhibition, a function of the wild-type FLT3 receptor that ensures the TKD will not be activated without ligand binding. This mutation leads to constitutive autophosphorylation and, consequently, activation of PI3K/AKT, RAS/ERK and STAT5 pathways [1].

The frequency of *FLT3*-ITD varies depending on the acute leukaemia subtype. Herein, we have also used online databases to determine the frequencies of alterations affecting *FLT3* in different leukaemic subgroups, i.e. Therapeutically Applicable Research to Generate Effective Treatment (TARGET; https://ocg.cancer.gov/programs/target/acute-lymphoblastic-leukemia) and The Cancer Genome Atlas (TCGA) available at http://www.cbioportal.org [9,10] (Fig. 1A). In AML, *FLT3*-ITD occurs in 12–20% of cases [6,11,12] (Fig. 1B and C). On the other hand, this mutation is less frequent in ALL [6] (Fig. 1D and E). Based on TARGET data, *FLT3*-ITD is observed only in T-cell ALL (T-ALL) corresponding to 2.9% of ALL cases (Fig. 1E). In the literature, *FLT3*-ITD has already been described in 2–4% of T-ALL cases. Of note, *FLT3* is more frequently altered in a particular T-ALL subtype known as early T-cell precursor ALL (ETP-ALL), i.e. *FLT3*-ITD occurs in 12–21% of ETP-ALL patients [2,13] (Fig. 1E).

2.2. Point mutations

Point mutations are also recurrent alterations in *FLT3* and they commonly occur in the TKD (*FLT3*-TKD). Alterations in the TKD of FLT3 receptor result in its ligand-independent phosphorylation due to the constitutive opening of the activation loop which then accommodates ATP allowing downstream protein binding. Similarly to *FLT3*-ITD, *FLT3*-TKD leads to cell proliferation due to activation of PI3K/AKT and RAS/ERK pathways, however, it does not result in STAT5 phosphorylation [5]. The most frequent mutations in TKD affects the codon 835 for an aspartate (D835) or the 836 for an isoleucine (I836) residue.

FLT3-TKD occurs in 3–9% of AML cases, with a remarkably higher frequency (14–16%) in those with *KMT2A* rearranged (*KMT2A*-r, aka *MLL*-r) [6,11,12] (Fig. 1B and C). In B-ALL, *FLT3*-TKD frequency was found in < 5% of patients in different series of cases [14] (Fig. 1D). However, there are two specific ALL subgroups in which *FLT3*-TKD is more common: ETP-ALL and *KMT2A*-r cases. In ETP-ALL, *FLT3*-TKD frequency varies greatly according to age groups: in adults 25% and in children 5% [13]. Focusing in *KMT2A*-r ALL, *FLT3*-TKD is mostly reported in infant cases, accounting for 12% of them [15]. Due to the small number of ETP-ALL and *KMT2A*-r ALL cases in the TARGET database, it was not possible to identify these frequencies in children and adults.

FLT3-JMD and *FLT3*-TMD are another class of GOF point mutations which were recently described [16]. *FLT3*-JMD have been found in paediatric cases, corresponding to 1.4% in AML, 2.1% in *KMT2A*-r AML and 2.4% in B-ALL patients (Fig. 1C and D).

3. *FLT3* overexpression and acute leukaemia

FLT3 expression is significantly higher in leukaemia when compared to other types of cancer [1] and since the 90's high levels of this gene have already been reported in AML and ALL cases. Thus, over the years, *FLT3* overexpression has been described as a recurrent event in acute leukaemia characterised by higher levels of *FLT3* expression in these patients when compared with normal bone marrow samples (CD34-positive haematopoietic stem cells) or defined by median and mean measures as cut-off point (Fig. 2). The recent findings related to this alteration will be further detailed in the following topics.

3.1. Acute myeloid leukaemia

FLT3 is significantly upregulated in a high proportion of AML cases when those are compared to normal haematopoietic cells [17]. In addition, this event seems to be associated with *KMT2A*- and *NMP1*-like signatures [1]. Although it is possible to identify these upregulated cases (Fig. 2A and B), only those expressing extremely high transcript levels of *FLT3* present phosphorylation of the protein [17]. Studies have shown that the membrane expression level of FLT3 receptor is not correlated with the transcript level, however, analysis using the total cellular protein level in leukaemic cells demonstrated proportional gene expression levels [11,17]. Thus, as an alternative process of activation, the significant increase of FLT3 on the cell surface, due to *FLT3* overexpression, might facilitate ligand-independent dimerization and, consequently, activation of the receptor. Other possible mechanism is that *FLT3* overexpression can induce the expression of FLT3 ligand leading to phosphorylation of the receptor by an autocrine mechanism [5,17].

Recent overall survival (OS) analyses of AML patients with normal karyotype have shown that those with high *FLT3* expression (top half above median) have poorer OS when compared to patients with low expression [1]. Similarly, *FLT3* overexpression among *FLT3*-ITD-negative patients (> 200,000 copies/μgRNA) was associated with an unfavourable prognosis in young and adult AML cases by univariate and multivariate OS analysis [17]. Although Tarlock K et al. have not observed difference in the OS in paediatric AML cases according to *FLT3* status, they demonstrated that low transcript expression was associated with a superior 3-year event-free survival compared to high transcript expression (fourth quartile) [11].

3.2. Acute lymphoblastic leukaemia

FLT3 abnormalities that lead to this gene overexpression have been described in ALL in few recent studies [18–21] (Fig. 2C and D). Despite the paucity of available data in ALL, *FLT3* overexpression has been identified in particular high-risk subgroups of B-ALL and T-ALL cases, i.e. *KMT2A*-r and ETP-ALL [2–4]. Although it has already been demonstrated that elevated expression of *FLT3* is associated with a poorer outcome in B-ALL cases [22,23], further studies should be performed to establish the prognostic role of *FLT3* overexpression in ALL.

3.3. KMT2A-rearranged leukaemia

KMT2A-r occurs in 10% of all acute leukaemias regardless the age group and are frequent in infant ALL (iALL, ≤12 months of age, ~80%) and in a much broader age range of AML patients [24]. *KMT2A*-r leukaemias present a specific gene expression profile, with *FLT3* being one of the most upregulated genes in this leukaemic subtype [25]. *FLT3* upregulation in *KMT2A*-r has been correlated to high FLT3 protein levels, high phosphorylation and activation of the protein by different groups [26–28]. Additionally, a recent study demonstrated that *de novo* GOF mutations in *FLT3* and *NRAS* led to an increased phosphorylation of AKT, ERK1/2 and STAT5 which accelerates the disease onset of *KMT2A*-r leukaemias [20].

FLT3 overexpression in *KMT2A*-r cases was recently evaluated by a few studies [11,15]. Because *FLT3* GOF mutations occur only in ~9% (3–18%) of *KMT2A*-r patients [15,24], for a considerable amount of those cases, other mechanisms ought to be involved in this gene overexpression. Endorsing this observation, *in vitro* investigations have demonstrated that, in a *KMT2A*-r context, *FLT3* overexpression is

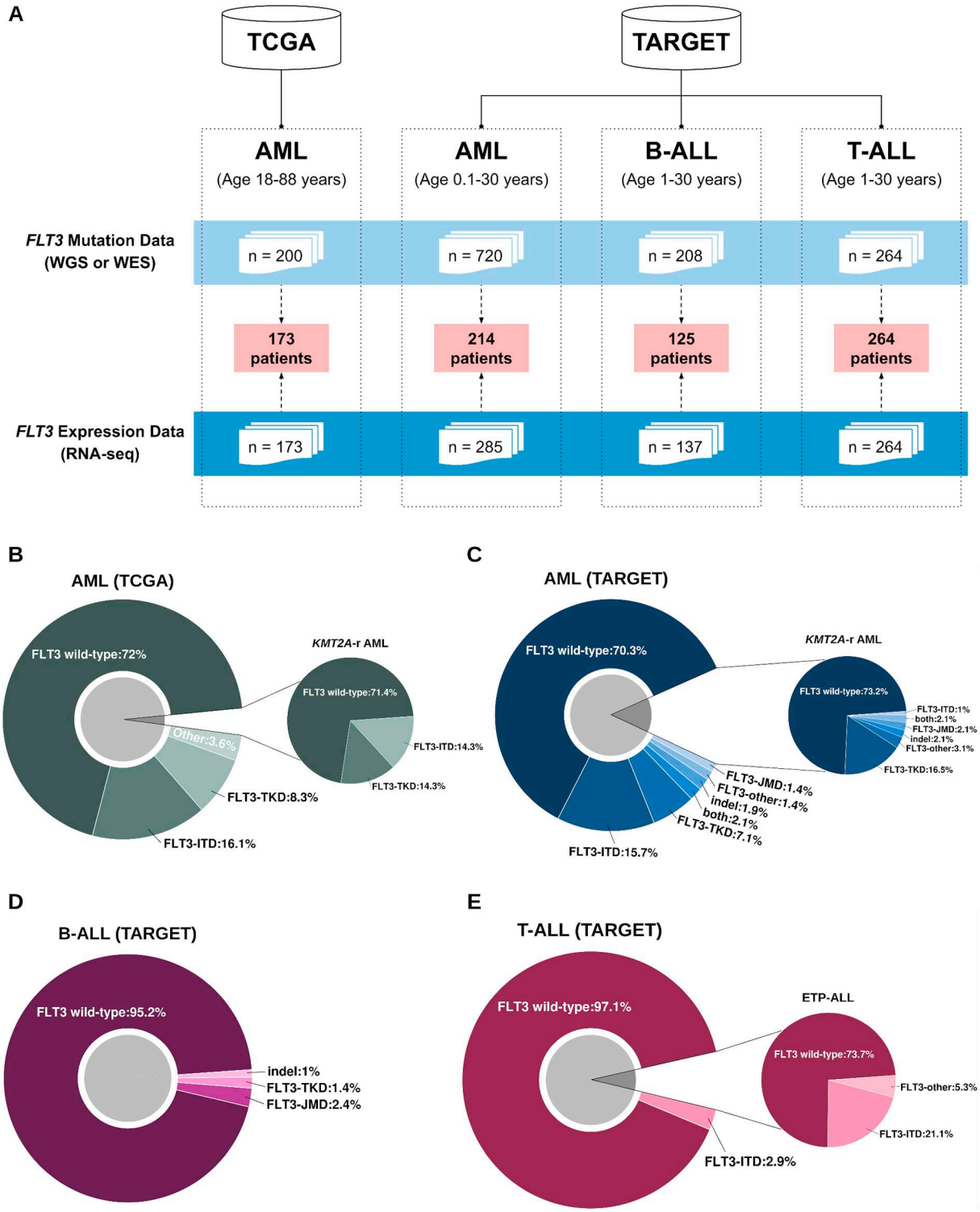

Fig. 1. A workflow of data provenance for this study and frequency of *FLT3* mutations in different AML and ALL subgroups. (A) A flowchart to illustrate the databases accessed, number of cases evaluated by leukaemia subtype and type of data. In our study, the frequencies displayed in the figures were calculated based on the data available in the TCGA (AML) or TARGET (AML and ALL). Mutations frequency were estimated considering all cases genotyped either by whole genome sequencing (WGS) or whole exome sequencing (WES), whereas for gene expression quantification, only patients with known *FLT3* genotype were included in our analysis, corresponding to the numbers in the light red (pink) rectangles. Data was mainly accessed through the cBioPortal for Cancer Genomics and TARGET website. For cases lacking genotype information in the portal, we used the data provided as supplemental material in the consortium papers [2,14,16]. Frequency of *FLT3* mutations (B) in AML based on TCGA data highlighting the *KMT2A*-r subtype; (C) TARGET data for AML highlighting the *KMT2A*-r subtype; (D) TARGET data for B-ALL and (E) TARGET data for T-ALL highlighting the ETP-ALL cases. *FLT3* mutations were classified according to either the type of mutation or affected domain: *FLT3*-ITD, internal tandem duplication that occur in JMD; *FLT3*-TKD, point mutations in TKD; *FLT3*-JMD, point mutations in JMD; *FLT3*-other, point mutations that occur outside JMD and TKD; both, co-occurrence of ITD and point mutation; indel, small insertions/deletions. Point mutations in TMD were not observed.

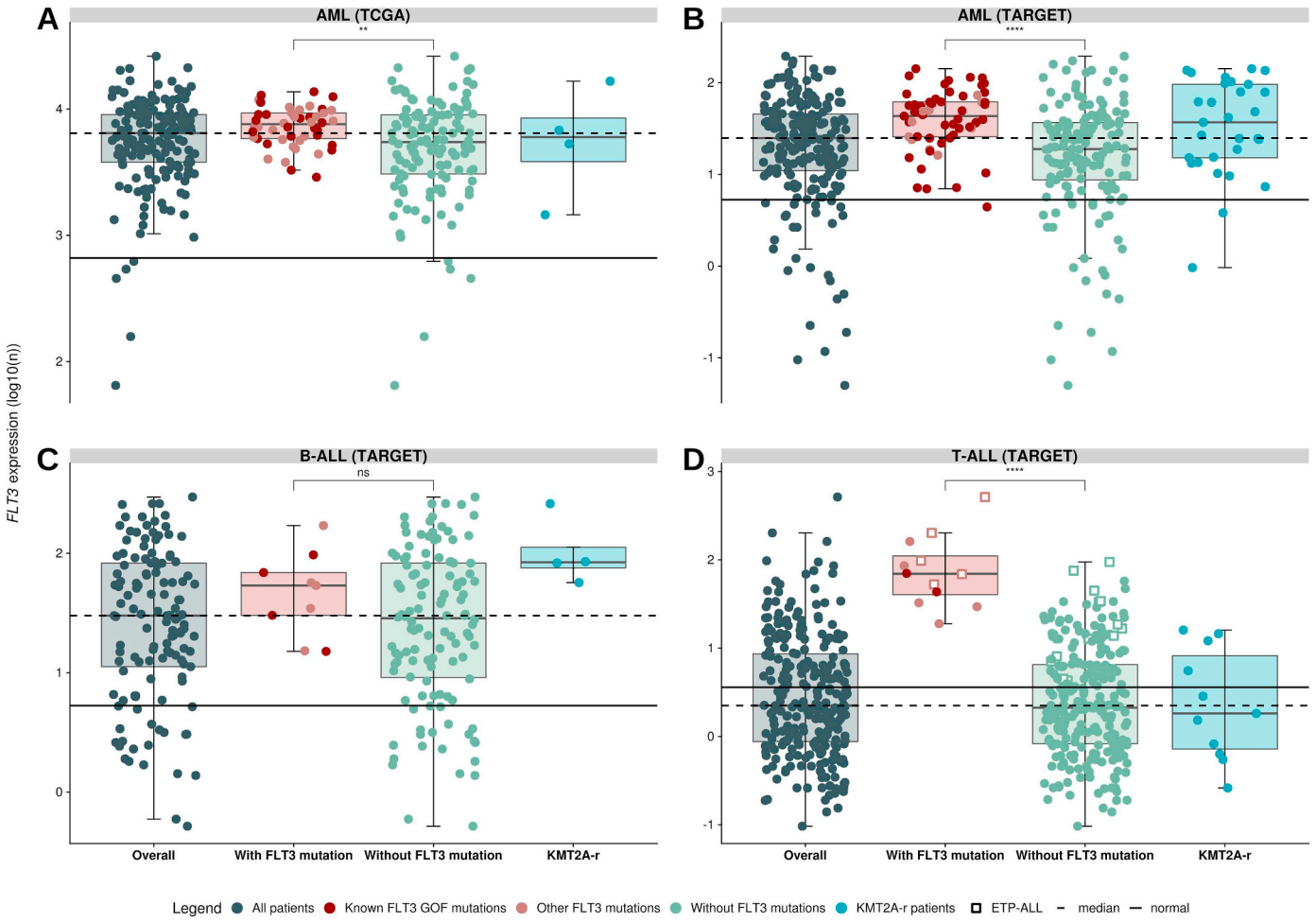

Fig. 2. *FLT3* expression pattern according to genotype among different acute leukaemias. Each boxplot shows the *FLT3* expression for (A) AML (TCGA); n = Normalized count according to cbioportal; (B) AML (TARGET); n = Reads per kilobase million (RPKM); (C) B-ALL (TARGET), n = RPKM; and D) T-ALL (TARGET), n = Fragments per kilobase million (FPKM). Gene expression was evaluated according to the presence (dark and light red) or absence (dark and light green) of *FLT3* mutations. We also show the *FLT3* expression pattern in *KMT2A*-r cases (blue). Dashed lines represent the overall median, which was used as the cut-off point to classify patients with *FLT3* overexpression. Solid lines represent *FLT3* expression in normal haematopoietic sites extracted from: human bone marrow RNA-seq data (ArrayExpress Archive #E-MTAB-1733) to compare with AML or B-ALL samples; human thymus RNA-seq data (Sequence Read Archive #SRX982644) to compare with T-ALL samples. Dark red dots and squares represent *FLT3* expression in cases with known GOF mutations (The Clinical Knowledgebase Core) and ETP-ALLs, respectively. Mean differences were tested with wilcoxon test and *p*-values < .05 were considered statistically significant. ns, non-significant; $^{**}p < .01$; $^{***}p < .001$. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

associated with phosphorylation and activation of the protein, even in the absence of these GOF mutations [26,28].

With respect to prognosis, both the presence of *KMT2A*-r and *FLT3* overexpression are commonly described as markers of poor outcome in acute leukaemias [16,29]. However, whereas *KMT2A*-r is mostly shown as a marker for dismal prognosis (in AML it may vary depending on the translocation partner gene), the data for *FLT3* still seems to be a bit controversial. For example, a recent study on paediatric *KMT2A*-r ALL showed that, differently from other studies in the infant subgroup, *FLT3* upregulation was associated with good prognosis [15]. The same study also discusses the relevance of *FLT3* expression irrespective of the presence of mutations, once it is quite challenging to establish a correlation between *FLT3* mutations and ALL outcome due to the lower mutation frequency and insufficient studies in this leukaemic subtype. For the AML scenario, the clinical significance of the association between *KMT2A*-r and *FLT3* expression is unknown, with one recent study in paediatric AML showing no prognostic relevance [11]. While not all studies indicate the prognostic value of *FLT3*, these prognostic findings highlight the potential therapeutic benefit of targeting FLT3 in *KMT2A*-r patients.

3.4. Known and emerging mechanisms leading to FLT3 overexpression

As commented above, *FLT3* activating abnormalities are not as commonly observed in ALL as in newly diagnosed AML cases. *FLT3* GOF mutations (point mutations and ITDs) lead to constitutive activation of the receptor and are the most studied mechanisms associated with *FLT3* overexpression [1]. *FLT3* GOFs have been identified in 3–6% ALL and ~15–35% of de novo AML cases, with missense point mutations of the TKD being predominantly found in ALL and ITD of the juxtamembrane domain in AML [1,30,31]. Some leukaemic subtypes exhibit a higher frequency of *FLT3* GOFs such as: high hyperdiploid B-ALL, *BCR-ABL1*-like (Ph-like) B-ALL, ETP-ALL cases and normal karyotype AML [21,32].

Mutations in genes encoding epigenetic regulators are relatively common in acute leukaemia. In the *FLT3* scenario, a recent study showed that inactivating mutations of the Polycomb Repressive Complex 2 (PRC2) core components (*EZH2*, *SUZ12* and EED) are novel oncogenic events involved in *FLT3* overexpression in T-ALL patients. PRC2 complex catalyses the trimethylation of H3 at lysine 27 (H3K27me3) and acts as a critical regulator of normal haematopoiesis [33]. The study showed that *EZH2* inactivation led to loss of H3K27me3

Table 1
Overview of recent published data regarding the clinical use of FLT3 inhibitors in acute leukaemias.

Drug	Clinical phase	Patients	*FLT3* status investigated		Treatment rationale	Response	Ref
			Mutations	Expression			
First-generation inhibitors							
Midostaurin (Type I)	Phase III	Newly diagnosed *FLT3*-mutated AML (n = 717; 18–59 yrs. of age)	ITD and TKD	No	Pts received standard chemotherapy plus either midostaurin or placebo and were randomized according to the type and burden of *FLT3* mutation	OS and EFS were significantly better in the midostaurin than in the placebo group (p = .009 and p = .002, respectively)	[52]
	Phase II	Newly diagnosed *FLT3*-ITD mutated AML (n = 284; 18–70 yrs. of age)	ITD and TKD	No	Midostaurin was given in combination with chemotherapy including stem cell transplantation and single-agent maintenance therapy	CR rate including CRi after induction therapy was 76.4%; EFS and OS at 2 yrs. were 39% and 34%; and 53% and 46% in younger and older patients, respectively	[53]
	Phase I/II	Paediatric pts. with relapsed or refractory *FLT3*-mutated AML or *KMT2A*-r ALL (n = 22; 3 months-18 yrs. of age)	ITD and TKD	No	Oral midostaurin was administered; *FLT3* mutation was not required for pts. with *KMT2A*-r ALL	Single-agent midostaurin had limited clinical activity in previously treated children with *FLT3*-mutated AML or *KMT2A*-r ALL	[54]
Sorafenib (Type II)	Phase II	Newly diagnosed AML (n = 267; ≤60 yrs. of age)	ITD	No	Pts were randomized to receive either sorafenib or placebo as add-on to standard treatment in a double blinded fashion irrespective of *FLT3* status	Longer EFS and clinically relevant 36% risk reduction for relapse or death. 5-year OS rate of 61% vs 52% (p = .28) for the sorafenib vs placebo arms, respectively	[55]
	Phase II	Newly diagnosed *FLT3*-ITD mutated AML (n = 27; ≥ 60 yrs. of age)	ITD	No	*FLT3*-ITD mutated pts. received a median of 3 treatment cycles of sorafenib combined with 5-azacytidine	Overall response rate of 78% with 14.5 months of median response duration and median OS of 8.3 months	[56]
	Phase I/II	Relapsed and/or refractory AML or pts. > 70 with previously untreated disease (phase I, n = 15; phase II, n = 20; ≥ 18 yrs. of age)	ITD and TKD	No	Phase I, the safe dose was determined for both sorafenib and vorinostat; Phase II, all patients received at least two lines of chemotherapy and the safe dose of sorafenib, vorinostat and bortezomib	Overall responses with pts. from both phases: 3% CR; 16% CRi and 5%PR; all responders in both phases harboured *FLT3*-ITD mutation	[57]
Lestaurtinib (Type I)	Phase III	Infant *KMT2A*-r ALL (n = 218; ≤ 1 yrs. of age)	ITD and TKD	Yes	The inhibitor was administered regardless of *FLT3* status	For all risk groups evaluated the addition of lestaurtinib did not improve outcome	[58]
	Phase III	Newly diagnosed *FLT3*-mutated AML (n = 500; ≤ 60 yrs. of age)	ITD, TKD and unknown type of mutation	No	Pts were randomly assigned between lestaurtinib and control: 74% had ITD, 23% TKD, and 2% both types	No significant differences were seen in OS or EFS	[59]
Next-generation inhibitors							
Crenolanib (Type I)	Phase I	Relapsed or refractory *FLT3*-mutated AML (n = 69; ≥ 18 yrs. of age)	ITD and TKD	No	*FLT3*-mutated pts. received crenolanib as a single-agent in 3 divided doses	Overall response rate of 50% among pts. without prior *FLT3* inhibitor treatment; overall response rate of 31% among pts. previously treated with *FLT3* inhibitors	[60]
	Phase I	Relapsed or refractory *FLT3*-mutated AML (n = 13; ≥ 18 yrs. of age)	ITD and TKD	No	*FLT3*-mutated pts. were treated with salvage idarubicin and high-dose ara-C followed by crenolanib	Full doses of crenolanib could be safely combined with standard chemotherapies; overall response rate of 36%	[61]
	Phase II	Newly diagnosed *FLT3*-mutated AML (n = 26; ≥ 18 yrs. of age)	ITD and TKD	No	*FLT3*-mutated pts. were treated with high-dose ara-C and either daunorubicin or idarubicin followed by crenolanib	CR rate of 88% with an overall CR/CRi rate of 96% were observed	[62]
Gilteritinib (Type I)	Phase I/II	Relapsed or refractory AML (n = 252, ≥ 18 yrs. of age)	ITD and TKD	No	Pts received different doses of gilteritinib irrespective of *FLT3* status	Overall response rate was 40% (CRc rate: 30%).	[63]
	Phase I/II	Relapsed and/or refractory *FLT3*-mutated AML (n = 191, ≥ 18 yrs. of age)	ITD and TKD	No	Pts received gilteritinib at FLT3-inhibitory doses	Trend toward improved OS and complete morphologic remission rates among pts. with response without differentiation	[64]

(*continued on next page*)

Table 1 (*continued*)

Drug	Clinical phase	Patients	*FLT3* status investigated		Treatment rationale	Response	Ref
			Mutations	Expression			
Quizartinib (Type II)	Phase I	Relapsed AML or relapsed *KMT2A*-r-ALL (n = 22; ≤ 21 yrs. of age)	ITD and TKD	Yes	The inhibitor was administered regardless of *FLT3* status	For the 17 pts. evaluated: 2 CR, 1 CRp, 1 CRi, 10 SD, and 3 PD; 7 of which were *FLT3*-ITD: 1 CR, 1 CRp, 1 CRi, and 4 SD	[39]
	Phase I/II	In phase I, relapsed or refractory AML (n = 22); in phase II, pts. > 60 yrs. of age with untreated AML or any age receiving first salvage treatment for AML (*n* = 49)	ITD	No	In phase I, pts. were eligible irrespective of *FLT3* mutation; in phase II, pts. with *FLT3*-ITD were eligible	Overall response rate of 75% using the combination of quizartinib with azacytidine or low-dose cytarabine; moreover, 92% of older pts. in the frontline arm achieved 83% of CRc rate with a median OS of 18.6 months	[65]
	Phase I	Newly diagnosed AML (*n* = 19; 18–60 yrs. of age)	ITD	No	Pts were eligible irrespective of *FLT3* mutation	The safety and efficacy of quizartinib in combination with intensive chemotherapy was confirmed	[66]
	Phase II	Relapsed or refractory AML (*n* = 157 in cohort 1; ≤ 60 yrs. of age; and *n* = 176 in cohort 2; ≥ 18 yrs. of age)	ITD	No	Pts received quizartinib regardless of *FLT3* status; the initial 17 pts. received 200 mg per day but subsequently, doses were amended (135 mg for men and 90 mg for women)	In cohort 1, 56% and 36% of pts. with and without *FLT3*-ITD achieved CRc, respectively. In cohort 2, 46% and 30% of pts. with and without *FLT3*-ITD achieved CRc, respectively	[67]
	Phase IIB	Relapsed or refractory *FLT3*-ITD-mutated AML (*n* = 76, ≥ 18 yrs. of age)	ITD	No	Pts with *FLT3*-ITD were randomly assigned to 30- or 60-mg/day doses of single-agent oral quizartinib	Higher median OS (20.9 and 27.3 weeks), duration of CRc (4.2 and 9.1 weeks), and bridge to transplant rates (32% and 42%) in the 60-mg group	[68]

Abbreviations: ALL, acute lymphoblastic leukaemia; AML, acute myeloid leukaemia; CR, complete remission; CRc, composite complete remission; CRi, complete response with incomplete neutrophil and platelet recovery; CRp, complete remission with incomplete platelet recovery; EFS, event free survival; *KMT2A*-r, *KMT2A*-rearranged; OR, overall response; OS, overall survival; PD, progress disease; PR, partial remission; Pts, patients; SD stable disease, yrs., years. Obs: First- and second generation defines the drug discovery phase of *FLT3* inhibitors. Types I and II describes the interaction with *FLT3*, i.e. type I inhibitors are active in either *FLT3*-ITD or *FLT3*-TKD, while type II acts only in *FLT3*-ITD leukaemias.

at the transcription start site of the *FLT3* gene resulting in an increased *FLT3* expression and phosphorylation [34]. In light of these recent discoveries, one might argue that EZH2 inhibition could be investigated in clinical trials for *FLT3*-upregulated cases.

For AML, a recent study showed a negative correlation between *FLT3* expression and its promoter methylation level. It was also observed that *DNMT1* haploinsufficiency significantly increased the expression of *Flt3* in mouse leukaemia cells, suggesting that hypomethylation of *FLT3*, due to *DNMT1* alteration, may be a potential mechanism resulting in *FLT3* upregulation [1].

Another mechanism that has been described as a mediator of *FLT3* transcription in AML is the activity of PRMT5. The regulation loop involves miR-29b, which is silenced via dimethylation of histone 4 arginine residue H4R3 by the transcriptional repressor complex formed by PRMT5 and Sp1. Since *Sp1* is also a target of miR-29b, the miR silencing leads to increased *Sp1* transcription, resulting in *FLT3* transcriptional activation [35]. Authors discussed that as *FLT3* is commonly mutated in the AML scenario, the use of a specific inhibitor of PRMT5 could be a plausible treatment strategy for *FLT3*-driven AMLs.

FLT3 expression has been shown to be regulated by transcription factors such as proto-oncogene *MYB* and the CCAAT/enhancer binding protein C/EBPα. Also, *CEBPA* biallelic mutations are associated with a decreased *FLT3* expression level [36]. In the post-transcriptional regulation level, MSI2 protein has been suggested to be an important modulator of *FLT3* expression by physically binding to the *FLT3* mRNA transcripts. Both genes are significantly co-regulated in AML and loss of *MSI2* leads to down-regulation of the FLT3 receptor [37].

4. Current therapeutic approaches using *FLT3* inhibitors

Some subgroups of acute leukaemia with high expression of *FLT3* tend to be aggressive, particularly *KMT2A*-r infant ALL and *FLT3*-ITD AMLs (modulated by occurrence of *WT1* and *NPM1* mutations and *NUP98* translocations) [16,18]. For those particular groups the use of specific *FLT3* inhibitors could work as an opportunity to improve disease outcomes. For ALL, fewer studies have been performed so far and clinical trials are less prominent than for AML. Currently, the most used *FLT3* inhibitors include first-generation (e.g. sorafenib, midostaurin, lestaurtinib) and next-generation (e.g. crenolanib, quizartinib, gilteritinib) drugs. In addition to FLT3, those inhibitors can also target other key pathways (JAK, KIT, PDGFR, RAF, among others) [38]. In Table 1, we provide an overview of FLT3 inhibitors data focusing on clinical trial studies with results published from 2016 onwards. As shown by their treatment rationale, even though studies consistently evaluated *FLT3* mutational status, many patients were treated with FLT3 inhibitors regardless the presence of ITD/TKD. It remains an open question whether *FLT3* wild-type patients might benefit from these TK inhibitors. A possible explanation for the successful use of *FLT3* inhibitors in patients lacking *FLT3* mutations could be the existence of a *FLT3* overexpression profile due to other molecular mechanisms. Unfortunately, because very few clinical studies analyse *FLT3* expression levels, no definitive conclusions can be drawn so far.

Despite the paucity of recent published studies investigating *FLT3* inhibitors in ALL, Català A et al. evaluated the correlation between *FLT3* expression and cytarabine uptake in paediatric *KMT2A*-r ALL. Their results demonstrated that FLT3 inhibitors should be administered after cytarabine in order to induce higher cytotoxicity in leukaemic cells. In summary, they conclude that the order of drug administration impacted on treatment efficacy since FLT3 regulates cytarabine transport [18]. Another two publications also addressed the topic in 2016, one of them was a phase I study that established the safe and biologically active dose of quizartinib (this type II inhibitor is currently in phase 3 clinical trials) at 60 mg/m^2/day, which led to complete inhibition of FLT3 phosphorylation when combined with intensive chemotherapy and did not result in additive toxicity in those patients. The complete inhibition of FLT3 phosphorylation was observed in all

patients regardless *FLT3* mutational status [39]. The second investigation determined that the introduction of lestaurtinib to post-induction regimen did not improve the outcome for infants with *KMT2A*-r ALL [40]. Three studies experimentally evaluated the role of midostaurin in ALL models and due to the lack of clinical data were not included in Table 1. Two of them were performed by the same group [41,42], and they initially showed that FLT3 activation was responsible for inducing chemoresistance in *KMT2A*-r ALL and that this could be partially reverted by treatment with FLT3 inhibitor [41]. Then subsequently they observed that a pre-treatment with CXCR7 inhibitor accelerated cytosine-arabinoside-induced apoptosis of *KMT2A*-r ALL cells [42]. The third one observed that the combination of midostaurin and p21-activated kinases inhibitors has a synergistic effect on growth inhibition and apoptosis in childhood ALL [43]. Collectively these studies highlight that the combined use of FLT3 inhibitors and other target-drugs might become a useful alternative to treat *FLT3*-driven ALL.

The scenario is very different for AML with over 40 clinical-trials currently evaluating the impact of FLT3 inhibitors in the treatment of both children and adult patients [44]. Therefore, this vast amount of recent data illustrates how extensively this theme has been explored and the full translational potential of acknowledging the mechanisms into *FLT3*-driven AML for future clinical benefit [45–47]. The following reviews were elegantly written and thoroughly discussed the matter, constituting a great source for up-to-date information on the use of FLT3 inhibitors in AML [38,44,48]. Of note, a recent clinical investigation showed that, due to AML clonal heterogeneity, monotherapy with crenolanib might result in drug resistance, so drug combinations (with drugs targeting other alterations) are an alternative to restore crenolanib sensitivity [47]. In this regard, considering the imposing underlying clonal diversity found in the majority of AML patients harbouring *FLT3*-GOF mutations, it is important to keep in mind that all the data regarding the use of FLT3 inhibitors should be considered with caution. In other words, aware that some studies have already reported the occurrence of clinical resistance to FLT3 inhibitors [7,47,49–51], these findings might translate the extremely complex scenario of treating distinct patients at different time points (diagnosis/untreated or relapsed/refractory disease). Furthermore, for the development of new therapeutic approaches, it is critical to further contemplate the use of combined therapies that could then be more efficient in eradicating leukaemic cells, as well as, in controlling clonal evolution-driven resistance.

5. Closing remarks

Different studies have shown that *FLT3*-related acute leukaemias are associated with treatment failure and, therefore, contribute to a poorer prognosis for patients. However, most investigations evaluated only patients with AML, in which *FLT3* abnormalities are frequently found. In ALL, although fewer studies have been performed, some specific subtypes may also benefit from a better understanding of the biology behind *FLT3* aberrant expression. Our Mini-Review shines a light on the importance of using a better classifier for *FLT3*-driven leukaemias, i.e. to evaluate *FLT3* expression levels instead of simply looking for GOF mutations. In that regard, it is also critical to define a proper and well-established cut-off able to accurately categorise patients into the *FLT3* overexpression group. Overall this new approach would not only outline a 'new' biomarker for those leukaemias, but significantly impact on the success of FLT3 inhibitors treatment. At last, we believe that the emerging mechanisms of gene deregulation discussed herein could provide relevant insights into the development of new therapy strategies.

Declaration of Competing Interest

The authors declare no conflict of interest.

Acknowledgements

The authors would like to thank the Bioinformatics Core Facility (INCA-RJ) for their support. Authors apologise for the inevitable omissions due to space restrictions in this mini-review.

CP and MBM are supported by the Ministry of Health (INCA-Brazil). MB is supported by Brazilian National Counsel of Technological and Scientific Development-CNPq (28/2018#427380/2018-9) and Fundação Carlos Chagas Filho de Amparo à Pesquisa do Estado do Rio de Janeiro-FAPERJ (E_26/211.334/2016) research grants. ME is supported by CNPq (PQ-2014#304142/2014-0) and FAPERJ-JCNE (E_26/201.539/2014) research grants.

References

[1] J. Cheng, L. Qu, J. Wang, L. Cheng, Y. Wang, High expression of FLT3 is a risk factor in leukemia, Mol. Med. Rep. 17 (2018) 2885–2892, https://doi.org/10.3892/mmr.2017.8232.
[2] Y. Liu, J. Easton, Y. Shao, J. Maciaszek, Z. Wang, M.R. Wilkinson, et al., The genomic landscape of pediatric and young adult T-lineage acute lymphoblastic leukemia, Nat. Genet. 49 (2017) 1211–1218, https://doi.org/10.1038/ng.3909.
[3] S.A. Armstrong, J.E. Staunton, L.B. Silverman, R. Pieters, M.L. den Boer, M.D. Minden, et al., MLL translocations specify a distinct gene expression profile that distinguishes a unique leukemia, Nat. Genet. 30 (2002) 41–47, https://doi.org/10.1038/ng765.
[4] S. Chiaretti, X. Li, R. Gentleman, A. Vitale, K.S. Wang, F. Mandelli, et al., Gene expression profiles of B-lineage adult acute lymphocytic leukemia reveal genetic patterns that identify lineage derivation and distinct mechanisms of transformation, Clin. Cancer Res. 11 (2005) 7209–7219, https://doi.org/10.1158/1078-0432.CCR-04-2165.
[5] P. Tsapogas, C.J. Mooney, G. Brown, A. Rolink, The cytokine Flt3-ligand in normal and malignant hematopoiesis, Int. J. Mol. Sci. 18 (2017), https://doi.org/10.3390/ijms18061115.
[6] A. Andersson, K. Paulsson, H. Lilljebjörn, C. Lassen, B. Strömbeck, J. Heldrup, et al., FLT3 mutations in a 10 year consecutive series of 177 childhood acute leukemias and their impact on global gene expression patterns, Genes Chromosom. Cancer 47 (2008) 64–70, https://doi.org/10.1002/gcc.20508.
[7] A.L. Paguirigan, J. Smith, S. Meshinchi, M. Carroll, C. Maley, J.P. Radich, Single-cell genotyping demonstrates complex clonal diversity in acute myeloid leukemia, Sci. Transl. Med. 7 (2015) 281re2, https://doi.org/10.1126/scitranslmed.aaa0763.
[8] N. Potter, F. Miraki-Moud, L. Ermini, I. Titley, G. Vijayaraghavan, E. Papaemmanuil, et al., Single cell analysis of clonal architecture in acute myeloid leukaemia, Leukemia. 33 (2019) 1113–1123, https://doi.org/10.1038/s41375-018-0319-2.
[9] E. Cerami, J. Gao, U. Dogrusoz, B.E. Gross, S.O. Sumer, B.A. Aksoy, et al., The cBio cancer genomics portal: an open platform for exploring multidimensional cancer genomics data, Cancer Discov. 2 (2012) 401–404, https://doi.org/10.1158/2159-8290.CD-12-0095.
[10] J. Gao, B.A. Aksoy, U. Dogrusoz, G. Dresdner, B. Gross, S.O. Sumer, et al., Integrative analysis of complex cancer genomics and clinical profiles using the cBioPortal, Sci. Signal. 6 (2013) pl1, https://doi.org/10.1126/scisignal.2004088.
[11] K. Tarlock, T.A. Alonzo, M.R. Loken, R.B. Gerbing, R.E. Ries, R. Aplenc, et al., Disease characteristics and prognostic implications of cell-surface FLT3 receptor (CD135) expression in pediatric acute myeloid leukemia: a report from the Children's Oncology Group, Clin. Cancer Res. 23 (2017) 3649–3656, https://doi.org/10.1158/1078-0432.CCR-16-2353.
[12] H. Döhner, E. Estey, D. Grimwade, S. Amadori, F.R. Appelbaum, T. Büchner, et al., Diagnosis and management of AML in adults: 2017 ELN recommendations from an international expert panel, Blood. 129 (2017) 424–447, https://doi.org/10.1182/blood-2016-08-733196.
[13] M. Neumann, S. Heesch, N. Gökbuget, S. Schwartz, C. Schlee, O. Benlasfer, et al., Clinical and molecular characterization of early T-cell precursor leukemia: a high-risk subgroup in adult T-ALL with a high frequency of FLT3 mutations, Blood Cancer J. 2 (2012) e55, , https://doi.org/10.1038/bcj.2011.49.
[14] X. Ma, Y. Liu, Y. Liu, L.B. Alexandrov, M.N. Edmonson, C. Gawad, et al., Pan-cancer genome and transcriptome analyses of 1,699 paediatric leukaemias and solid tumours, Nature. 555 (2018) 371–376, https://doi.org/10.1038/nature25795.
[15] H. Fedders, A. Alsadeq, J. Schmäh, F. Vogiatzi, M. Zimmermann, A. Möricke, et al., The role of constitutive activation of FMS-related tyrosine kinase-3 and NRas/KRas mutational status in infants with KMT2A-rearranged acute lymphoblastic leukemia, Haematologica. 102 (2017) e438–e442, https://doi.org/10.3324/haematol.2017.169870.
[16] H. Bolouri, J.E. Farrar, T. Triche, R.E. Ries, E.L. Lim, T.A. Alonzo, et al., The molecular landscape of pediatric acute myeloid leukemia reveals recurrent structural alterations and age-specific mutational interactions, Nat. Med. 24 (2018) 103–112, https://doi.org/10.1038/nm.4439.
[17] K. Ozeki, H. Kiyoi, Y. Hirose, M. Iwai, M. Ninomiya, Y. Kodera, et al., Biologic and clinical significance of the FLT3 transcript level in acute myeloid leukemia, Blood. 103 (2004) 1901–1908, https://doi.org/10.1182/blood-2003-06-1845.
[18] A. Català, M. Pastor-Anglada, L. Caviedes-Cárdenas, R. Malatesta, S. Rives, N. Vega-García, et al., FLT3 is implicated in cytarabine transport by human equilibrative

nucleoside transporter 1 in pediatric acute leukemia, Oncotarget. 7 (2016) 49786–49799, https://doi.org/10.18632/oncotarget.10448.
[19] A.J. de Smith, J. Ojha, S.S. Francis, E. Sanders, A.A. Endicott, H.M. Hansen, et al., Clonal and microclonal mutational heterogeneity in high hyperdiploid acute lymphoblastic leukemia, Oncotarget. 7 (2016) 72733–72745, https://doi.org/10.18632/oncotarget.12238.
[20] A. Hyrenius-Wittsten, M. Pilheden, H. Sturesson, J. Hansson, M.P. Walsh, G. Song, et al., De novo activating mutations drive clonal evolution and enhance clonal fitness in KMT2A-rearranged leukemia, Nat. Commun. 9 (2018) 1770, https://doi.org/10.1038/s41467-018-04180-1.
[21] M. Mohseni, H. Uludag, J.M. Brandwein, Advances in biology of acute lymphoblastic leukemia (ALL) and therapeutic implications, Am. J. Blood Res. 8 (2018) 29–56.
[22] H. Kang, C.S. Wilson, R.C. Harvey, I.-M. Chen, M.H. Murphy, S.R. Atlas, et al., Gene expression profiles predictive of outcome and age in infant acute lymphoblastic leukemia: a Children's Oncology Group study, Blood. 119 (2012) 1872–1881, https://doi.org/10.1182/blood-2011-10-382861.
[23] I. Garza-Veloz, M.L. Martinez-Fierro, J.C. Jaime-Perez, K. Carrillo-Sanchez, M.G. Ramos-Del Hoyo, A. Lugo-Trampe, et al., Identification of differentially expressed genes associated with prognosis of B acute lymphoblastic leukemia, Dis. Markers 2015 (2015) 828145, https://doi.org/10.1155/2015/828145.
[24] A.C. Winters, K.M. Bernt, MLL-rearranged leukemias-an update on science and clinical approaches, Front. Pediatr. 5 (2017) 4, https://doi.org/10.3389/fped.2017.00004.
[25] E.-J. Yeoh, M.E. Ross, S.A. Shurtleff, W.K. Williams, D. Patel, R. Mahfouz, et al., Classification, subtype discovery, and prediction of outcome in pediatric acute lymphoblastic leukemia by gene expression profiling, Cancer Cell 1 (2002) 133–143, https://doi.org/10.1016/S1535-6108(02)00032-6.
[26] S.A. Armstrong, A.L. Kung, M.E. Mabon, L.B. Silverman, R.W. Stam, M.L. Den Boer, et al., Inhibition of FLT3 in MLL. Validation of a therapeutic target identified by gene expression based classification, Cancer Cell. 3 (2003) 173–183.
[27] P. Brown, M. Levis, S. Shurtleff, D. Campana, J. Downing, D. Small, FLT3 inhibition selectively kills childhood acute lymphoblastic leukemia cells with high levels of FLT3 expression, Blood. 105 (2005) 812–820, https://doi.org/10.1182/blood-2004-06-2498.
[28] R.W. Stam, M.L. den Boer, P. Schneider, P. Nollau, M. Horstmann, H.B. Beverloo, et al., Targeting FLT3 in primary MLL-gene-rearranged infant acute lymphoblastic leukemia, Blood, 106 2005, pp. 2484–2490, , https://doi.org/10.1182/blood-2004-09-3667.
[29] R.W. Stam, P. Schneider, P. de Lorenzo, M.G. Valsecchi, M.L. den Boer, R. Pieters, Prognostic significance of high-level FLT3 expression in MLL-rearranged infant acute lymphoblastic leukemia, Blood. 110 (2007) 2774–2775, https://doi.org/10.1182/blood-2007-05-091934.
[30] L.-W. Ding, Q.-Y. Sun, K.-T. Tan, W. Chien, A. Mayakonda, A.E.J. Yeoh, et al., Mutational landscape of pediatric acute lymphoblastic leukemia, Cancer Res. 77 (2017) 390–400, https://doi.org/10.1158/0008-5472.CAN-16-1303.
[31] A. Elshoury, A. Przespolewski, J. Baron, E.S. Wang, Advancing treatment of acute myeloid leukemia: the future of FLT3 inhibitors, Expert. Rev. Anticancer. Ther. 19 (2019) 273–286, https://doi.org/10.1080/14737140.2019.1573679.
[32] E. Papaemmanuil, H. Döhner, P.J. Campbell, Genomic classification in acute myeloid leukemia, N. Engl. J. Med. 375 (2016) 900–901, https://doi.org/10.1056/NEJMc1608739.
[33] A. Iwama, Polycomb repressive complexes in hematological malignancies, Blood. 130 (2017) 23–29, https://doi.org/10.1182/blood-2017-02-739490.
[34] J. Zhang, Y. Zhang, M. Zhang, C. Liu, X. Liu, J. Yin, et al., FLT3 pathway is a potential therapeutic target for PRC2-mutated T-cell acute lymphoblastic leukemia, Blood. 132 (2018) 2520–2524, https://doi.org/10.1182/blood-2018-04-845628.
[35] S.S. Tarighat, R. Santhanam, D. Frankhouser, H.S. Radomska, H. Lai, M. Anghelina, et al., The dual epigenetic role of PRMT5 in acute myeloid leukemia: gene activation and repression via histone arginine methylation, Leukemia. 30 (2016) 789–799, https://doi.org/10.1038/leu.2015.308.
[36] G. Volpe, D.S. Walton, W. Del Pozzo, P. Garcia, E. Dassé, L.P. O'Neill, et al., C/EBPα and MYB regulate FLT3 expression in AML, Leukemia. 27 (2013) 1487–1496, https://doi.org/10.1038/leu.2013.23.
[37] A. Hattori, D. McSkimming, N. Kannan, T. Ito, RNA binding protein MSI2 positively regulates FLT3 expression in myeloid leukemia, Leuk. Res. 54 (2017) 47–54, https://doi.org/10.1016/j.leukres.2017.01.015.
[38] N. Daver, R.F. Schlenk, N.H. Russell, M.J. Levis, Targeting FLT3 mutations in AML: review of current knowledge and evidence, Leukemia 33 (2019) 299–312, https://doi.org/10.1038/s41375-018-0357-9.
[39] T.M. Cooper, J. Cassar, E. Eckroth, J. Malvar, R. Sposto, P. Gaynon, et al., A phase I study of quizartinib combined with chemotherapy in relapsed childhood leukemia: a therapeutic advances in childhood leukemia & lymphoma (TACL) study, Clin. Cancer Res. 22 (2016) 4014–4022, https://doi.org/10.1158/1078-0432.CCR-15-1998.
[40] Abstracts From the 48th Congress of the International Society of Paediatric Oncology (SIOP) Dublin, Ireland October 19–22, 2016, Pediatr. Blood Cancer 63 (Suppl. 3) (2016) S5–S321, https://doi.org/10.1002/pbc.26233.
[41] Y. Furuichi, K. Goi, T. Inukai, H. Sato, A. Nemoto, K. Takahashi, et al., Fms-like tyrosine kinase 3 ligand stimulation induces MLL-rearranged leukemia cells into quiescence resistant to antileukemic agents, Cancer Res. 67 (2007) 9852–9861, https://doi.org/10.1158/0008-5472.CAN-07-0105.
[42] N. Ando, Y. Furuichi, S. Kasai, M. Tamai, D. Harama, K. Kagami, et al., Chemosensitivity is differentially regulated by the SDF-1/CXCR4 and SDF-1/CXCR7 axes in acute lymphoblastic leukemia with MLL gene rearrangements, Leuk. Res. 75 (2018) 36–44, https://doi.org/10.1016/j.leukres.2018.11.001.
[43] I.-K. Siekmann, K. Dierck, S. Prall, M. Klokow, J. Strauss, S. Buhs, et al., Combined inhibition of receptor tyrosine and p21-activated kinases as a therapeutic strategy in childhood ALL, Blood Adv. 2 (2018) 2554–2567, https://doi.org/10.1182/bloodadvances.2018020693.
[44] A.N. Sexauer, S.K. Tasian, Targeting FLT3 signaling in childhood acute myeloid leukemia, Front. Pediatr. 5 (2017) 248, https://doi.org/10.3389/fped.2017.00248.
[45] N.R. Mathew, F. Baumgartner, L. Braun, D. O'Sullivan, S. Thomas, M. Waterhouse, et al., Sorafenib promotes graft-versus-leukemia activity in mice and humans through IL-15 production in FLT3-ITD-mutant leukemia cells, Nat. Med. 24 (2018) 282–291, https://doi.org/10.1038/nm.4484.
[46] S. Maifrede, M. Nieborowska-Skorska, K. Sullivan-Reed, Y. Dasgupta, P. Podszywalow-Bartnicka, B.V. Le, et al., Tyrosine kinase inhibitor-induced defects in DNA repair sensitize FLT3(ITD)-positive leukemia cells to PARP1 inhibitors, Blood. 132 (2018) 67–77, https://doi.org/10.1182/blood-2018-02-834895.
[47] H. Zhang, S. Savage, A.R. Schultz, D. Bottomly, L. White, E. Segerdell, et al., Clinical resistance to crenolanib in acute myeloid leukemia due to diverse molecular mechanisms, Nat. Commun. 10 (2019) 244, https://doi.org/10.1038/s41467-018-08263-x.
[48] I.S. Tiong, A.H. Wei, New drugs creating new challenges in acute myeloid leukemia, Genes Chromosomes Cancer (2019), https://doi.org/10.1002/gcc.22750.
[49] F. Ravandi, M.L. Alattar, M.R. Grunwald, M.A. Rudek, T. Rajkhowa, M.A. Richie, et al., Phase 2 study of azacytidine plus sorafenib in patients with acute myeloid leukemia and FLT-3 internal tandem duplication mutation, Blood. 121 (2013) 4655–4662, https://doi.org/10.1182/blood-2013-01-480228.
[50] C.C. Smith, A. Paguirigan, G.R. Jeschke, K.C. Lin, E. Massi, T. Tarver, et al., Heterogeneous resistance to quizartinib in acute myeloid leukemia revealed by single-cell analysis, Blood. 130 (2017) 48–58, https://doi.org/10.1182/blood-2016-04-711820.
[51] N.J. Short, H. Kantarjian, F. Ravandi, N. Daver, Emerging treatment paradigms with FLT3 inhibitors in acute myeloid leukemia, Ther. Adv. Hematol. 10 (2019), https://doi.org/10.1177/2040620719827310 2040620719827310.
[52] R.M. Stone, S.J. Mandrekar, B.L. Sanford, K. Laumann, S. Geyer, C.D. Bloomfield, et al., Midostaurin plus chemotherapy for acute myeloid leukemia with a FLT3 mutation, N. Engl. J. Med. 377 (2017) 454–464, https://doi.org/10.1056/NEJMoa1614359.
[53] R.F. Schlenk, D. Weber, W. Fiedler, H.R. Salih, G. Wulf, H. Salwender, et al., Midostaurin added to chemotherapy and continued single-agent maintenance therapy in acute myeloid leukemia with FLT3-ITD, Blood. 133 (2019) 840–851, https://doi.org/10.1182/blood-2018-08-869453.
[54] C.M. Zwaan, S. Söderhäll, B. Brethon, M. Luciani, C. Rizzari, R.W. Stam, et al., A phase 1/2, open-label, dose-escalation study of midostaurin in children with relapsed or refractory acute leukaemia, Br. J. Haematol. 185 (2019) 623–627, https://doi.org/10.1111/bjh.15593.
[55] C. Rollig, H. Serve, A. Hüttmann, R. Noppeney, The addition of sorafenib to standard AML treatment results in a substantial reduction in relapse risk and improved survival. Updated Results from Long-Term Follow-up of the Randomized-Controlled Soraml Trial, Blood 130 (2017) 721.
[56] M. Ohanian, G. Garcia-Manero, M. Levis, E. Jabbour, N. Daver, G. Borthakur, et al., Sorafenib combined with 5-azacytidine in older patients with untreated FLT3-ITD mutated acute myeloid leukemia, Am. J. Hematol. 93 (2018) 1136–1141, https://doi.org/10.1002/ajh.25198.
[57] H. Sayar, L.D. Cripe, A.N. Saliba, M. Abu Zaid, H. Konig, H.S. Boswell, Combination of sorafenib, vorinostat and bortezomib for the treatment of poor-risk AML: report of two consecutive clinical trials, Leuk. Res. 77 (2019) 30–33, https://doi.org/10.1016/j.leukres.2018.12.011.
[58] P. Brown, J. Kairalla, C. Wang, Z. Dreyer, W. Salzer, Addition of FLT3 inhibitor lestaurtinib to post-induction chemotherapy does not improve outcomes in Mll-rearranged infant acute lymphoblastic leukemia (ALL): AALL0631, a Children's oncology group study, Pediatr. Blood Cancer 63 (2016) S7.
[59] S. Knapper, N. Russell, A. Gilkes, R.K. Hills, R.E. Gale, J.D. Cavenagh, et al., A randomized assessment of adding the kinase inhibitor lestaurtinib to first-line chemotherapy for FLT3-mutated AML, Blood. 129 (2017) 1143–1154, https://doi.org/10.1182/blood-2016-07-730648.
[60] J.E. Cortes, H.M. Kantarjian, T.M. Kadia, G. Borthakur, Crenolanib besylate, a type I pan-FLT3 inhibitor, to demonstrate clinical activity in multiply relapsed FLT3-ITD and D835 AML, J. Clin. Oncol. 34 (2016) 7008.
[61] M. Ohanian, H.M. Kantarjian, G. Borthakur, T.M. Kadia, Efficacy of a type I FLT3 inhibitor, crenolanib, with idarubicin and high-dose Ara-C in multiply relapsed/refractory FLT3+ AML, Blood. 128 (2016) 2744.
[62] E.S. Wang, R.M. Stone, M.S. Tallman, R.B. Walter, Crenolanib, a type I FLT3 TKI, can be safely combined with cytarabine and anthracycline induction chemotherapy and results in high response rates in patients with newly diagnosed FLT3 mutant acute myeloid leukemia (AML), Blood. 128 (2016) 1071.
[63] A.E. Perl, J.K. Altman, J. Cortes, C. Smith, M. Litzow, M.R. Baer, et al., Selective inhibition of FLT3 by gilteritinib in relapsed or refractory acute myeloid leukaemia: a multicentre, first-in-human, open-label, phase 1-2 study, Lancet Oncol. 18 (2017) 1061–1075, https://doi.org/10.1016/S1470-2045(17)30416-3.
[64] C.M. McMahon, J. Canaani, B. Rea, R.L. Sargent, J.N. Qualtieri, C.D. Watt, et al., Gilteritinib induces differentiation in relapsed and refractory FLT3-mutated acute myeloid leukemia, Blood Adv. 3 (2019) 1581–1585, https://doi.org/10.1182/bloodadvances.2018029496.
[65] M. Swaminathan, H.M. Kantarjian, N. Daver, G. Borthakur, The combination of quizartinib with azacitidine or low dose cytarabine is highly active in patients (pts) with FLT3-ITD mutated myeloid Leukemias: interim report of a phase I/II trial, Blood. 130 (2017) 723.
[66] J.K. Altman, J.M. Foran, K.W. Pratz, D. Trone, J.E. Cortes, M.S. Tallman, Phase 1

study of quizartinib in combination with induction and consolidation chemotherapy in patients with newly diagnosed acute myeloid leukemia, Am. J. Hematol. 93 (2018) 213–221, https://doi.org/10.1002/ajh.24974.

[67] J. Cortes, A.E. Perl, H. Döhner, H. Kantarjian, G. Martinelli, T. Kovacsovics, et al., Quizartinib, an FLT3 inhibitor, as monotherapy in patients with relapsed or refractory acute myeloid leukaemia: an open-label, multicentre, single-arm, phase 2 trial, Lancet Oncol. 19 (2018) 889–903, https://doi.org/10.1016/S1470-2045(18)30240-7.

[68] J.E. Cortes, M.S. Tallman, G.J. Schiller, D. Trone, G. Gammon, S.L. Goldberg, et al., Phase 2b study of 2 dosing regimens of quizartinib monotherapy in FLT3-ITD-mutated, relapsed or refractory AML, Blood. 132 (2018) 598–607, https://doi.org/10.1182/blood-2018-01-821629.