Venetoclax, a new player in the treatment of children with high-risk myeloid malignancies?

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Abstract:
Venetoclax selectively inhibits BCL-2 and restores apoptotic signaling of hematological malignant cells. Venetoclax in combination with hypomethylating and low-dose cytotoxic agents has revolutionized the management of elderly patients affected by acute myeloid leukemia (AML), as well as that of patients unfit to receive intensive chemotherapy. In a single phase 1 pediatric trial conducted on relapsed/refractory AML, the combination of venetoclax with intensive chemotherapy was shown to be safe and yielded promising response rates. In addition, several retrospective studies in children with AML reported that venetoclax combined with hypomethylating agents and cytotoxic drugs appears a safe and efficacious bridge to transplant. Promising results on the use of venetoclax combinations in advanced myelodysplastic syndromes (MDS) and therapy-related MDS/AML have also been reported in small case series. This review summarizes the available current knowledge about venetoclax use in childhood high-risk myeloid neoplasms, discussing a possible integration of BCL-2 inhibition in the current treatment algorithm of these children. It also focuses on specific genetic subgroups potentially associated with response in preclinical and clinical studies.

1 **Venetoclax, a new player in the treatment of children with high-risk myeloid malignancies?**

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26

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28 - Venetoclax combined with hypomethylating or cytotoxic therapies is a safe and effective bridge to
29   HCT in children with relapsed/refractory AML
30 - In pediatric MDS-EB and therapy-related MDS/AML, venetoclax plus hypomethylating agents
31   represents a potential option to reduce disease burden pre-HCT

32

Abstract

Venetoclax selectively inhibits BCL-2 and restores apoptotic signaling of hematological malignant cells. Venetoclax in combination with hypomethylating and low-dose cytotoxic agents has revolutionized the management of elderly patients affected by acute myeloid leukemia (AML), as well as that of patients unfit to receive intensive chemotherapy. In a single phase 1 pediatric trial conducted on relapsed/refractory AML, the combination of venetoclax with intensive chemotherapy was shown to be safe and yielded promising response rates. In addition, several retrospective studies in children with AML reported that venetoclax combined with hypomethylating agents and cytotoxic drugs appears a safe and efficacious bridge to transplant. Promising results on the use of venetoclax combinations in advanced myelodysplastic syndromes (MDS) and therapy-related MDS/AML have also been reported in small case series. This review summarizes the available current knowledge about venetoclax use in childhood high-risk myeloid neoplasms, discussing a possible integration of BCL-2 inhibition in the current treatment algorithm of these children. It also focuses on specific genetic subgroups potentially associated with response in preclinical and clinical studies.

Introduction

Overexpression of anti-apoptotic proteins of the B-cell lymphoma 2 (BCL-2) family, including BCL-2, BCL-XL, and MCL-1, is one of the primary mechanisms hematological cancers employ to escape cell death signaling[1]. Venetoclax (ABT-199) is a compound that selectively inhibits BCL-2, mimicking the function of BH3-only proteins (a BH3-mimetic), and restoring apoptosis signaling[2]. After its first clinical application in chronic lymphocytic leukemia[3], venetoclax has shown efficacy in acute myeloid leukemia (AML) models. Myeloid blasts rely on Bcl-2 for survival, and the overexpression of Bcl-2 is responsible for resistance to chemotherapy[4]. Conversely, normal hematopoietic stem cells are dependent on MCL-1, making venetoclax an agent capable of more selective in killing AML cells, while sparing healthy bone marrow components[4,5]. Venetoclax, in combination with hypomethylating agents (HMAs) such as azacytidine or decitabine, eradicates quiescent myeloid leukemia stem cells that overexpress Bcl-2 by abrogating cellular oxidative phosphorylation, suggesting an effect on cell metabolism beyond the classical pro-apoptotic signal[6,7] (see **Figure 1**). Venetoclax with azacytidine and low-dose cytarabine was demonstrated to be effective with an acceptable safety profile in patients with newly diagnosed AML[8,9], leading to the Food and Drug Administration (FDA) approval in the U.S. for this indication in 2020. In a few years, venetoclax containing regimens have significantly modified the management of older patients with AML, as well as of those patients unfit to receive intensive therapies, resulting in similar survival compared to CPX-351 (a dual-drug liposomal encapsulation of cytarabine and daunorubicin), with lower infections and inpatient length of stay in real-world observational analysis[10]. After these results, interest emerged in testing venetoclax therapies in younger patients. Several experiences have been reported so far and clinical trials are currently ongoing in pediatric myeloid neoplasms.

In this review, we will critically evaluate and summarize current evidence regarding the treatment of pediatric high-risk myeloid diseases, including relapsed/refractory AML (r/r AML), therapy-related MDS/AML (t-MDS/AML) and advanced MDS (or MDS with excess of blasts – MDS-EB). We will also discuss how venetoclax combination therapies can be integrated into the management of these disorders, often outside defined recommendations (see **Figure 2**). In addition, we will focus on identifying specific subgroups of interest, speculating on genetic lesions associated with peculiar venetoclax sensitivity or resistance in pediatric myeloid diseases.

Venetoclax as bridge to transplant in pediatric relapsed/refractory AML

Childhood AML is a genetically heterogeneous disease, with significant biological differences compared to adults. Genomic characterization plays a critical role in the management of pediatric AML, ensuring a more precise risk stratification and tailored treatment. Standard AML chemotherapy is not selective and does not ensure adequate response in all patients, due to the biological heterogeneity of this disease, being also associated with high rate of treatment-related toxicities[11]. Integration of genomic characterization and measurable residual disease (MRD) assessment in the treatment of pediatric AML, have improved clinical outcomes, with overall survival (OS) rates now approaching 70%[12,13]. Unfortunately, disease relapse

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87   represents the major cause of treatment failure, affecting 30-40% of patients[12]. No universal standard of care

88   exists regarding the management of r/r AML and poor OS rates, ranging from 20 to 40%, have been

89   reported[14,15]. Standard intensive chemotherapy, with or without the addition of gemtuzumab ozogamicin

90   (GO), is often employed during re-induction therapy of children with r/r AML, with response rate ranging

91   between 20-80% and OS of 20-40%[16,17]. Remarkably, AML cells develop resistance to anticancer drugs

92   through a series of cytogenetic events upon exposure to chemotherapy, demonstrating the importance of

93   adopting drugs that cause total remission in an early phase of disease preventing the development of

94   refractory clones[18]. In recent years, major efforts to modify treatment protocols have been made, by

95   incorporating novel targeted therapies and redesigning existing therapeutics, as is the case of CPX-351[19].

96   Administration of one CPX-351 cycle followed by standard chemotherapy in patients with r/r AML resulted

97   in CR rates of 81% with encouraging OS at 2 years of 52.7%[20]. Other novel strategies are currently being

98   tested with promising results, including monoclonal antibodies and cellular therapies, such as CAR.CD123-

99   NK cells[21,22]. Moreover, genomic analysis at the time of relapse with extensive characterization of clonal

100   evolution of AML, can help in identifying novel molecular therapeutic target[29].

101   Regimens containing venetoclax plus HMAs or low-dose chemotherapy are rarely used in newly diagnosed

102   childhood AML as first-line approach, considering that children are generally fit to tolerate more intensive

103   regimens. However, these approaches have increasingly been applied to children, especially in heavily

104   pretreated r/r AML cases, where they can benefit from less intensive regimens. In children, venetoclax has

105   been tested in combination with the intensive chemotherapy regimens typically adopted in pediatric

106   hematology[25]. First robust data on safety and efficacy of venetoclax with high-dose chemotherapy, came

107   from the VENAML phase 1 dose-escalating trial (NCT03194932) that enrolled 38 patients with r/r AML and

108   identified the recommended phase 2 dose (RP2D) of 360 mg/m2 (600 mg max) of venetoclax in combination

109   with high-dose cytarabine with or without idarubicin. Seventy and 80% of patients treated with RP2D

110   achieved complete response (CR) and CR/partial response (PR), respectively, with treatment-related death

111   occurring in only one patient[26]. Following the publication of these data, several retrospective series and

112   individual cases, predominantly from 2023, have evaluated the role of venetoclax combinations in young

113   patients with myeloid diseases, particularly r/r AML, as summarized in **Table 1**[27–36]. Variable response rates

114   were reported, with CR ranging from 10% to 92% and overall response rate (ORR) from 42% to 92%.

115   Patient populations largely vary across different studies and in each study cohort, but generally include

116   heavily pre-treated patients. Different dosage and length of venetoclax cycles were reported, with suboptimal

117   responses in studies adopting doses lower than RP2D[32]. Moreover, different drug partners have been

118   described, making it difficult to compare these studies and to draw solid conclusions. Analysis of selected

119   venetoclax combinations, particularly with HMAs, is highly awaited, considering the high efficacy of this

120   approach and the general good toxicity profile of this combination, being also applicable in outpatient

121   setting[32,33]. This combination could have a particular interest as bridge to hematopoietic cell transplantation

122   (HCT), reducing toxicities in the immediate pre-transplant phase[27,31–33]. Other agents have also been

123   combined with these regimens, including mostly GO and FLT3-inhibitors, confirming preclinical studies on

124   the synergistic effect of FLT3 and Bcl-2 inhibition[34,37]. When available, analysis on blast percentage at the

125   time of therapy shows a satisfactory efficacy of these approaches even in the presence of a high disease

126   burden[26,31,33]. Notably, some patients with mixed phenotype acute leukemia (MPAL) were included in these

127   studies, demonstrating favorable clinical outcomes when bridged to HCT after venetoclax, with both

128   hypomethylating and cytotoxic agents[26,28,38–42]. A variable percentage of patients (25-100%) was bridged to

129   HCT after achieving the maximum best response with one or two cycles[27,30,31,33]. Considering the dismal

130   prognosis of these diseases, outcome in transplanted patients was generally satisfactory, 50-70% of them

131   being alive and disease-free at the last follow-up[30,31,33]. In one study, venetoclax has been incorporated with

132   daratumumab in the preparative myeloablative regimen of children with refractory AML who received αβ T-

133   cell-depleted haploidentical HCT. No increased TRM was reported, with 2-year OS and EFS of 65 and 44%,

134   respectively[43]. When analyzed, no detrimental effect on engraftment/kinetics of neutrophil recovery or on the

135   occurrence of graft-versus-host disease (GvHD) after HCT was reported[30,43]. In all studies, toxicities were

136   generally manageable with no reported toxicity-related death. Dose-escalation during the first two days of

137   the first cycle was generally adopted in order to avoid tumor lysis syndrome. The most frequent adverse

138   event was neutropenia, often prolonged or profound and associated with severe infection[29,32], requiring

139   venetoclax premature interruption.

140   Due to the absence of published phase 3 trials, recommending the optimal duration of venetoclax cycles is

141   challenging. Various ongoing trials are currently employing either 21- or 28-day cycles. Regarding the

142   number of cycles, results of the phase 1 trial indicates that a good response (>50% blast reduction) during the

143   first cycle, is associated with high probability to achieve a CR with MRD negativity at the end of second

144   cycle[26]. In the study by Niswander and colleagues on azacytidine plus venetoclax combination, 4/12 patients

145   who achieved MRD negativity after first cycle, did not maintain the remission after the second cycle[33]. In our

146   experience, patients achieving CR after the first cycle with different combinations, maintained CR in 5/8

147   cases, while 7/8 with PR after the first cycle, successfully achieved CR after the second one[31].

148   Recommended criteria for venetoclax interruption are also lacking. We generally consider drug interruption

149   only in the case of clinically significant infection or other severe adverse events. If possible, we attempt to

150   temporarily interrupt venetoclax and restart as soon as possible when clinical conditions permit it, in order to

151   administer at least 21 days of therapy. Interestingly, patients not achieving complete hematological recovery

152   after therapy were successfully transplanted with general favorable outcome[30,31,33]. Regarding anti-infective

153   supportive measures, antibacterial and antiviral prophylaxis is not routinely performed[32], while antifungal

154   prophylaxis active against both yeasts and molds has to be administered considering the expected prolonged

155   course of neutropenia, with dose reduction in case of azole coadministration.

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157   **A potential role of venetoclax in pediatric *de novo* MDS and therapy-related MDS/AML**

158   Childhood MDS present unique biological characteristics that differ significantly from MDS in the elderly[44].

159   Particularly, MDS with excess of blasts (MDS-EB) are defined as presence of 5-19% blasts according to the

160   recent International Consensus Classification (ICC)[45]. Differentiating between MDS-EB and AML is crucial

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161 for selecting appropriate therapeutic strategies, as treatment approaches vary significantly between the two

162 conditions[44,46]. However, no standard recommendation exists on the best therapeutic approach to advanced

163 MDS/MDS-EB or AML that evolves from MDS, defined in adults as myelodysplastic-related or MDR-

164 AML[47]. Importantly, pediatric MDS can arise from a germline predisposition condition, which frequently

165 present excessive and unique risk of toxicities secondary to treatment[48,49]. In advanced MDS, conventional

166 AML-chemotherapy alone resulted in high risk of treatment-related toxicities and long-term survival lower

167 than 30%[50,51]. More favorable outcomes have been reported with allogeneic HCT, but patients given HCT as

168 first therapy without any bridge had a substantial risk of relapse[52]. For clinicians managing children with

169 advanced MDS/MDS-EB, the role of therapy as bridge to HCT, remains the most controversial issue, that

170 has not been investigated in a systematic manner so far[53]. In the largest EWOG study, intensive AML-

171 chemotherapy before HCT did not impact on relapse or TRM, this resulting in comparable OS or EFS in

172 patients receiving or not chemotherapy. In the MDR-AML subgroup, intensive chemotherapy was associated

173 with lower risk of relapse, leading to improved EFS, even if not statistically significant[54]. In a recent

174 retrospective study on 36 children with MDS, a blast count $\geq$ 5% and having received pre-HCT

175 chemotherapy, were both significantly associated with inferior OS (54% versus 87%), due to increased risk

176 of relapse, while patients achieving MRD-negative status before HCT showed improved OS (63.9% versus

177 33.3%) in a mixed population of patients with primary and secondary MDS[55,56]. Similarly to primary MDS,

178 therapy-related or post-cytotoxic therapy MDS/AML (t-MDS/AML) represent a difficult-to-treat condition

179 in which the optimal management has not been fully identified[45]. Patients with these conditions frequently

180 present a poor biological response to conventional chemotherapy and a high risk of treatment-related

181 toxicities. Time from diagnosis to HCT has been demonstrated to be the only significant prognostic factor[47],

182 suggesting to test novel approaches as bridge to HCT, such as CPX-351[57].

183 These considerations highlight the importance of finding interventions able to control disease burden, while

184 avoid intensive chemotherapy, in order to improve overall outcomes[55]. Currently, intensive chemotherapy is

185 not routinely recommended for childhood advanced MDS. However, cytoreduction is often necessary in

186 cases with an excess of blasts, and the role of novel, less intensive agents such as HMAs or targeted

187 therapies remains to be fully elucidated[47]. Azacytidine was well tolerated with variable response in some

188 retrospective series of pediatric primary MDS[58,59]. However, results on patients with treatment-*naïve* primary

189 advanced MDS receiving azacytidine in the AZA-JMML-001 trial showed poor response, suggesting the

190 ineffectiveness of HMAs as monotherapy[60]. The synergistic effect of venetoclax plus HMA was tested in

191 adult MDS with encouraging clinical benefits and this experience was translated to the pediatric setting[61].

192 For instance, few cases of childhood primary advanced MDS and MDR-AML receiving venetoclax-

193 containing regimens have been reported and are summarized in **Table 2**[27–29,31,42,62–64]. In *de novo* MDS,

194 results differ in terms of response rate, but these strategies seem to represent a potential effective bridge to

195 HCT. Patients who do not proceed to HCT after therapy, almost invariably relapse, highlighting the need to

196 transplant these patients at the time of best response[29,31,62,63]. Furthermore, when MDS progress to MDR-

197 AML, the efficacy of venetoclax is lower, even when used in combination with cytotoxic therapy[28,29].

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Interestingly, venetoclax combinations showed activity in patients with MDR-AML in the context of Schwachman-Diamond syndrome and Fanconi anemia, representing a fascinating opportunity to limit toxicities in these peculiar conditions[28,63,64]. Among the reported cases of t-MDS/AML, 9/13 patients achieved CR/PR with venetoclax combinations; 8 were bridged to HCT and were reported to be alive and disease-free. In light of these results, venetoclax plus HMA can represent a valid alternative option compared to other more intensive therapies[31].

Some clinical issues still remain unresolved; they pertain to the use of venetoclax-containing regimens in this peculiar population. The optimal number of venetoclax cycles in these disorders has not been definitively elucidated, and these combinations should be tested in rigorously conducted trials. In case of poor response to a first cycle, a second one should be avoided, and other alternatives should be explored. In case of PR or CR, a second cycle before HCT seems to be an opportunity to consider. Lastly, the best clinical endpoint to assess the efficacy of the different approaches, either blast reduction before HCT or post-HCT outcomes, remain undefined and this limits to some extent the opportunity to clearly define the best treatment option and the best partner drug to be used with venetoclax. MRD-negative remission, morphologic CR (blasts < 5%), PR (blasts 5-20%) or stable disease with lack of leukemic progression, are adopted as required criteria to proceed to HCT in different centers[65]. Defining the treatment algorithm of these diseases represents an unmet need for the pediatric hematology community[47].

Identifying genetic lesions predictive of response in AML and MDS

With the wider clinical use of venetoclax in pediatric hematology, it became increasingly important to identify recurrent genetic abnormalities that can help predict the response to venetoclax therapy[25]. Importantly, no mechanistic link between genetic lesions and venetoclax response has been demonstrated so far; however, specific molecular subtypes have been investigated in clinical reports. Rearrangements of the *KMT2A* gene are frequent in pediatric and infant leukemia, being generally associated with an aggressive clinical course[66,67]. In the ICC classification, the presence of ≥ 10% of blasts is sufficient for the diagnosis of *KMT2A*-rearranged AML[53]. Revumenib, a potent and selective oral inhibitor of the menin-*KMT2A* interaction, has shown promising remission rates with favorable toxicity profile, in patients with *KMT2A*-rearranged AML refractory to multiple previous lines[68]. Six of 12 patients with *KMT2A* rearrangements in phase 1 trial on venetoclax plus chemotherapy, responded to therapy (5 with CR/CRi)[26]. In the retrospective study that we published, eight patients presented *KMT2A* rearrangements; of them, six and one achieved CR and PR, respectively[31]. CR of 40% was achieved in 17 *KMT2A*-rearranged AML included in another report[32]. Two of eight patients with *KMT2A*-rearranged acute leukemias who received a cycle of venetoclax plus azacytidine, achieved MRD-negativity[33]. *In vitro* models showed high response rates to venetoclax plus azacytidine in lymphoblastic *KMT2A*-rearranged acute leukemia[69]. Moreover, adult *KMT2A*-rearranged AML seem to be sensitive to this combination[70]. The role of the association of venetoclax with menin inhibitors is currently under investigation with preliminary results of phase 1-2 study on HMA plus

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235   venetoclax and revumenib reporting high efficacy in *KMT2A*-rearranged, NPM1-mutated and NUP98-

236   rearranged AML[71]. These results confirmed preclinical studies showing a synergistic lethal effect of menin

237   plus Bcl-2 inhibition in AML lines[72]. Moreover, novel compounds, such as bromodomain inhibitor, I-

238   BET151, sunitinib or thioridazine, have been shown to decrease Bcl-2 expression and significantly

239   synergized with venetoclax, enhancing blast death in *KMT2A*-rearranged myeloid cell lines[73].

240   The role of venetoclax in AML with FLT3 aberrations is more controversial. FLT3-ITD is common in

241   children with AML, with a prognostic negative effect in patients treated with conventional multi-agent

242   chemotherapy[74]. FLT3-inhibitors are currently adopted in adult AML and favorable results have also been

243   observed in a Children Oncology Group (COG) report on pediatric patients receiving sorafenib in

244   combination with chemotherapy[75]. Encouraging results have been reported with gilteritinib and quizartinib

245   and pediatric trials are currently ongoing[76,77]. Adult FLT3-ITD AML patients showed limited response to

246   venetoclax-containing treatments, and these results were confirmed in the pediatric phase 1 trial in which

247   none of the five children with FLT3-AML responded to therapy[26]. At the same time, preclinical tests show a

248   synergistic effect of venetoclax with FLT3-inhibitors[37]. Some pediatric reports have incorporated these

249   drugs into venetoclax combinations, resulting in improved response rates[31,32,34], suggesting to test the

250   "triplet" approach (venetoclax plus FLT3-inhibitor plus cytotoxic drugs or HMA) in this genetic subgroup.

251   *CBFA2T3::GLIS2* fusion gene, resulting from the cryptic inversion of chromosome 16, defines a rare

252   subtype of AML that is peculiarly diagnosed in young children and is characterized by an aggressive clinical

253   course with OS ranging between 10 and 30%[78]. In very recent years, novel therapies have been tested in this

254   setting. Particularly, the identification of FOLR1 as target for CAR-T cells and monoclonal antibody (STRO-

255   002) has the potential to modify the management paradigm[79,80]. While four patients with AML with

256   *CBFA2T3::GLIS2* fusions in two different studies[29,31] did not respond to therapy, venetoclax plus azacytidine

257   resulted effective in treating molecular relapse of CBFA2T3::GLIS2 AML post-transplant[81] and in achieving

258   MRD-negative remission in three of four children with CBFA2T3::GLIS2 AML[33]. Interestingly, preclinical

259   tests show that dual BCL-2 family protein inhibition is necessary to treat these diseases, combining

260   venetoclax with MCL-1 or BCL-XL inhibitors[82,83]. Integration of pro-apoptotic agents with novel target

261   therapies will represent a fascinating opportunity that warrants future investigations.

262   Mutations of NPM1 and IDH1/2 are associated with good response to venetoclax in adult AML[84,85]. IDH1/2

263   mutations are rare in children, being detected in less than 3% of pediatric AML[86] while mutations of NPM1

264   are found in approximately 5-8% of cases[87]. Mutant NPM1 demonstrated a critical oncogenic mechanism in

265   AML, associated with upregulation of HOX genes in a menin-dependent manner[88]. Cooperation with

266   KMT2A complex is responsible for the sensitivity to menin inhibitors observed in NPM1-mutated AML[68,89].

267   Favorable response rates to venetoclax were confirmed in one retrospective pediatric study in NPM1-

268   mutated AML[32], and in one case of NPM1-mutated MDS/AML in a patient with Fanconi anemia[64].

269   Interestingly, NPM1 mutations have been reported in 14 of 235 pediatric MDS-EB of the EWOG registry,

270   with potential implications on the management of these rare entities[90].

271 Mutations of TP53 confer resistance to venetoclax in adult AML[91]. Numbers among the pediatric reports are
272 low with surprisingly favorable results in phase 1 trial[26] that were not confirmed in other reports, showing a
273 general association of TP53 mutations with diseases resistant to therapy or at high risk of relapse after
274 HCT[27,32]. PTPN11 mutations also resulted associated to venetoclax-resistant pediatric AML, confirming
275 adult reports[26,32,92]. Venetoclax combinations has to be considered with caution in these genetic subgroups
276 and other therapies, if available, should be preferred[25,93].

277 Regarding MDS-EB, only preliminary results have been reported so far, regarding the susceptibility to Bcl-2
278 inhibition of specific genetic subgroups. A recent study showed particularly high BCL-2 expression in
279 GATA2 MDS-EB compared to GATA2 refractory cytopenia of childhood, suggesting deregulation of
280 apoptosis as a potential driver to disease progression of GATA2 disease to overt MDS and AML and
281 providing biological evidence for the use of venetoclax therapies in this disease[94]. Moreover, pediatric
282 myeloid hematology is currently facing the increasingly understanding of the role of tandem duplications in
283 exon 13 of UBTF (UBTF-TDs) gene [95]. UBTF-mutated AML cases present a distinct genetic profile and are
284 associated with poor outcome[96]. In adult AML, UBTF-TD has been associated with myelodysplastic
285 features, lower response rates to induction therapy and worse survival compared to UBTF-wt[96,97]
286 Preliminary data from a German cohort of children revealed that UBTF-TDs are present in nearly a third of
287 pediatric MDS-EB and are associated with worse outcome post-HCT compared to UBTF-wild type MDS[98].
288 The poor prognosis of UBTF patients in both AML and MDS setting, despite the use of allogeneic HCT,
289 suggests that conventional treatment algorithms need to be revised in the management of these patients
290 carrying this molecular abnormality. Preliminary data suggest a role for menin inhibitors in UBTF-TD
291 AML[99]. The possible role of BCL-2 inhibition may warrant further exploration, in light of the overexpression
292 of HOX genes in these diseases, a biomarker for sensitivity to Bcl-2 inhibitors, and the genetic expression
293 profile overlapping with NPM1-mutated diseases[89,97,100]. A single case report has been recently published
294 describing a patient with UBTF-TD MDS experiencing relapse following two HCT procedures and showing
295 a dramatic optimal response to venetoclax plus azacytidine[101].

296

297 **Future directions**
298 Despite the promising results we summarized and discussed, several questions remain to be addressed for
299 optimizing the use of venetoclax in pediatric myeloid neoplasms. First, we urgently need prospective studies
300 aimed at obtaining regulatory approval, taking into consideration the lack of a pediatric formulation available
301 and the limited effectiveness of intensive chemotherapy alone in children with r/r AML. In this perspective,
302 several clinical trials are currently ongoing (as reviewed in[25]) and preliminary findings of venetoclax in
303 combination with intensive chemotherapy plus GO or CPX-351 are certainly promising[102,103]. Moreover,
304 factors predictive of response should be investigated systematically in large pediatric cohorts and will
305 potentially help clinicians in the future determine the best therapeutic approach on a single-patient basis.
306 These factors include both the genetic lesions previously discussed, as well as pharmaco-typing assays that
307 are becoming part of clinical practice in recent years. In this regard, BH3 profiling is a functional assay that

measures apoptotic priming and determines dependence on BCL-2, BCL-XL or MCL-1 by the relative release of cytochrome-c by mitochondria. As an exploratory objective of the pediatric phase 1 trial, AML blasts of patients with BCL-2 dependence presented major reduction in circulating blasts and higher CR rates compared to samples with BCL-XL dependance[26]. *Ex vivo* drug sensitivity screening of blasts to venetoclax with exposure to serial drug dilution has also been used in some reports and generally correlates with clinical response to Bcl-2 inhibition in pediatric acute leukemia[35,39,104]. Different mechanisms of resistance to venetoclax have been identified in recent years, including down-regulation of the pro-apoptotic proteins BIM and BAX secondary to venetoclax exposition, acquisition of MCL-1 or BCL-XL dependence of myeloid blasts, and acquisition of BCL-2 mutations[105–107]. Possible strategies to overcome venetoclax resistance are currently been tested[108–111] and have been extensively reviewed in[112]. Finally, interest is emerging in testing venetoclax therapies in other potential pediatric myeloid settings, including the management of AML molecular relapse after HCT[81] and different diseases such as chronic myeloid leukemia[113] and juvenile myelomonocytic leukemia[28,114]. Future studies will also have to dissect the optimal duration of venetoclax treatment and the number of cycles to be administered.

Conclusions

The integration of venetoclax into clinical practice represents a potential opportunity to enhance the clinical care of pediatric patients with myeloid diseases. Bcl-2 inhibition provides a potential option that can be considered in different conditions. In r/r AML and t-MDS/AML venetoclax in combination with both cytotoxic therapies and HMAs can be used as bridge to HCT, depending on the clinical condition of the patient and considering the impact of genetic characterization in predicting response. In advanced MDS, a peculiar setting that lacks largely validated therapeutic options, venetoclax plus azacytidine certainly represents a promising approach, potentially reaching the ambitious goal of reducing disease burden pre-HCT, while avoiding intensive AML-type chemotherapy.

345 **Conflict of Interest Disclosures**

346 The authors declare no competing financial interests.

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Table 1. Venetoclax therapies in pediatric myeloid diseases. rel-AML, relapsed AML; refr-AML, refractory AML; MPAL, mixed-phenotype acute leukemia; R2PD, recommended phase 2 dose; ORR, overall response rate; CR, complete response; PR, partial response; MRD, measurable residual disease; CRi, complete response with incomplete recovery; NR, non-response; TF, treatment failure; SD, stable disease; PD, progression of disease; AEs, adverse events; BSI, bloodstream infection; IFI, invasive fungal infection; MDR-AML, myelodysplastic related AML; SDS, Shwachman-Diamond syndrome; N/A, not available; N/E, not evaluable; TKI, tyrosine kinase inhibitor

Study	Pts,n (age, range)	Disease	Combination therapies	Venetoclax	Response	HCT post ven	Survival post HCT	Toxicities
Karol 2020 Lancet Oncol	38 (10 yrs, 3-22)	rel-AML (33), refr-AML (4), refr-MPAL (1) [KMT2Ar (12); FLT3 (5); TP53 (4)] [median disease burden: 33% (18-68]]	Cytarabine (1000mg/m²/dose for 8 doses) +/- idarubicin (12mg/m² as a single dose)	240 or 360mg/m²/day (28 days) † [2 cycles]	In 35 pts evaluable for response: ORR 69% (24), CR (20; 13 MRD neg; 4 CRi); PR (4); NR (11) In 20 pts treated at R2PD: ORR 80%, CR/CRi (14), PR (2), NR (4)	16 of 20 pts achieving CR	N/A ^	Grade III-IV AEs: febrile neutropenia (25), BSI (6), IFI (6)
Winters 2021 PBC	8 (11 yrs, 2-20)	MDS-EB (2) t-MDS/AML (1) r/r AML (5) [FLT3 (3), TP53 (2)]	Azacytidine (75 mg/m²/day days 1-7)	Adult-equivalent dose of 800 mg (28 days) [median n cycles: 1 (1-9)]	CR 75% (6; 1 CRi) NR 25% (2)	4 + 2 pending/8	N/A	Grade III-IV neutropenia (8)
Bobeff 2023 Children	5 (national survey: age < 10 years)	MDR-AML (2), rel-AML (1), t-AML (1) refr-MPAL (1) [KMT2Ar (2)]	Cytarabine + idarubicin (2), cytarabine (1), cytarabine + azacitidine (2), azacitidine (1)	360 mg/m²/day (28 days) [median n cycles: 1 (1-2)]	CR 60% (3; 1 CRi), NR 40% (2)	2/5	1 alive disease-free	N/A
Marinoff 2023 PBC	10 (10 yrs, 1-29)	t-MDS/AML (2), r/r AML (6), MDS (1), MDR-AML (1) » [GATA2 (1), KMT2Ar (2), GLIS2 (1), SDS (1)]	Cytarabine (1000 mg/m²/dose for 8 doses) (5), decitabine (20 mg/m²/day for 5 days) (4), azacitidine (1)	Adult-equivalent dose of 400 mg [median n cycles: 1 (1-3)]	CR 10% (1), PR/SD 50% (5), NR/PD 40% (4)	2/10	1/2 alive disease-free	Grade III-IV AEs: cytopenia, infections (5)
Pfeiffer 2023 BMT	28 (13, 1-21)	Refr-AML (5); rel-AML (23) [adverse genetics in 12]	Cytarabine (17), cytarabine + idarubicin (5), cytarabine + azacitidine (3), decitabine (2), azacitidine (1)	240-360 mg/m²/day [median n cycles: 2 (1-7)]	CR 92% (26) (2 CRi), PR/NR 18% (2)	28/28	20/28 alive disease-free, 8 relapse ‡	N/A (no impact on GVHD incidence or neutrophil and platelet engraftment)
Trabal 2023 Cancers	43 (18, 1-21)	r/r AML (43) [KMT2Ar (17), FLT3-ITD (10), NPM1 (4), TP53 (3), IDH1/2 (2)]	HMA (37), cytotoxic agents (6) [+ trametinib (1), gemtuzumab ozogamicin (7), TKI (5), MCL-1 inhibitor (1)]	median dose 93 mg/m2/day (28 days cycles; effective duration median 14 days) [median n cycles: 2 (1-9)]	CR 37% (16, 6 Cri), PR 5% (2), NR 51% (22) N/E. 7% (3)	11/43	6/11 alive disease-free ¶	Grade III neutropenia / febrile neutropenia (49%)
Masetti 2023 Bl Adv	31 (10.2, 1.3-17.4)	MDS-EB (4), rel-AML (11), refr AML (7), t-MDS/AML (9) [KMT2Ar (8), FLT3 (5)] [median disease	HMA (19), cytotoxic agents (9), HMA + cytotoxic (3) [+ gilteritinib (1)]	median 350 mg/m2/day (28 days) [median n cycles 2 (1-15)]	CR 51.6% (16; 6 MRD neg; 5 CRi), PR 19.4% (6), NR 25.8% (8)	20/31	15/20 alive disease-free •	Grade III-IV cytopenia (4), IFI (3) (one TF due to severe pancytopenia)

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		burden 20% (0-80)]						
Niswander 2023 Hematologica	29 (8, 0-19)	r/r AML (27), MPAL (2) [KMT2Ar (8), GLIS2 (4), FLT3 (1)] [median disease burden 10.5% (0.01-91.5)]	Azacitidine 100 mg/m2 (days 1-5) [+ gemtuzumab ozogamicin (9)]	Adult-equivalent dose of 800 mg (28 days) [median n cycles 2 (0-6)]	CR with MRD neg 44.8% (13)	12/29	7/12 alive disease-free	Severe cytopenia (7), bacteremia (6), IFI (2)

† RP2D of venetoclax plus chemotherapy = 360mg/m^2/day (600mg max)

ˆ 1y-OS (whole cohort): 20/38 dead

» one patient with Shwachman-Diamond syndrome (SDS) who developed AML

‡ median f-up 344 days (111–1056) from HCT: 1-y OS 80.5%, 1-y EFS 69.2%, CI of relapse at 1 year post-HCT 30.8% and at 2 years post-HCT 43.2%

¶ median OS and EFS duration 8.7 months (range 0.2–53 months) and 3.7 (range 0.1–53 months)

• 30-months estimated OS after the start of venetoclax treatment 29.9% in the whole cohort and 74.4% for patients undergoing HCT

667 **Table 2. Patients with primary advanced MDS or t-MDS/AML treated with venetoclax combination**

668 **therapies**. VEN, venetoclax; AZA, azacytidine; CR, complete response; MRD, measurable residual disease;

669 N/A, not available; RAEB, refractory anemia with excess of blasts; RAEB-t, refractory anemia with excess

670 of blasts in transformation; SDS, Shwachman-Diamond syndrome; NF1, neurofibromatosis type 1; Mon7,

671 monosomy 7; FLA, fludarabine, cytarabine; IDA-FLA, fludarabine, cytarabine, idarubicin; ARA-C,

672 cytarabine; PD, progression of disease; DEC, decitabine; FA, Fanconi anemia; TRM, transplant-related

673 mortality; CRi, complete response with incomplete recovery

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Study	Age (years)	Diagnosis	Genetics	Previous lines	Venetoclax combination therapy	Response	HCT	Outcome (disease status/cause of death)
Winters 2021	7	MDS-EB/RAEB in SDS	Mon7, ETV6, GATA2	0	VEN + AZA (1 cycle)	CR (morphological <5%; cytogenetic monosomy 7 10%)	Yes	Alive; disease-free
Marinoff 2023	17	MDS	GATA2 germline	1	VEN + AZA	NR	Yes	Dead; relapse after HCT
Masetti 2023	15	MDS-EB	LIG4 and SH2B3 germline, mon7, RIT1, EZH2, SETBP1, ASXL1, ETV6	0	VEN + cytotoxic (IDA-FLA) (1 cycle)	CR	Yes	Alive; disease-free
Masetti 2023	14	MDS-EB	Del7q	4	VEN + DEC (5 cycles)	CR¶	Yes	Dead; TRM
Winters 2021	8	MDR-AML/RAEB-t in NF1	Del17p/loss TP53, ASXL1, TET2	0	VEN + AZA (3 cycles)	CR (morphological <5%; cytogenetic persistence del17p/loss TP53)	Yes	Alive; relapse cytogenetic del17/TP53 and MRD posˆ
Bobeff 2023	<6	MDR-AML in NF1	Mon7	1	VEN + cytotoxic therapy (IDA-FLA) (1 cycle)	CR	Yes	Dead; relapse post HCT
Bobeff 2023	6-10	MDR-AML in familial platelet disorder	RUNX1	4	VEN + cytotoxic therapy (Idarubicin + ARA-C) (1 cycle)	NR	No	Dead; PD before HCT
Marinoff 2023	14	MDR-AML in SDS	IDH1, KMT2A	0	VEN + DEC	CR (MRD neg)	Yes	Alive; disease-free
Raedler 2020	16	MDR-AML/RAEB-t	Complex karyotype	2	VEN + DEC (4 cycles)	CR (for 10 months, then molecular relapse)	No	Alive; molecular relapse‡
Naviglio 2023	14	MDR-AML in SDS	neg	2	VEN + AZA (1 cycle)	PR	No	Dead; PD before HCT
Wen 2023	3	MDR-AML	Complex karyotype, NRAS	1	VEN + AZA	CR (MRD neg)	Yes	Alive; disease-free
Ma 2023	7	MDS/MDR-AML in FA•	NPM1, GATA2, WT1	1	VEN+AZA (2 cycles)	CR (MRD neg)	Yes	Alive; disease-free
Masetti 2023	17	MDR-AML	FLT3, WT1	1	VEN + AZA (1 cyle)	NR	Yes	Dead; TRM
Masetti 2023	14	MDR-AML	WT1	2	VEN + AZA (1 cycle) + VEN + ARA-C (1 cycle)	CR	Yes	Dead; relapse post HCT
Bobeff 2023	6-10	t-AML	KMT2A, t (9;11)	1	VEN + ARA-C (1 cycle)	CR (MRD neg)	Yes	Alive; disease-free
Marinoff 2023	17	t-AML	Mon7, t (7;11), PTPN11, SED2, RUNX1, BCOR	3	VEN + DEC	PR	No	Dead; PD before HCT
Winters 2021	11	t-MDS/AML	RUNX1	1	VEN + AZA (9 cycles)	CR (MRD neg)	No	Alive; disease-free†
Marinoff 2023	9	t-MDS	PTPN11	1	VEN + DEC	PR (stable disease)	No	Dead; PD before HCT
Masetti 2023	7	t-MDS/AML	T (11;17), KMT2A	1	VEN + IDA-FLA (2 cycles)	CRi	Yes	Alive; disease-free

Masetti 2023	5	t-MDS/AML	t (9;11), SDHC, KMT2A	1	VEN + IDA-FLA (2 cycles)	CRi	Yes	Alive; disease-free
Masetti 2023	1	t-MDS/AML	t (4;11), KMT2A	1	VEN + ARA-C + idarubicin	NR	No	Dead; PD before HCT
Masetti 2023	10	t-MDS/AML	Mon7	1	VEN + AZA (15 cycles)	PR (stable disease) ††	Yes	Alive; disease-free
Masetti 2023	10	t-MDS/AML	Del3q, PTPN11, WT1	1	VEN + AZA	NR	Yes	Alive; disease-free
Masetti 2023	6	t-MDS/AML	t (11;19), KMT2A	2	VEN + AZA (5 cycles)	PR	No	Dead; PD before HCT
Masetti 2023	9	t-MDS/AML	Mon7, TP53	1	VEN + AZA (1 cycle)	PR	Yes	Alive; disease-free
Masetti 2023	14	t-MDS/AML	Mon7, CBL, KRAS, ASXL2	1	VEN + AZA (2 cycles)	CR	Yes	Alive; disease-free
Masetti 2023	6	t-MDS/AML	t (9;11), KMT2A	1	VEN + FLA (1 cycle); VEN + AZA (1 cycle)	CR (MRD neg)	Yes	Alive; disease-free

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676 † maintaining MRD neg after 9 cycles

677 ˆ receiving AZA post-HCT

678 ‡ therapy ongoing

679 • previous HCT for FA; donor-cell derived leukemia (DCL) 43 months after HCT

680 ¶ CR after two cycles, maintained for 10 months, then relapse, other 3 cycles with response, bridged to HCT

681 †† stable disease maintained for 15 cycles, then relapse, received AML-type induction therapy, bridged to HCT

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685 **Figure 1. Mechanism of action of venetoclax (ABT-199) in myeloid malignant cells**. In blast cells,

686 venetoclax inhibits BCL-2 protein, thereby reducing the inhibitory effect of BCL-2 on the proapoptotic

687 complex. Venetoclax also inhibits oxidative phosphorylation (OXPHOS) in the mitochondria. Upregulation

688 of MCL-1 or BCL-XL, metabolic reprogramming or BCL-2/BAX mutations can occur in blast cells as a

689 compensatory effect and escape mechanism (red text and arrows). Normal cells, which rely on MCL-1

690 signaling, are less sensitive to venetoclax inhibition. HMA, hypomethylating agents

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693 **Figure 2. Clinical indications of venetoclax combination therapies and possible predictors of response**.

694 The lower section of the figure illustrates the clinical settings where various venetoclax combinations may be

695 applied. In the upper section, the relationship between genetic drivers, BH3 profiling results and sensitivity

696 to therapy is depicted. On the left, genes with higher resistance potential are presented, while on the right,

697 genes associated with higher sensitivity are highlighted; r/r AML, relapsed/refractory AML; MDS-EB, MDS

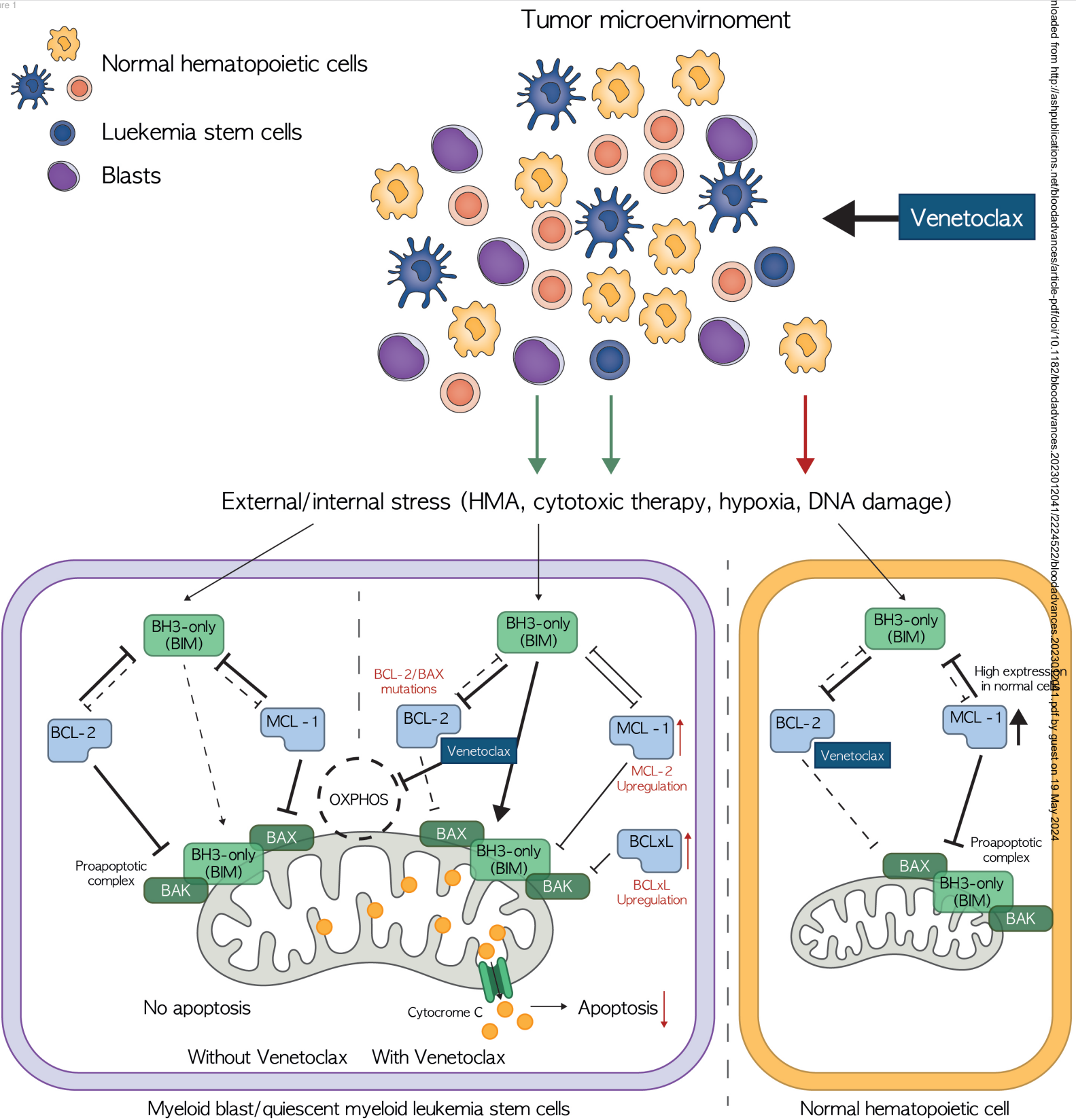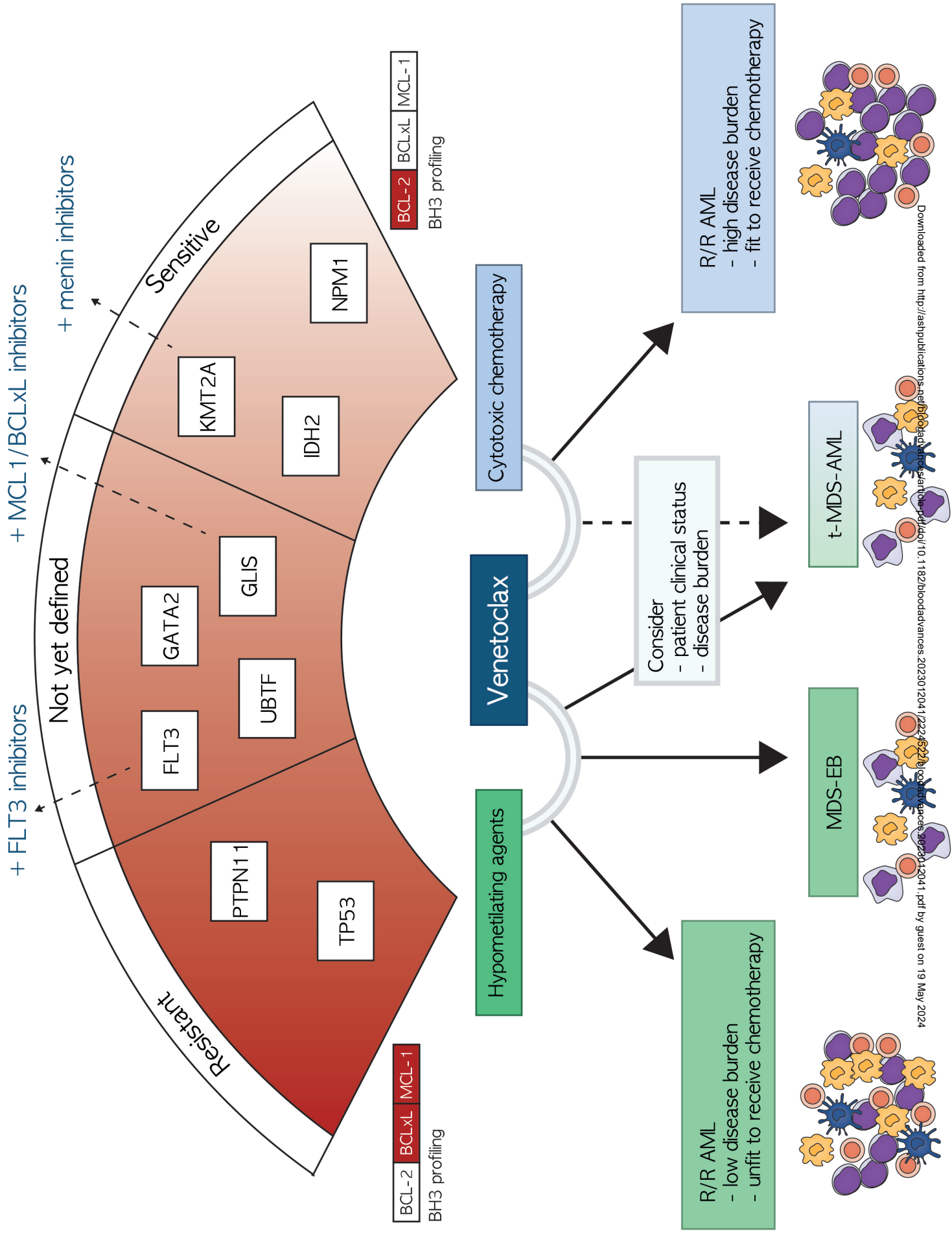698 with excess of blasts; t-MDS/AML, therapy-related MDS/AML

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Figure 1

Figure 2