Venetoclax and azacytidine in childhood primary advanced myelodysplastic syndromes, refractory/relapsed acute myeloid leukemia and therapy-related myeloid neoplasms

Francesco Baccelli,[1,2] Pietro Merli,[3] Martina Pigazzi,[4,5] Barbara Buldini,[4,5] Marco Becilli,[3] Katia Girardi,[3] Valeria Paganelli,[3] Gennaro Pagano,[1,2] Davide Leardini,[1,2] Edoardo Muratore,[1] Francesca Vendemini,[6] Giuliana Beneduce,[7] Manuela Tumino,[4] Francesco Saglio,[8] Elena Sieni,[9] Elena Mastrodicasa,[10] Marianna Maffeis,[11] Francesco Paolo Tambaro,[7] Riccardo Masetti[1,2#] and Franco Locatelli[3,12#]

[1]Pediatric Hematology and Oncology, IRCCS Azienda Ospedaliero-Universitaria di Bologna, Bologna; [2]Department of Medical and Surgical Sciences (DIMEC), University of Bologna, Bologna; [3]Department of Pediatric Hematology/Oncology, Cell and Gene Therapy, IRCCS Ospedale Pediatrico Bambino Gesù, Roma; [4]Department of Women's and Children's Health, Onco-hematology Laboratory and Clinic, University of Padova, Padova; [5]Foundation "Istituto Ricerca Pediatrica" (IRP), Padua; [6]Fondazione IRCCS San Gerardo dei Tintori, Monza; [7]AORN Santobono-Pausillipon, Napoli; [8]Ospedale Regina Margherita, Torino; [9]Azienda Ospedaliera Universitaria Meyer, Firenze; [10]Azienda Ospedaliera Perugia, Perugia; [11]Pediatric Oncohematology and Bone Marrow Transplant Unit, Children's Hospital, ASST Spedali Civili of Brescia, Brescia and [12]Catholic University of the Sacred Heart, Roma, Italy

[#]RM and FL contributed equally as senior authors.

Correspondence: R. Masetti
riccardo.masetti5@unibo.it

Received: February 25, 2026.
Accepted: May 14, 2026.
Early view: May 21, 2026.

https://doi.org/10.3324/haematol.2026.300775

©2026 Ferrata Storti Foundation
Published under a CC BY-NC license

Supplementary methods

BM trephine material of MDS-EB and t-MDS/AML were centrally reviewed at the Department of Pathology of Bambino Gesù Children's Hospital in Rome. Immunophenotypic and molecular analysis were centrally performed at the Pediatric Onco-Haematology Laboratory of the University of Padua. Immunophenotyping was performed according to WHO criteria, using a direct immunofluorescence technique with 8-12 colour combinations[1,2]. For genetic characterization, RNA was extracted from all samples at diagnosis from mononuclear cells isolated by density gradient. Reverse transcription polymerase chain reaction (RT-PCR) was performed to amplify target sequences. Amplification products were analyzed by agarose gel electrophoresis and/or capillary electrophoresis using the 3500 Dx Genetic Analyzer, and/or sequencing[3–7]. All DNA samples of patients were screened for the presence of *UBTF*-TD by polymerase chain reaction (PCR) with specific primers previously validated to detect the presence of tandem duplications (*UBTF* forward (5'FAM): CTGGGCTCCCTGGCAGCAC; *UBTF* reverse: GCCCGCCAAGGGGAAGAGG). PCR products were diluted and analyzed by capillary electrophoresis on a 3500Dx Genetic Analyzer using GeneMapper software. A wild-type peak at 270±1 bp was expected, while additional peaks indicated UBTF-TD. The allelic ratio was calculated as the ratio of mutant to wild-type peak areas. Positive, negative, and non-template controls were included to ensure assay specificity and exclude contamination. Further details were previously reported[8].

1. Dworzak MN, Buldini B, Gaipa G, et al. AIEOP-BFM consensus guidelines 2016 for flow cytometric immunophenotyping of Pediatric acute lymphoblastic leukemia. Cytometry B. Clin. Cytom. 2018;94(1):82–93.
2. Maurer-granofszky M, Schumich A, Buldini B, et al. An Extensive Quality Control and Quality Assurance (QC / QA) Program Significantly Improves Inter-Laboratory Concordance Rates of Flow-Cytometric Minimal Residual Disease Assessment in Acute Lymphoblastic Leukemia : An I-BFM-FLOW-Network Report. 2021;
3. Pigazzi M, Manara E, Bisio V, et al. Screening of novel genetic aberrations in pediatric acute myeloid leukemia: a report from the AIEOP AML-2002 study group. Blood. 2012;120(18):3860–3862.
4. van Dongen JJ, Macintyre EA, Gabert JA, et al. Standardized RT-PCR analysis of fusion gene transcripts from chromosome aberrations in acute leukemia for detection of minimal residual disease. Report of the BIOMED-1 Concerted Action: investigation of minimal residual disease in acute leukemia. Leukemia. 1999;13(12):1901–1928.
5. Bisio V, Zampini M, Tregnago C, et al. NUP98-fusion transcripts characterize different biological entities within acute myeloid leukemia: a report from the AIEOP-AML group. Leukemia. 2017;31(4):974–977.
6. Pigazzi M, Masetti R, Bresolin S, et al. MLL partner genes drive distinct gene expression profiles and genomic alterations in pediatric acute myeloid leukemia: an AIEOP study. Leukemia. 2011;25(3):560–563.
7. Pigazzi M, Manara E, Buldini B, et al. Minimal residual disease monitored after induction therapy by RQ-PCR can contribute to tailor treatment of patients with t(8;21) RUNX1-RUNX1T1 rearrangement. Haematologica. 2015;100(3):e99-101.
8. Merli P, Masetti R, Pigazzi M, et al. Sensitivity of Pediatric Myelodysplastic Syndromes With Excess of Blasts With UBTF–TD to Venetoclax/Azacitidine. Am. J. Hematol. 2025;1–5.

Supplementary results _ *disease burden prior to HCT*

Among the 33 patients successfully bridged to HCT, 26 (78.8%) reached the transplant with 0% BM blasts. In this subgroup, only 1 relapse was observed (alongside 3 deaths due to transplant-related mortality - TRM). In contrast, among the 7 patients with morphologically detectable disease (range 1-13% blasts), 3 relapses occurred. Focusing on the MDS-EB and t-MDS/AML subgroup (n=14), 11 patients had 0% blasts at HCT and one of these patients relapsed after transplant, while three patients were transplanted with morphological disease and none experienced relapses.

Supplementary Table S1. Univariate and multivariate analysis of factors associated to 1-year event-free survival (EFS)

	1-y EFS			
	Univariate		**Multivariate**	
Risk factors	**HR (95% CI)**	**p**	**HR (95% CI)**	**p**
Sex	0.535 (0.227-1.261)	0.153		
r/r AML diagnosis	0.313 (0.105-0.935)	**0.037** *	2.354 (0.751-7.379)	0.142
MDS-EB diagnosis	2.641 (0.614-11.356)	0.192		
t-MDS/AML	2.518 (0.585-10.839)	0.215		
HOX dysregulation	0.942 (0.399-2.224)	0.892		
KMT2 rearrangements	0.560 (0.231-1.355)	0.198		
No HCT previous to ven/aza	2.900 (1.157-7.266)	**0.023** *	1.079 (0.323-3.605)	0.902
CR achieved	3.708 (1.490-9.226)	**0.005** *	0.345 (0.130-0.916)	**0.033**
Bridged to HCT	0.098 (0.035-0.272)	**<0.0001**	7.303 (2.139-24.935)	**0.002**

EFS, event-free survival; HR, hazard ratio; CI, confidence interval; r/r AML, relapsed/refractory acute myeloid leukemia; MDS-EB, myelodysplastic syndromes with excess of blasts; t-MDS/AML, therapy-related MDS or AML; CR, complete response; HCT, hematopoietic cell transplantation

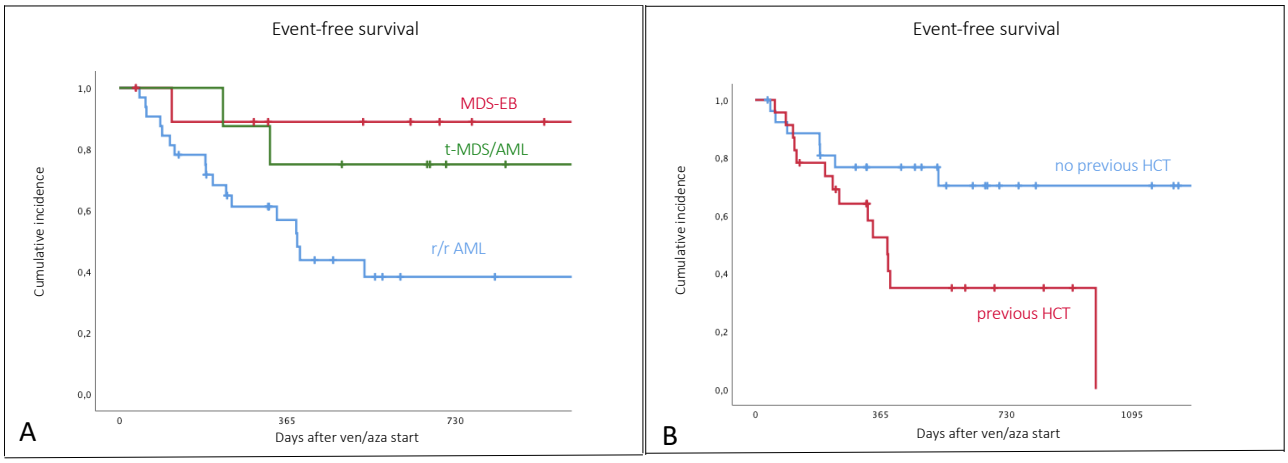
* diagnosis of r/r AML, previous HCT status and CR achievement were not associated with EFS when analysis was restricted to patients bridged to HCT in Cox regression analysis

Supplementary Table S2. Transplant characteristics in patients undergoing hematopoietic cell transplantation (HCT) after ven/aza

Patients (n = 33)	**n (% or range)**
Conditioning, n (%)	
- Bu-based	- 17
- Treo-based	- 6
- TBI-based	- 10
Donor, n (%)	
- MSD	- 9
- MUD	- 12
- UCB	- 1
- haploidentical	- 11
Interval between ven/aza start and HCT (days), median (range)	97 (range, 33 – 933)
BM blast percentage before HCT, median (range)	0 (0 – 13%)*

Bu, busulfan; Treo, treosulfan; TBI, total-body irradiation; MSD, matched sibling donor; MUD, matched unrelated donor; UCB, umbilical cord blood

Supplementary Figure S1. Kaplan-Mayer estimates of EFS in patients receiving ven/aza. (A) EFS according to diagnosis and (B) according to previous transplantation status (B).



EFS, event-free survival; r/r AML: relapsed/refractory acute myeloid leukemia; MDS-EB: myelodysplastic syndrome with excess of blasts; t-MDS/AML, therapy-related MDS/AML; HCT, hematopoietic cell transplantation