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Haematology

Supplementary appendix

This appendix formed part of the original submission and has been peer reviewed. We post it as supplied by the authors.

Supplement to: Russo D, Polverelli N, Bernardi S, et al. Venetoclax plus decitabine as a bridge to allogeneic haematopoietic stem-cell transplantation in older patients with acute myeloid leukaemia (VEN-DEC GITMO): final report of a multicentre, single-arm, phase 2 trial. *Lancet Haematol* 2024; published online Sept 20. https://doi.org/10.1016/S2352-3026(24)00241-2.

APPENDIX

Statistical Design and analysis

The study was designed as a Simon optimal two-stage Phase II clinical trial, incorporating a planned futility check. Considering the existing literature indicating that fewer than 10% of elderly (≥60 <75 years) AML patients reach the goal allo-SCT, primarily due to non-response to conventional chemotherapy (NR or PR) and/or treatment-related toxicity, the null hypothesis (conventional chemotherapy) was tested with $p \leq 0.10$ against the alternative (VEN-DEC) with $p \geq 0.20$. The anticipated sample size was set at 89 patients, with an early termination probability of 0.647 when the true proportion is 0.1. The alpha error was 0.0478, and the beta value was 0.1982. If three or fewer patients were submitted to allo-SCT in the first 30 enrolled patients, the trial would be terminated for futility. Factoring in an overall dropout rate (screening failures, etc.) of 12%, an additional 70 patients were required for a total of 100 patients. The null hypothesis would be rejected if 14 or more patients treated with VEN-DEC were submitted to allo-SCT in CR/CRi/MLFS.

NGS analysis

NGS analysis was performed for research purpose by CREA Laboratory in ASST Spedali Civili of Brescia. Approximately, 10 ml EDTA of BM were collected at patients' enrolment and at least 200 ng of DNA were locally extracted in every participating Center.

Both manual and automatized extraction were allowed, based on the expertise of the participating Center. BM DNA samples for NGS analysis were stored at -20°C by the Participating Center and centralized in Brescia every 6 months, in dry ice, by express courier.

Genomic analysis by NGS was gene-panel based (CE-IVD Myeloid Solution by Sophia Genetics) and conducted as correlative study aiming to explore the mutational status of 30 genes commonly involved in AML.

NGS analysis was performed following manufacturer's instructions. Briefly, the DNA was tagmented at 300bp and enriched for the 30 targeted genes by probes hybridization. Three different pools of 8 patients were contemporary sequenced per each run by MiSeq platform using a High-output flow-cell (V3, 600 cycles).

Bioinformatic analysis was performed by Sophia DDM® software on .fastq files. Sophia DDM is an artificial intelligence-based platform which performs the call, annotation, and classification of the variants taking advantage of machine- and deep-learning. Three different patented algorithms are included in Sophia DDM. The analysis of the sequencing data was automatically carried out with fixed parameters (2,5% Limit of Detection, 2000X Coverage, 99% Coverage Uniformity). NGS analysis allowed the identification and characterization of the pathogenicity levels of Single Nucleotide Variants (SNVs), small insertions or deletions (Indels), Internal Tandem Duplications (ITDs), and Copy Number Variants (CNVs). Polymorphisms, benign and potentially benign significance mutations were excluded from additional analysis, while malignant, potentially malignant and unknown significance mutations were retained.

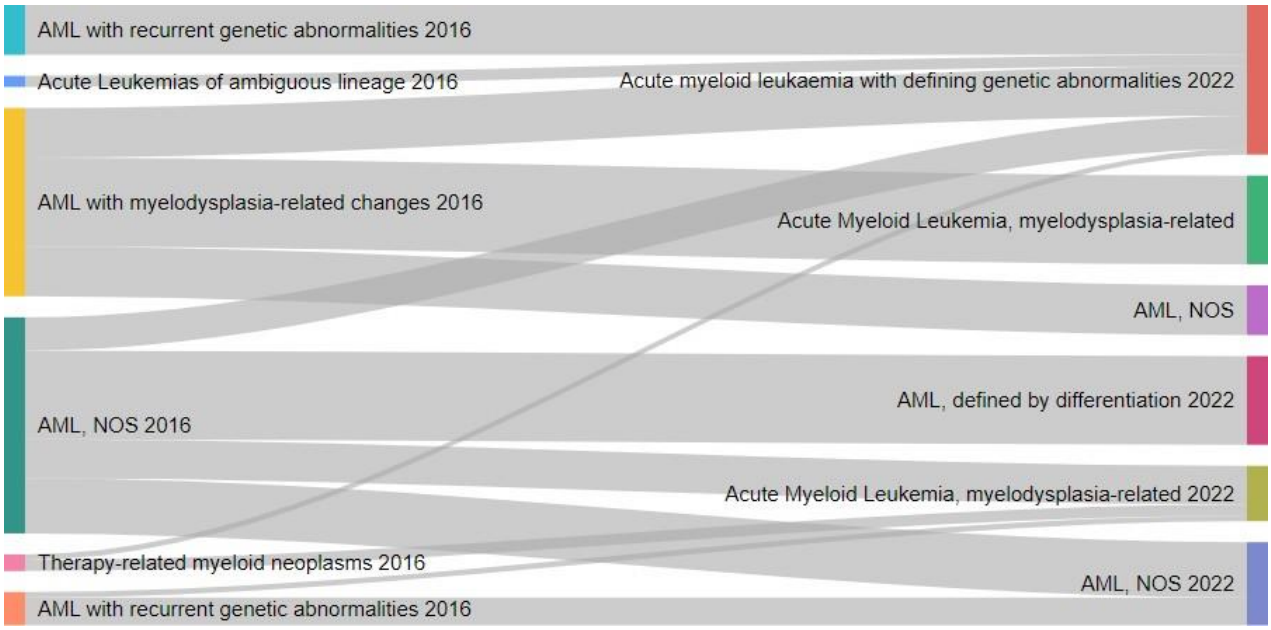
Clustering biological functions

The clustering rationale stemmed from the Cancer Genome Atlas Research Network's findings, which identified six distinct subnetworks within a genome-wide protein–protein interaction network. These subnetworks were delineated

using the HotNet algorithm, focusing on specific gene groups, namely: (i) Epigenetic modifiers (DNMT3A, IDH1, IDH2, TET2), (ii) Chromatin modifiers (ASXL1, EZH2, SETBP1), (iii) Splicing factors (SRSF2, SF3B1, U2AF1, ZRSR2), (iv) Oncogenes (TP53, WT1), (v) Signaling molecules (ABL1, BCL, BRAF, CALR, CSF3R, FLT3, HRAS, JAK2, KIT, KRAS, NRAS, PTPN11), (vi) Myeloid Transcriptional Factors (CEBPA, ETV6, MPL, RUNX1), and (vii) NPM1.

Citation from:

Genomic and Epigenomic Landscapes of Adult De Novo Acute Myeloid Leukemia. New England Journal of Medicine. 2013 May 30;368(22):2059–74. Available from: http://www.nejm.org/doi/10.1056/NEJMoa1301689



Appendix Figure 1: Diagnosis distribution according to WHO 2016 vs WHO 2022

Appendix Figure 2: ELN risk stratification, 2016 vs 2022.

Venetoclax Dose	Modified Venetoclax Dose if co-administered with a Moderate CYP3A or P-gp Inhibitor	Modified Venetoclax Dose if co-administered with a Strong CYP3A Inhibitor
100 mg (Cycle 1 Day 1 only)	50 mg	10 mg
200 mg (Cycle 1 Day 2 only)	100 mg	20 mg
400 mg	200 mg	50 mg

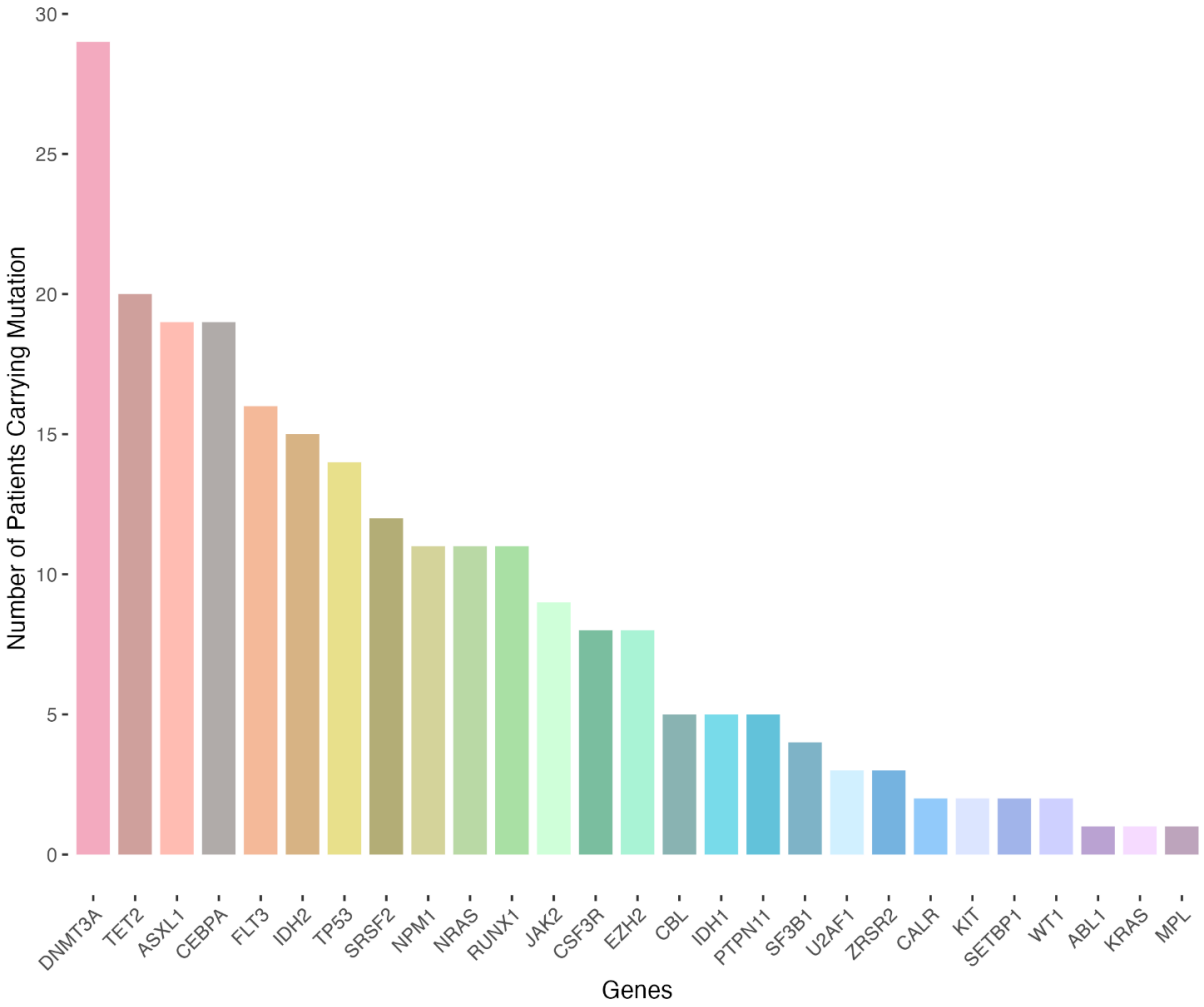
Appendix Table 1: Dosages of Venetoclax intake when taken in the absence of CYP3A inhibitors or in the presence of moderate/strong CYP3A inhibitors. In case of discontinuation of inhibitors wait for 2 to 3 days before Venetoclax dose is increased back to the previous dose level. Ramp-up is not required upon discontinuation of the inhibitor.

Comorbidities	N° (%)
Pulmonary	27 (29%)
Diabetes	11 (12%)
Prior Solid Tumor	6 (6%)
Psychiatric Disturbance	6 (6%)
Cerebrovascular Disease	4 (4%)
Obesity	3 (3%)
Cardiac disfunction	2 (2%)
Heart Valve Disease	2 (2%)
Infection	2 (2%)
Arrhythmia	1 (1%)
Hepatic dysfunction	1 (1%)
Peptic Ulcer	1 (1%)
Rheumatologic	1 (1%)
Inflammatory Bowel Disease	0 (0%)
Renal condition	0 (0%)

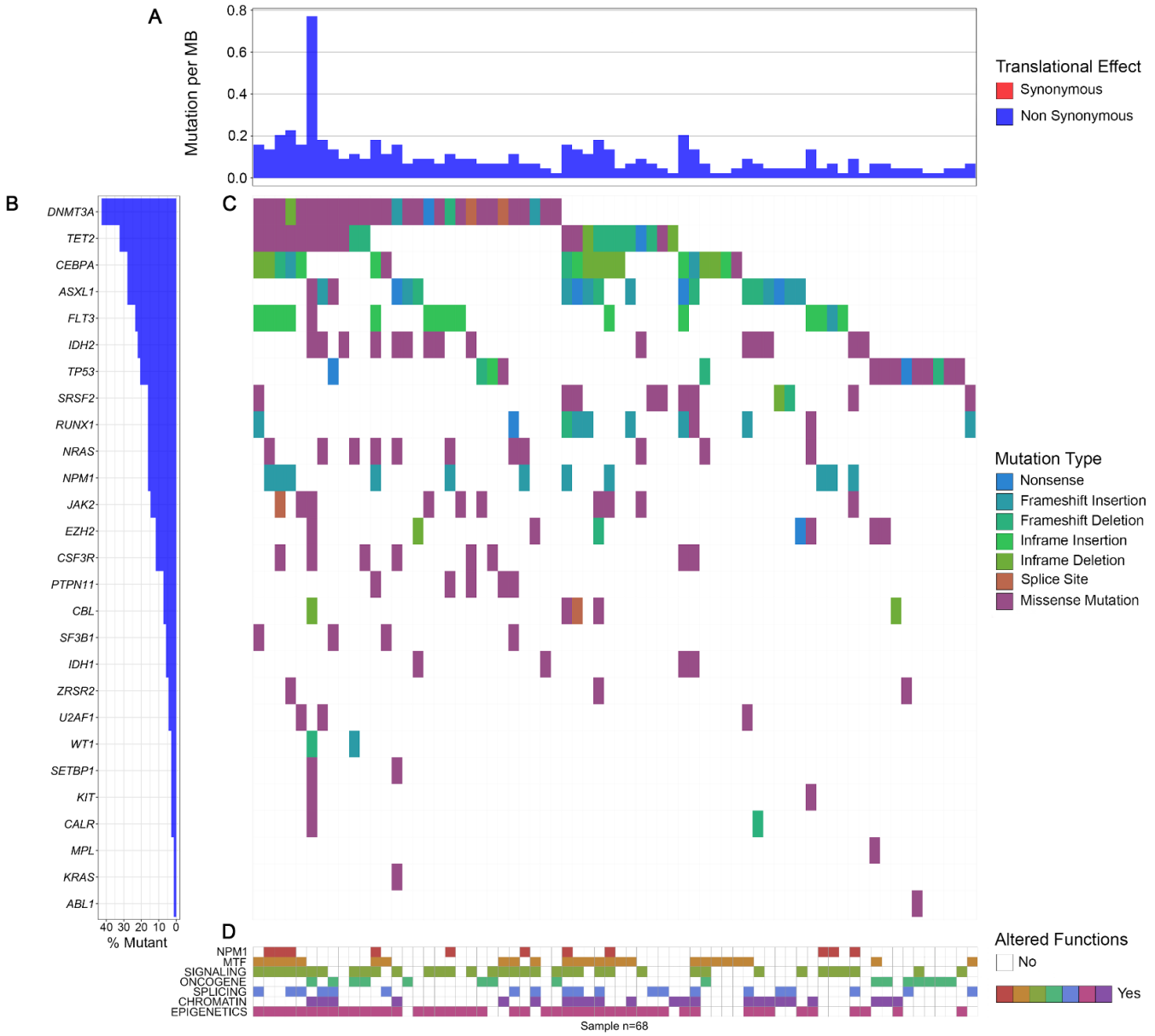
Appendix Table 2: Comorbidities and other clinical evaluations.

Gene	Patients' Mutation, n° (%) (n=69)	Frequency of Non-Unique Mutation, n° (%) (n=292)
DNMT3A	29 (42%)	34 (12%)
TET2	20 (29%)	36 (12%)
ASXL1	19 (28%)	23 (8%)
CEBPA	19 (28%)	22 (8%)
FLT3	16 (23%)	20 (7%)
IDH2	15 (22%)	15 (5%)
TP53	14 (20%)	17 (6%)
SRSF2	12 (17 %)	12 (4%)
NPM1	11 (16%)	11 (4%)
NRAS	11 (16%)	13 (4%)
RUNX1	11 (16%)	15 (5%)
JAK2	9 (13%)	11 (4%)
EZH2	8 (12%)	10 (3%)
CSF3R	8 (12%)	9 (3%)
CBL	5 (7%)	5 (2%)
IDH1	5 (7%)	5 (2%)
PTPN11	5 (7%)	5 (2%)
SF3B1	4 (6%)	4 (1%)
U2AF1	3 (4%)	3 (1%)
ZRSR2	3 (4%)	4 (1%)
CALR	2 (3%)	2 (1%)
KIT	2 (3%)	3 (1%)
SETBP1	2 (3%)	7 (2%)
WT1	2 (3%)	3 (1%)
ABL1	1 (1%)	1 (1%)
KRAS	1 (1%)	1 (1%)
MPL	1 (1%)	1 (1%)
BRAF	0 (0%)	0 (0%)
ETV6	0 (0%)	0 (0%)
HRAS	0 (0%)	0 (0%)

Appendix Table 3: List of the mutations discovered by NGS analysis.



Appendix Figure 3: List of mutations according to NGS analysis.



Appendix Figure 4: Molecular landscape of the 69 analyzed patients. Only *non-synonymous* mutations were considered (A) The Plot on the top reports the ration of mutations found every megabases (MB) in each patient. (B) The chart on the left refers to the portion of patients carrying at least one mutation on the corresponding gene. (C) The central diagram represents, by column, the molecular profile of each patient (68). 1/69 patient was excluded due to absence of mutations. (D) Functional clustering of gene mutations and their functions. Note that MTF stands for Myeloid Transcriptional Factor.

Time	Number of CR°	Cytogenetik	RT-qPCR	Flow Cytometry
Enrollment		41 K+	58 PCR+	59 IF+
After C2	60	9/41 (22%)*	15/58 (26%)	34/59 (58%)^
After C2-C4	64	10/41 (24%)*	16/58 (28%)	37/59 (63%)^
Before allo-HSCT	53	18/41 (44%)*	19/58 (33%)	38/59 (64%)^
		*18 pts NE	0 pts NE	^14 pts NE

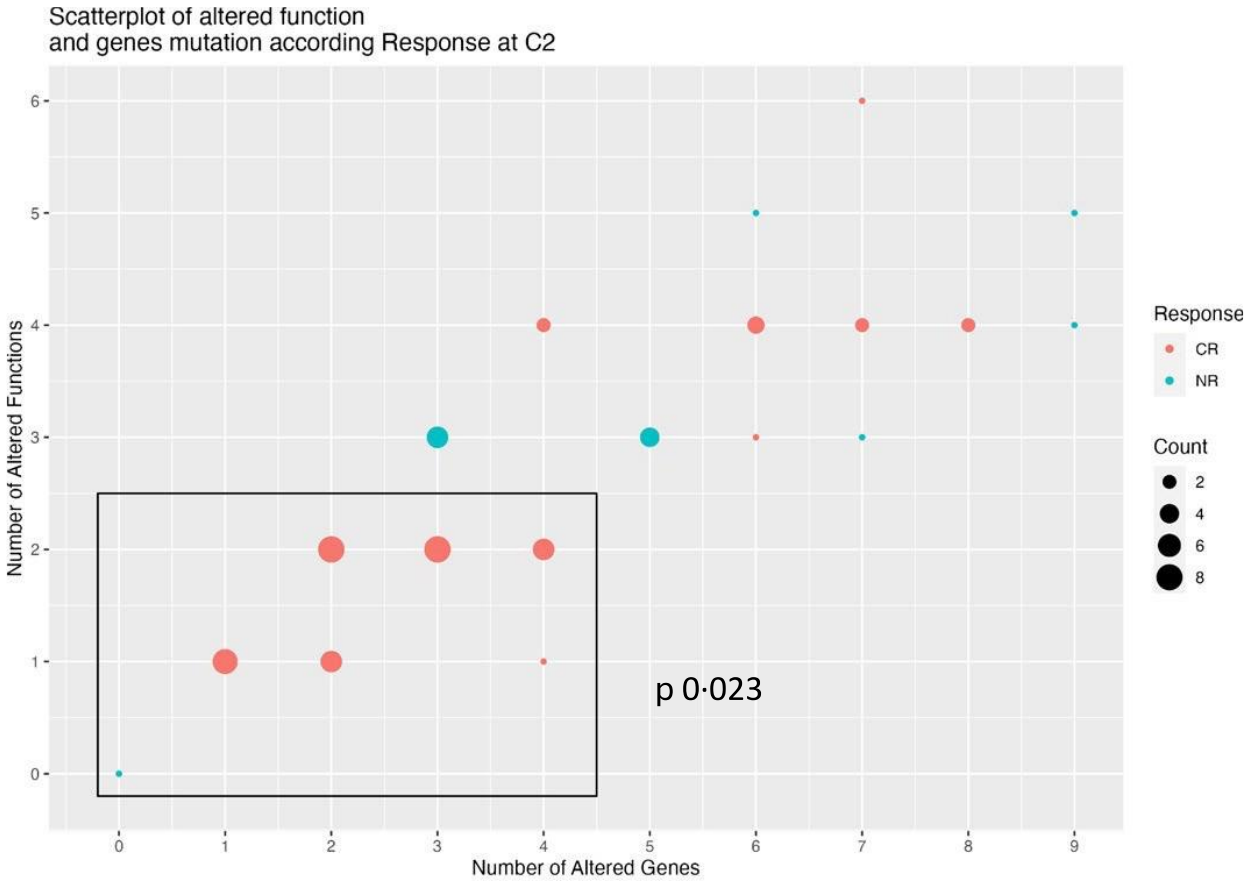
Appendix Table 4: Proportion of patients that achieves MRD negativity at the time of first CR (after cycle 2 and after cycle 24) and before allo-HSCT. CR° = CR/CRi/MLFS; NE = Not Eligible; K = karyotype. Pts = patients. IF = immunophenotype. PCR=Polymerase Chain Reaction.

	After C2	After C2-C4	Before allo-HSCT
K+PCR+IF	**Pts Evaluable = 13** Pts MRD-neg = 4 /13 (31%)	**Pts Evaluable = 9** Pts MRD-neg = 3 /9 (33%)	**Pts Evaluable = 6** Pts MRD-neg = 2 /6 (33%)
K+IF	**Pts Evaluable = 13** Pts MRD-neg = 5 /13 (38%)	**Pts Evaluable = 11** Pts MRD-neg = 4 /11 (36%)	**Pts Evaluable = 10** Pts MRD-neg = 2 /10 (20%)
K+PCR	**Pts Evaluable = 15** Pts MRD-neg = 5 /13 (38%)	**Pts Evaluable = 11** Pts MRD-neg = 7 /13 (53%)	**Pts Evaluable = 7** Pts MRD-neg = 3 /7 (43%)
PCR+IF	**Pts Evaluable = 29** Pts MRD-neg = 4 /29 (14%)	**Pts Evaluable = 19** Pts MRD-neg = 7 /19 (37%)	**Pts Evaluable = 12** Pts MRD-neg = 1 /12 (0.8%)

Appendix Table 4a: Level of concordance/discordance between cytogenetics, RT-qPCR and flow cytometry at different time-points. K = karyotype. IF = immunophenotype. PCR=Polymerase Chain Reaction.

	Responder	Non-responder	p
Karyotype	**(56 pts)**	**(18 pts)**	
Normal	29 (52%)	9 (50%)	ns
Abnormal	27 (48%)	9 (50%)	
Complex (≥ 3)	10 (18%)	6 (33%)	
Molecular Biology	**(60 pts)**	**(18 pts)**	
NPM1	7 (12%)	1 (6%)	ns
FLT3-ITD	14 (23%)	2 (11%)	
FLT-TKD	0 (0%)	0 (0%)	
WT1 overexpression	15 (25%)	7 (39%)	
NGS	**(44 pts)**	**(16 pts)**	
DNMT3A	17 (39%)	7 (44%)	ns
TET2	12 (27%)	5 (31%)	
FLT3	12 (27%)	2 (13%)	
IDH2	12 (27%)	2 (13%)	
ASXL1	11 (25%)	7 (44%)	
CEBPA	9 (21%)	6 (38%)	
SRSF2	8 (18%)	3 (19%)	
NPM1	8 (18%)	1 (6%)	
JAK2	8 (18%)	1 (6%)	
TP53	7 (16%)	4 (25%)	
EZH2	7 (16%)	0 (0%)	
NRAS	5 (11%)	4 (25%)	
RUNX1	5 (11%)	4 (25%)	
CBL	4 (9%)	0 (0%)	
CSF3R	2 (5%)	4 (25%)	0.04
IDH1	2 (5%)	3 (19%)	ns
PTPN11	2 (5%)	1 (6%)	
SF3B1	2 (5%)	1 (6%)	
U2AF1	2 (5%)	1 (6%)	
CALR	2 (5%)	0 (0%)	
KIT	2 (5%)	0 (0%)	
ZRSR2	1 (2%)	1 (6%)	
SETBP1	1 (2%)	1 (6%)	
WT1	1 (2%)	1 (6%)	
ABL1	0 (0%)	1 (6%)	
KRAS	0 (0%)	1 (6%)	

Appendix Table 5: Correlation between Cytogenetic, molecular and mutational features and Response to VEN-DEC assessed after C2.



Appendix Figure 5: Calculated cut-off based on number of altered genes and altered functions for each patient analyzed with NGS. Two patients were excluded due to (i) absence of mutations and (ii) excessed number of mutations. The latter patient is not included in the graphical representation only for aesthetic purpose, while both are included in the statical analysis. The box identifies patients below cut-off value (≤2 altered functions and ≤5 altered genes).

	Patients	**Decitabine**		**Venetoclax**	
		Dose	***n° pts (%)***	***Dose***	***n° pts (%)***
Cycle 1	93	100%	88 (95%)	100%	31 (34%)
		≥75%	2 (2%)	≥75%	6 (6%)
		≥50%	1 (1%)	≥50%	5 (5%)
		<50%	2 (2%)	≤50%	51 (55%)
		Total	93 (100%)	Total	93 (100%)
Discontinuation after 1st cycle: 12 pts (13%)					
Cycle 2	81	100%	75 (93%)	100%	26 (32%)
		≥75%	3 (4%)	≥75%	10 (12%)
		≥50%	1 (1%)	≥50%	9 (12%)
		<50%	2 (2%)	≤50%	36 (44%)
		Total	81 (100%)	Total	81 (100%)
Discontinuation after 2nd cycle: 8 pts (9%)					
Allo-SCT after 2nd cycle: 9 (11%)					
Cycle 3	64	100%	63 (98%)	100%	20 (30%)
		≥75%	0 (0%)	≥75%	8 (13%)
		≥50%	0 (0%)	≥50%	8 (13%)
		<50%	1 (2%)	<50%	28 (44%)
		Total	64 (100%)	Total	64 (100%)
Discontinuation after 3rd cycle: 5 (8%)					
Allo-SCT after 3rd cycle: 22 (34%)					
Cycle 4	37	100%	35 (94%)	100%	8 (22%)
		≥75%	1 (3%)	≥75%	5 (14%)
		≥50%	0 (0%)	≥50%	5 (14%)
		<50%	1 (3%)	≤50%	19 (50%)
		Total	37 (100%)	Total	37 (100%)
Discontinuation after 4th cycle: 15 (41%)					
Allo-SCT after 4th cycle: 22 (59%)					

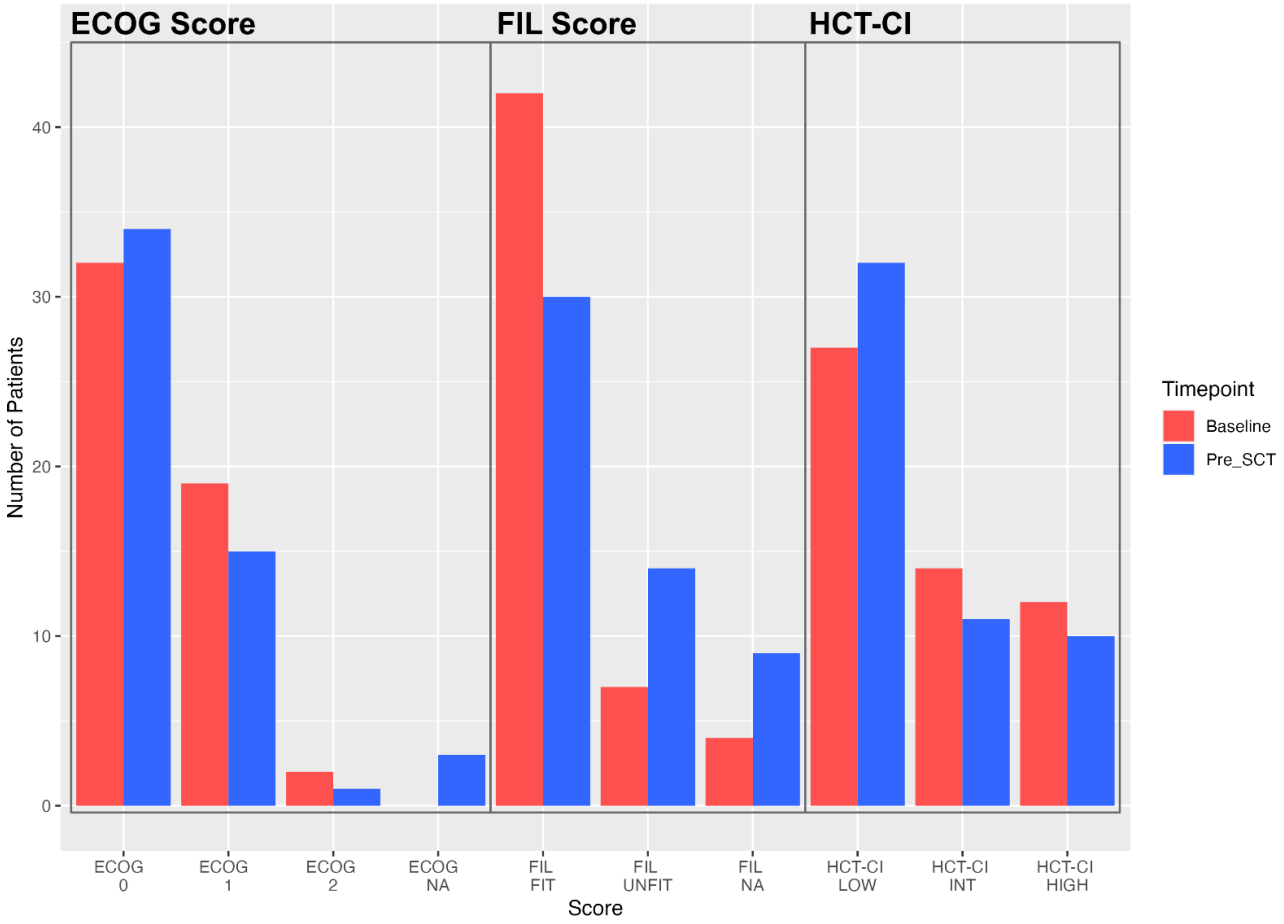
Appendix Table 6: Distribution of patients according to Venetoclax and Decitabine time and dose administration at each cycle.

Delay reason(s)	**Delay C1-C2 N° of event**	**Delay C2-C3 N° of event**	**Delay C3-C4 N° of event**
Neutropenia	24	13	9
Logistic reasons	6	6	0
Infection	5	3	2
FUO	3	2	1
Medical decision	1	4	4
Thrombocytopenia	1	1	0
Hyperbilirubinemia	0	1	0
Myocarditis	0	1	0

Appendix Table 7: Causes for VEN-DEC cycling delay.

	Cycle 1		Cycle 2		Cycle 3		Cycle 4	
	G 3-4	**G 5**	**G 3-4**	**G 5**	**G 3-4**	**G 5**	**G 3-4**	**G 5**
All AEs (N°=104)	50 (48%)	5 (5%)	19 (18%)	4 (4%)	13 (13%)	0	13 (13%)	0
Hematologic AEs								
Neutropenia	9 (18%)	0	12 (63%)	0	6 (46%)	0	5 (38%)	0
Thrombocytopenia	0	1 (20%)	2 (11%)	0	0	0	0	0
Anemia	11 (22%)	0	0	0	0	0	1 (8%)	0
Nonhematologic AEs								
Infections	1 (2%)	3 (60%)	1 (5%)	4 (100%)	2 (15%)	0	3 (23%)	0
Neutropenic Fever	11 (22%)	0	3 (16%)	0	4 (30%)	0	3 (23%)	0
Heart	1 (2%)	1 (20%)	1 (5%)	0	1 (8%)	0	0	0
Kidney	1 (2%)	0	0	0	0	0	0	0
Upper GI	1 (2%)	0	0	0	0	0	0	0
Mucositis	1 (2%)	0	0	0	0	0	0	0
Enterocolitis	1 (2%)	0	0	0	0	0	0	0
Suspected neoplasia	0	0	0	0	0	0	1 (8%)	0

Appendix Table 8: List of adverse events (AEs) with a grade ≥3 per CTCAE at each cycle. A total of 104 AEs was observed in our cohort. In the table they are distributed as per single cycle and as hematologic/nonhematologic events. The percentages refer to the total of observed AEs. The only grade 1-2 adverse event occurred in ≥10% of patients was the fever of unknown origin (FUO), irrespective of neutropenia. Twenty episodes of grade 1-2 FUO were observed in 13 patients and they were distributed as follows: 10 during C1, 4 during C2, 5 during C3 and 1 during C4.

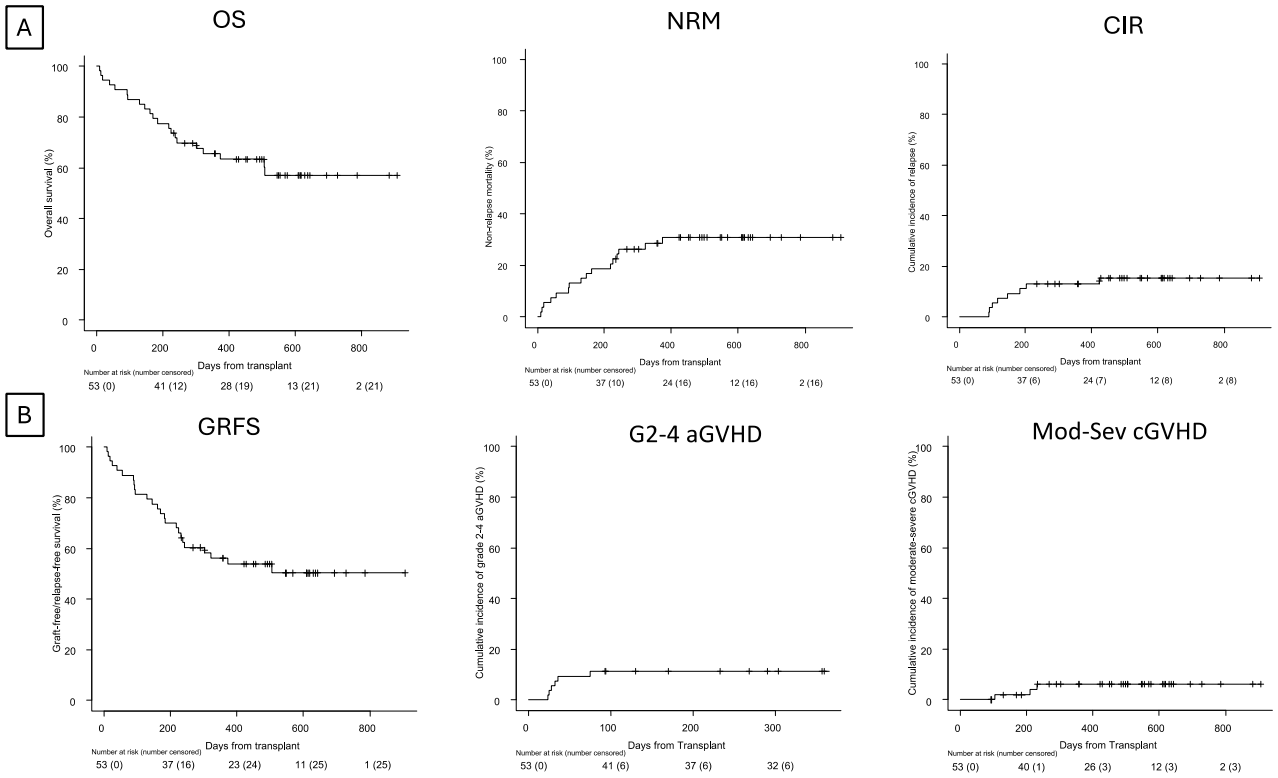


Appendix Figure 6: Comparison patients' fitness in terms of ECOG, HCT-CI and FIL score.

ECOG performance status (Eastern Cooperative Oncology Group), HCT-CI (Hematopoietic stem cell transplantation specific comorbidity index) and FIL score (Lymphoma Italian Foundation score).

Transplant features	**N=53**
Patient sex	
Male	29 (55%)
Female	24 (45%)
Donor type	
Matched Unrelated	24 (45%)
Matched sibling	6 (11%)
Haploidentical	23 (44%)
Stem Cell source	
Peripheral Blood	49 (93%)
Bone Marrow	4 (7%)
Conditioning Intensity Regimen	
Myeloablative Reduced Intensity	36 (68%)
Reduced Intensity	17 (32%)

Appendix Table 9: Transplant features.



Appendix Figure 7: Allo-HSCT Outcome. (A) On the left, the Overall Survival (OS); in the middle, the Non relapse Mortality curve (NRM); while on the right the Cumulative Incidence of Relapse (CIR). (B) On the left, Graft-free relapse survival (GRFS); in the middle, the cumulative incidence of Grade 2-4 acute Graft Versus Host Disease (G2-4 aGVHD); and on the right, the cumulative incidence of moderate severe Chronic Graft Versus Host Disease (Mode-Sev cGVHD). Overall survival and GRFS were assessed using the Kaplan-Meier estimator. A Fine-Gray regression model for competing risks was utilized for cumulative incidence of aGVHD and cGVHD, NRM and CIR calculation.

Transplant Outcomes	**N° patients (%)**
Graft failure	0 (0%)
Acute GVHD any Grade - Grade II – IV	14 (26%) 6 (11%)
Chronic GVHD any Grade - Grade moderate-severe	5 (9%) 3 (6%)
Relapse after allo-SCT - Before 100 days after allo-SCT - From 100 to 180 days after allo-SCT - After 180 days after allo-SCT	8 (15%) 3 (6%) 2 (4%) 3 (6%)
Graft-versus-Host Disease (GVHD)-free relapse-free survival (GRFS) - 1 year after allo-HSCT - 2 years after allo-HSCT	 56 % 50 %
Non-Relapse Mortality - NRM - Before 100 days after allo-SCT - From 100 to 180 days after allo-SCT - After 180 days after allo-SCT	16 (30%) 7 (18%) 2 (4%) 7 (18%)
NRM causes n° total - Infection - Hemorrhage - GvHD - VOD - Thrombosis - PLTD - Neurological toxicity - Respiratory failure	16 (30%) 6 (38%) 3 (19%) 2 (13%) 1 (6%) 1 (6%) 1 (6%) 1 (6%) 1 (6%)

Appendix table 10: Transplant Outcomes on 53 transplanted patients

List of Centers:

- Centro Trapianti Midollo Osseo; Azienda Sanitaria Locale di Pescara – PI: S. Santarone - 12 patients.
- Unit of Blood Diseases and Bone Marrow Transplantation, Cell Therapies and Hematology Research Program, Department of Clinical and Experimental Science, University of Brescia, ASST Spedali Civili di Brescia - PI: D. Russo - 10 patients.
- MTN Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico - ASST Fatebenefratelli-Sacco, University of Milan – PI: F. Onida - 9 patients.
- Bone Marrow Transplantation Unit, Palermo – PI: L. Castagna - 8 patients.
- Humanitas Clinical and Research Center, IRCCS, Rozzano – PI: S. Bramanti - 6 patients.
- IRCCS Ospedale Casa Sollievo della Sofferenza, Foggia – PI: A.M. Carella - 6 patients.
- Department of Hematology, S. Croce e Carle Hospital, Cuneo – PI: R. Sorasio – 6 patients.
- Centro Unico Trapianti and Division of Hematology, Grande Ospedale Metropolitano Bianchi Melacrino Morelli, Reggio Calabria – PI: M. Martino - 5 patients.
- Clinica di Ematologia Azienda Ospedaliero Universitaria delle Marche, Ancona – PI: A. Olivieri - 5 patients.
- U.O. Ematologia e Terapie Cellulari, IRCCS Azienda Ospedaliera Universitaria San Martino, Genova – PI: G. Beltrami - 4 patients.
- IRCCS Azienda Ospedaliero-Universitaria di Bologna, Istituto di Ematologia "Seràgnoli", Bologna – PI: A. Curti - 4 patients.
- Division of Hematology and BMT Unit, A.O.U. Policlinico G. Rodolico - S. Marco, Catania – PI: C. Vetro - 4 patients.
- Department of Hematology, ASST Grande Ospedale Metropolitano Niguarda, Milano – PI: V. Mancini - 3 patients.
- Hematology Department, Fondazione IRCCS San Gerardo dei Tintori, Monza – PI: E. Teruzzi - 3 patients.
- Unit of Hematology and Bone Marrow Transplantation, I.R.C.C.S. Ospedale San Raffaele, Milan – PI: M. Bernardi - 2 patients.
- UOC Hematology, Mazzoni Hospital-Ascoli Piceno, Ascoli Piceno – PI: P. Galieni - 2 patients.
- UOC Ematologia, Bari – PI: P. Musto - 2 patients.
- Department of Hematology, Stem Cell Transplant Unit, Policlinico Tor Vergata, Rome – PI: R. Ceretti - 1 patient.
- University of Torino, AOU Città Della Salute E Della Scienza Di Torino, Torino – PI: L. Giaccone - 1 patient.
- Unit of Haematology and Bone Marrow Transplantation, "Ospedale Dell'Angelo", Venezia Mestre – PI: C. Skert - 1 patient.