Data Supplement

Table S1. Baseline characteristics of patients ≤ 60 years

	8d Venetoclax (n=8)	11d Venetoclax (n=6)	14d Venetoclax (n=6)	All Cohorts (n=20)
Age, yrs median (range)	47.5 (27-59)	55 (43-59)	53 (42-59)	53 (27-59)
Sex, male n (%)	5 (62.5)	5 (83.3)	3 (50)	19 (56)
Race, n (%)				
white*	3 (37.5)	1 (16.7)	1 (16.7)	5 (25)
non-white*	5 (62.5)	5 (83.3)	5 (83.3)	15 (75)
WBC at diagnosis, $x10^3$/uL, median (range)	15.2 (1.6 – 153.9)	13.7 (1.4 - 343.6)	26.7 (1.2 – 92.9)	19.4 (1.2 - 343.6)
WBC on Day 1, $x10^3$/uL median (range)	4.5 (1.3 – 16.3)	7.9 (1.7 – 14.2)	3 (1.1 – 24.1)	4 (1.1 – 24.1)
Secondary AML, n (%)	1 (12.5)	1 (16.7)	-	2 (10)
Cytogenetic risk, n (%)				
Favorable	2 (25)	-	1 (16.7)	3 (15)
Intermediate	3 (37.5)	5 (83.3)	3 (50)	11 (55)
Adverse	3 (37.5)	1 (16.7)	2 (33.3)	6 (30)
ELN 2022 risk, n (%)				
Favorable	4 (50)	2 (33.3)	3 (50)	9 (45)
Intermediate	1 (12.5)	1 (16.7)	1 (16.7)	3 (15)
Adverse	3 (37.5)	3 (50)	2 (33.3)	8 (40)

Table S2. Baseline characteristics of patients >60 years

	8d Venetoclax (n=6)	11d Venetoclax (n=3)	14d Venetoclax (n=5)	All Cohorts (n=14)
Age, yrs median (range)	65 (60-69)	65 (62-71)	69 (61-71)	66 (60-71)
Sex, male n (%)	3 (50)	1 (33.3)	2 (40)	6 (42.9)
Race, n (%)				
white*	4 (66.7)	2 (66.7)	2 (40)	8 (57.1)
non-white*	2 (33.3)	1 (33.3)	3 (60)	6 (42.9)
WBC at diagnosis, $x10^3$/uL median (range)	38.9 (2.1 – 223.6)	3.4 (3.3 - 28.6)	15.1 (1.3 – 241.7)	16.4 (1.3 – 241.7)
WBC on Day 1, $x10^3$/uL median (range)	6 (1.4 – 22.3)	4.7 (3 – 24.4)	1.9 (0.7 – 10.2)	3.8 (0.7 – 24.4)
Secondary AML, n (%)	2 (33.3)	-	-	2 (14.3)
Cytogenetic risk, n (%)				
Favorable	-	2 (66.7)	-	2 (14.3)
Intermediate	4 (66.7)	1 (33.3)	4 (80)	9 (64.3)
Adverse	2 (33.3)	-	1 (20)	3 (21.4)
ELN 2022 risk, n (%)				
Favorable	1 (16.7)	2 (66.7)	1 (20)	4 (28.6)
Intermediate	-	1 (33.3)	2 (40)	3 (21.4)
Adverse	5 (83.3)	-	2 (40)	7 (50.0)

Table S3. Most common treatment emergent adverse effects in patients ≤60 years

TEAEs ≥5%	8d Venetoclax (n=8)		11d Venetoclax (n=6)		14d Venetoclax (n=6)		All Cohorts (n=20)	
	Any Grade	Grade ≥3	Any Grade	Grade ≥3	Any Grade	Grade ≥3	Any Grade	Grade ≥3
	n (%)							
WBC/ANC Decreased	8 (100)	8 (100)	6 (100)	6 (100)	6 (100)	6 (100)	20 (100)	20 (100)
Anemia	8 (100)	8 (100)	6 (100)	6 (100)	6 (100)	6 (100)	20 (100)	20 (100)
PLT Decreased	8 (100)	8 (100)	6 (100)	6 (100)	6 (100)	6 (100)	20 (100)	20 (100)
Neutropenic Fever	8 (100)	8 (100)	6 (100)	6 (100)	6 (100)	6 (100)	20 (100)	20 (100)
Neutropenic Enterocolitis	0	0	1 (16.7)	1 (16.7)	3 (50)	3 (50)	4 (20)	4 (20)
Sepsis/Bacteremia	1 (12.5)	1 (12.5)	0	0	1 (16.7)	1 (16.7)	2 (10)	2 (10)
Lung Infection	2 (25)	2 (25)	1 (16.7)	1 (16.7)	2 (33)	2 (33)	5 (25)	5 (25)
Soft Tissue Infection	1 (12.5)	1 (12.5)	0	0	0	0	1 (5)	1 (5)
Skin Infection	1 (12.5)	1 (12.5)	1 (16.7)	0	0	0	2 (10)	1 (5)
Infectious Colitis	0	0	0	0	1 (16.7)	1 (16.7)	1 (5)	1 (5)
Hypokalemia	5 (62.5)	2 (25)	3 (50)	1 (16.7)	6 (100)	2 (33)	14 (70)	5 (25)
Hyperkalemia	0	0	0	0	1 (16.7)	0	1 (5)	0
Hyponatremia	0	0	0	0	5 (83)	0	5 (25)	0
Abdominal Pain	1 (12.5)	0	0	0	0	0	1 (5)	0
Alk Phos Increased	3 (37.5)	0	0	0	4 (67)	0	7 (35)	0
ALT Increased	5 (62.5)	0	5 (83)	0	4 (67)	1 (16.7)	14 (70)	1 (5)
AST Increased	2 (25)	0	2 (33)	0	3 (50)	0	7 (35)	0
Bilirubin Increased	5 (62.5)	1 (12.5)	5 (83)	0	5 (83)	0	15 (75)	1 (5)

Ileus	1 (12.5)	0	0	0	0	0	1 (5)	0
Nausea	6 (75)	0	2 (33)	0	4 (67)	0	12 (60)	0
Vomiting	3 (37.5)	0	0	0	4 (67)	0	7 (35)	0
Diarrhea	4 (50)	0	4 (67)	0	3 (50)	0	11 (55)	0
Wheezing	1 (12.5)	0	0	0	0	0	1 (5)	0
Urinary Retention	2 (25)	0	0	0	0	0	2 (10)	0
Mucositis Oral	1 (12.5)	1 (12.5)	3 (50)	1 (16.7)	0	0	4 (20)	2 (10)
Rectal Pain	0	0	0	0	1 (16.7)	0	1 (5)	0
Back Pain	2 (25)	1 (12.5)	0	0	0	0	2 (10)	1 (5)
Headache	1 (12.5)	0	1 (16.7)	0	0	0	2 (10)	0
Epistaxis	1 (12.5)	1 (12.5)	0	0	0	0	1 (5)	1 (5)
Constipation	0	0	1 (16.7)	0	0	0	1 (5)	0
Maculopapular Rash	2 (25)	0	0	0	0	0	2 (10)	0
Infusion Related Reaction	0	0	2 (33)	1 (16.7)	1 (16.7)	0	3 (15)	1 (5)
Fungemia	0	0	0	0	1 (16.7)	1 (16.7)	1 (5)	1 (5)
Toothache	0	0	1 (16.7)	0	0	0	1 (5)	0
Lower GI Hemorrhage	0	0	1 (16.7)	1 (16.7)	0	0	1 (5)	1 (5)
Syncope	0	0	0	0	1 (16.7)	1 (16.7)	1 (5)	1 (5)
Generalized Muscle Weakness	1 (12.5)	1 (12.5)	0	0	0	0	1 (5)	1 (5)
Menorrhagia	0	0	0	0	1 (16.7)	0	1 (5)	0
Gluteal Hematoma	0	0	0	0	1 (16.7)	1 (16.7)	1 (5)	1 (5)

WBC: white blood cells, ANC: absolute neutrophil count, PLT: platelets, AST: aspartate aminotransferase, ALT, alanine aminotransferase, Alk Phos: alkaline phosphatase

Table S4. Most common treatment emergent adverse effects in patients >60 years

TEAEs ≥5%	8d Venetoclax (n=6)		11d Venetoclax (n=3)		14d Venetoclax (n=5)		All Cohorts (n=14)	
	Any Grade	Grade ≥3	Any Grade	Grade ≥3	Any Grade	Grade ≥3	Any Grade	Grade ≥3
	n (%)							
WBC/ANC Decreased	6 (100)	6 (100)	3 (100)	3 (100)	5 (100)	5 (100)	14 (100)	14 (100)
Anemia	6 (100)	6 (100)	3 (100)	3 (100)	5 (100)	5 (100)	14 (100)	14 (100)
PLT Decreased	6 (100)	6 (100)	3 (100)	3 (100)	5 (100)	5 (100)	14 (100)	14 (100)
Neutropenic Fever	6 (100)	6 (100)	3 (100)	3 (100)	5 (100)	5 (100)	14 (100)	14 (100)
Neutropenic Enterocolitis	3 (50)	3 (50)	0	0	1 (20)	1 (20)	4 (29)	4 (29)
Sepsis/Bacteremia	4 (67)	4 (67)	0	0	4 (80)	4 (80)	8 (57)	8 (57)
Lung Infection	0	0	0	0	1 (20)	1 (20)	1 (7)	1 (7)
Infectious Colitis	1 (16.7)	0	0	0	1 (20)	1 (20)	2 (14)	1 (7)
Joint Infection	0	0	0	0	1 (20)	1 (20)	1 (7)	1 (7)
Soft Tissue Infection	1 (16.7)	1 (16.7)	0	0	0	0	1 (7)	1 (7)
Hypokalemia	5 (83)	3 (50)	3 (100)	0	3 (60)	1 (20)	11 (79)	4 (29)
Hyperkalemia	1 (16.7)	0	0	0	0	0	1 (7)	0
Hyponatremia	6 (100)	1 (16.7)	2 (67)	0	1 (20)	0	9 (64)	1 (7)
Hypernatremia	1 (16.7)	0	0	0	0	0	1 (7)	0
Thromboembolic Event	2 (33)	0	0	0	0	0	2 (14)	0
Upper Respiratory Infection	1 (16.7)	0	0	0	0	0	1 (7)	0
Chest Pain	1 (16.7)	0	0	0	0	0	1 (7)	0
Edema Limbs	1 (16.7)	0	0	0	0	0	1 (7)	0

Abdominal Pain	1 (16.7)	0	0	0	0	0	1 (7)	0
Alk Phos Increased	2 (33)	0	0	0	3 (60)	0	5 (36)	0
ALT Increased	6 (100)	0	2 (67)	1 (33)	5 (100)	2 (40)	13 (93)	3 (21)
AST Increased	5 (83)	0	1 (33)	1 (33)	4 (80)	2 (40)	10 (71)	3 (21)
Bilirubin Increased	5 (83)	0	2 (67)	0	4 (80)	1 (20)	11 (79)	1 (7)
Maculopapular Rash	0	0	2 (67)	0	1 (20)	0	3 (21)	0
Nausea	1 (16.7)	0	1 (33)	0	2 (40)	0	4 (29)	0
Vomiting	0	0	0	0	1 (20)	0	1 (7)	0
Diarrhea	2 (33)	0	2 (67)	0	0	0	4 (29)	0
Mucositis Oral	0	0	1 (33)	0	1 (20)	1 (20)	2 (14)	1 (7)
Rectal Pain	0	0	1 (33)	0	0	0	1 (7)	0
Rectal Fistula	0	0	1 (33)	0	0	0	1 (7)	0
Back Pain	0	0	1 (33)	0	0	0	1 (7)	0
Headache	0	0	1 (33)	0	0	0	1 (7)	0
Constipation	1 (16.7)	0	2 (67)	0	0	0	3 (21)	0
Dizziness	0	0	1 (33)	0	0	0	1 (7)	0
Supraventricular Tachycardia	0	0	0	0	1 (20)	0	1 (7)	0
Atrial Fibrillation	0	0	0	0	1 (20)	1 (20)	1 (7)	1 (7)
Syncope	0	0	0	0	1 (20)	1 (20)	1 (7)	1 (7)
Generalized Muscle Weakness	0	0	0	0	1 (20)	1 (20)	1 (7)	1 (7)

WBC: white blood cells, ANC: absolute neutrophil count, PLT: platelets, AST: aspartate aminotransferase, ALT, alanine aminotransferase, Alk Phos: alkaline phosphatase

Table S5. Incidence of neutropenic enterocolitis by age, venetoclax duration and pre-treatment cytoreduction

	≤ 60 yrs			>60 yrs		
	Ven 8d (n=8)	Ven 11d (n=6)	Ven 14d (n=6)	Ven 8d (n=6)	Ven 11d (n=3)	Ven 14d (n=5)
Incidence, n (%)	-	1 (17)	3 (50)	3 (50)	-	1 (20)
Cytoreduction (n)						
Hydroxyurea			2			
Cytarabine		1				
Hydroxyurea + Cytarabine				1		
Median time to symptom onset, days (range)		10	13 (9-20)	8 (7-9)		10

	Combined age groups			
	Ven 8d (n=14)	Ven 11d (n=9)	Ven 14d (n=11)	All (n=34)
Incidence, n (%)	3 (42)	1 (11)	4 (36)	8 (23.5)
Cytoreduction (n)				
Hydroxyurea			2	2
Cytarabine		1		1
Hydroxyurea + Cytarabine	1			1
Median time to symptom onset, days (range)	8 (7-9)	10	13 (9-20)	9 (7-20)

3/5 of pts cytoreduced with AraC did not develop NEC
10/12 pts cytoreduced with hydroxyurea did not develop NEC

Table S6. Responses by molecular subgroup and ELN 2022 risk category

	CBF (n=5)	*NPM1* (n=13)	*FLT3*-ITD (n=7)	*TP53* (n=5)	ELN22 Favorable (n=13)	ELN22 Intermediate (n=6)	ELN22 Adverse (n=15)
Response				n (%)			
No response	-	-	1 (14)	3 (60)	-	-	5 (33)
CR/CRh	5 (100)	13 (100)	6 (86)	2 (40)	13 (100)	6 (100)	10 (67)
MFC-MRD-neg CR	5 (100)	13 (100)	5/6 (83)	1 (20)	13 (100)	6 (100)	6/10 (60)
Mol-MRD-neg CR	5* (100)	9/10* (90)	3/6 (50)	N/A	10/10 (100)	3/5 (60)	-

CR: complete remission; CRh complete remission with partial hematologic recovery; CRc_{MRD-}: MRD negative composite CR by multiparameter flow cytometry (Hematologics Inc); LOD: limit of detection

Table S7. Median time to hematologic recovery in consolidation phase.

Consolidation Phase (IDAC* +/- Ven**)	Time to ANC* ≥1.0K/uL (days)	Time to PLT* ≥100K/uL (days)
Cycle 1 (n=23)	19 (16-43)	25 (18-134)
Cycle 2 (n=15)	19.5 (15-25)	27 (19-46)
Cycle 3 (n=14)	19.5 (13-31)	30.5 (17-47)
Cycle 4 (n=6)	20 (16-28)	33 (22-44)

*IDAC: intermediate dose cytarabine, ANC : absolute neutrophil count, PLT : platelets
**3 patients >60 years received IDAC alone after 2/7 hematologic DLTs occurred during C1 of consolidation in the older age group

Table S8. Most common (≥5%) treatment related adverse events during 1st cycle of consolidation, by age group

≤60 years	Post Cycle 1 Consolidation (n=13)	
	Any Grade	Grade ≥3
	n (%)	
WBC/ANC Decreased	13 (100)	12 (92)
Anemia	13 (100)	13 (100)
PLT Decreased	13 (100)	13 (100)
Neutropenic Fever	1 (8)	1 (8)
Neutropenic Sepsis/Bacteremia	1 (8)	1 (8)
Fatigue	7 (54)	0
Alk Phos Increased	2 (15)	0
ALT Increased	8 (62)	0
AST Increased	3 (23)	0
Bilirubin Increased	6 (46)	0
Troponin I Increased	1 (8)	0
Hypotension	1 (8)	1 (8)
Nausea	1 (8)	0
Vomiting	1 (8)	0
Constipation	1 (8)	0
Rectal Pain	1 (8)	0
Hemorrhoidal Bleeding	1 (8)	0
Presyncope	1 (8)	0
Syncope	1 (8)	1 (8)
Tachycardia	1 (8)	0
Bone Pain	1 (8)	1 (8)
Cough	1 (8)	0
Flu Like Symptoms	1 (8)	0
Insomnia	1 (8)	0
Hyperglycemia	1 (8)	0
Headache	1 (8)	0

Nail Discoloration	1 (8)	0
Menorrhagia	1 (8)	0
Oral Mucosal Bleeding	1 (8)	0
>60 years	n (%)	
WBC/ANC Decreased	10 (100)	10 (100)
Anemia	10 (100)	10 (100)
PLT Decreased	10 (100)	10 (100)
Neutropenic Fever	2 (10)	2 (10)
Lung Infection	1 (10)	1 (10)
Skin Infection	1 (10)	0
Hyperkalemia	4 (40)	0
Hyperglycemia	1 (10)	0
Abdominal Pain	1 (10)	0
Intracranial Hemorrhage	1 (10)	1 (10)
Fatigue	6 (60)	2 (20)
Alk Phos Increased	5 (50)	0
ALT Increased	6 (60)	0
Bilirubin Increased	7 (70)	0
Nausea	3 (30)	0
Vomiting	1 (10)	0
Diarrhea	1 (10)	0
Anorexia	1 (10)	1 (10)
GERD	1 (10)	0
Mucositis Oral	1 (10)	0
Rectal Pain	1 (10)	0
Hemorrhoidal Bleeding	1 (10)	0
Chills	1 (10)	0
Non cardiac chest pain	1 (10)	0
Dyspnea	1 (10)	1 (10)
Pruritus	1 (10)	0
Edema, Limb	2 (20)	0

Table S9. Demographic and disease characteristics of refractory and relapsed patients

	sex	Age, yrs	race	ethnicity	sAML	Karyotype/FISH	NGS	Outcome
					Relapsed			
1	M	34	white	hispanic	-	inv(16)	*KIT, FLT3 TKD, WT1*	Off protocol due to hematologic DLT after cycle 1 consolidation. Subsequent treatment with IDAC+GO with long delays, CMR at EOT, MRD relapse 2.5 months after EOT
2	F	36	black	non-hispanic	-	normal	*NPM1, FLT3 TKD, TET2*	PCR neg CR after 2nd consolidation with IDAC/Ven. Relapse 12 months after initial diagnosis
3	M	58	black	non-hispanic	-	de5q, del7q, trisomy 21, del2, del16, del17, der(7)	*TP53, NF1*	MFC-MRD(+) post-induction. Morphologic relapse after 1st consolidation. Death in relapse.
4	M	43	black	non-hispanic	-	normal	*NRAS, WT1, U2AF1*	MFC-MRD negative, post induction and during consolidation but U2AF1 persistent by commercial NGS. Relapse post alloSCT 10 months after initial diagnosis.
5	M	67	white	non-hispanic	Prior CMML	trisomy 8	*RUNX1, ASXL1, FLT3 TKD, KRAS, EZH2, TET2*	MFC-MRD neg post-induction and 1st consolidation pre-alloSCT, relapse post alloSCT almost 13 months post initial diagnosis
6	M	60	black	hispanic	-	de5q, del7q, del12p	*TP53, SRSF2*	MFC-MRD-neg post induction, MRD relapse peri-consolidation cycle 4, morphologic relapse during alloSCT admission, pre-engraftment

Refractory								
1	F	53	white	hispanic	Breast Ca s/p CRT	del5q	*TP53*	Died of leukemia
2	M	52	white	hispanic	Prior MDS refractory to HMA/Ven on trial	del4q, del20q, add17p, del21, 2-3 marker chromosomes	*DNMT3A, TP53*	Refractory to induction. AlloSCT with active disease with no response. Died of leukemia
3	F	59	white	hispanic	-	Del3q, del5q, del17q, add7p, -13, der(16;21), -18, add21p, del22q	*TP53, NRAS*	Refractory to induction, died of leukemia
4	F	63	asian	non-hispanic	Ovarian Ca s/p chemotherapy	del20q	*FLT3 ITD, U2AF1*	Refractory to induction, MLFS s/p HMA/Ven/Gilteritinib, death in aplasia
5	M	67	white	non-hispanic	-	del7q	*SF3B1, ETV6-NTRK3* fusion	Refractory to induction, remission with HMA plus NTRK3i, alloSCT, relapse post alloSCT; died of leukemia

sAML: secondary AML, DLT: dose limiting toxicity, IDAC: intermediate dose cytarabine, GO: gemtuzumab ozogamicin, CMR: complete molecular remission, CR: complete remission, EOT: end of treatment, MRD: measurable residual disease, MFC: multiparameter flow cytometry, HMA: hypomethylating agent, alloSCT: allogeneic transplantation, NTRK3i: NTRK3 inhibitor

Figure Legends

Figure S1. Study schema

Figure S2. CONSORT flow diagram of the progress through the phases of the study

Figure S3. Reverse Kaplan Meier plot of time to neutrophil and platelet count recovery for all responding patients (A,B) and by venetoclax-duration cohort (C,D)

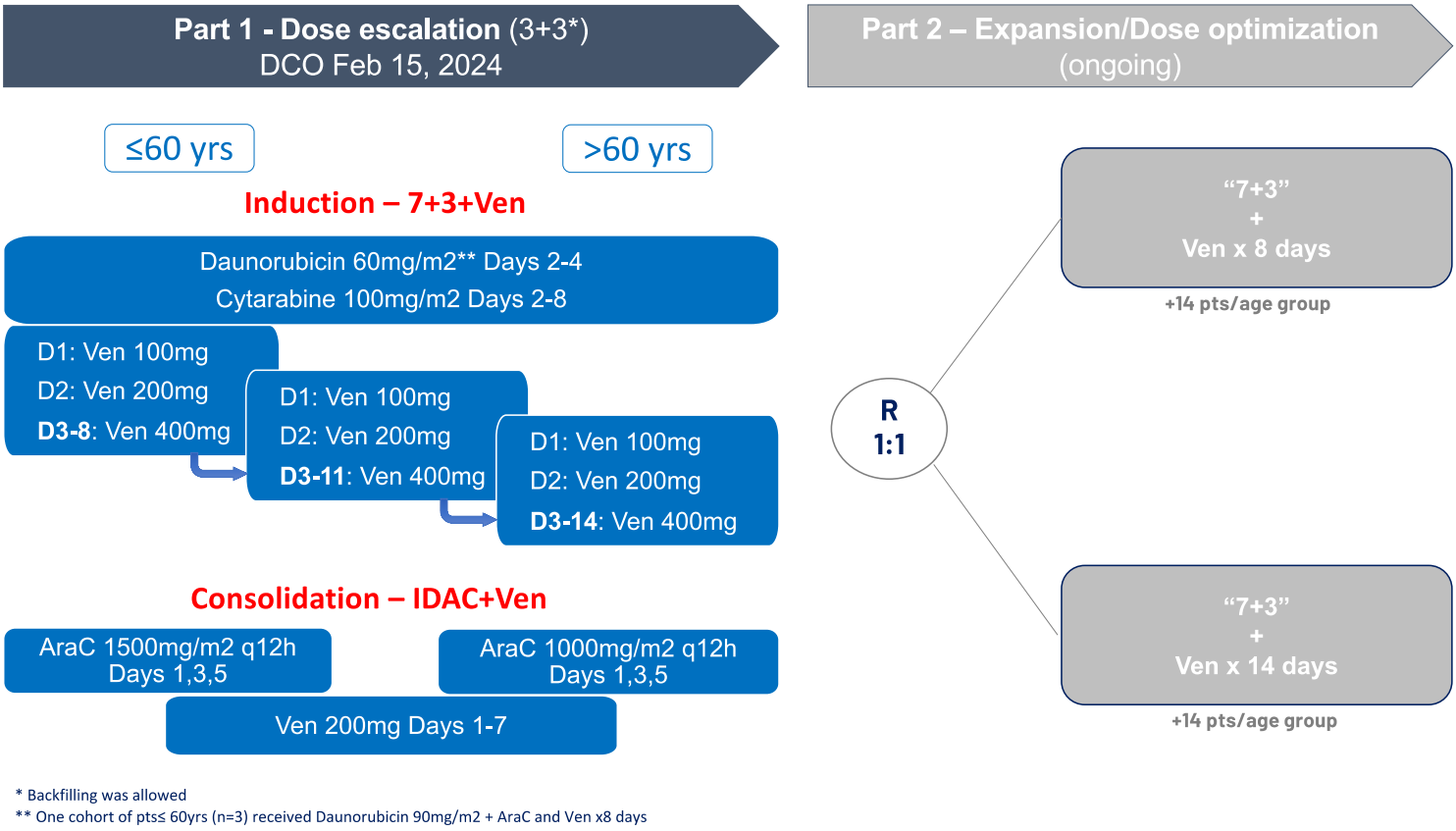
Figure S4. Oncoprint of individual patients' (each column) mutations, cytogenetic-risk, ELN 2022-risk and response to induction Ven plus "7+3" chemotherapy

Figure S5. Kaplan-Meier plot of OS (A) and EFS (B) for the entire patient population

Figure S6. Swimmer plot of patients' course and response durability; each bar is an individual patient. CR/CRh: complete response/incomplete hematologic recovery, AlloSCT: allogeneic stem cell transplantation

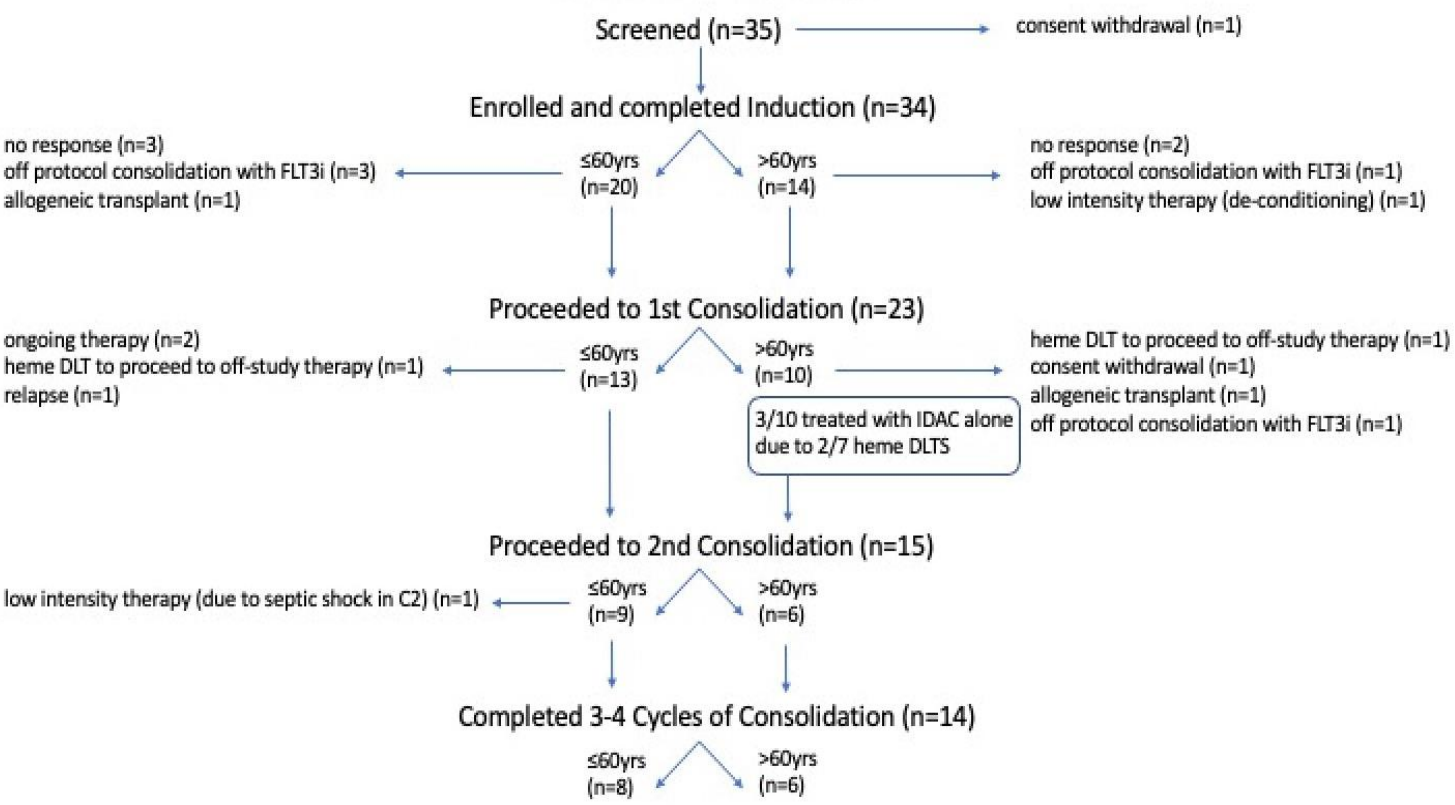
Figure S7. Kaplan-Meier plot of OS (A) and EFS (B) by ELN 2022 risk-category

Figure S8. Kaplan-Meier plot of EFS (A) and OS (B) of ELN 2022 adverse-risk patients, by TP53–mutation status

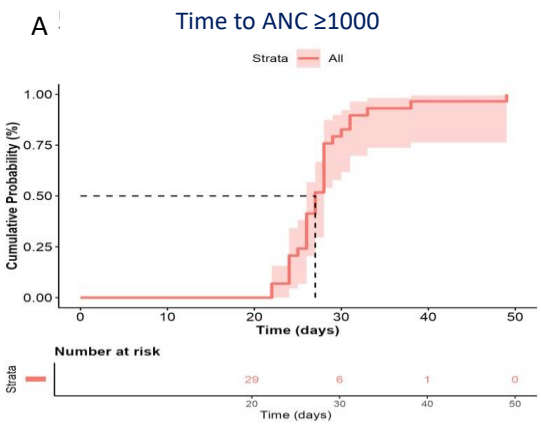


* Backfilling was allowed

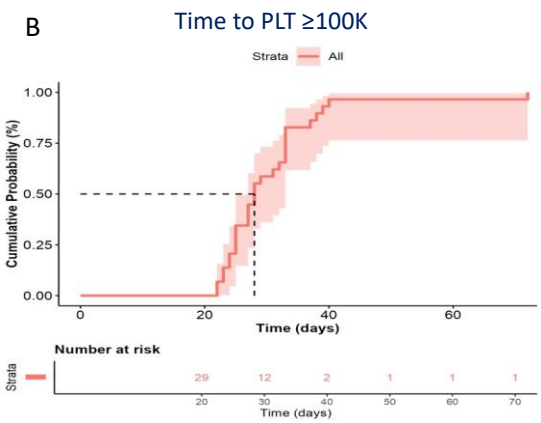
** One cohort of pts≤ 60yrs (n=3) received Daunorubicin 90mg/m2 + AraC and Ven x8 days


Screened (n=35)
consent withdrawal (n=1)
Enrolled and completed Induction (n=34)
no response (n=3)
off protocol consolidation with FLT3i (n=3)
allogeneic transplant (n=1)
≤60yrs (n=20)
>60yrs (n=14)
no response (n=2)
off protocol consolidation with FLT3i (n=1)
low intensity therapy (de-conditioning) (n=1)
Proceeded to 1st Consolidation (n=23)
ongoing therapy (n=2)
heme DLT to proceed to off-study therapy (n=1)
relapse (n=1)
≤60yrs (n=13)
>60yrs (n=10)
heme DLT to proceed to off-study therapy (n=1)
consent withdrawal (n=1)
allogeneic transplant (n=1)
off protocol consolidation with FLT3i (n=1)
3/10 treated with IDAC alone due to 2/7 heme DLTS
Proceeded to 2nd Consolidation (n=15)
low intensity therapy (due to septic shock in C2) (n=1)
≤60yrs (n=9)
>60yrs (n=6)
Completed 3-4 Cycles of Consolidation (n=14)
≤60yrs (n=8)
>60yrs (n=6)

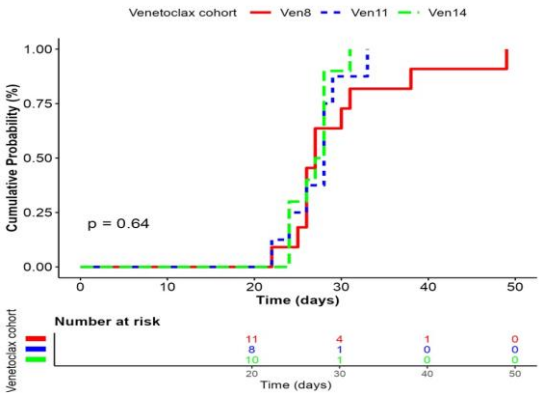
A


Time to ANC ≥1000
Strata All
Cumulative Probability (%)
1.00
0.75
0.50
0.25
0.00
0 10 20 30 40 50
Time (days)
Number at risk
Strata
29 6 1 0
20 30 40 50
Time (days)

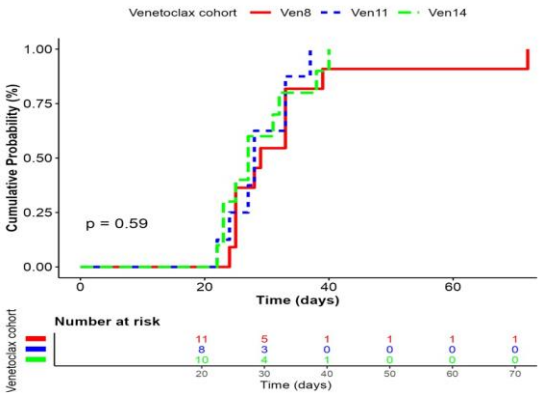
B

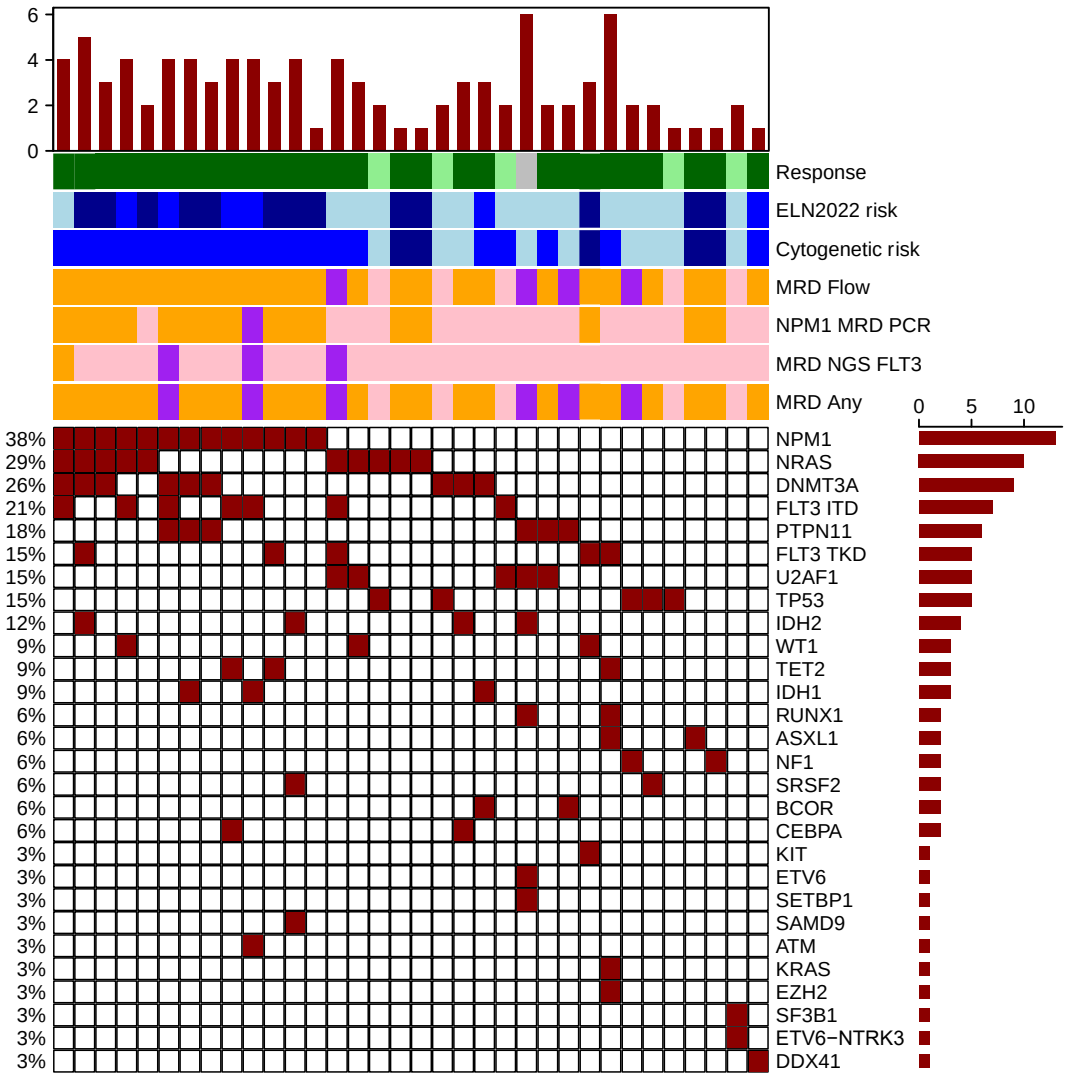

Time to PLT ≥100K
Strata All
Cumulative Probability (%)
1.00
0.75
0.50
0.25
0.00
0 20 40 60
Time (days)
Number at risk
Strata
29 12 2 1 1 1
20 30 40 50 60 70
Time (days)

C


Time to ANC ≥1000
Venetoclax cohort Ven8 Ven11 Ven14
Cumulative Probability (%)
1.00
0.75
0.50
0.25
0.00
p = 0.64
0 10 20 30 40 50
Time (days)
Number at risk
Venetoclax cohort
11 4 1 0
8 1 0 0
10 1 0 0
20 30 40 50
Time (days)

D


Time to PLT ≥100K
Venetoclax cohort Ven8 Ven11 Ven14
Cumulative Probability (%)
1.00
0.75
0.50
0.25
0.00
p = 0.59
0 20 40 60
Time (days)
Number at risk
Venetoclax cohort
11 5 1 1 1 1
8 3 0 0 0 0
10 4 1 0 0 0
20 30 40 50 60 70
Time (days)



Mutation
- Mutation

Response
- CR
- CRh
- No response

ELN2022 risk
- Poor
- Intermediate
- Favorable

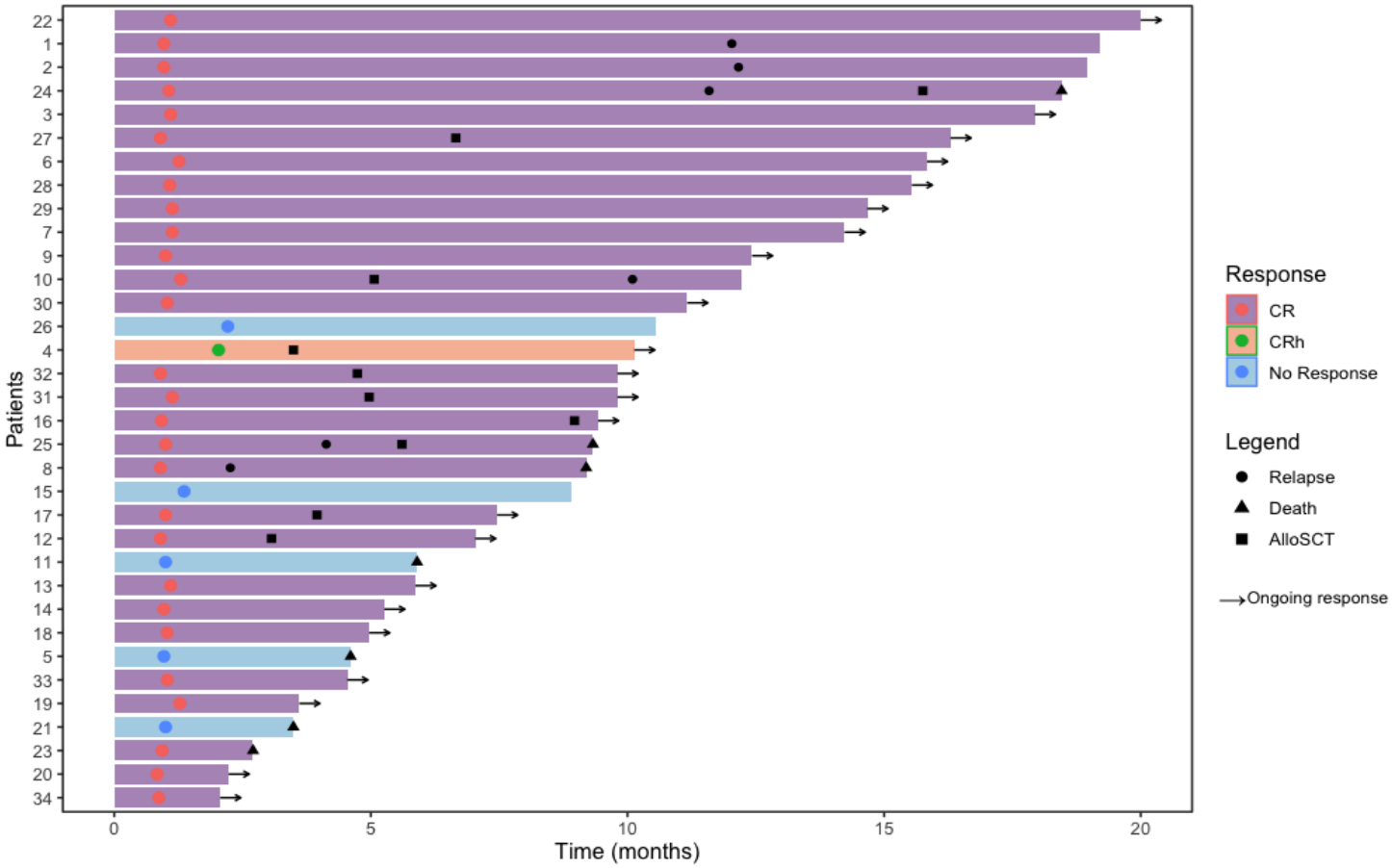
Cytogenetic risk
- Favorable
- Intermediate
- Poor

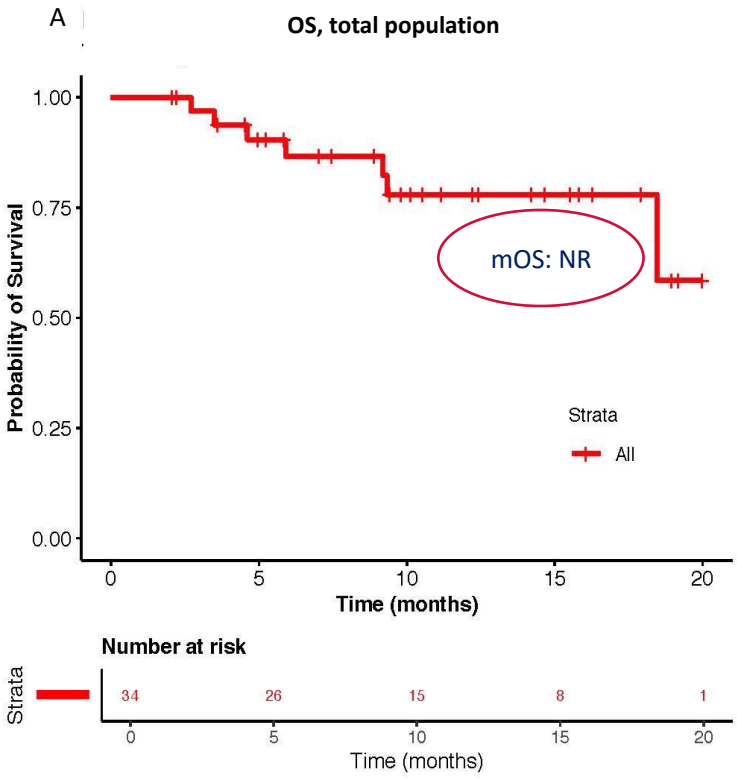
MRD Flow
- MRD NA
- MRD−negative
- MRD−positive

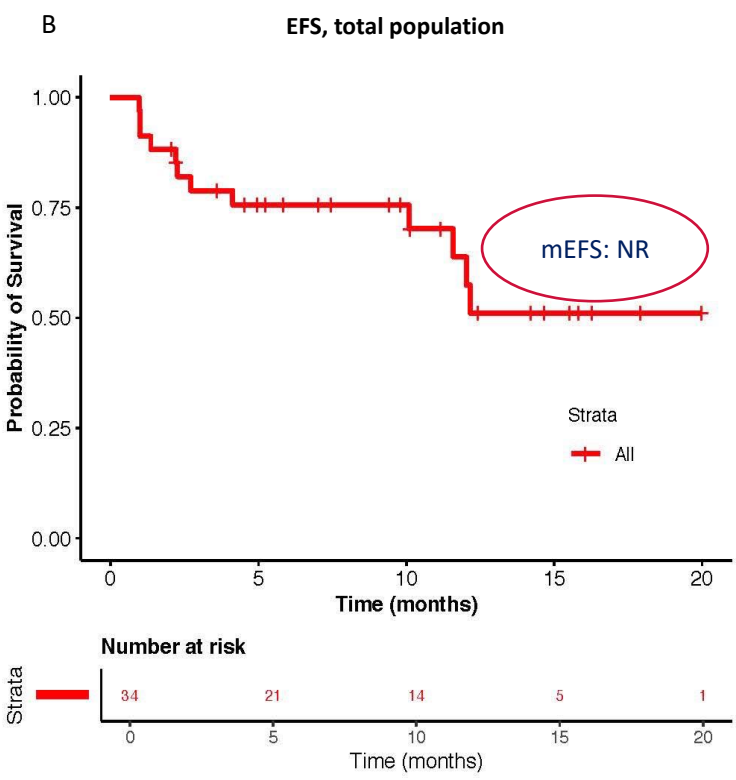
NPM1 MRD PCR
- MRD NA
- MRD−negative
- MRD−positive

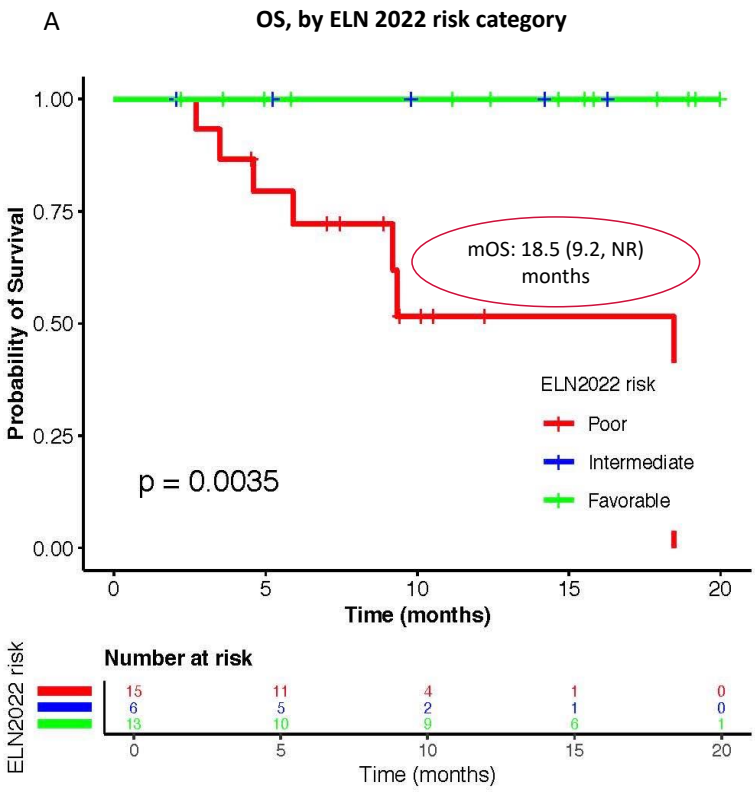
MRD NGS FLT3
- MRD NA
- MRD−negative
- MRD−positive

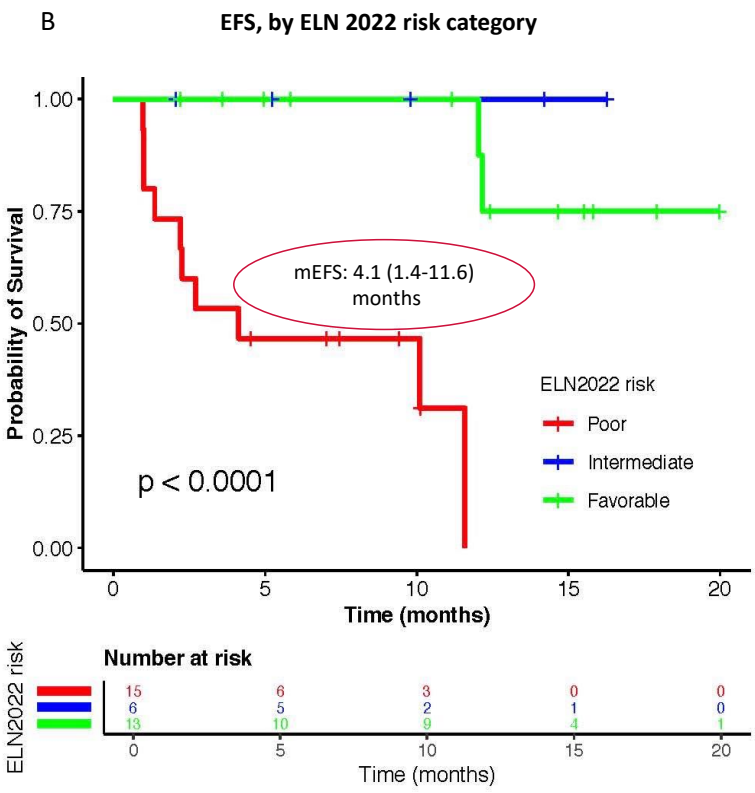
MRD Any
- MRD NA
- MRD−negative
- MRD−positive

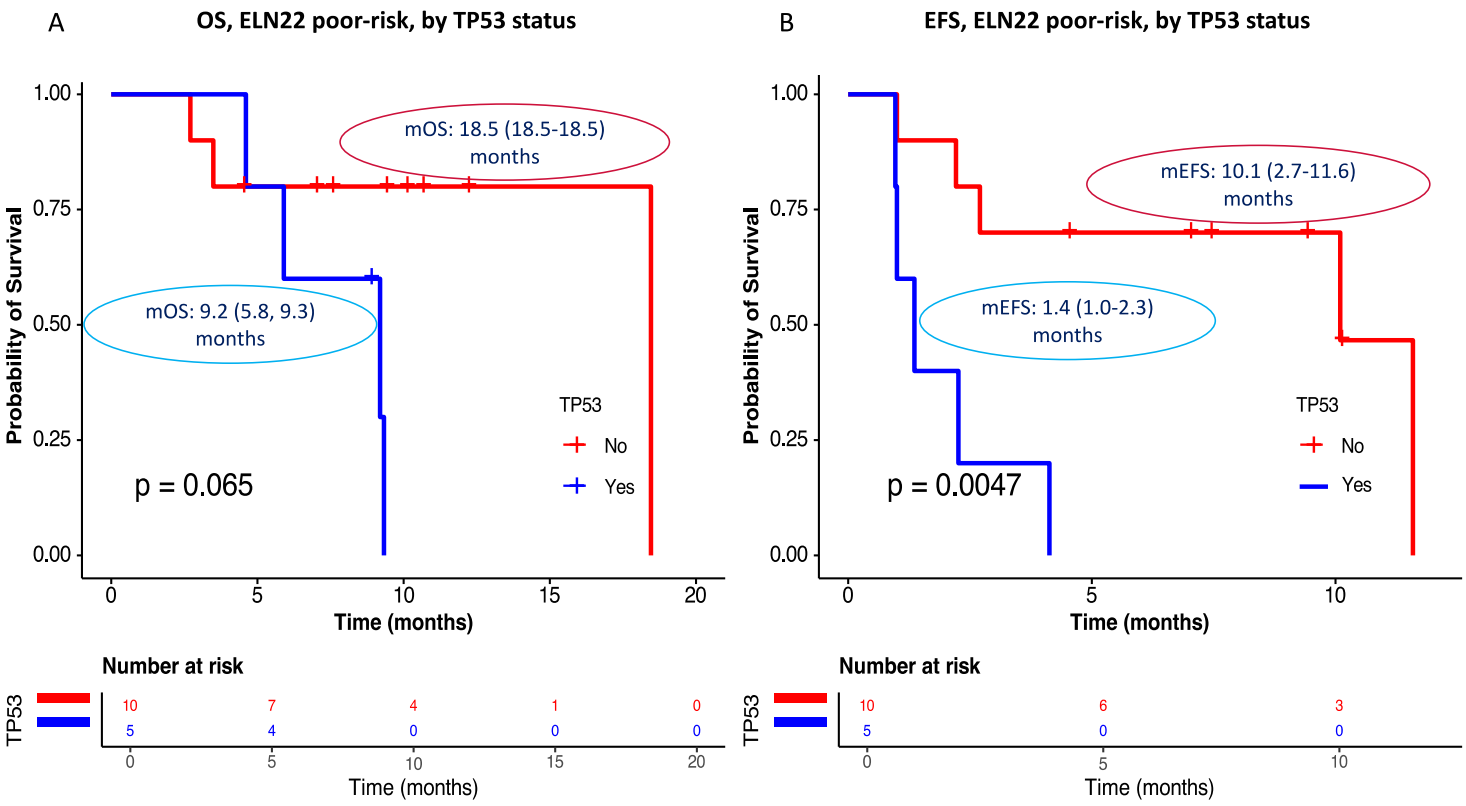

Patients
22
1
2
24
3
27
6
28
29
7
9
10
30
26
4
32
31
16
25
8
15
17
12
11
13
14
18
5
33
19
21
23
20
34
0
5
10
15
20
Time (months)
Response
CR
CRh
No Response
Legend
Relapse
Death
AlloSCT
Ongoing response


A
OS, total population
Probability of Survival
1.00
0.75
0.50
0.25
0.00
mOS: NR
Strata
All
0
5
10
15
20
Time (months)
Number at risk
Strata
34
26
15
8
1
0
5
10
15
20
Time (months)


B
EFS, total population
Probability of Survival
1.00
0.75
0.50
0.25
0.00
mEFS: NR
Strata
All
0
5
10
15
20
Time (months)
Number at risk
Strata
34
21
14
5
1
0
5
10
15
20
Time (months)


A
OS, by ELN 2022 risk category
Probability of Survival
1.00
0.75
0.50
0.25
0.00
mOS: 18.5 (9.2, NR) months
ELN2022 risk
Poor
Intermediate
Favorable
p = 0.0035
0
5
10
15
20
Time (months)
ELN2022 risk
Number at risk
15 11 4 1 0
6 5 2 1 0
13 10 9 6 1
0
5
10
15
20
Time (months)


B
EFS, by ELN 2022 risk category
Probability of Survival
1.00
0.75
0.50
0.25
0.00
mEFS: 4.1 (1.4-11.6) months
ELN2022 risk
Poor
Intermediate
Favorable
p < 0.0001
0
5
10
15
20
Time (months)
ELN2022 risk
Number at risk
15 6 3 0 0
6 5 2 1 0
13 10 9 4 1
0
5
10
15
20
Time (months)


A
OS, ELN22 poor-risk, by TP53 status
Probability of Survival
1.00
0.75
0.50
0.25
0.00
mOS: 18.5 (18.5-18.5) months
mOS: 9.2 (5.8, 9.3) months
p = 0.065
TP53
No
Yes
0
5
10
15
20
Time (months)
Number at risk
TP53
10
7
4
1
0
5
4
0
0
0
Time (months)
B
EFS, ELN22 poor-risk, by TP53 status
Probability of Survival
1.00
0.75
0.50
0.25
0.00
mEFS: 10.1 (2.7-11.6) months
mEFS: 1.4 (1.0-2.3) months
p = 0.0047
TP53
No
Yes
0
5
10
Time (months)
Number at risk
TP53
10
6
3
5
0
0
0
5
10
Time (months)

PHASE 1B STUDY OF VENETOCLAX IN COMBINATION WITH STANDARD INTENSIVE CHEMOTHERAPY WITH DAUNORUBICIN PLUS CYTARABINE FOLLOWED BY HIGH-DOSE CYTARABINE IN ADULT PATIENTS WITH NEWLY DIAGNOSED ACUTE MYELOID LEUKEMIA AND ADVANCED MYELODYSPLASTIC SYNDROME

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Aditi Shastri, MD

Biostatistician :

Mimi Kim, ScD

Oncology Fellow

David Levitz, MD

Research Nurses:

Karen Fehn, RN

Anne Munoz, RN

Study Coordinators:

Aradhika Dhawan

Joel Victor

SYNOPSIS

Protocol Title	Phase 1b study of venetoclax in combination with standard intensive chemotherapy with daunorubicin plus cytarabine followed by high-dose cytarabine in adult patients with newly diagnosed acute myeloid leukemia.
Brief Title	Venetoclax combined with intensive chemotherapy in newly diagnosed AML
Objectives	<u>Primary:</u> • To evaluate the safety and tolerability and determine the dose-limiting toxicity and the maximum tolerated dose (MTD) of the combination of daunorubicin & cytarabine chemotherapy plus venetoclax for patients with AML <u>Secondary:</u> • To assess efficacy by response per 2022 ELN and revised IWG criteria • To determine additional response parameters: CR/CRi and CR/CRh rates • To determine time to response variables including overall survival (OS), event-free survival (EFS) and duration of response (DOR) <u>Exploratory Objectives:</u> • To comprehensively analyze LSC and assess rates of LSC eradication by means of MFC and single cell sequencing (“high resolution MRD assay”) • To determine the protein expression of BCL-2 family members and the reliance of leukemic cells on differing members of the BH3 family at the time of diagnosis and

	relapse and explore association of such observations with response to venetoclax when combined with chemotherapy
Study Design	This is a Phase 1b, open-label study evaluating Venetoclax in combination with intensive induction and consolidation chemotherapy in previously untreated, adult patients with acute myeloid leukemia. In Part 1, the dose escalation phase, the safety and tolerability of the combination with Venetoclax at different doses and duration will inform the appropriate dose(s) and regimen(s) for Part 2. In Part 2, the dose expansion phase, a maximum of 28 additional patients will be randomized 1:1 to the MTD determined in Part 1 and the starting dose (assuming the MTD is not the starting dose), to further evaluate the safety and efficacy of the study drug combination.
Study Entry Criteria	Inclusion Criteria: • New diagnosis of AML by WHO criteria. Patients with higher risk MDS (R-IPSS>3.5) and 10% blasts or more, or proliferative (WBC ≥ 13 x 10^9/L) CMML-2 are also eligible at the discretion of the PI. Patients having received any prior hypomethylating agent with or without BCL2 inhibitor therapy for MDS/AML are also eligible at the discretion of the PI. • Patients ≥ 18 to ≤ 75 years. • Eastern Cooperative Oncology Group (ECOG) Performance Status of ≤2 • Adequate renal function including creatinine clearance > 30 mL/min based on the Cockcroft Gault equation. • Adequate hepatic function including total bilirubin < 1.5x ULN unless increase is due to Gilbert's disease or leukemic involvement, and AST and/or ALT < 3x ULN unless considered due to leukemic involvement • Ability to understand and provide signed informed consent

	Male subjects must agree to refrain from unprotected sex and sperm donation from initial study drug administration until 90 days after the last dose of study drug. Exclusion Criteria: • Patients with t(15;17) karyotypic abnormality or acute promyelocytic leukemia (FAB class M3-AML) • Subject has known active CNS involvement with AML • Patients with New York Heart Association (NYHA) Class III or IV congestive heart failure or LVEF <45% by echocardiogram or multi-gated acquisition (MUGA) scan • Patients with a history of myocardial infarction within the last 6 months or unstable / uncontrolled angina pectoris or history of severe and/or uncontrolled ventricular arrhythmias • Patients with uncontrolled infection with human immunodeficiency virus (HIV) or active Hepatitis B or C • Patients with known dysphagia, short-gut syndrome, or other conditions that would affect the ingestion or gastrointestinal absorption of drugs administered orally. • Subject has any other significant medical or psychiatric history that in the opinion of the investigator would adversely affect participation in this study. • Subject has a white blood cell count > 25 x 10^9/L. (Note: Hydroxyurea and/or cytarabine (1 or 2 doses; up to 2 is permitted to meet this criterion.) • Nursing women, women of childbearing potential (WOCBP) with positive urine pregnancy test, or women of childbearing potential who are not willing to maintain adequate contraception Appropriate method(s) of contraception include oral or injectable hormonal birth control, IUD, and double barrier methods (for example a condom in combination with a spermicide).
Treatment Plan	Induction Phase

	Dose Escalation Cohorts: A minimum of 3 patients will be treated in each cohort (dose level) sequentially in a 3+3 design. Patients will receive the Venetoclax plus daunorubicin/cytarabine combination as shown below (see dose adjustment guidelines for renal insufficiency or liver dysfunction in Table 1): *A. Patients aged ≤ 60 years* Cohort 1A: Daunorubicin 60mg/m2 intravenously (IV) daily on days 2 to 4 Cytarabine 100mg/m2 IV daily on days 2 to 8 Venetoclax 100mg orally on day 1, 200mg on day 2, 400mg on D3 and till day 8 Cohort 2A: Daunorubicin 90mg/m2 intravenously (IV) daily on days 2 to 4 Cytarabine 100mg/m2 IV daily on days 2 to 8 Venetoclax 100mg orally on day 1, 200mg on day 2, 400mg on D3 and till day 8 Cohort 3A: Daunorubicin 60mg/m2 intravenously (IV) daily on days 2 to 4 Cytarabine 100mg/m2 IV daily on days 2 to 8 Venetoclax 100mg orally on day 1, 200mg on day 2, 400mg on D3 and till day 11

	Cohort 4A: Daunorubicin 60mg/m2 intravenously (IV) daily on days 2 to 4 Cytarabine 100mg/m2 IV daily on days 2 to 8 Venetoclax 100mg orally on day 1, 200mg on day 2, 400mg on D3 and till day 14 At the time of this amendment, no DLTs in induction phase have been observed and Cohort 3A has completed enrollment. However, the daunorubicin dose of 90mg/m2 will not be further studied due to recently reported results of no superiority over the dose of 60mg/m2. *B. Patients >60 years* Cohort 1B: Daunorubicin 60mg/m2 intravenously (IV) daily on days 2 to 4 Cytarabine 100mg/m2 IV daily on days 2 to 8 Venetoclax 100mg orally on day 1, 200mg on day 2, 400mg on D3 and till day 8 Cohort 2B: Daunorubicin 60mg/m2 intravenously (IV) daily on days 2 to 4 Cytarabine 100mg/m2 IV daily on days 2 to 8 Venetoclax 100mg orally on day 1, 200mg on day 2, 400mg on day 3 and till day 11 Cohort 3B: Daunorubicin 60mg/m2 intravenously (IV) daily on days 2 to 4

	Cytarabine 100mg/m2 IV daily on days 2 to 8 Venetoclax 100mg orally on day 1, 200mg on day 2, 400mg on D3 and till day 14 Expansion Cohort(s): A maximum of 28 additional patients aged ≤60 years and 28 patients >60 years will be randomized (1:1) to the MTD and the starting dose (assuming the MTD is not the starting dose), to further evaluate safety and efficacy of the study drug combination and identify the optimal phase 2 dose. Consolidation Phase: Patients who achieve CRc post induction will proceed to consolidation therapy with high-dose cytarabine in combination with escalating doses of Venetoclax. The 3+3 algorithm will be applied for dose escalation/de-escalation of Venetoclax in combination with Cytarabine. As of February 2023, there have been 2/6 hematologic DLTs in consolidation cohort 1B, therefore we will no longer give Venetoclax in combination with high-dose cytarabine during the consolidation phase, in pts >60 yrs of age. We will also not explore dose escalation of Venetoclax during consolidation in the 60-year-old or younger age-group before RP2D of induction is determined. All subjects ≤60yrs will be treated at the consolidation Cohort 1A dose. *A. Patients ≤60 years* Consolidation cohort 1A: Cytarabine 1.5gr/m2 every 12 hours on days 1,3,5 Venetoclax 200mg on Days 1-7

	B. Patients >60 years Consolidation cohort 1B: Cytarabine 1gr/m2 every 12 hours on days 1,3,5
Sample Size Determination	A minimum of 3 patients will be treated in each cohort (dose level) sequentially in a 3+3 design. Additional 3 subjects may be backfilled to lower dose levels so that each cohort will reach a total of 6 subjects. Once the MTD is reached, a maximum of 28 additional patients will be randomized 1:1 to the MTD or the starting dose (assuming the MTD is not the starting dose) for a total of up to 20 patients (6 from Part 1, 20 from Part 2) treated at each of those dose levels. A maximum of 52 patients (including backfill and expansion cohorts) 60 years or younger and 46 patients older than 60 years may be enrolled in this Phase 1b study.

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1.0 OBJECTIVES

1.1 Primary Objectives

The primary objectives of this study are to evaluate the safety and tolerability and determine the dose-limiting toxicity and the maximum tolerated dose (MTD) of the combination of daunorubicin & cytarabine chemotherapy plus venetoclax for patients with AML (Phase 1b).

1.2 Secondary Objectives

The secondary objectives of the study are:

- To assess efficacy by response per 2022 ELN and revised IWG criteria
- To determine additional response parameters: CR/CRi and CR/CRh rates
- To determine time to response variables including overall survival (OS), event-free survival (EFS) and duration of response (DOR)

1.3 Exploratory Objectives

- To comprehensively analyze LSC and assess rates of LSC eradication by means of MFC and single cell sequencing ("high resolution MRD assay")
- To determine the protein expression of BCL-2 family members and the reliance of leukemic cells on differing members of the BH3 family at the time of diagnosis and relapse and explore association of such observations with response to venetoclax when combined with chemotherapy

2.0 BACKGROUND/RATIONALE:

2.1 *Overview of Acute Myeloid Leukemia and present therapeutic landscape*

Acute myeloid leukemia (AML) is the most common acute leukemia in adults diagnosed at a median age of 68 years. Only about 30% of patients with newly diagnosed AML enjoy long-term survival with current therapies, while the majority will succumb to their illness (Cancer.gov). Patients' survival is highly dependent on the depth of response to the treatment employed, with complete remission (CR) historically used as the major therapeutic milestone, while additional prognostic value is increasingly identified in the presence of measurable residual disease

(MRD) (Walter, Kantarjian et al. 2010, Percival, Wang et al. 2021). However, even patients with MRD negative complete remissions relapse due to remaining chemotherapy-resistant pre-leukemic and leukemic stem cells (LSCs). Eradication of residual disease at the LSC level is the ultimate goal of anti-leukemia therapy, efforts to develop well-reproducible LSC MRD assays are ongoing and persistence of residual LSC measured by such assays seems to provide additional prognostic information (Buccisano, Palmieri et al. 2019).

Since the 1970s the standard of care in the treatment of fit, primarily younger, AML patients has been induction intensive chemotherapy with the combination of cytarabine with anthracyclines (daunorubicin, idarubicin), the so called “3+7” regimen. With this regimen about 70% of younger (≤60 years of age) and 60% of older (>60 yrs) fit AML patients achieve complete remission (Fernandez, Sun et al. 2009, Löwenberg, Ossenkoppele et al. 2009). The rates of MRD negativity in CR, as measured by multiparameter flow cytometry (MFC) are less well defined due to its recent implementation, but are reported to range between 30-50% (Freeman, Hills et al. 2018, Paietta 2021). Identifying therapies able to induce deeper responses that translate to sustained remission and higher cure rates represents an area of unmet need.

Recent advances in the understanding of AML pathophysiology have led to the discovery of novel agents, which have since 2017 been added to the treatment armamentarium against AML. Overexpression of the anti-apoptotic protein BCL-2 has been reported in AML blasts and LSCs and has been associated with drug resistance and worse outcomes (Lagadinou, Sach et al. 2013). Venetoclax is a potent, selective small molecule inhibitor of BCL-2 that demonstrated activity against AML, both as single agent in relapsed/refractory AML and in frontline combination therapy approaches, FDA approved since 2018 in combination with hypomethylating agents or low-dose cytarabine (DiNardo, Jonas et al. 2020, FDA.gov 2020, Wei, Montesinos et al. 2020). The combination of Venetoclax with hypomethylating agents is shown to not only eradicate AML blasts but also target and eliminate LSCs, leading to sustained, durable responses (Pollyea, Stevens et al. 2018).

It is reasonable to consider that a combination of venetoclax with chemotherapy can have a synergistic effect. Genotoxic agents induce apoptosis and Venetoclax reduces the apoptotic threshold, while Venetoclax resistance via MCL-1 upregulation could be overcome by the addition of chemotherapy, shown to decrease levels of MCL-1 (Niu, Zhao et al. 2016). Venetoclax was recently combined with intensive chemotherapy in early phase trials and the

initial reported results are encouraging in terms of both safety and efficacy (DiNardo, Lachowiez et al. , Chua, Roberts et al. 2020, Stone, DeAngelo et al. 2020)

We propose a phase 1b study of venetoclax in combination with standard intensive age-adjusted induction daunorubicin/cytarabine chemotherapy followed by high-dose cytarabine consolidation in all patients with newly diagnosed AML, fit for aggressive chemotherapy. We hypothesize that the combination of Venetoclax with chemotherapy will result in higher rates of deep, MRD-negative remission than what has been historically reported with chemotherapy alone. This study offers a unique opportunity to utilize an eloquent, highly-sensitive LSC MRD assay to test the efficacy of Venetoclax in eliminating LSCs when combined with conventional chemotherapy.

2.2 *Bcl-2 and Clinical experience with Venetoclax in AML*

The BCL-2 family consists of multiple proteins, functionally classified as either anti-apoptotic or pro-apoptotic proteins. The anti-apoptotic BCL-2 proteins include BCL-2, BCL-XL, MCL-1, BCL-W, BFL-1/A1 and possibly BCL-B (Chipuk, Moldoveanu et al. 2010). The pro-apoptotic proteins can be divided into effector proteins and BH3-only proteins. The anti-apoptotic BCL-2 proteins can protect cells from apoptosis by directly binding to or antagonizing pro-apoptotic proteins (Cheng, Wei et al. 2001).

A number of early studies showed that BCL-2 was overexpressed in CD34+ AML cells and was associated with poor prognosis and resistance to chemotherapy (Karakas, Maurer et al. 1998, Cheng, Wei et al. 2001). Subsequently, a better understanding of the intrinsic apoptosis pathway led to the development of small molecules that mimic the BH3-domain, common in all pro-apoptotic BCL-2 family proteins, thereby disrupting the interaction of pro-apoptotic and anti-apoptotic proteins, setting free the former to eventually induce apoptosis. This drug development culminated in the development of Venetoclax, a potent, selective small molecule inhibitor of BCL-2.

2.2.1 *Venetoclax monotherapy in AML*

A phase II, single-arm study evaluated treatment with venetoclax, at the dose of 800mg daily in patients with relapsed/refractory acute myelogenous leukemia (AML) or unfit for intensive

chemotherapy. The overall response rate by IWG criteria was 19%. BH3 profiling was consistent with on-target BCL2 inhibition. Common adverse events included nausea, diarrhea and vomiting (all grades), febrile neutropenia and hypokalemia (grade 3/4). On the basis of this modest clinical activity of venetoclax in advanced AML along with preclinical data supporting synergy with other agents (Tsao, Shi et al. 2012, Bogenberger, Delman et al. 2015, Niu, Zhao et al. 2016, Teh, Nguyen et al. 2018), Venetoclax was subsequently studied in combination with hypomethylating agents and low-dose cytarabine.

2.2.2 *Venetoclax combinations in elderly AML patients*

A multicenter phase 1b trial of Venetoclax in combination with a hypomethylating agent (HMA), azacitidine or decitabine, in elderly patients newly diagnosed with AML, non-fit for intensive induction chemotherapy showed acceptable tolerability with a composite complete remission (CR+CRi) rate of 73% in the venetoclax 400 mg + HMA cohort. Common adverse events (>30%) included nausea, diarrhea, constipation, febrile neutropenia, fatigue, hypokalemia, decreased appetite, and decreased white blood cell count. No tumor lysis syndrome was observed. The median overall survival (mOS) was 17.5 months (DiNardo, Pratz et al. 2019). An international phase 1b/2 trial of Venetoclax in combination with low-dose cytarabine (LDAC) in a similar AML population treated 82 patients in the recommended phase 2 dose of Venetoclax 600mg and showed similar toxicity profile as in combination with HMA, while the combination achieved a CR/CRi rate of 56% and resulted in a mOS of 13.5 months (Wei, Strickland et al. 2019). Based on the unprecedented efficacy of those combinations in a historically difficult-to-treat population of AML, Venetoclax was FDA approved in 2018 for use in combination with azacitidine, decitabine or low-dose cytarabine in elderly, unfit for intensive chemotherapy AML patients. Subsequently, two confirmatory phase 3 randomized placebo-controlled trials of Venetoclax in combination with Azacitidine (VIALE-A) or LDAC (VIALE-C) were performed to evaluate efficacy and safety of the combinations compared to placebo plus azacitidine and LDAC, respectively in elderly or unfit-for-intensive chemotherapy, newly diagnosed AML patients. With a primary endpoint of overall survival, VIALE-A showed significantly superior mOS in the azacitidine–venetoclax group (14.7 months) compared to the control group (9.6 months) (HR 0.66; 95% CI, 0.52 to 0.85; P<0.001). The incidence of composite complete remission was higher with azacitidine–venetoclax than with the control regimen (CR/CRi 66.4% vs. 28.3%; P<0.001) (DiNardo, Jonas et al. 2020). Similarly, the combination of Venetoclax plus LDAC in VIALE-C demonstrated a 30% reduction in risk of death compared to LDAC plus

placebo with median OS of 8.4 months for the venetoclax arm (HR, 0.70; 95% CI, 0.50-0.98; P = 0.04). Complete remission (CR) plus CR with incomplete blood count recovery rates were 48% and 13% for venetoclax plus LDAC and LDAC plus placebo, respectively, while key grade ≥3 adverse events (venetoclax vs LDAC alone) were febrile neutropenia (32% vs 29%), neutropenia (47% vs 16%), and thrombocytopenia (45% vs 37%) (Wei, Montesinos et al. 2020).

2.2.3 *Clinical safety of Venetoclax in currently approved indications*

Venetoclax is a potent, selective small molecule inhibitor of BCL-2, currently FDA approved for the treatment of chronic lymphocytic leukemia/small lymphocytic lymphoma (CLL/SLL), as well as in combination with azacitidine/decitabine or low-dose cytarabine in AML patients unfit for intensive chemotherapy due to advanced age or comorbidities.

The safety profile of venetoclax is well described. In CLL/SLL, the most common adverse reactions (≥20%) for Venetoclax when given in combination with obinutuzumab or rituximab or as monotherapy were neutropenia, thrombocytopenia, anemia, diarrhea, nausea, upper respiratory tract infection, cough, musculoskeletal pain, fatigue, and edema.

In AML, the most common adverse reactions (≥30%) in combination with azacitidine or decitabine or LDAC were nausea, diarrhea, thrombocytopenia, constipation, neutropenia, febrile neutropenia, fatigue, vomiting, edema, pyrexia, pneumonia, dyspnea, hemorrhage, anemia, rash, abdominal pain, sepsis, musculoskeletal pain, dizziness, cough, oropharyngeal pain, and hypotension.

Risk of TLS has been identified, primarily in CLL patients with high burden of disease and a slow dose ramp-up has been adopted. TLS was observed in only 1.1% of AML patients treated with venetoclax in combination with azacitidine/decitabine/low-dose cytarabine when risk was mitigated by cytoreduction to WBC≤25,000/m^3 prior to venetoclax administration and a 3-4 day dose ramp-up depending on target dose (400mg or 600mg). (Inc 2021)

Notable serious adverse effects of venetoclax in combination with azacitidine or low-dose cytarabine in newly diagnosed older AML patients in VIALE-A and VIALE-C were febrile neutropenia (30% and 32%, respectively), pneumonia (13%, respectively) and sepsis (6%).

2.2.4 *Recent Venetoclax combinations with intensive chemotherapy in AML*

In the relapsed/refractory (R/R) setting Venetoclax was combined with FLA-Ida chemotherapy in a study by Shahswar R. et al. Venetoclax was dosed at 100mg daily due to concurrent azole antifungals/strong CYP3A4 inhibitors and given for 7 days (days 1-7) (Shahswar, Beutel et al. 2020). Thirteen patients with R/R AML were treated and an improved composite CR rate of 69% was seen without additional hematologic toxicity compared to a historical control of R/R AML patients treated with FLA-Ida chemotherapy alone.

In a phase 1b dose-escalation study by Chua et al. Venetoclax was combined with attenuated "5+2" chemotherapy (idarubicin 12mg/m2 x 2 days and cytarabine 100mg/m2 x 5 days) in 51 newly diagnosed (ND) elderly (≥65 years of age) AML patients. Venetoclax was given for a total of 14 days and the dose was escalated to 600mg without reaching a maximum tolerated dose (MTD), however cumulative hematologic toxicity in terms of delayed platelet recovery was seen with each consolidation course leading to decreased dose density and intensity (Chua, Roberts et al. 2020). Induction mortality rates were low (6%), while CR/CRi rates in denovo AML (97%) compared favorably to historical remission rates with standard of care "7+3" chemotherapy (75%) (Castaigne, Pautas et al. 2012).

Venetoclax was tested in combination with the FLAG-Ida regimen by DiNardo et al. in a phase 1b/2 study in both ND and R/R AML patients. The recommended phase 2 dose for Venetoclax was 400mg daily given for a total of 14 days during induction and 7 days in consolidation cycles. Twenty-nine ND and thirty-nine R/R AML patients were treated with a median age of 46 years (20-73). Median time to count recovery following induction was 31 and 37 days in ND and R/R patients, respectively. Thirty- and sixty-day mortality was 0% and 4.4%, with no deaths on study seen in ND patients. Key grade 3 and 4 adverse effects occurring in ≥ 10% of patients included febrile neutropenia (50%), bacteremia (35%), pneumonia (28%), and sepsis (12%). Composite CR was achieved by 90% of ND patients, 96% of whose attained MRD negativity (DiNardo, Lachowiez et al. 2021).

Finally, the "3+7" regimen with daunorubicin 60mg/m2 x 3 days plus cytarabine 200mg/m2 x 7 days was combined with Venetoclax by Stone et al. in younger (≤60 years) patients with ND AML in a phase 1 clinical trial to determine MTD. Venetoclax was given in escalating doses for a total of 11 days (Days 1-11). In an abstract presentation of the first 10 patients treated, Venetoclax MTD was reached at 400mg. The median time to neutrophil and platelet recovery was 33 days and 29 days, respectively. All 10 patients achieved CRc (9CR/1CRi) while 6/8 attained MRD negativity by flow cytometry (Stone, DeAngelo et al. 2020).

These studies suggest improved efficacy of chemotherapy when it is combined with venetoclax but also highlight the fact that the optimal dose and schedule of venetoclax is yet to be defined in order to avoid excess toxicity from myelosuppression.

2.3 *Rationale for daunorubicin/cytarabine dosing in proposed study*

2.3.1 Two doses of Daunorubicin (06 JUN 2022 – 31 MAR 2023)

The preferred approach for remission induction in newly diagnosed patients with AML is a 7-day continuous infusion of cytarabine and anthracycline treatment on days 1 to 3, which is commonly referred to as "3+7" therapy. The dose of cytarabine as well as the choice and dose of anthracycline vary among different institutions, but no clear superiority of one over the other has been clearly shown. In an attempt to improve the outcomes achieved with this regimen different doses of these two drugs have been investigated in randomized controlled trials (RCT). In regard to daunorubicin dose, the ECOG 1900 study showed superiority of daunorubicin 90 vs 45mg/m2 x 3 days in combination with continuous cytarabine at 100mg/m2 x 7 days, both in terms of CR rates and OS in patients with newly diagnosed AML, younger than or equal to 60 years of age (Fernandez, Sun et al. 2009). Similar results were seen when the two daunorubicin doses were compared in a Korean RCT (Lee, Joo et al. 2011). The same doses of daunorubicin were compared in patients with AML above the age of 60 years, but no significant difference was seen in OS except for the age-subgroup of 60-65 years. The subsequent multicenter AML17 study by NCRI found no difference in efficacy between daunorubicin 90 and 60mg/m2 daily doses in combination with cytarabine (Burnett, Russell et al. 2015), although per protocol patients in both arms received another 50mg/m2 x3 days of daunorubicin in a second induction, making it difficult to compare the results with those of single induction done in aforementioned studies. The daunorubicin dose currently recommended by NCCN, ESMO, ELN and other societies is at least 60mg/m2 (60- 90mg/m2) x3 days in combination with either 100 or 200mg/m2 cytarabine in continuous 7-day infusion.

In our institution, we have historically followed the ECOG 1900 dosing schema for ND AML patients ≤60 years old and do not exceed daunorubicin 60mg/m2 x3 days for patients >60 years of age fit for intensive chemotherapy. In this trial, given the myelosuppressive potential of venetoclax, we will explore both doses of daunorubicin (60 and 90mg/m2 x3 days) in combination with venetoclax, starting with the lower daunorubicin dose for those aged ≤60yrs. In the >60 years

of age group, we will explore the combination of daunorubicin 60mg/m2 x 3days and cytarabine 100mg/m2 x 7 days with escalating doses of Venetoclax.

2.3.2 Daunorubicin 90mg/m2 dose cohorts discontinued

New data from the DaunoDouble multicenter prospective phase III trial presented at the 64th ASH Annual Meeting and Exposition showed similar efficacy of both doses of daunorubicin, 60mg/m2 (Dauno60) and 90mg/m2 (Dauno90), when combined with 7 days of cytarabine 100mg/m2 in induction chemotherapy for newly diagnosed younger AML patients (median age: 52 years). Comparing Dauno60 and Dauno90, CR rates after the end of induction were similar (89.6% vs 88.5%) (p=0.691), and after a median follow-up of 43.6 months, 3-y RFS was 53.8% vs 50.1% (p=0.561) and 3-y OS 65.2% vs 58.3% (p=0.196) (Röllig, Steffen et al. 2022). This data further supports the equivalency of the two daunorubicin doses previously reported by the AML 17 study by NCRI (Burnett, Russell et al. 2015). At the time of this amendment and as of 02.03.2023, we have completed enrollment of Cohort 2A with Daunorubicin 90mg/m2 with no observed dose limiting toxicity. However, based on the results of DaunoDouble and AML17, we will no longer explore the Dauno90 dose in subsequent cohorts of patients with AML 60 years of age or younger. Venetoclax dose escalation will continue as previously planned in combination with Dauno60.

2.3.3 Cytarabine dose in consolidation

Those who achieve CR/CRi after induction will proceed to consolidation chemotherapy with cytarabine. When cytarabine doses >1,500mg/m2 were combined with Venetoclax as part of the FLAG-Ida/Ven regimen excessive myelosuppression was noted (DiNardo, Lachowiez et al. 2021). In addition to increased toxicity, the failure of high doses of cytarabine to improve survival argues against its continued use, and preference of intermediate doses is reflected in ELN 2017 and 2022 management recommendations (Döhner, Estey et al. 2017, Döhner, Wei et al. 2022). In this study, we will use cytarabine consolidation doses of 1,500mg/m2 (1,000mg/m2 if aged >60 years per institutional guidelines) on days 1,3,5 along with escalating doses of Venetoclax.

2.4 *Rationale for inclusion of FLT3 mutated AML*

The current standard of care for the treatment of FLT3 mutated AML in patients younger than 60 years of age is the combination of 3+7 chemotherapy with the non-selective, multi-kinase/FLT3 inhibitor midostaurin, based on the results of a phase 3 randomized placebo-controlled trial (RATIFY) that showed a significantly longer OS and EFS in the midostaurin group compared to placebo (HR 0.78; one-sided P=0.009 and HR 0.78; one-sided P=0.002, respectively) (Stone, Mandrekar et al. 2017). The daunorubicin dose used in the RATIFY trial was 60mg/m2 x3 days. Despite survival benefit, about 37% of midostaurin-chemotherapy treated patients did not survive at 4 years, highlighting the need for further improvement in available treatments of this aggressive AML subtype. Mature, post-hoc analysis of ECOG1900 showed that FLT3 AML was one of the molecular subgroups particularly benefited from daunorubicin 90mg/m2 (vs 45mg/m2) dose (Luskin, Lee et al. 2016). Similar benefit was seen for FLT3 mutated AML in a later, intention to treat analysis of the UK NCRI AML17 trial of daunorubicin 90 vs 60mg/m2, while the magnitude of the benefit was similar to the benefit delivered by the addition of midostaurin to the daunorubicin 60mg/m2 dose in the RATIFY trial (Burnett, Russell et al. 2016). Taken together these data suggest the potentially important role of daunorubicin 90mg/m2 in improving outcomes in FLT3 mutated disease. Moreover, although there was survival benefit with addition of midostaurin to the chemotherapy backbone, the CR rates were similar to placebo arm (58.9% [95% CI, 53.6 to 64.0] in the midostaurin group vs 53.5% [95% CI, 48.2 to 58.8] in the placebo group, P=0.15), suggesting that the benefit from midostaurin was derived by means of a greater depth of remission beyond morphologic CR, but also highlighting the modest activity of the superior combination (midostaurin-chemo). There is therefore, significant room for improvement of the efficacy of the current standard of care therapy for FLT3 mutated AML.

As described above, Venetoclax appears to be one of the most efficacious novel antileukemic agents in the armamentarium against AML and the preliminary results of the combination with intensive chemotherapy has produced unprecedented, high rates of complete remission. In addition, in combination with hypomethylating agents (HMA), Venetoclax has shown high rates of remission (CR+CRi) in FLT3-mutated AML in unfit patients, highlighting its activity in FLT3 mutated AML. It is therefore conceivable that Venetoclax can be a better partner than midostaurin to conventional 3+7 chemotherapy, especially when the daunorubicin dose is 90mg/m2.

2.5 *Rationale for exploratory objectives*

AML originates from a rare population of cells, termed leukemic stem cells (LSCs) or leukemia-initiating cells, which are capable of self-renewal, proliferation and differentiation into malignant blasts. At least some of these cells persist after treatment and are probably responsible for disease relapse (Hope, Jin et al. 2004, van Rhenen, Feller et al. 2005, Moshaver, van Rhenen et al. 2008). Those cells are difficult to differentiate from normal hematopoietic stem and progenitor cells (HSPCs) and there is an ongoing effort to develop sensitive and reproducible assays to identify and monitor those cells at diagnosis of AML and during the treatment course.

Investigators at Einstein/Montefiore have developed assays that can detect pre-leukemic and leukemic stem cells at an unprecedented level of resolution. Using multicolor flow cytometry optimized to work with primary samples, and a combination of novel leukemic stem cell markers, we can achieve depth of sequencing at 1 million x and detect very low levels of mutant cells in patients' marrow (Chen, Kao et al. 2019, Sridharan, Schinke et al. 2019). We have used these assays in ongoing trials to detect whether therapeutics have the ability to inhibit leukemic stem cells (Santini, Valcárcel et al. 2019). We will perform ultra-high resolution MRD assessment in this trial and determine whether the addition of Venetoclax to standard chemotherapy can result in elimination of rare leukemic stem cells clones.

Mitochondrial priming assays are an established method of evaluating cancer cell susceptibility to chemotherapeutic agents (Ni Chonghaile, Sarosiek et al. 2011). These assays rely on the common final pathway to cell apoptosis; although treatments work by a variety of mechanisms to damage the cancer cells, they ultimately lead to cancer cell apoptosis via activation of the Bax/Bak pathway, mitochondrial depolarization, and activation of the caspase cascade. When a cell culture exposed to drug demonstrates mitochondrial depolarization ex-vivo, this is called "mitochondrial priming" as the cells are prepared to undergo apoptosis.

BCL-2 represents one of a family of proteins distinguished by protein domain BH3 (BCL-2 Homology Domain 3) that either upregulate or downregulate the Bax/Bak pathway. Different leukemic cells lines have been found to have reliance on differing members of the BH3 family and this may explain in part the variations in efficacy of treatment amongst patients. For example, in a phase II study of venetoclax monotherapy for AML, in-vitro sensitivity to BCL-2 correlated with longer duration on venetoclax therapy. Furthermore, functional BH3 profiling was also weakly associated with time on therapy although this was just above statistical significance (Konopleva, Pollyea et al. 2016). Our goal is to measure the mitochondrial outer membrane permeabilization (MOMP) of patient leukemic cells exposed to various BH3 peptides as well as venetoclax. This reflects how well the cell has been primed towards apoptosis and how

dependent the cells are on the BCL-2 pathway (Fraser, Ryan et al. 2019). We will then correlate these findings with patient outcomes including overall response.

Single cell mass cytometry (Cytometry Time of Flight, **CyTOF**) enables analysis of multiple cell surface and intracellular parameters per cell, in longitudinal samples collected over the course of therapy. Leukemia stem cells (LSCs) have been implicated in chemoresistance, altered mitochondrial metabolism, and BCL-2 activity (Zhang, Zhou et al. 2017). Identifying therapies that target LSCs will be important to obtain durable remissions. We will use CyTOF apoptosis assay to determine which apoptotic pathways patient-specific samples are dependent on, whether different apoptotic proteins are present in LSCs vs. bulk leukemia cells, and whether these results correlate with response or survival, or mutation patterns. We will further evaluate whether the combination of venetoclax and chemotherapy is able to reduce BCL-2 expressing LSC populations.

3.0 STUDY ELIGIBILITY

3.1 Inclusion Criteria

- New diagnosis of AML by WHO criteria. Patients with intermediate and higher risk MDS (R-IPSS>3.5) and 10% blasts or more, or proliferative (WBC ≥ 13 x 10^9/L) CMML-2, are also eligible at the discretion of the PI. Patients having received any prior hypomethylating agent with or without BCL2 inhibitor therapy for MDS/AML are also eligible at the discretion of the PI.
- Patients ≥ 18 to ≤ 75 years.
- Eastern Cooperative Oncology Group (ECOG) Performance Status of ≤2
- Adequate renal function including creatinine clearance > 30 mL/min based on the Cockcroft Gault equation.
- Adequate hepatic function including total bilirubin < 1.5x ULN unless increase is due to Gilbert's disease or leukemic involvement, and AST and/or ALT < 3x ULN unless considered due to leukemic involvement
- Ability to understand and provide signed informed consent
- Male subjects must agree to refrain from unprotected sex and sperm donation from initial study drug administration until 90 days after the last dose of study drug.

3.2 Exclusion Criteria

- Patients with t(15;17) karyotypic abnormality or acute promyelocytic leukemia (FAB class M3-AML)
- Subject has known active CNS involvement with AML
- Patients with New York Heart Association (NYHA) Class III or IV congestive heart failure or LVEF <45% by echocardiogram or multi-gated acquisition (MUGA) scan
- Patients with a history of myocardial infarction within the last 6 months or unstable / uncontrolled angina pectoris or history of severe and/or uncontrolled ventricular arrhythmias
- Patients with uncontrolled infection with human immunodeficiency virus (HIV) or active Hepatitis B or C
- Patients with known dysphagia, short-gut syndrome, or other conditions that would affect the ingestion or gastrointestinal absorption of drugs administered orally.
- Subject has any other significant medical or psychiatric history that in the opinion of the investigator would adversely affect participation in this study.
- Subject has a white blood cell count > 25 x 10^9/L. (Note: Hydroxyurea and/or cytarabine (1 or 2 doses; up to 2 is permitted to meet this criterion.)
- Nursing women, women of childbearing potential (WOCBP) with positive urine pregnancy test, or women of childbearing potential who are not willing to maintain adequate contraception
    - Appropriate method(s) of contraception include oral or injectable hormonal birth control, IUD, and double barrier methods (for example a condom in combination with a spermicide).

3.3 Enrollment on Study

Subjects will be recruited at Montefiore/Einstein Cancer Center. All subjects who consent will be registered in REDCap Database. For those subjects who are consented, but not enrolled, the reason for exclusion must be recorded.

All subjects will be registered through Montefiore/Einstein Cancer Center and will be provided a study number by contacting the study coordinator listed on the cover page.

4.0 TREATMENT PLAN

4.1 Overview and Overall Design:

This is a Phase 1b, open-label study evaluating Venetoclax in combination with intensive induction and consolidation chemotherapy in previously untreated, adult patients with acute myeloid leukemia. In Part 1, the safety and tolerability of the combination with Venetoclax at different doses and duration will determine the maximum tolerated dose (MTD), and inform the appropriate dose(s) and regimen(s) for Part 2. In Part 2, an additional 28 patients aged ≤60 years and 28 patients >60 years will be randomized 1:1 to the MTD and a dose level below MTD (assuming MTD is not the starting dose), to further evaluate the safety and efficacy of the study drug combination and identify the optimal phase 2 dose.

Venetoclax will be given orally once a daily for the duration determined in each dose cohort. Because of the risk of tumor lysis syndrome, there will be a dose ramp-up as per Venetoclax package insert.

4.1.1 Dose Escalation Phase

A minimum of 3 patients will be treated in each cohort (dose level) sequentially in a 3+3 design. Patients will receive the Venetoclax /daunorubicin/cytarabine combination as shown below (see dose adjustment guidelines for renal insufficiency or liver dysfunction in Table 1):

A. Patients aged ≤60 years

Cohort 1A:

Daunorubicin 60mg/m2 intravenously (IV) daily on days 2 to 4

Cytarabine 100mg/m2 IV daily on days 2 to 8

Venetoclax 100mg orally on day 1, 200mg on day 2, 400mg on D3 and till day 8

Cohort 2A:

Daunorubicin 90mg/m2 intravenously (IV) daily on days 2 to 4

Cytarabine 100mg/m2 IV daily on days 2 to 8

Venetoclax 100mg orally on day 1, 200mg on day 2, 400mg on D3 and till day 8

Cohort 3A:

Daunorubicin 60mg/m2 intravenously (IV) daily on days 2 to 4

Cytarabine 100mg/m2 IV daily on days 2 to 8

Venetoclax 100mg orally on day 1, 200mg on day 2, 400mg on D3 and till day 11

Cohort 4A:

Daunorubicin 60mg/m2 intravenously (IV) daily on days 2 to 4

Cytarabine 100mg/m2 IV daily on days 2 to 8

Venetoclax 100mg orally on day 1, 200mg on day 2, 400mg on D3 and till day 14

B. Patients >60 years

Cohort 1B:

Daunorubicin 60mg/m2 intravenously (IV) daily on days 2 to 4

Cytarabine 100mg/m2 IV daily on days 2 to 8

Venetoclax 100mg orally on day 1, 200mg on day 2, 400mg on D3 and till day 8

Cohort 2B:

Daunorubicin 60mg/m2 intravenously (IV) daily on days 2 to 4

Cytarabine 100mg/m2 IV daily on days 2 to 8

Venetoclax 100mg orally on day 1, 200mg on day 2, 400mg on day 3 and till day 11

Cohort 3B:

Daunorubicin 60mg/m2 intravenously (IV) daily on days 2 to 4

Cytarabine 100mg/m2 IV daily on days 2 to 8

Venetoclax 100mg orally on day 1, 200mg on day 2, 400mg on D3 and till day 14

The following is a description of the dose escalation/de-escalation rule (**Figures 1&2**):

- If 0/3 patients experience dose limiting toxicities (DLTs) in the first treatment cycles, there will be dose escalation. The next cohort of 3 patients will be enrolled at the next higher dose.
- If 1/3 patients experience DLTs in the first treatment cycles, 3 additional patients will be enrolled at the same dose. If no additional patients experience DLTs in the first treatment cycle, i.e. 1/6 patients experience DLTs, dose will be escalated. The next cohort of 3 patients will be enrolled at the next higher dose.
- Additional 3 subjects will be backfilled to lower dose levels if only 3 at that dose level so that each cohort may reach a total of 6 subjects. These additional subjects at each dose level will have the purpose of generating additional safety data, and will not undergo formal DLT observation.
- If ≥2/3 or ≥2/6 patients experience DLTs the MTD has been exceeded. No additional patients will be treated at that dose and regimen. A new cohort of three patients will be enrolled at the next lower dose if at that dose only three patients were previously treated.
- Non-evaluable patients will be replaced to ensure at least 3 patients will be evaluable in each phase 1 dose level.

Dose level 0 will be defined as the combination of chemotherapy with the shortest duration of Venetoclax (Ven 400mg for a total of 8 days including duration of dose ramp-up) (**Table 1**).

If dose level 0 is too toxic (i.e. ≥2/3 or ≥2/6 patients experience DLTs), the study will be stopped. MTD is defined as the highest dose at which no more than one patient out of 6 patients (i.e. 0/3 or 1/6) experience DLTs. In total, up to a maximum of 24 patients (including backfill cohorts) equal or below the age of 60 years and 18 patients between 61 and 75 years of age may be enrolled in Phase 1b part of the study and their data may be evaluated.

4.1.2 Dose Expansion Phase:

In both the aged ≤60 years and age >60 years cohorts, up to 28 additional patients will be randomized 1:1 at the MTD and the starting dose to yield a maximum of 20 patients total at

each of those two dose levels (14 from dose expansion + 6 from dose escalation phase), to further evaluate safety and efficacy of the study drug combination and identify the optimal phase 2 dose {FDA, 2022-D-2827 #44}. Randomization will be used in this Phase 1 trial to achieve balance in patient characteristics across dose groups (Iasonos and O'Quigley 2021). The computer-generated randomization list for each age cohort will be provided by the study statistician. Bayesian toxicity monitoring will be used to terminate any of the dose expansion cohorts early for unacceptable toxicity using the stopping boundaries specified in section 12.0.

Table 1: Dose escalation/de-escalation for venetoclax in combination with daunorubicin/cytarabine in younger (≤60 yrs) and older (>60 yrs) patients.

Dose level	**Venetoclax**	**Daunorubicin**		**Cytarabine**		**Dose level**
		≤ 60 yrs	**>60yrs**	**≤ 60 yrs**	**>60yrs**	
3	400mg x14 days	60mg/m2 x3 days	60mg/m2 x3 days	100mg/m2 x7 days		2
2	400mg x11 days	60mg/m2 x3 days	60mg/m2 x3 days	100mg/m2 x7 days		1
1	400mg x8 days	90mg/m2 x3 days		100mg/m2 x7 days		
0 (starting dose)	400mg x8 days	60mg/m2 x3 days	60mg/m2 x3 days	100mg/m2 x7 days		0 (starting dose)

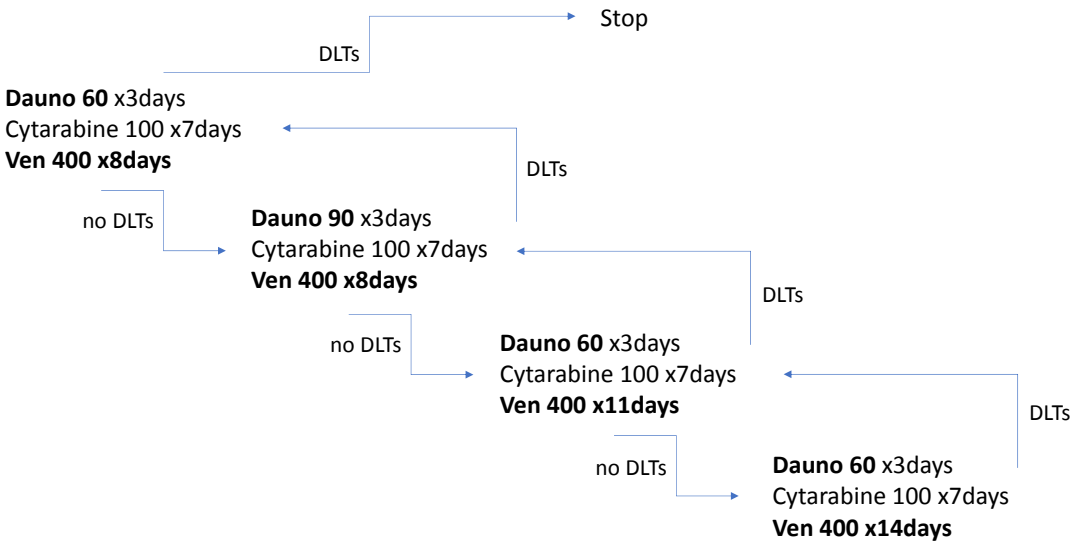

Figure 1. Schema of dose-escalation/de-escalation in patients ≤60 years of age.

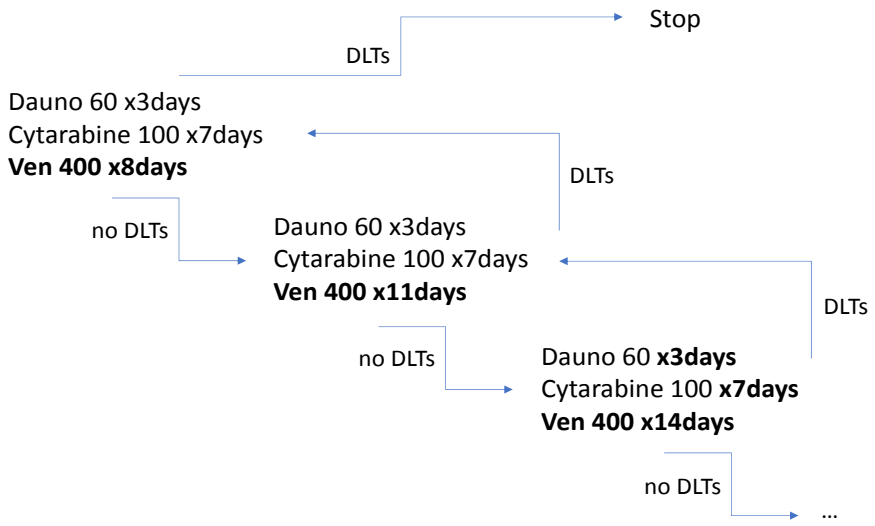

Figure 2. Schema of dose-escalation/de-escalation in patients >60 years of age.

4.2 Stopping rule for FLT3 mutated AML patients

We plan to use Venetoclax in combination with daunorubicin and cytarabine without waiting for the results of molecular analysis on FLT3 mutational status. Post induction and once in complete remission patients can be treated with consolidation chemotherapy plus midostaurin off trial at the discretion of the treating physician. After the first 3 patients with FLT3 mutated AML are treated in each daunorubicin dose cohort, we will evaluate the rate of CR/CRi, and if CR/CRi is seen in less than 4/6 patients (benchmark CR/CRi rates of 7+3 plus midostaurin per RATIFY trial were 58.9%[95%CI 53.6 to 64.0]) (Stone, Mandrekar et al. 2017), the trial will be amended to exclude further enrollment of patients with FLT3 mutation.

4.3 Duration of Therapy

Patients will receive one cycle of induction chemotherapy as described. An interim bone marrow evaluation will be performed between days 14-21 from chemotherapy initiation. Patients who will not achieve marrow hypoplasia (cellularity <20% of which less than 5% are residual blasts) will receive a second induction, as allowed by their clinical circumstances. Second induction chemotherapy will be comprised of daunorubicin 60mg/m2 x 2 days (45mg/m2 x 2 days in patients initially treated with daunorubicin 90mg/m2) and cytarabine 100mg/m2 x 5 days along with Venetoclax 400mg for 7 days, whether younger or older than 60 years of age, and disease assessment will be performed on day 28 (up to day 42) of second induction. Patients who do not achieve CR/CRi after a second induction will be taken off protocol and will be treated per treating physician's discretion. Patients who achieve CRc will proceed to consolidation therapy with high-dose cytarabine in combination with escalating doses of Venetoclax (200-400mg) for up to four 28(-42)-day cycles, as shown below (see dose adjustment guidelines for renal insufficiency or liver dysfunction in **Table 2**); consolidation with allogeneic transplantation off protocol is allowed per physician's discretion. The 3+3 algorithm will be applied for dose escalation/de-escalation of Venetoclax in combination with High-Dose Cytarabine. Each cycle of cytarabine will last up to 42 days.

As of February 2023, there have been 2/6 hematologic DLTs in consolidation cohort 1B, therefore Venetoclax will no longer be given in combination with high-dose cytarabine during the

consolidation phase, in pts >60 yrs of age. In addition, we will not explore dose escalation of Venetoclax during consolidation in the 60-year-old or younger age-group before RP2D of induction is determined. All subjects ≤60yrs will be treated at the consolidation Cohort 1A dose.

A. Patients ≤60 years

Consolidation cohort 1A:

Cytarabine 1.5gr/m2 every 12 hours on days 1,3,5

Venetoclax 200mg on Days 1-7

B. Patients >60 years

Consolidation cohort 1B:

Cytarabine 1gr/m2 every 12 hours on days 1,3,5

4.4 Response Evaluation

All participants should undergo response evaluations between Day 28 and Day 42 of first and/or second induction course. Unless there is clear evidence of progressive disease in the blood, bone marrow aspiration is required and bone marrow biopsy is strongly encouraged. In cases with hypocellular marrows (<10% cellularity), repeat bone marrow examinations should be considered when there is evidence of hematopoietic recovery. If multiple bone marrow examinations are performed, the last examination will be used to classify the patient's response. The final response will be determined no later than day 42 from the start of therapy.

4.5 Definitions of Dose Limiting Toxicity (DLT)

Toxicities will be graded according to the CTEP Common Terminology Criteria for Adverse Events (CTCAE) version 5.0. DLT is based on toxicities that are deemed to be

at least possibly attributable to venetoclax. Attribution to venetoclax will be determined by the treating physician, in consultation with the PI, after consideration of the timing of the event, biologic plausibility, clinical judgment, and alternative causes. AEs commonly related to AML will

not be considered a DLT (Appendix). DLTs will be determined during the first 28 days and 42 days of a course for non-hematologic and hematologic toxicities, respectively.

Table 2. Dose adjustment guidelines for renal insufficiency or liver dysfunction

<table>
<tr><td></td><td colspan="2">Induction Chemotherapy (3+7)</td></tr>
<tr><td></td><td>Cytarabine</td><td>Daunorubicin</td></tr>
<tr><td>CrCl >30ml/min</td><td>No dose adjustment</td><td>No dose adjustment</td></tr>
<tr><td>Serum bilirubin 1.2 to 3 mg/dL*</td><td rowspan="3">Administer 50% of dose if serum bilirubin>2mg/dL; may increase subsequent doses in the absence of toxicities</td><td>Administer 75% of dose</td></tr>
<tr><td>Serum bilirubin 3.1 to 5 mg/dL*</td><td>Administer 50% of dose</td></tr>
<tr><td>Serum bilirubin >5 mg/dL*</td><td>Avoid use</td></tr>
<tr><td rowspan="2"></td><td colspan="2">Consolidation cycles</td></tr>
<tr><td colspan="2">Cytarabine</td></tr>
<tr><td></td><td>≤60 yrs (1,500mg/m2/dose)</td><td>>60 yrs (1,000mg/m2/dose)</td></tr>
<tr><td>CrCl 46-60ml/min</td><td colspan="2">Administer 60% of dose</td></tr>
<tr><td>CcCl 30-45</td><td colspan="2">Administer 50% of dose</td></tr>
<tr><td>Serum bilirubin > 2 mg/dL</td><td colspan="2">Administer 50% of dose; may increase subsequent doses in the absence of toxicities</td></tr>
</table>

* unless increase is due to Gilbert's disease or leukemic involvement

4.5.1 Non-Hematologic DLT

DLT is defined as any Grade 3 or higher non-hematologic event occurring during the first cycle (i.e. the first 28 days) that is at least possibly attributable to venetoclax, unless the event is

clearly due to extraneous causes or disease progression, confirmed Hy's law case, with the exception of the following:

• CTCAE Grade 3 or 4 AST or ALT that resolves to < Grade 2 within 14 days

• Grade 3 nausea, vomiting or diarrhea that can be managed to < Grade 3 with standard antiemetic or antidiarrheal medications and do not require tube feeding, total parenteral nutrition, or prolong hospitalization.

• Grade 3 or 4 electrolyte abnormalities will only be considered DLT if possibly related to study drug and do not resolve to grade ≤ 1 within 72 hours with or without intervention. Venetoclax interruption for up to 72 hours following transient (< 48 hours) chemical changes and laboratory TLS may be allowed and may not be considered a DLT, as this is an expected event with AML therapy

• Grade 3 biochemical abnormalities of amylase or lipase without clinical evidence of pancreatitis that resolve to grade ≤ 1 within 72 hours.

4.5.2 Hematologic DLT

Hematologic DLT is defined as Grade > 3 neutropenia and/or thrombocytopenia with no greater than 5% marrow blasts lasting for 6 weeks or more after the start of a course unless the delay in count recovery is due to another identifiable factor, such as documented myelosuppressive infection. Anemia will not be considered for the definition of DLT.

4.6 Concomitant Therapy and Supportive Care

4.6.1 Concomitant Therapy

Patients must be instructed not to take any additional medications (including over-the-counter products) during the study. Venetoclax is predominantly metabolized by CYP3A4 and data suggest that there are strong interactions with azoles and rifampin. Therefore, patients will not receive azoles (including ketoconazole, fluconazole, itraconazole, voriconazole, or posaconazole) for antifungal prophylaxis during the administration of venetoclax. Patients may not receive rifampin during the administration of venetoclax. Other medications that are known

to be inhibitors of CYP3A4 should be used with caution due to a potential increase in venetoclax exposure.

In the case of patients who require azole therapy for optimal management of active or prior fungal infections venetoclax dose will be reduced according to the azole used (**table 2**).

The use of other anti-leukemic agents is not permitted while on study therapy with the exception of intrathecal chemotherapy for prophylactic use or for controlled CNS leukemia.

Whenever possible, patients should discontinue proton pump inhibitors, antacids, and H2-receptor antagonists which increase the gastric pH and may reduce absorption of venetoclax resulting in decreased systemic exposure. Discontinuation is not required but is strongly recommended.

Concomitant medications will be captured in REDCap Database daily for the duration of Venetoclax administration, then weekly and in the case of SAEs.

Table 2. Dose Modifications for Venetoclax when Co-administered with 'Azole' Drugs

Assigned Venetoclax Dose	Venetoclax plus moderate CYP3A4 inhibitor (i.e fluconazole, isavuconazole)	Venetoclax plus strong CYP3A4 inhibitor (i.e voriconazole, Posaconazole)*
400mg	200mg	100mg (70mg with Posaconazole)
200mg	100mg	50mg

* Per FDA label, venetoclax dose should be reduced by 50% in the setting of moderate CYP3A4 inhibitors and 75% in the setting of strong CYP3A inhibitors. Posaconazole specifically requires a venetoclax reduction to 70 mg daily.

4.6.2 Prophylaxis and Treatment of Tumor Lysis Syndrome

The venetoclax dose titration schema utilized in the AML studies performed to date to mitigate the risk of tumor lysis syndrome (TLS) will be employed. All patients will be hospitalized for the entirety of induction chemotherapy till blood count recovery, starting at least on day -1 of

treatment initiation. Venetoclax will be administered at a dose of 100 mg on day 1, 200 mg on day 2, and either 400 mg on day 3 onwards. To mitigate the risk for TLS, subjects must be receiving tumor lysis prophylaxis, including hydration (oral, intravenous) and treatment with a uric acid reducing agent (allopurinol, rasburicase) prior to and during the venetoclax ramp up period.

TLS chemistry tests (potassium, uric acid, creatinine, calcium and phosphorus) will be obtained prior to dosing and 6-8 hours after each day of a new venetoclax dose during cycle 1 ramp up only. TLS chemistry test results will be reviewed by the investigator in real time and prior to the subject's next dose to ensure appropriate management. TLS chemistry tests will be monitored for 24 hours after reaching final dose. Increased laboratory monitoring and reduction of venetoclax starting dose might be considered in patients with risk factors for TLS (e.g., circulating blasts, high burden of leukemia involvement in bone marrow, elevated pretreatment lactate dehydrogenase levels, or reduced renal function). Venetoclax should be held in patients who develop clinically significant TLS, including, but not limited to grade 3 or greater hyperkalemia, grade 3 or greater acute kidney injury, and grade 3 TLS. Venetoclax should be resumed at the planned dose when electrolyte abnormalities related to TLS resolve or return to baseline. Venetoclax interruption for up to 72 hours following transient (< 48 hours) chemical changes and laboratory TLS will be allowed and will not require a dose reduction.

4.6.3. Supportive Care

Supportive measures including blood and platelet transfusions, antimicrobials, and analgesics are permitted. Supportive care investigational agents that are not for treatment of hematologic malignancy (i.e. anti-infective prophylaxis or therapeutics) are allowed.

Vomited doses: If a venetoclax dose is vomited within one hour of ingestion, it will be replaced. If vomiting occurs more than 1 hour after dosing, it will be considered a complete dose.

Growth factors: The routine use of growth factors is discouraged during the induction phase, but may be used in cases of documented infection or sepsis during periods of neutropenia, after

consultation with the Principal Investigator. Routine use of GCSF (filgrastim, pegfilgrastim) is allowed, as per institutional practices, during consolidation cycles.

Prophylaxis for fungal infections: Because patients with AML during induction chemotherapy are at high risk for fungal infections, all participants should receive antifungal prophylaxis according to institutional guidelines. Because azoles may not be given during venetoclax administration, all patients should receive prophylactic micafungin or caspofungin.

Prophylaxis for bacterial infections: Because patients with AML are also at high risk for bacterial infections, all participants should receive antibiotic prophylaxis according to institutional guidelines. We recommend starting prophylactic antibiotics when the ANC ≤ 1000 and falling or predicted to fall and continued until the ANC ≥ 500 and rising.

Management of febrile neutropenia: All patients with fever ≥ 38.3° C on a single occasion or ≥ 38.0°C that persists for one hour should be hospitalized and treated immediately with broad spectrum antibiotics according to institutional guidelines.

5.0 DRUG INFORMATION

5.1 Venetoclax (Venclexta®, formerly ABT-199)

AVAILABILITY:

Commercially available

DOSAGE AND ROUTE OF ADMINISTRATION:

Orally; see Treatment Plan sections 4.1.1 and 4.2.

FORMULATION:

Tablet formulation (10 and 50 and 100 mg)

GUIDELINES FOR ADMINISTRATION:

Each dose of Venetoclax will be taken with approximately 240 mL of water, within 30 minutes after the completion of a meal, preferably breakfast. If vomiting occurs within 15 minutes of taking Venetoclax and all expelled tablets are still intact, another dose may be taken. Otherwise, no replacement dose is to be taken. In cases where a dose of Venetoclax is missed or forgotten, the subject should take the dose as soon as possible, ensuring the dose is taken within 8 hours of the missed dose with food. Otherwise, the dose should not be taken.

TOXICITY:

In CLL/SLL, the most common adverse reactions (≥20%) for Venetoclax when given in combination with obinutuzumab or rituximab or as monotherapy were neutropenia, thrombocytopenia, anemia, diarrhea, nausea, upper respiratory tract infection, cough, musculoskeletal pain, fatigue, and edema.

In AML, the most common adverse reactions (≥30%) in combination with azacitidine or decitabine or LDAC were nausea, diarrhea, thrombocytopenia, constipation, neutropenia, febrile neutropenia, fatigue, vomiting, edema, pyrexia, pneumonia, dyspnea, hemorrhage, anemia, rash, abdominal pain, sepsis, musculoskeletal pain, dizziness, cough, oropharyngeal pain, and hypotension.

Risk of TLS has been identified, primarily in CLL patients with high burden of disease and a slow dose ramp-up has been adopted. TLS was observed in only 1.1% of AML patients treated with venetoclax in combination with azacitidine/decitabine/low-dose cytarabine

5.2 Cytarabine (Cytosine arabinoside, Ara-C, Cytosar®)

AVAILABILITY:

Commercially available.

Please refer to the FDA-approved package insert for cytarabine for product information, extensive preparation instructions, and a comprehensive list of adverse events.

DOSAGE AND ROUTE OF ADMINISTRATION:

During induction cytarabine will be administered intravenously at a dose of 100mg/m2/dose in 1,000mL of normal saline in a 24-hour infusion. During consolidation cytarabine will be administered intravenously at the dose of 3,000mg/m2/dose (1,000mg/m2 in patients >60 years of age) every 12 hours in 250ml of normal saline over 2 hours. See Treatment Plan sections 4.1.1 and 4.2. See dose adjustment guidelines for renal insufficiency or liver dysfunction provided in Table 1.

FORMULATION AND STABILITY:

Cytarabine for Injection is available in vials of 100 mg, 500 mg, 1 g, and 2 g containing a sterile powder for reconstitution. It is also available at a 20 mg/mL concentration with benzyl alcohol (25 mL per vial) or as a preservative free solution (5 mL, 50 mL per vial), and at a 100 mg/mL concentration with benzyl alcohol (20 mL vial) or as preservative free solution (20 mL vial). Hydrochloric acid and/or sodium hydroxide may be added to adjust the pH. Store at 25°C (77°F); excursions permitted to 15°-30°C (59°-86°F). Cytarabine solutions should be protected from light. When reconstituted with Bacteriostatic Water for Injection, cytarabine is stable for 48 hours at room temperature. Solutions reconstituted without a preservative should be used immediately. Discard if solution appears hazy. Diluted solutions in D5W or NS are stable for 8 days at room temperature; however, the diluted cytarabine should be used within 24 hours for sterility concerns. Refer to the package insert for complete information.

TOXICITY: Most common (>20%) nausea, vomiting, anorexia, conjunctivitis (with high dose), myelosuppression, stomatitis, alopecia.

5.3 Daunorubicin (Daunomycin, Daunorubicin hydrochloride, Cerubidine®)

AVAILABILITY:

Commercially available.

Please refer to the FDA-approved package insert for daunorubicin for product information, extensive preparation instructions, and a comprehensive list of adverse events.

DOSAGE AND ROUTE OF ADMINISTRATION:

Daunorubicin will be administered at the dose of 90mg/m2 (or 60mg/m2) in short intravenous infusion (15 minutes). See treatment plan section 4.1.1. See dose adjustment guidelines for renal insufficiency or liver dysfunction provided in Table 1.

Daunorubicin is a potent vesicant, frequent monitoring of intravenous access is necessary to avoid extravasation. It is best to administer IV infusion doses through a central venous access device.

FORMULATION AND STABILITY:

Daunorubicin for injection is available as a lyophilized powder in glass vials; 20 mg base daunorubicin activity. Reconstituted solutions are stable for 24 hours at room temperature and for 48 hours when refrigerated. Protect from light to prevent photodeactivation. Color change from red to blue/purple indicates decomposition. Stable in PVC and nylon filter; absorption occurs with cellulose, acetate/nitrate and polytetrafluoroethylene filters.

TOXICITY:

Myelosuppression, fever, chills, hyperuricemia, stomatitis (mild initially, may increase with prolonged therapy), nausea/vomiting (mile to moderate), alopecia, hyperpigmentation, elevations in liver enzymes (transient), red urine for 24–48 hours, extravasation with necrosis, cardiotoxicity (dose dependent), CHF, fatigue, dyspnea on exertion, arrhythmias, cardiomegaly, at cumulative dose of 550 mg/m2, 1–2% CHF.

6.0 EVALUATIONS, TESTS, OBSERVATIONS

6.1 Pre-Treatment Clinical Evaluations

- Medical history, medication history
- Physical exam with vital signs, height, weight, and BSA, performance status evaluation
- Complete blood count with differential

- Chemistry profile: glucose, electrolytes, BUN, creatinine, LDH, uric acid, bilirubin, SGOT, SGPT, calcium, phosphorous, magnesium, total protein and albumin
- Clotting times (PT, PTT) and fibrinogen
- Electrocardiogram, echocardiogram (repeat echocardiogram is required for patients with baseline LVEF<50% in need of re-induction chemotherapy prior to additional anthracycline chemotherapy)
- Bone marrow evaluation for morphology, immunophenotyping, cytogenetics, molecular diagnosis, and minimal residual disease (MRD) as well as leukemia stem cell profiling. Morphologic examination and MRD are required for all patients. Immunophenotyping, cytogenetic analysis, and molecular analysis should be performed. For patients with elevated leukocyte counts and high blast percentages, and patients too ill to undergo bone marrow aspirate, all diagnostic studies may be performed on blood rather than bone marrow. A portion of these samples will be used for research purposes. An additional amount of aspirate (or blood) will be taken for biobanking.
- Pregnancy test of females of childbearing potential
- Hepatitis B and C serologies (HBsAg, total HBcAb, HCV ab)
- HIV blood screening test
    - HIV viral load if HIV-positive

6.2 Evaluations during Therapy

Required studies	Schedule
Physical exam, vital signs	Daily during first week of induction and first consolidation cycle, then weekly, at end of treatment and as clinical indicated
CBC with differential	Daily during first week of induction and first consolidation cycle, then weekly, at end of treatment and as clinical indicated
Tumor lysis labs (potassium, calcium, phosphate, uric acid, LDH and creatinine)	Prior to the first dose, every 6-8 hours until 24 hours after the target dose is given, and then daily till completion of intravenous chemotherapy. Subsequently

	at least weekly during induction, then as clinically indicated
BUN, creatinine, electrolytes, SGOT, SGPT, bilirubin	Daily during first week of induction and first consolidation cycle, then weekly, at end of treatment and as clinical indicated
Bone marrow aspirate and biopsy for morphology, immunophenotype, cytogenetic and molecular analysis, MRD profiling, LSC profiling, BH3 and CyTOF profiling	Prior to day 1 (within 7 days) and at the suspicion of relapsing disease
Bone marrow aspirate and/or biopsy for response assessment, including MRD, LSC MRD and CyTOF profiling	On day 14-21 after start of chemotherapy, at the end of induction (day 28 and 42), prior to every cycle of chemotherapy and at the end of therapy

6.3 Response Evaluations during Therapy

All participants should undergo response evaluations at the end of induction chemotherapy (between days 28 and 42). Unless there is clear evidence of progressive disease in the blood, bone marrow aspiration (BMA) is required and bone marrow biopsy is strongly encouraged. In cases with hypocellular marrows (<10 % cellularity), repeat bone marrow examinations should be considered when there is evidence of hematopoietic recovery. If multiple bone marrow examinations are performed, the last examination will be used to classify the patient's response. The final response will be determined no later than day 42 from the start of induction therapy. Clinical BMA should be repeated to assess response prior to each cycle of additional therapy (end of induction chemotherapy BMA can be used for pre-consolidation 1 BMA).

6.4 MRD by multiparameter flow cytometry

Multiparameter flow cytometry MRD assay by Hematologics, Inc. will be used at baseline, the end of induction chemotherapy and with all subsequent BMA.

6.5 Correlative studies

6.5.1 High resolution MRD assay (Steidl lab, Konopleva lab)

Leukemia stem cell (LSC) profiling, sorting and quantification will be performed in bone marrow samples at baseline, at the end of induction chemotherapy and with all subsequent BMA. For patients who are in remission at the end of therapy and subsequently relapse, these studies will also be performed at the time of relapse.

Using multicolor flow cytometry optimized to work with primary samples, and a combination of novel leukemic stem cell markers, we can achieve depth of sequencing at 1 million x and detect very low levels of mutant cells in patients' marrow (Chen, Kao et al. 2019, Sridharan, Schinke et al. 2019). We have used these assays in ongoing trials to detect whether therapeutics have the ability to inhibit leukemic stem cells (Santini, Valcárcel et al. 2019). Additionally, clonal architecture of MRD cells will be evaluated by scDNA sequencing analysis using Mission Bio Tapestri system, at Dr Konopleva's lab. In this trial we will perform ultra-high resolution MRD assessment to determine whether the addition of Venetoclax to standard chemotherapy can result in elimination of rare leukemic stem cells clones.

6.5.2 BH3 profiling (Konopleva and Gavathiotis lab)

Leukemic cells from bone marrow aspirates (or blood when there are circulating blasts and aspirate not obtained) at diagnosis and at progression of disease will be analyzed for reliance on various members of BH3 family. This protocol is based on previously published work by Fraser et al (Fraser, Ryan et al. 2019).

Our goal is to measure the mitochondrial outer membrane permeabilization (MOMP) of patients' leukemic cells exposed to various BH3 peptides as well as venetoclax. This reflects how well the cell has been primed towards apoptosis and how dependent the cells are on the BCL-2 pathway. We will then correlate these findings with patient outcomes including overall response.

MOMP allows release of cytochrome c from the mitochondria into the cytoplasm where it activates the caspase cascade leading to apoptosis. The HT-DBP method measures MOMP by measuring retained cytochrome c. With FACS, cell surface marking with intracellular stains makes the method suitable for mixtures of different cell types found in primary tissue samples which allows for specifying activity of leukemic cells.

6.5.3 CyTOF profiling (Konopleva lab)

Single cell mass cytometry (Cytometry Time of Flight, **CyTOF**) is a transformative technology that substitutes rare earth element lanthanides for fluorophores as labels for antibodies that are quantified by time-of-flight mass spectrometer.(Agrawal, Hanfstein et al. 2014) CyTOF enables measurement of up to 100 parameters per cell without correction of spectral overlap. The Konopleva lab has developed a single-cell mass cytometry (CyTOF) panel that includes different leukemia stem/progenitor (LSC) and intracellular signaling markers, and has applied this panel to identify variability in phenotypes and activation of signal transduction pathways in primary AML samples.(Han, Qiu et al. 2015) Since the initial publication, the panel was optimized and now includes a total of 36 markers that characterize: (1) AML LSCs and progenitors, (2) minimal residual disease (MRD), (3) intracellular signaling pathways and (4) anti-apoptotic proteins. CyTOF will be used to observe changes in the levels of BCL-2 and associated proteins in bulk AML cells and in LSCs before therapy, at time of response assessment, upon recovery from consolidation (if applicable) and at relapse if feasible.

6.6 Evaluations after Completion of Therapy

When a participant discontinues the study, a final visit will be conducted. Following discontinuation of the study treatment, the participant will be treated according to the investigator's or treating physician's discretion. After the end-of-therapy visit, patients who have not progressed/relapsed or initiated subsequent anticancer therapy will be followed every 3 months for response assessment for up to 3 years, until the initiation of subsequent anticancer therapy, or until disease progression/relapse, whichever occurs first. All patients will be followed to document the start of subsequent anticancer therapy and overall survival (OS) every 3 months for up to 3 years after discontinuation of study drug. If a participant discontinues from the study due to an adverse event considered related to study treatment, a follow-up visit should be conducted no later than 30 days after the last dose of protocol therapy. Safety assessments are recommended at least every 14 days, until all toxicities resolve, return to baseline or become clinically satisfactory, stable, or are considered irreversible.

7.0 EVALUATION CRITERIA

7.1 Response Criteria:

Clinical activity of “3+7” with venetoclax will be assessed based on 2022 ELN recommendations (Döhner, Wei et al. 2022) and revised IWG response criteria for AML (Cheson, Bennett et al. 2003) with an overall response rate (ORR) defined as CR + CRi + PR.

CR: Absolute neutrophil count > 1,000/μL, platelets ≥ 100,000/μL, red cell transfusion independence, and bone marrow with < 5% blasts.

CRi: Bone marrow with < 5% blasts, with peripheral neutrophils of < 1,000/μL or platelets≤ 100,000/μL.

CRh: Bone marrow with < 5% blasts, with peripheral neutrophils > 500 and platelets >50,000/μL.

PR: All of the hematologic values for a CR but with a decrease of at least 50% in the percentage of blasts to 5% to 25% in the bone marrow aspirate.

HR: Improvement in hematologic parameters that do not meet criteria for PR (i.e., transfusion independence and /or decline in incidence of febrile neutropenia and related hospitalizations).

MLFS: Bone marrow blasts <5%, absence of blasts with Auer rods, absence of

extramedullary disease, no hematologic recovery required.

CR_{MRD-}, CRi_{MRD-}, CRh_{MRD-}: CR, CRh or CRi with MRD below a defined threshold for a genetic marker by qPCR, or by MFC. MFC-MRD positivity is defined as ≥ 0.1% of CD45 expressing cells with the target immunophenotype. Response with MRD detection at low-level (CR_{MRD-LL}) is included in this category of CR, CRh or CRi without MRD. CR_{MRD-LL} is currently only defined for NPM1-mutant and CBF-AML. In NPM1-mutated and CBF-AML, CR with molecular MRD detectable at low-level (CRMRD-LL) defined as < 2% is designated as negative for MRD.

CR = complete remission; CRi = CR with incomplete hematologic recovery; CRh = CR with partial hematologic recovery; PR = partial remission; HR = hematologic response, MLFS = morphologic leukemia-free state CR_{MRD-}, CRi_{MRD-}, CRh_{MRD-} = CR, CRi, CRh without minimal residual disease

Bone marrow blast assessments are measured by standard differential and/or flow cytometry for aberrant clonal blasts as available and appropriate. MRD assessment is measured by 10-color multiparameter flow cytometry.

Additional analyses of response including CR/CRi rate and CR/CRh rate. The depth of remission with exploratory analyses of LSC MRD negativity by flow cytometry and single cell sequencing will also be evaluated.

For each subject, response to therapy, duration of response, event-free survival, and overall survival will be calculated. The duration of response (DoR) is defined as the number of days from the date of initial response (CRi or better) to the date of first documented disease progression/relapse or death, whichever occurs first. Event-free survival (EFS) is defined as the number of days from the date of treatment initiation (i.e., C1D1) to the date of documented treatment failure, relapses from CR, or death from any cause, whichever occurs first, and will be calculated for all patients. In the event that neither disease progression nor death is documented prior to study termination, analysis cutoff, or the start of confounding anticancer therapy, these endpoints will be censored at the date of last tumor assessment date.

7.2 Toxicity Evaluation Criteria

This study will utilize the Common Terminology Criteria for Adverse Events v5.0 (CTCAE) of the National Cancer Institute (NCI) for toxicity and performance reporting. A copy of the current version of the CTCAE can be downloaded from the Cancer Therapy Evaluation Program home page (http://ctep.info.nih.gov). Additionally, toxicities will be reported on the appropriate data collection screens.

8.0 OFF-TREATMENT AND OFF-STUDY CRITERIA

8.1 Off-Treatment Criteria

• Development of a DLT

• Development of unacceptable toxicity during treatment outside the DLT period that would otherwise qualify for DLT

• No response to therapy

• Relapse

• Second malignancy

• Treatment with other antineoplastic therapy or HSCT

• Refusal of further protocol therapy by participant

• Completion of protocol therapy and evaluation period

8.2 Off-Study Criteria

• Death

• Lost to follow up

• Withdrawal of consent

9.0 SAFETY AND ADVERSE EVENT REPORTING

9.1 Reporting Adverse Experiences and Deaths

Only “unanticipated problems involving risks to participants or others” referred to hereafter as “unanticipated problems” are required to be reported to the Einstein IRB promptly (no event later than 10 working days) after the investigator first learns of the unanticipated problem. Only adverse events that constitute unanticipated problems are reportable to the Einstein IRB. This includes any event that in the PI’s opinion was:

• Unexpected (in terms of nature, severity, or frequency) given (1) the research procedures that are described in the protocol-related documents, such as the IRB-approved research protocol and informed consent document, as well as other relevant information available about the research; (2) the observed rate of occurrence (compared to a credible baseline for comparison); and (3) the characteristics of the subject population being studied; and

• Related or possibly related to participation in the research; and

• Serious; or if not serious suggests that the research places subjects or others at a greater risk of harm (including physical, psychological, economic, or social harm) than was previously known or recognized.

Unrelated, expected deaths that occur after patient has completed protocol treatment do not require reporting to the IRB. Though death is “serious”, the event must meet the other two requirements of “related or possibly related” and “unexpected/unanticipated” to be considered reportable. However, all deaths while on active protocol treatment (or within 30 days of last protocol treatment) must be reported to the IRB. Deaths meeting reporting requirements are to be reported immediately to the Einstein IRB, but in no event later than 48 hours after the investigator first learns of the death.

The following definitions apply with respect to reporting adverse experiences:

Serious adverse event (SAE): Any adverse event temporally associated with the subject’s participation in research that meets any of the following criteria:

• results in death

• is life-threatening (places the subject at immediate risk of death from the event)

• requires inpatient hospitalization or prolongation of existing hospitalization;

• results in a persistent or significant disability/incapacity

• results in a congenital anomaly/birth defect

• results in a secondary or concurrent cancer

• any other adverse event that, based upon appropriate medical judgment, may jeopardize the subject’s health and may require medical or surgical intervention to prevent one of the other outcomes listed in this definition

Unexpected adverse event:

• Any adverse event for which the specificity or severity is not consistent with the protocol related documents and other relevant sources of information, such as product labeling and package inserts; or if it does appear in such documents, an event in which the specificity, severity or duration is not consistent with the risk information included therein; or

• The observed rate of occurrence is a clinically significant increase in the expected rate (based on a credible baseline rate for comparison); or

• The occurrence is not consistent with the expected natural progression of any underlying disease, disorder, or condition of the subject(s) experiencing the adverse event and the subject's predisposing risk factor profile for the adverse event.

Unanticipated problem involving risks to subjects or others: An unanticipated problem involving risks to subjects or others is an event, which was not expected to occur and which increases the degree of risk posed to research participants.

Such events, in general, meet all of the following criteria:

• unexpected

• related or possibly related to participation in the research

• suggests that the research places subjects or others at a greater risk of harm (including physical, psychological, economic, or social harm) than was previously known or recognized.

Although some adverse events will qualify as unanticipated problems involving risks to subjects or others, some will not; and there may be other unanticipated problems that go beyond the definitions of serious and/or unexpected adverse events. Examples of unanticipated problems involving risks to subjects or others include improperly staging a participant's tumor resulting in the participant being assigned to an incorrect arm of the research study; the theft of a research computer containing confidential subject information; and the contamination of a study drug. Unanticipated problems generally will warrant consideration of substantive changes in the research protocol or informed consent process/document or other corrective actions in order to protect the safety, welfare, or rights of subjects or others.

This is an investigator-initiated study. The PI and Montefiore/Einstein are conducting the study and acting as the sponsor. Therefore, the legal/ethical obligations of the PI include both those of a sponsor and those of an investigator.

9.4 Recording Adverse Events and Serious Adverse Events

AEs will be evaluated and documented by the clinical staff and investigators throughout inpatient hospitalizations and each outpatient visit. CRAs are responsible for reviewing documentation related to AEs and entering directly into the protocol-specific database for all clinically significant non-hematologic adverse events grade 2, and all grade 3 or higher. The data to be recorded are 1) the event description, 2) the NCI CTCAE v5.0 code and grade, 3) the onset date, 4) the resolution date (or ongoing if it has not resolved at time of off study), 4) action

taken for event, 5) patient outcome 6) attribution (relationship) of AE to protocol treatment/interventions, 7) if AE was expected or unexpected, and 8) comments, if applicable. AEs that are classified as serious, unexpected, and at least possibly related will be notated as such in the database as SAEs. These events will be reported expeditiously to the Einstein IRB within the timeframes as described above.

Attribution is the relationship between an adverse event or serious adverse event and the study treatment. Attribution will be assigned as follows:

• Definite – The adverse event is clearly related to the study treatment.

• Probable – The adverse event is likely related to the study treatment.

• Possible – The adverse event may be related to the study treatment.

• Unlikely – The adverse event is doubtfully related to the study treatment.

• Unrelated – The adverse event is clearly NOT related to the study treatment.

Cumulative summary of Grade 2 clinically relevant events and all Grades 2-5 events will be reported as part of the progress reports to IRB at the time of continuing review. Specific data entry instructions for AEs and other protocol-related data will be documented in protocol-specific data entry guidelines, which will be developed and maintained by study team. The study team will meet regularly to discuss AEs. The PI will review AE reports generated from the research database, and corrections will be made if applicable. Once the information is final the PI will sign and date reports, to acknowledge his review and approval of the AE as entered in the research database.

10.0 REGULATORY CONSIDERATIONS

10.1 Protection of Human Subjects

The Investigator must ensure that patients or their legally acceptable representatives are clearly and fully informed about the purpose, potential risks and other critical issues regarding clinical trials in which they volunteer to participate. Preparation of the consent form is the responsibility of the Investigator and must include all elements required by CFR 21 Part 50.25 and the local IRB.

10.2 Compliance with the Protocol

Protocol compliance will be assessed as well as the accuracy and completeness of all data points for the first three study enrollees, then at least one participant per dose level for the phase I cohort quarterly and at least 15% of participants in the expansion cohort every six months.

Regulatory compliance will be reviewed every six months during active enrollment. Accrual will be tracked continuously, and the appropriateness of SAE reporting will be assessed on all participants. The Eligibility Coordinator(s) will verify 100% of the informed consent documentation on all participants and verify 100% of participants' eligibility status within 5 working days of the completion of enrollment.

Continuing reviews by the IRB will occur at least annually. In addition, SAE reports in TRACKS are reviewed in a timely manner by the IRB.

11.0 DATA HANDLING AND RECORD KEEPING

11.1 Confidentiality

Information about study subjects will be kept confidential and managed according to the requirements of the Health Insurance Portability and Accountability Act of 1996 (HIPAA). Those regulations require a signed subject authorization informing the subject of the following:

- What protected health information (PHI) will be collected from subjects in this study
- Who will have access to that information and why
- Who will use or disclose that information
- The rights of a research subject to revoke their authorization for use of their PHI.
- In the event that a subject revokes authorization to collect or use PHI, the investigator, by regulation, retains the ability to use all information collected prior to the revocation of subject authorization. For subjects that have revoked authorization to collect or use PHI, attempts should be made to obtain permission to collect at least vital status (i.e. that the subject is alive) at the end of their scheduled study period.

11.2 Source Documents

Source data is all information, original records of clinical findings, observations, or other activities in a clinical trial necessary for the reconstruction and evaluation of the trial. Source data are contained in source documents Examples of these original documents, and data records include: hospital records, clinical and office charts, laboratory notes, memoranda, subjects' diaries or evaluation checklists, pharmacy dispensing records, recorded data from automated instruments, copies or transcriptions certified after verification as being accurate and complete, microfiches, photographic negatives, microfilm or magnetic media, x-rays, subject files, and records kept at the pharmacy, at the laboratories, and at medico-technical departments involved in the clinical trial.

11.3 Data Safety and Monitoring Board

The Albert Einstein College of Medicine/Albert Einstein Cancer Center Data Safety Monitoring Committee (DSMC) has the responsibility for ensuring data and safety monitoring along with the PI, who is ultimately responsible for the ongoing monitoring and safety of clinical protocols. The primary functions of the AECC DSMC are as follows:

1. To review and ensure protocol compliance with dose escalation in phase I trials

2. To review/assure protocol compliance for all trials that have two-stage phase II designs,

3. Reviewing all internal and external serious adverse reports, investigator alerts, action letters, and other safety reports for trials being performed at AECC- affiliated institutions

4. To implement and to determine the adequacy of DSM plans of all approved protocols.

The DSMC is an independent committee and meets on a monthly basis. During its monthly meeting, the DSMC will review serious (grade 3 or higher) adverse events from this study. In the event that the DSMC decides that a protocol revision is warranted, the committee will immediately notify the principal investigator of this study. The DSMC has the authority to close trials to patient accrual should the risk to patients be excessive or outweigh the potential benefits of the study. All study suspensions and closures will be forwarded to the IRB/CCI and study sponsor from the DSMC. The DSMC will review all safety data from Phase 1b before the study proceeds to an expansion cohort.

12.0 STATISTICAL METHODS

12.1 Statistical design

This is a phase 1b, dose escalation study of “3+7” chemotherapy + venetoclax in patients with AML. The primary objective is to evaluate the safety and tolerability of the combination therapy and determine the maximum tolerated dose (MTD). Once MTD is determined, additional patients will be treated in expansion cohorts to further assess safety and clinical activity of the combination at the MTD and a dose below the MTD.

12.2 Sample Size

In Part 1 of the study (dose escalation), up to a maximum of 24 patients (including backfill cohorts) equal or below the age of 60 years and 18 patients between 61 and 75 years of age may be enrolled. Once the MTD is reached, and assuming the MTD is not the starting dose, a maximum of 28 additional patients in each age group will be enrolled in Part 2 (dose expansion phase) and randomized 1:1 ratio to the MTD for that age group and the starting dose, to further evaluate safety and efficacy of the study drug combination and identify the optimal phase 2 dose. The intention of expanding more than one dose is to further study a dose other than MTD as a potentially safe and effective alternative to the MTD, in line with FDA Project Optimus{FDA, 2022-D-2827 #44}. Randomization will be used in this Phase 1 trial to achieve balance in patient characteristics across dose groups (Iasonos and O'Quigley 2021). The computer-generated randomization list for each age cohort will be provided by the study statistician. The total sample size at each of these dose levels in each age group will be 20 patients (14 from dose expansion phase + 6 from dose escalation phase). If the MTD is the starting dose, then a maximum of 14 additional patients will be enrolled in an expansion cohort at that dose only for a total of 20 patients. The sample size of the expansion cohort was based primarily on feasibility considerations. Formal power calculations were not performed because hypothesis testing will not be conducted to compare dose groups; the selection of the recommended Phase 2 dose will be based on considering both the overall safety and response profiles as stated in section 12.3.1. However, to indicate the precision with which the DLT rate can be estimated with a maximum total of 20 patients, if 3 DLTs are observed, the estimated DLT rate is 15% with a corresponding 90% confidence interval (CI) by the Clopper-Pearson method of 4.2% to 34.4%; if 4 DLTs are observed (20%), the 90% CI will be 7.1% to 40.1%.

Bayesian toxicity monitoring (https://trialdesign.org/one-page-shell.html#BTOX) will be used to terminate early any of the dose expansion cohorts for excessive toxicity. The maximum allowable DLT rate in this phase is assumed to be 20%. Early stopping of a dose expansion will

occur if the posterior probability that the DLT rate exceeds this maximum allowable rate is 80% or greater. The toxicity stopping boundaries and summary of operating characteristics of this monitoring plan are given below. If the true DLT rate is 30% or higher, the probability of stopping the expansion cohort early is at least 68%:

Toxicity Stopping Boundaries

# Patients (inclusive)	# Toxicities (inclusive) are considered too toxic	Actions
3	2 - 3	Early stopping
6	2 - 6	Early stopping
9	3 - 9	Early stopping
12	4 - 12	Early stopping
14	5 - 14	Reach to Nmax

Summary of Operating Characteristics

Scenario	Prob.Of.Tox	Prob.Early.Stop	Prob.Declare.Tox	Avg.N.Patients	Avg.N.Tox	C
1	0.1	0.1279	0.1283	12.9432	1.2943	
2	0.2	0.4084	0.4123	10.6744	2.1349	
3	0.3	0.6862	0.6951	8.2951	2.4885	
4	0.5	0.9622	0.9677	5.0854	2.5427	

Assuming two doses are evaluated in the expansion phase in each age cohort, if enrollment into the dose expansion cohort at the MTD is stopped early due to excessive toxicity, then all subsequent subjects will be assigned only to the dose below the MTD until either the maximum size of the expansion cohort of 14 patients or the stopping boundary is reached for that cohort. If the dose below the MTD is terminated first for excessive toxicity, then enrollment at the MTD will also be terminated at the same time.

12.3 Statistical analysis

12.3.1 Primary Endpoint Analysis

The number and percentage of patients with AEs, including AEs leading to dose modifications or discontinuations, and serious AEs (SAEs) will be summarized overall and by age group and dose level. Laboratory values and changes in values from baseline will be summarized descriptively by week, and shift tables will be provided showing changes in National Cancer Institute (NCI) CTCAE (version 5) grade from baseline to worst grade postbaseline. Descriptive summaries will be provided for vital sign values and changes in values from baseline. The number and percentage of patients with dose modifications will be summarized. The DLT rate and corresponding 90% CI will be estimated using the Clopper-Pearson method with all available data at the MTD, and if relevant, also for a dose below. If two dose levels are evaluated in the dose expansion phase, the groups will not be formally compared statistically given the limited sample sizes. The selection of the RP2D will be based on considering both the overall safety and response profiles.

12.3.2 Secondary Endpoint Analysis

The rates of CR, MRD-negative CR/CRi/CRh (CR/CRi/CRh$_{MRD-}$), CR/CRi , CR/CRh, OR and 95% Cis will be estimated using the Clopper-Pearson method.

Kaplan-Meier estimates of median PFS and OS, median duration of CR, median duration of CR/CRi, and median duration of CR/CRh will also be computed as well as corresponding 95% CIs.

12.3.3 Analysis of exploratory objective

The frequency/percentage of LSCs present measured by “high-resolution LSC MRD” at baseline and after every treatment cycle will be summarized with descriptive statistics.

13.0 OBTAINING INFORMED CONSENT

13.1 Informed Consent Prior to Research Interventions

Before a subject’s participation in the clinical study, the investigator is responsible for obtaining written informed consent from the subject or legally acceptable representative after adequate explanation of the aims, methods, anticipated benefits, and potential hazards of the study and

before any protocol-specific screening procedures or any investigational products are administered. A legally acceptable representative is an individual or other body authorized under applicable law to consent, on behalf of a prospective subject, to the subject's participation in the clinical study.

The acquisition of informed consent should be documented in the subject's medical records, and the informed consent form should be signed and personally dated by the subject or a legally acceptable representative and by the person who conducted the informed consent discussion. The original signed informed consent form should be retained in accordance with institutional policy, and a copy of the signed consent form should be provided to the subject or legally acceptable representative.

The study team would be seeking a waiver of consent to access records for recruitment from the IRB.

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APPENDIX: ADVERSE EVENTS COMMONLY ASSOCIATED WITH LEUKEMIA

Fever

Fatigue

Dyspnea

Pain, all types

Thrombocytopenia

Anemia

Neutropenia

Infection (bacterial, viral, fungal)

Neutropenic infection

Neutropenic sepsis

Oral candidiasis

Stomatitis

Periodontal infection

Tooth infection, abscess

Upper respiratory tract infection

Sinusitis, Rhinitis

Bronchitis (bacterial, viral)

Bronchitis chronic

Pneumonia (bacterial, viral, fungal)

Catheter site cellulitis

Herpes zoster disseminated, multi-dermatomal

Herpes zoster

Herpes simplex (oral, genital)

Skin candida

Urinary tract infection bacterial, fungal

Genitourinary tract infection (viral, bacterial, fungal)

Gastroenteritis

Enterocolitis

Malignant disease progression, including death

Hyperleukocytosis, including the symptomatic form (leukostasis)

Leukemic infiltration brain

Malignant pleural effusion

Chloroma

Bleeding

Gingival bleeding

Mouth hemorrhage

Epistaxis

Hematuria

Injection site hemorrhage

Petechiae

Skin hemorrhage

Retinal hemorrhage

Population-Related Comorbidities

Hypertension

Rheumatoid arthritis/osteoarthritis

Hyperlipidemia

Peptic ulcer

Coronary artery disease

Valvular disease

Atrial fibrillation

Diabetes mellitus

Chronic obstructive pulmonary disease

Cerebrovascular accident

Transient ischemia attack