Supplemental Material

Table of Contents

Supplemental Table 1. Study Patient Disposition

Patient Disposition	N (%)
# total enrolled patients	27 (100)
# received Ven/FluBu2 conditioning	27 (100)
# did not initiate maintenance	5 (18.5)
Relapse, n (%)	3 (60)
Withdrawal, n (%)	1 (20)
Provider preference, n (%)	1 (20)
# received Ven/Aza maintenance	22 (81.5)
Dose Level 1 (42 days)	11 (50)
Dose Level 2 (28 days)	11 (50)
Death within 30 days of enrollment, n (%)	0 (0)
Death within 60 days of enrollment, n (%)	0 (0)

Supplemental Table 2. All treatment-emergent hematologic and non-hematologic toxicities (intent-to-treat, N=27)

Toxicity Grade CTCAE v5.0	Dose Level								All N=27			
	Dose Level 1 n=13				Dose Level 2 N=14							
	1	2	3	4	1	2	3	4	1	2	3	4
	N	N	N	N	N	N	N	N	N	N	N	N
HEMATOLOGIC												
Anemia	4	-	4	-	2	5	6	-	6	5	10	-
Neutrophil count decreased	-	2	1	8	1	-	1	11	1	2	2	19
Platelet count decreased	1	2	5	3	-	1	3	9	1	3	8	12
WBC decreased	-	-	4	7	-	-	1	12	-	-	5	19
NON-HEMATOLOGIC												
Abdominal pain	1	-	-	-	-	-	-	-	1	-	-	-
ALT increased	5	1	-	-	4	1	-	-	9	2	-	-
ALK Phos increased	2	-	-	-	2	-	-	-	4	-	-	-
Anorexia	1	1	-	-	3	-	-	-	4	1	-	-
Arthralgia	-	-	-	-	1	1	-	-	1	1	-	-
AST increased	2	-	-	-	4	-	-	-	6	-	-	-
Bloating	-	-	-	-	1	-	-	-	1	-	-	-
Blood bilirubin increased	1	1	-	-	-	-	-	-	1	1	-	-
Bone pain	-	-	-	-	1	-	-	-	1	-	-	-
Constipation	1	-	-	-	4	-	-	-	5	-	-	-
Cough	-	-	-	-	1	-	-	-	1	-	-	-
Creatinine increased	3	-	-	-	6	2	-	-	9	2	-	-
Diarrhea	7	2	-	-	8	1	-	-	15	3	-	-
Dizziness	1	-	-	-	2	-	-	-	3	-	-	-
Dysgeusia	3	-	-	-	2	-	-	-	5	-	-	-
Dyspepsia	-	-	-	-	2	-	-	-	2	-	-	-
Dyspnea	1	1	-	-	-	-	-	-	1	1	-	-
Dysuria	-	-	-	-	1	-	-	-	1	-	-	-
Fatigue	2	3	-	-	11	-	-	-	13	3	-	-
Febrile neutropenia	-	-	-	-	-	-	1	-	-	-	1	-
Headache	1	-	-	-	-	-	-	-	1	-	-	-
Hyperkalemia	1	-	-	-	1	1	-	-	2	1	-	-
Hyperuricemia	1	-	-	-	1	-	-	-	2	-	-	-
Hypocalcemia	-	-	-	-	3	1	-	-	3	1	-	-
Hypomagnesemia	1	1	-	-	4	-	-	-	5	1	-	-
Lip pain	1	-	-	-	-	-	-	-	1	-	-	-
Mucositis oral	-	-	-	-	4	-	-	-	4	-	-	-
Nail discoloration	-	-	-	-	1	-	-	-	1	-	-	-
Nausea	3	3	-	-	9	3	-	-	12	6	-	-
Parainfluenza infection	-	-	-	-	1	-	-	-	1	-	-	-
Peripheral sensory neuropathy	-	-	-	-	1	-	-	-	1	-	-	-
Pruritus	1	-	-	-	1	-	-	-	2	-	-	-
Rash maculo-papular	1	1	-	-	-	-	-	-	1	1	-	-
Urinary frequency	-	-	-	-	1	-	-	-	1	-	-	-
Vomiting	-	1	-	-	1	-	-	-	1	1	-	-
Weight loss	-	-	-	-	2	-	-	-	2	-	-	-

All 27 patients enrolled into trial are included regardless of maintenance receipt (intent-to-treat). ALT, Alanine aminotransferase; Alk Phos, Alkaline phosphatase; AST, Aspartate aminotransferase; WBC, White blood cell

Supplemental Table 3. Absolute neutrophil counts on maintenance. Median neutrophil counts (K/μL) and inter-quartile ranges are provided at each timepoint per dose level.

Absolute Neutrophil Count (K/μL)								
	Dose Level 1				Dose Level 2			
	N	Median	Q1	Q3	N	Median	Q1	Q3
Screen	11	3.3	2.2	7.5	11	4.3	3.6	5.7
C1D1	11	4.1	2.2	4.7	11	4.2	3.6	5.2
C1D8	11	2.7	2.5	4.1	11	3.6	2.7	4.7
C1D15	11	1.4	1.2	3.0	11	2.7	1.6	3.4
C1D22	11	1.1	0.54	2.1	11	2.2	0.97	2.8
C2	20	2.4	1.3	1.8	20	2.2	3.6	3.0
C3	14	2.1	1.6	1.7	20	2.3	3.0	2.8
C4	12	2.4	1.3	2.2	16	2.5	4.3	2.7
C5	10	2.5	1.6	2.1	15	2.6	3.5	3.1
C6	9	2.5	1.9	1.7	14	2.1	3.8	3.0
C7	9	2.4	1.3	5.1	12	2.5	0.98	3.9
C8	7	2.3	1.0	2.7	11	2.9	1.4	4.4
C9	-	-	-	-	12	2.5	1.8	3.8
C10	-	-	-	-	10	1.8	1.7	3.1
C11	-	-	-	-	10	2.1	1.9	6.1
C12	-	-	-	-	10	2.3	1.7	3.5

Day 1 and day 15 labs are shown at whole cycles for cycle 2 on-ward.

Supplemental Table 4. Platelet counts on maintenance. Median platelet counts (K/μL) and inter-quartile ranges are provided at each timepoint per dose level.

Platelet Level (K/μL)								
	Dose Level 1				Dose Level 2			
	N	Median	Q1	Q3	N	Median	Q1	Q3
Screen	11	97	85	140	11	150	99	202
C1D1	11	104	83	135	11	104	93	184
C1D8	11	104	78	122	11	124	80	171
C1D15	11	88	62	104	11	104	58	147
C1D22	11	115	68	127	11	128	106	179
C2	20	105	77	133	20	114	98	178
C3	14	109	76	147	20	120	90	180
C4	12	137	113	210	16	136	88	198
C5	9	117	95	212	16	113	82	200
C6	9	132	103	216	14	108	69	199
C7	9	148	97	230	12	115	91	195
C8	7	102	98	210	11	113	93	176
C9	-	-	-	-	12	119	92	177
C10	-	-	-	-	10	145	87	196
C11	-	-	-	-	10	151	95	199
C12	-	-	-	-	10	157	108	211

Day 1 and day 15 labs are shown at whole cycles for cycle 2 on-ward.

Supplemental Table 5. Individual Patient Chimerism Status

Study ID	Disease Type	Ven/Aza Maint. (Y/N)	D28 CD3+ T-cells (%)	D28 marrow chimerism (%)	D100 CD3+ T-cells (%)	D100 marrow chimerism (%)	6 mo. CD3+ T-cells (%)	6 mo. Marrow chimerism (%)	12 mo. CD3+ T-cells (%)	12 mo. marrow chimerism (%)	Relapse* (Y/N)
23	sAML	Yes	86	100	86	98	93	100	99	100	No
24	AML	Yes	49	n/a	84	99	100	100	100	100	Yes
25	AML	Yes	86	98	88	98	98	99	95	99	No
26	t-AML	Yes	91	100	99	97	99	85	-	-	Yes
27	MDS	No	19	97	79	12	-	-	-	-	Yes
28	MDS	Yes	87	100	95	99	86	72	-	-	Yes
29	AML	No	70	47	80	2	85	5	-	-	Yes
30	t-MDS	Yes	79	98	81	98	90	100	100	100	No
31	MDS	Yes	52	100	65	99	71	99	98	100	No
32	MDS	Yes	47	94	60	91	61	22	-	-	Yes
33	AML	Yes	93	n/a	97	99	99	100	100	100	No
34	MDS/ MPN	Yes	68	96	64	99	71	98	100	100	No
35	AML	Yes	16	99	38	85	-	-	-	-	Yes
36	AML	Yes	57	100	68	95	96	100	100	100	No
37	MDS	Yes	27	89	64	78	-	-	-	-	Yes
38	MDS	No	69	4	-	-	-	-	-	-	Yes
39	MDS	Yes	75	96	82	98	85	68	-	-	Yes
40	AML	Yes	87	99	99	100	100	100	100	100	No
41	t-MDS	Yes	100	98	100	96	100	100	100		Yes
42	MDS	Yes	49	98	91	97	100	100	n/a	100	No
43	MDS	No	48	85	82	72	-	-	-	-	Yes
44	t-AML	No	75	99	92	99	100	100	100	100	No
45	MDS	Yes	53	99	69	97	72	96	100	100	No
46	MDS	Yes	66	99	86	99	n/a	100	n/a	100	No
47	t-MDS	Yes	83	n/a	97	96	n/a	100	n/a	100	No
48	t-MDS	Yes	80	98	83	63	83	41	99	100	Yes
49	MDS	Yes	15	96	41	98	n/a	100	64	98	No

Supplemental Table 6. Individual Patient MRD Status

Study ID	Disease Type	Ven/Aza Maint. (Y/N)	TP53m at entry (Y/N)	Pre-HCT Flow MRD	Pre-HCT Molecular MRD	D28 Flow MRD	D28 Molecular MRD	D100 Flow MRD	D100 Molecular MRD	6 mo. Flow MRD	6 mo. Molecular MRD	12 mo. Flow MRD	12 mo. Molecular MRD	Relapse* (Y/N)
23	sAML	Yes	No	Positive	n/a	Negative	Negative	Negative	Negative	n/a	Negative	Negative	Negative	No
24	AML	Yes	Yes	Negative	Positive	n/a	Negative	Positive	Positive	Negative	Negative	Positive	Positive	Yes
25	AML	Yes	Yes	Negative	Positive	Negative	Positive	Negative	Negative	Negative	Negative	Negative	Negative	No
26	t-AML	Yes	Yes	Positive	Positive	Negative	Negative	Positive	Positive	n/a	n/a	n/a	n/a	Yes
27	MDS	No	No	Negative	Positive	n/a	Positive	n/a	n/a	n/a	n/a	n/a	n/a	Yes
28	MDS	Yes	Yes	Positive	Negative	Negative	Negative	Negative	Negative	Negative	Negative	n/a	n/a	Yes
29	AML	No	No	Positive	Positive	n/a	Positive	Positive	n/a	n/a	n/a	n/a	n/a	Yes
30	t-MDS	Yes	No	Positive	Positive	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	No
31	MDS	Yes	Yes	Negative	Positive	Negative	Negative	Negative	Negative	Negative	Positive	Negative	Negative	No
32	MDS	Yes	Yes	Positive	Positive	Negative	Positive	Positive	Positive	Positive	Positive	Positive	n/a	Yes
33	AML	Yes	No	Negative	Positive	Negative	Negative	Positive	Positive	Negative	Negative	Negative	Negative	No
34	MDS/ MPN	Yes	No	Negative	Positive	Negative	Positive	Negative	Positive	Negative	Positive	Negative	Negative	No
35	AML	Yes	No	Positive	Positive	Negative	Negative	Positive	Positive	n/a	n/a	n/a	n/a	Yes
36	AML	Yes	Yes	Positive	Positive	Negative	Negative	Negative	Negative	Negative	Negative	Negative	n/a	No
37	MDS	Yes	Yes	Positive	Positive	Negative	Positive	Negative	Positive	n/a	n/a	n/a	n/a	Yes
38	MDS	No	No	Positive	Positive	Positive	Positive	n/a	n/a	n/a	n/a	n/a	n/a	Yes
39	MDS	Yes	Yes	Negative	Positive	Negative	Negative	Negative	Positive	Positive	Positive	n/a	n/a	Yes
40	AML	Yes	Yes	Negative	Positive	Negative	Positive	Negative	Positive	Negative	Negative	Negative	Positive	No
41	t-MDS	Yes	Yes	Positive	Positive	Positive	Negative	Positive	Positive	n/a	n/a	n/a	n/a	Yes
42	MDS	Yes	No	Positive	Positive	Negative	Negative	Positive	Positive	Negative	Negative	Negative	Negative	No
43	MDS	No	Yes	Positive	Positive	Positive	Positive	Positive	n/a	n/a	n/a	n/a	n/a	Yes
44	t-AML	No	No	Negative	Positive	Negative	Positive	Negative	Negative	Negative	Negative	Negative	Negative	No
45	MDS	Yes	Yes	Positive	Positive	Negative	Positive	Negative	Positive	Negative	Positive	Negative	Negative	No
46	MDS	Yes	No	Negative	Positive	Negative	Positive	Negative	Negative	Negative	Negative	Negative	Negative	No
47	t-MDS	Yes	Yes	Positive	Positive	Negative	Negative	Positive	Positive	Negative	Negative	Negative	Negative	No
48	t-MDS	Yes	Yes	Negative	Positive	Positive	Negative	Positive	Positive	Positive	n/a	n/a	n/a	Yes
49	MDS	Yes	Yes	Negative	Positive	Negative	Positive	Negative	Negative	Negative	n/a	Negative	n/a	No

*At time of July 31, 2023 data-cut

Disconcordant flow/molecular MRD at matched timepoints are shaded in light blue.

Positive flow MRD in purple indicates cases where MRD was detected below <0.1%.

Supplemental Table 7. QOL assessment during Ven/Aza maintenance therapy according to FACT-BMT. Surveys were conducted at screening (on first day of conditioning/ pre-transplant; study entry) and then on cycle 1 day 1 (C1D1), cycle 2 day 1 (C2D1), and cycle 4 day 1 (C4D1) of Ven/Aza maintenance.

FACT-BMT

	Baseline			C1D1			C2D1			C4D1		
	N	Mean	Std	N	Mean	Std	N	Mean	Std	N	Mean	Std
PWB	13	22.9	4.5	15	21.7	3.8	13	20.0	5.8	13	23.7	3.7
SWB	13	26.6	2.1	15	23.7	3.5	13	24.7	3.6	13	25.5	3.2
EWB	13	19.0	4.1	15	19.3	3.2	12	19.8	3.1	13	19.5	3.3
FWB	13	17.3	4.9	15	12.6	4.3	12	14.7	6.0	12	19.3	4.8
FACTG	13	85.8	11.4	15	77.2	10.8	12	78.9	14.3	12	88.8	11.1
BMTS	13	28.6	5.1	15	27.3	5.4	13	26.2	6.1	13	29.3	4.5
TOTAL	13	114.4	14.8	15	104.6	15.0	12	104.7	19.8	12	118.7	14.5
TOI	13	68.8	11.7	15	61.6	10.9	12	60.0	15.6	12	73.2	10.7

FACT-BMT differences between baseline and C1D1, C2D1, or C4D1

	Baseline-C1D1				Baseline -C2D1				Baseline -C4D1			
	N	Mean	Std	p-value	N	Mean	Std	p-value	N	Mean	Std	p-value
d_PWB	10	1.03	4.75	0.73	8	1.49	3.16	1	8	1	4.34	1
d_SWB	10	2.7	2.58	0.039	8	2.23	3.16	0.13	8	2.1	3.58	0.38
d_EWB	10	0.9	2.92	0.29	8	-0.63	1.92	1	8	-1.38	3.02	0.45
d_FWB	10	5.74	3.43	0.002	8	4.16	4.41	0.13	8	-0.33	5.34	1
d_FACTG	10	10.37	8.35	0.02	8	7.25	10.13	0.29	8	1.4	10.76	0.73
d_BMTS	10	0.86	4.28	1	8	0.92	4.23	1	8	-0.16	4.36	1
d_TOTAL	10	11.22	12	0.11	8	8.17	12.65	0.29	8	1.24	12.35	1
d_TOI	10	7.62	9.14	0.11	8	6.57	8.8	0.29	8	0.52	9.74	1

Supplemental Table 8. Gene List.

ASXL1
ATM
BCOR
BCORL1
BRAF
CALR
CBL
CEBPA
CREBBP
CSF3R
CSNK1A1
CUX1
DDX41
DNMT3A
EP300
ETNK1
ETV6
EZH2
FLT3
GATA2
GNAS
GNB1
IDH1
IDH2
IKZF1
JAK2
KIT
KRAS
MPL
MYC
NF1
NFE2
NPM1
NRAS
PDS5B
PHF6
PPM1D
PRPF8
PTPN11
RAD21
RIT1
RUNX1
SETBP1
SF3B1
SH2B3
SMC1A
SMC3
SRSF2

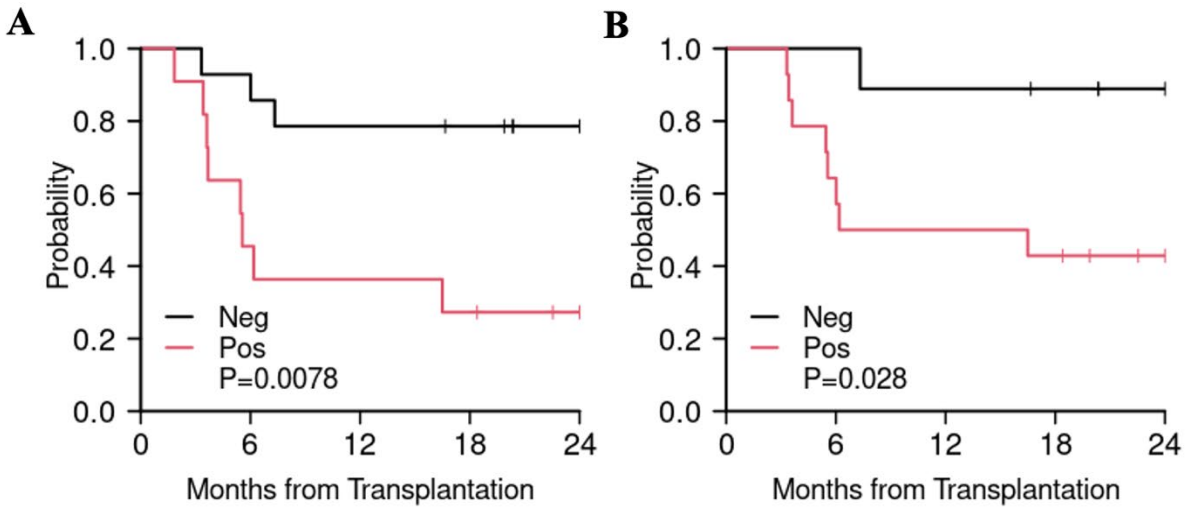
STAG2
TERT
TET2
TP53
U2AF1
WT1
ZRSR2

Supplemental Table 9. Conjugated monoclonal antibodies used in immune monitoring panels.

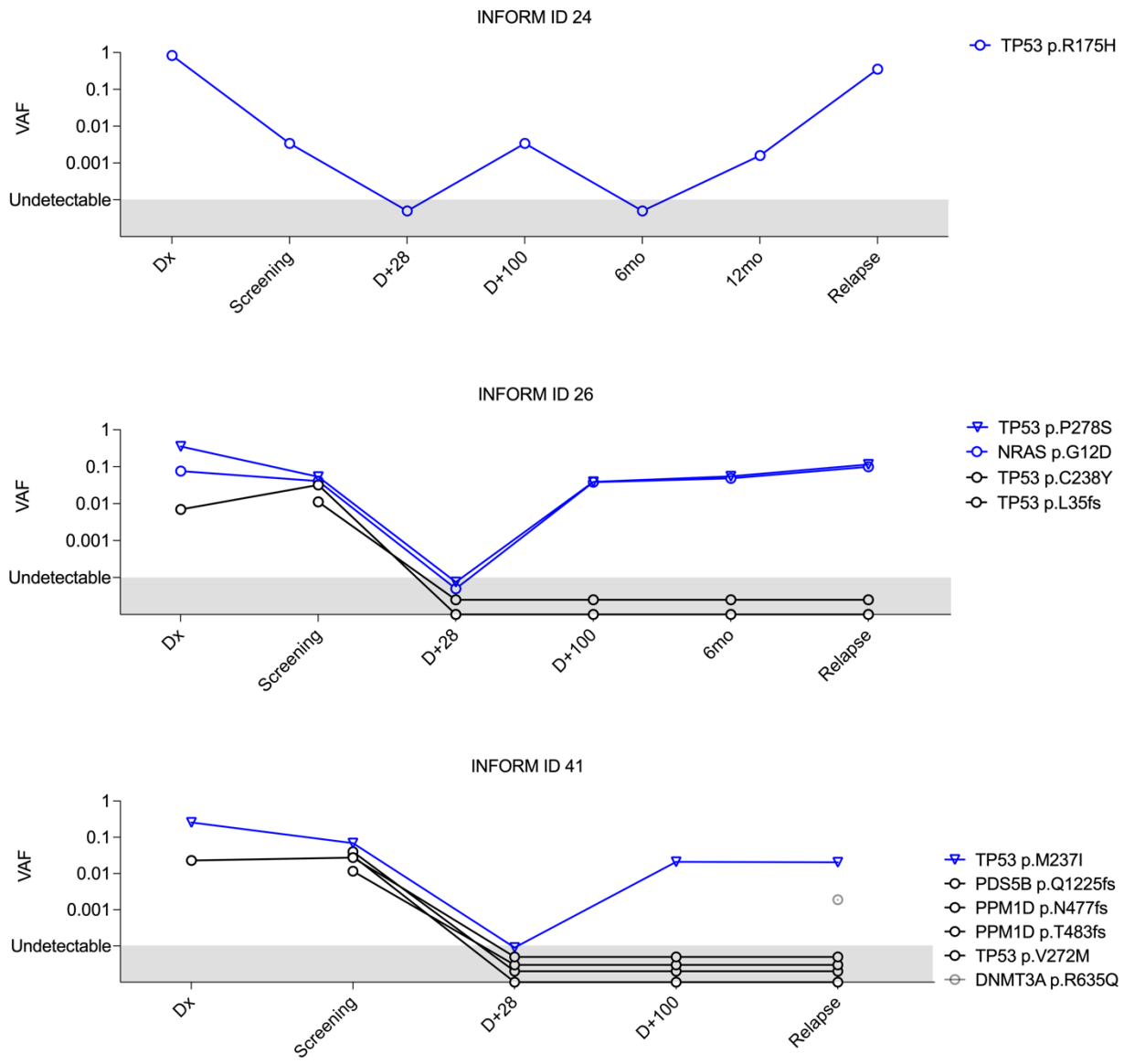
TUBE	TARGET	FLUOROCHROME	CLONE
T Cells	**CD25**	FITC	m-A251
	PD-1	PE	J105
	CD95	PeCF594	DX2
	TCR αβ	PeCy 7	IP26
	CD127	APC	eBioRDR5
	CD8	Alexa 700	RPA-T8
	TCR γδ	APC Vio 770	11F2
	TIM 3	BV421	F38-2E2
	CD4	BV510	RPA-T4
	CD45RA	BV605	HL100
	CD31	BV650	M-T605
	CCR7	BV711	G043-H7
	CD3	BV786	UCHT1
B Cells	**BAFF-R CD268**	FITC	8A7
	IgD	PE	IA6-2
	CD27	PeCy 5	O323
	CD38	PeCy 7	HIT2
	CD19	APC	HIB19
	CD20	APC Cy 7	2H7
	CD5	BV711	UCHT2
	CD45	BV510	H130
	CD6	BV650	M-T605
	CD3	BV786	UCHT1
	CD166	BV421	3A6
NK Cells	**CD16**	FITC	3G8
	PD-1	PE	J105
	CD95	PeCF594	DX2
	NKG2D	APC	1D11
	CD8	Alexa 700	RPA-T8
	CD3	BV786	UCHT1
	CD56	BV605	NCAM
	CD6	BV650	M-T605
	CD57	PerCP Cy5.5	HNK-1
	NKG2A	BV421	131411
	KIRs (CD158a/CD158b/CD158e)	PeCy7	HP-MA4/DX27/DX9
Dendritic Cells	**CD123**	FITC	AC145
	CD141	PE	1A4
	CD11c	PeCy 5	B-LY6
	LINEAGE (CD3/CD19/CD56/CD14)	PeCy 7	SK7/SJ25C1/B159/M5E2
	CD86	APC	2331 (FUN1)
	CD45	APC Cy 7	2D1
	CD14	BV605	M5E2
	HLA-DR	BV510	G46-6
	PD-L1	BV786	MIH1
	CD166	BV421	3A6

Supplemental Figures

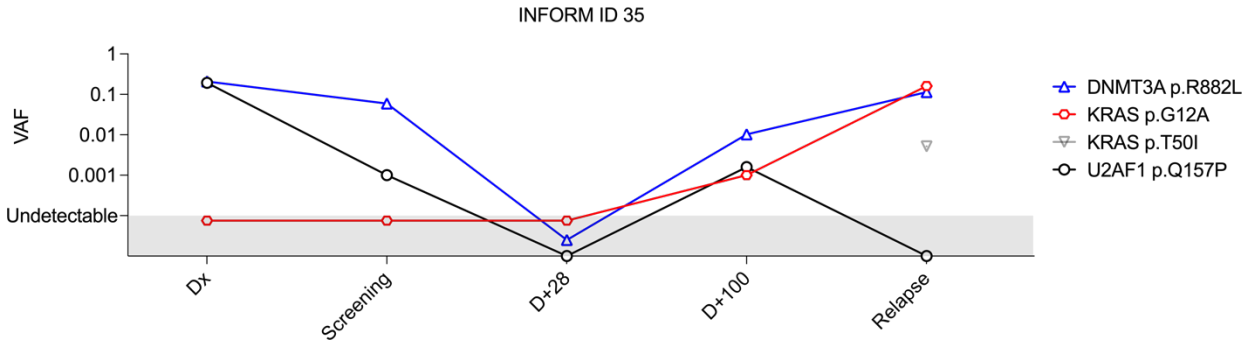
Supplemental Figure 1. Flow- and Molecular-MRD. Swimmer plot for intent-to-treat cohort (N=27) and time to death or last known alive. The accompanying heatmap indicates disease status over time (D0, D28, D100, 6M, 12M), flow and molecular (or NGS)-MRD status, maintenance receipt, and survival status. D0, day 0; D28, day 28 after transplant; D100, day 100 after transplant; 6M, 6 months after transplant; and 12M, 12 months after transplant. Relapse indicated by red filled triangle.



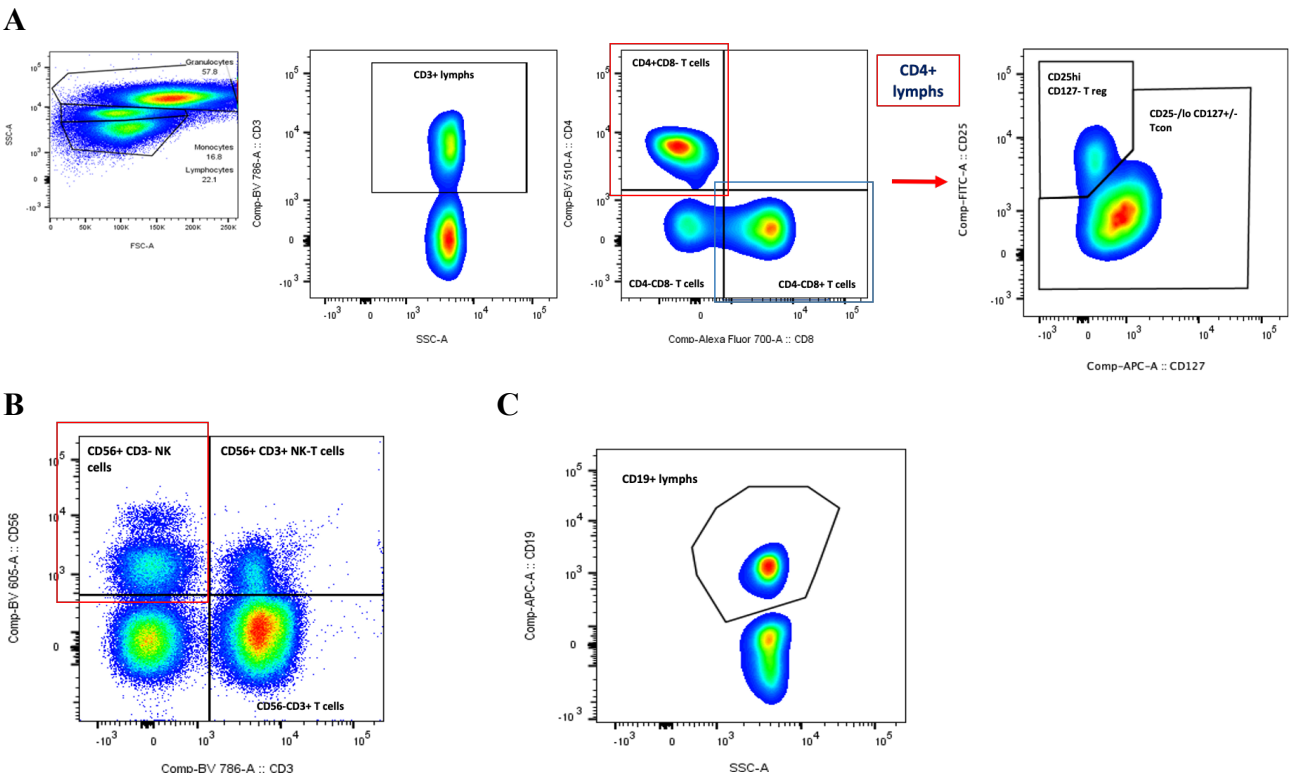
Supplemental Figure 2. Flow and Molecular MRD and Outcome on Maintenance. Shown are K-M curves based on MRD status. PFS based on (A) flow MRD status at day +100 and (B) molecular MRD at day +100.



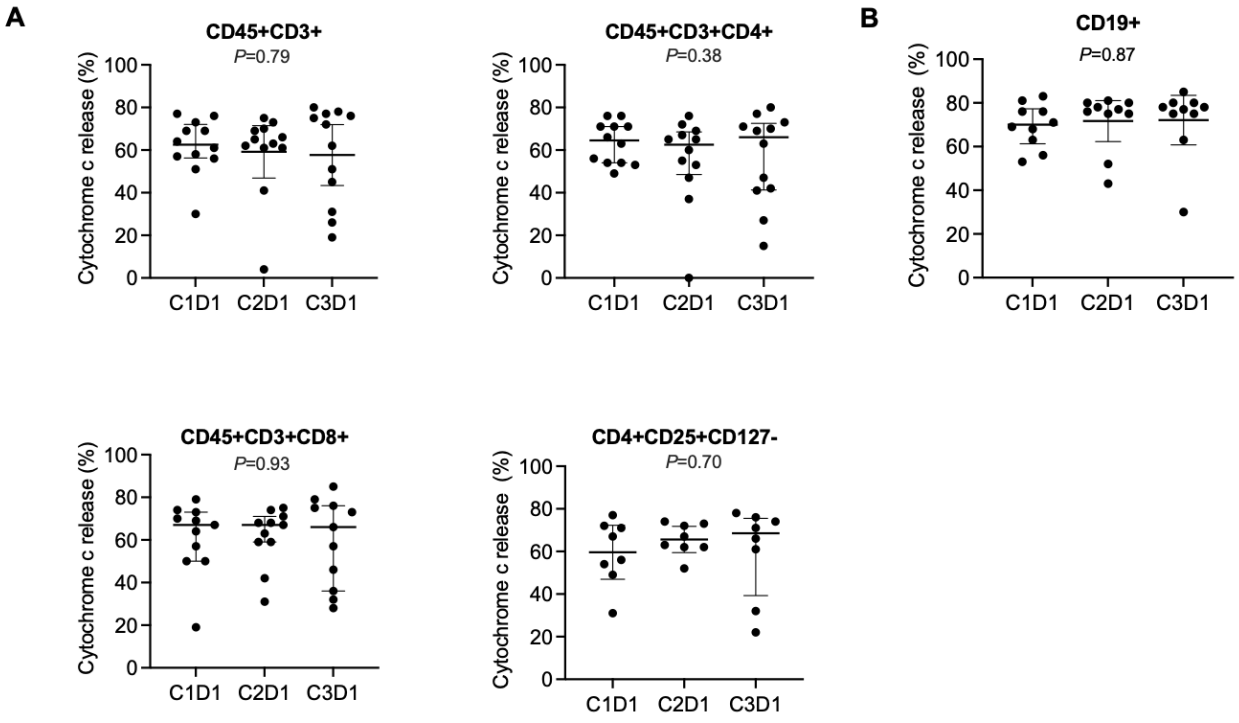
Supplemental Figure 3. Day 28 Molecular MRD negativity in *TP53*-mutated disease. Three patient examples including **(A)** ID 24, **(B)** ID 26, and **(C)** ID 41 with *TP53*-mutated disease and negative molecular MRD assessment at day +28 who subsequently relapsed, suggesting negative molecular MRD at an early time point such as day +28 may be falsely reassuring. Blue symbols and lines indicate pre-alloSCT mutations detected at relapse, black symbols indicate pre-alloSCT mutations not identified at relapse, with gray variants indicating newly detected variants at relapse.



Supplemental Figure 4. Detection of subclonal KRAS mutations at time of relapse after transplant. Emergence of new mutations at day +100 preceding relapse that include MAPK signaling genes (KRAS) that were not previously detectable. Blue symbols and lines indicate pre-alloSCT mutations detected at relapse, black symbols indicate pre-alloSCT mutations not identified at relapse, gray indicates newly detected variants at relapse, and red symbols indicate mutations present at post-HSCT time-points and at relapse.



Supplemental Figure 5. Immune profiling on maintenance. A) Gating strategy for T cell subset analysis. Three major T cell populations, CD4Treg, CD4Tcon, and CD8 T cells, were defined as $CD3^{+}CD4^{+}CD8^{-}CD25^{+}CD127^{-}$, $CD3^{+}CD4^{+}CD8\text{-}CD25^{-/low}CD127^{+/-}$ cells and $CD3^{+}CD4^{-}CD8^{+}$, respectively. **B)** Gating strategy for Nk cells and Nk-T cells. NK cells were defined as CD3-CD56+ lymphocytes and divided in 4 subsets based on the expression of CD56 and CD16: $CD56^{bright}CD16^{-}$, $CD56^{dim}CD16^{+}$, $CD56^{dim}CD16^{-}$ and $CD56^{bright}CD16^{+}$ NK cells. NKT cells were defined as $CD3^{+}CD56^{+}$ lymphocytes. **C)** Gating strategy for B cells. B cells were defined as CD45+CD19+ lymphocytes. See **Supplemental Table 9** for markers used in immune monitoring panels.



Supplemental Figure 6. BH3 profiling immune cells subsets. Peripheral blood samples were collected from patients on Ven/Aza maintenance pre-dose on cycle 1 day 1 (C1D1), cycle 2 day 1 (C2D1), and cycle 3 day 1 (C3D1). Peripheral blood mononuclear cells (fresh) underwent BH3 profiling. At least 50 events/population per well were required for analysis. BH3 peptides (BIM BH3 peptide (0.1 μM)) were used to measure cytochrome c release (to indicate mitochondrial apoptotic priming sensitivity). One-way repeated measures anova for % cytochrome c release across the three timepoints was applied. **(A)** T cells subpopulations were gated using CD45+CD3+, CD45+CD3+CD4+, CD3+CD8+ and CD45+CD3+CD4+ CD25+ CD127- (T regulatory cell subset) immunophenotypic markers. **(B)** B cells populations were gated using CD45+CD19+ immunophenotypic markers. At each timepoint, the bar represents the median cytochrome c release (%) and inter-quartile ranges are provided.

Supplemental Methods

1. Clinical Trial Design

Eligible patient population details: Eligible patients were at least 18 years and included those with an Eastern Cooperative Oncology Group (ECOG) performance status (PS) of 0 to 2, adequate organ function, an available 8/8 HLA-matched related or unrelated donor with peripheral blood stem cells as source, and a diagnosis of one of the following: (1) high-risk AML defined as adverse risk by ELN 2017,[1] secondary/therapy-related or secondary-type ontogeny,[2] or persistent flow MRD (≥ 0.1%); (2) higher-risk MDS defined as International Prognostic Scoring System intermediate-2 or high at diagnosis,[3] therapy-related, or presence of a mutation in *TP53* or in the *RAS* pathway;[4] or (3) high-risk MDS/myeloproliferative neoplasm (MPN) defined as the presence of trisomy 8, chromosome 7 abnormalities, or complex karyotype or a mutation in *ASXL1*. Prior venetoclax therapy was allowed. Patients with MDS or MDS/MPN were required to enter the study with ≤10% marrow blasts, while those with AML were required to have <5% marrow blasts.

Conditioning treatment details: Venetoclax was dosed at 400 mg daily on day -8 to day -2 for a total of 7 doses. Fludarabine 30 mg/m2/day was administered as a bolus intravenous infusion over 30 minutes once a day for 4 days on day -5 to day -2. Busulfan 0.8 mg/kg was administered intravenously twice daily over 3 hours on day -5 to day -2 for a total of 8 doses.

Trial Design Details: A minimum of ten patients per maintenance dose schedule were planned accounting for up to 11% potential drop-out for early events. The recommended phase 2 dose

(RP2D) or best tolerated schedule was determined if ≤ 2 patients experienced DLT. The probability of dose escalation is 0.93 if the true but unknown rate of DLT is 10% but 0.17 if the rate is 40%. With 10 patients at the MTD level, the maximum width of 90% confidence interval for DLT will be within ± 28%.

2. Sequencing and mutational analysis

Clinical and research-level next-generation sequencing (NGS) assays were performed as previously described.[5] Samples were collected at diagnosis, screening (pre-transplant), and post-transplant timepoints. The clinical 88-gene targeted sequencing assay (sensitivity estimated to be 3%) was performed in a Clinical Laboratory Improvement Amendments–certified laboratory in real-time.[6] A research-level duplex NGS assay was used in screening (pre-transplant) and in post-transplant MRD surveillance samples allowing for more sensitive detection of persistent variants. Assays were performed in batches. Mutations identified at diagnosis or at >1% variant allele fraction in the screening/pre-SCT sample were evaluated for persistence after SCT. Any persistent mutation (at least two duplex alternative reads) was considered to be "molecular MRD-positive."

Duplex Next Generation Sequencing: We selected 45 genes for targeted sequencing based on their recurrent alteration in myeloid malignancies (**Supplemental Table 8**). Library construction, quantification, normalization, hybrid selection, cluster amplification, and sequencing were performed as previously described by our group in Garcia et al *Blood Advances* 2021, and are reproduced below.[5]

Library Construction, quantification, and normalization: 500ng genomic DNA was used as input for DNA fragmentation using a Covaris acoustic ultrasonicator, targeting 150 bp fragments. Library preparation was performed using the KAPA Biosystems (KAPA HyperPrep Kit with Library Amplification product KK8504) and IDT's duplex UMI adapters. The libraries were then paired with unique 8-base dual index sequences embedded within the p5 and p7 primers

(IDT) during PCR. Enzymatic cleanups were performed using Beckman Coulter AMPure XP beads. Libraries were quantified using the INvitrogen Quant-It broad range dsDNA quantification assay kit (Thermo Scientific) with a 1:200 PicoGreen dilution. Each library was normalized to 35ng/uL using Tris-HCl, 10 mM, pH 8.0.

Hybrid Selection, Cluster Amplification, and Sequencing: In solution hybrid selection was performed using IDT's XGen hybridization and wash kit, with creation of 12-plex pre-hybridization pools. Custom exome bait (TWIST bioscience) was added to the lyophilized pool prior to resuspension, followed by library normalization and hybridization performed using a Hamilton Starlet liquid handling platform and target capture on an Agilent Bravo automated platform. Post capture, a PCR was performed to amplify the captured DNA. Cluster amplification using Exclusion Amplification cluster chemistry and HISeq amplification was performed according to manufacturer's protocol (Illumina) and using HiSeq X flowcells. Flowcells were sequenced on v2 Sequencing-bySynthesis chemistry for HiSeq X flowcells. The flowcells were then analyzed using RTA v.2.7.3 or later. Each pool of whole genome libraries was run on paired 151 bp runs, reading the dual-indexed sequences to identify molecular indices and sequenced across the number of lanes needed to meet coverage for all libraries in the pool.

Variant calling: Reads were aligned with bwa-mem 0.7.15. Duplex consensus reads were called with fgbio 1.0 and realigned using bwa-mem. Consensus reads were required to have reads from both families αβ and βα, and consensus reads with Ns in excess of 5% of bases were discarded. Read one and two were soft-clipped from the 5′ end by 10 bases to reduce errors due to end repair. Single nucleotide and small insertion and deletion calling was performed with samtools-

0.1.18 mpileup and Varscan 2.2.3. Variants were annotated to include information about cDNA and amino acid changes, sequence depth, number, and percentage of reads supporting the variant allele, population allele frequency in the Genome Aggregation Database (gnomAD). Variants were excluded if they had fewer than two total duplex-reassembled alternate reads at the position or fell outside of the target coordinates, caused synonymous changes, or were recurrent small insertions/deletions at low variant allele fraction adjacent to homopolymer repeat regions. Individual single nucleotide substitutions and small insertions or deletions were evaluated as candidate drivers of myeloid malignancies based on gene-specific characteristics, then curated manually and classified as driver mutations based on genetic criteria and literature review. All interpretation of variants was blinded to clinical characteristics and thus agnostic to variables including age, sex, diagnosis, treatment status, and clinical outcomes; the genetic analysis was completed and locked prior to merging with any clinical data.

3. Flow cytometry

Flow cytometry staining for immune reconstitution analysis was performed on fresh whole blood samples collected at specific timepoints after transplantation (namely, 1 month, 2 months, 3 months, day +100, 6 months and 12 months). Overall, in the experimental cohort, flow cytometry data on paired samples were available on 26 patients of whom 22 received Ven/Aza maintenance on study. Flow cytometry data on a control cohort of 25 patients with AML/MDS that received a RIC matched transplantation with Flu/Bu and a standard GVHD prophylaxis with methotrexate (MTX)-tacrolimus (TAC), were used as comparison.

Four panels of directly conjugated monoclonal antibodies (**Supplemental Table 9**) were used to define functionally distinct immune cell subsets and homeostatic characteristics of each subset. After staining, cells were acquired on a Fortessa LSR flow cytometer (BD) and analyzed using FlowJo™ Software v10. Specific cell gating strategy and markers used for cell subset definition are described in **Supplemental Figure 5**. For analysis, both percentages and absolute numbers of the parental cells were considered. Absolute numbers were calculated from the complete blood cell count (CBC) performed on the same day as flow cytometry analysis.

4. BH3 profiling

BH3 profiling was performed using the Eppendorf epMotion 5075I automated pipetting platform (Eppendorf, Enfield, CT, UA).[7] Bone marrow mononuclear cells were exposed to synthetic BH3 peptides after plasma membrane permeabilization with digitonin and sensitivity to BH3 peptides was measured by cytochrome c release using an iQue Screener Plus VBR flow cytometer, which uses Forecyt version 9 software for acquisition, gating, and data export (Essen Bioscience/Sartorius, Ann Arbor, MI). Cytochrome c (Biolegend 983502) was stained overnight and the median fluorescence intensity (MFI) was calculated for each cell population. FMO served as a control well lacking the cytochrome c antibody. Cytochrome c release was calculated using the following formula:

$$MFI - MFI\ Cyto\ c\ Release = 1 - \frac{MFI_{sample} - MFI_{FMO}}{MFI_{buffer\ alone} - MFI_{FMO}}$$

Values reported are the average of two technical duplicates for every treatment. DMSO and alamethicin were used as negative and positive controls, respectively. Cells were gated based on the following: CD45+CD19+ for B-cells, CD45+CD3+CD8+ for CD8+ T-cells, CD45+CD3+CD4+ for CD4+ T-cells, and CD45+CD3+CD4+CD127-CD25+ for T-regulatory cells. A minimum of 50 events per population per well was required to calculate cytochrome c release. All antibodies were obtained from Biolegend, including cytochrome c FITC (983502), CD3 BV421 (300434), CD127 PE0Dazzle594 (351336), CD25 Alexa647 (356128), CD45 APC-Cy7 (368516), CD4 BV521 (357420), and CD8 BV605 (344742).

Supplemental References

1. Dohner H, Estey E, Grimwade D, et al. Diagnosis and management of AML in adults: 2017 ELN recommendations from an international expert panel. *Blood*. 2017;129(4):424-447.

2. Lindsley RC, Mar BG, Mazzola E, et al. Acute myeloid leukemia ontogeny is defined by distinct somatic mutations. *Blood*. 2015;125(9):1367-1376.

3. Greenberg P, Cox C, LeBeau MM, et al. International scoring system for evaluating prognosis in myelodysplastic syndromes. *Blood*. 1997;89(6):2079-2088.

4. Lindsley RC, Saber W, Mar BG, et al. Prognostic Mutations in Myelodysplastic Syndrome after Stem-Cell Transplantation. *N Engl J Med*. 2017;376(6):536-547.

5. Garcia JS, Kim HT, Murdock HM, et al. Adding venetoclax to fludarabine/busulfan RIC transplant for high-risk MDS and AML is feasible, safe, and active. *Blood Adv*. 2021;5(24):5536-5545.

6. Kluk MJ, Lindsley RC, Aster JC, et al. Validation and Implementation of a Custom Next-Generation Sequencing Clinical Assay for Hematologic Malignancies. *J Mol Diagn*. 2016;18(4):507-515.

7. Ryan J, Montero J, Rocco J, Letai A. iBH3: simple, fixable BH3 profiling to determine apoptotic priming in primary tissue by flow cytometry. *Biol Chem*. 2016;397(7):671-678.