ARTICLE - Acute Myeloid Leukemia

Higher-dose venetoclax with measurable residual disease-guided azacitidine discontinuation in newly diagnosed acute myeloid leukemia

Jonathan A. Gutman,[1] Amanda Winters,[2] Andrew Kent,[1] Maria Amaya,[1] Christine McMahon,[1] Clayton Smith,[1] Craig T. Jordan,[1] Brett Stevens,[1] Mohammad Minhajuddin,[1] Shanshan Pei,[1] Jeffrey Schowinsky,[3] Jennifer Tobin,[1] Kelly O'Brien,[1] Angela Falco,[1] Elizabeth Taylor,[1] Constance Brecl,[1] Katie Zhou,[1] Phuong Ho,[1] Connor Sohalski,[1] Jessica Dell-Martin,[1] Olivia Ondracek,[1] Diana Abbott[4] and Daniel A. Pollyea[1]

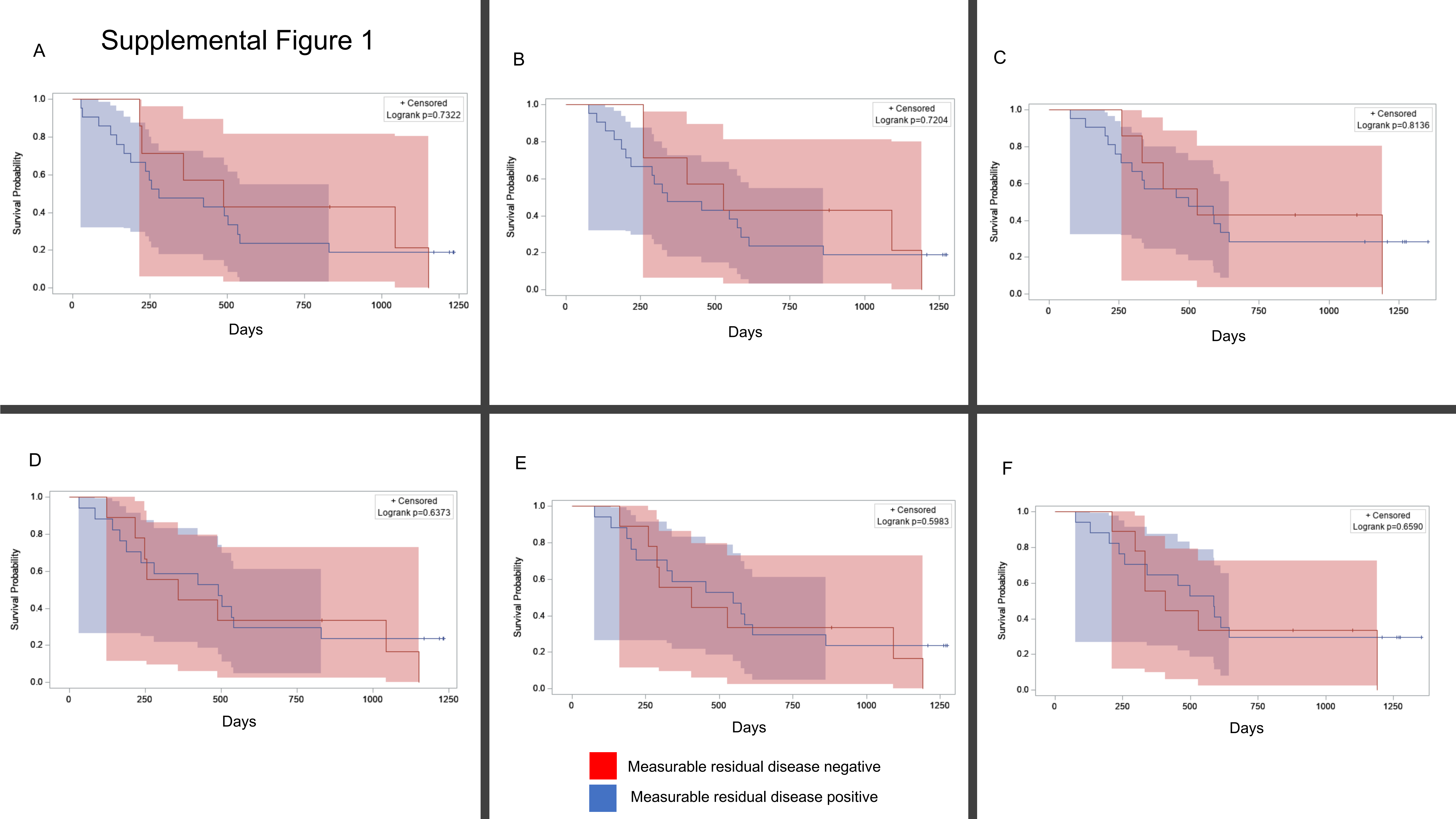
[1]Division of Hematology, Department of Medicine; [2]Center for Cancer and Blood Disorders, Department of Pediatrics; [3]Department of Pathology and [4]Center for Innovative Design and Analysis, Department of Biostatistics and Informatics, University of Colorado, Aurora, CO, USA

Correspondence: D.A. Pollyea
Daniel.pollyea@ucdenver.edu

Received: January 3, 2023.
Accepted: April 6, 2023.
Early view: April 13, 2023.

https://doi.org/10.3324/haematol.2023.282681

Supplemental Figure 1



Supplemental Table 1: Genes included in institutional next generation sequencing assay performed in all patients at baseline using disease sample from bone marrow or peripheral blood and for most patients a germline fingernail sample

ASXL1	*BCOR*	*BCORL1*	*BRAF*	*CALR*	*CBLS*	*CEBPA*
CSF3R	*CXCR4*	*DDX41*	*DNMT3A*	*ETV6*	*EZH2*	*FLT3*
GATA1	*GATA2*	*HRAS*	*IDH1*	*IDH2*	*JAK1*	*JAK2*
JAK3	*KDM6A*	*KIT*	*KMT2A*	*KRAS*	*MPL*	*MYD88*
NF1	*NOTCH1*	*NPM1*	*NRAS*	*PHF6*	*PPM1D*	*PTEN*
PTPN11	*RAD21*	*RUNX1*	*SETBP1*	*SFEB1*	*SH2B3*	*SRSF2*
STAG2	*STAT3*	*TET2*	*TP53*	*U2AF1*	*WT1*	*ZRSR2*

Supplemental Table 2: Genomic profile of each patient and which genes were monitored by ddPCR

Patient	Gene	Mutation	Variant Allele Frequency (%) or Allelic Ratio for *FLT3* ITD	Mutation(s) Monitored by ddPCR	Percentage of non DTA Baseline Genes Monitored for MRD (%)
001	*SF3B1*	K700E	36	Y	100
	RUNX1	R191X	35	Y	
	RUNX1	R91_T92insGGR	33	Y	
002	*IDH2*	R140Q	42	Y	50
	DNMT3A	R882H	41	N	
	PHF6	E337Lfs*4	54	Y	
	PHF6	Q71Hfs*22	8	N	
	BCORL1	G832Ffs*23	68	N	
003	*NPM1*	Type A	43	Y	100
	IDH2	R140Q	45	Y	
	DNMT3A	C494G	46	N	
004	*RUNX1*	R201X	13	Y	67
	RUNX1	V186Sfs*25	14	N	
	SRSF2	P95_R102del	26	Y	
005	*NPM1*	Type A	41	Y	100
	TET2	S136Rfs*8	22	N	
	TET2	L1886Ffs*6	43	N	
006	*FLT3*	ITD	8.06	N	50
	NPM1	Type A	47	Y	
007	*IDH1*	R132C	55	Y	67
	RUNX1	E22Gfs*180	33	N	
	DNMT3A	G543C	58	N	
	MYD88	V244I	51	Y	
008	*DNMT3A*	L901Sfs*15	32	N	100
	NPM1	Type DD1	44	Y	
	IDH1	R132G	43	Y	
009	*ASXL1*	R678Efs*36	21	N	50
	U2AF1	S34F	28	Y	
	BCOR	S373X	40	N	
	NRAS	G12D	15	Y	
	ETV6	M389T	23	N	
010	*RUNX1*	R166Q	56	Y	100
	RUNX1	W106S	10	Y	
	SRSF2	P95L	30	Y	
011	*TP53*	R306X	30	No response	
	TP53	S241F	38		

012	*IDH2* *TP53* *SRSF2*	R140Q R110L P95H	43 41 41	No response	
013	*TP53*	c.376-1G>A splice site	43	Y	100
014	*ASXL1* *ETV6* *SRSF2* *BRAF* *PTPN11* *KRAS*	G646Wfs*12 c.163+1G>A splice site P95H G469A Q510H Y64N	38 44 44 57 6 31	No response	
015	*TP53* *PTPN11*	R175H A72V	46 47	No response	
016	*NPM1* *PHF6* *TET2*	Type A I314T L1229R	38 45 87	Y Y N	100
017	*ASXL1* *IDH2* *SRSF2* *NRAS* *STAG2*	G710Efs*15 R140Q P95H G12V R953X	44 41 41 13 33	N Y Y Y N	75
018	*FLT3* *DNMT3A* *SF3B1* *STAG2*	ITD R771X K666M G1227Vfs*13	0.54 48 46 89	N N Y N	33
019	*ASXL1* *U2AF1*	C506Lfs*14 S34F	41 44	No response	
020	*U2AF1*	S34F	31	No response	
021	No mutations				
022	*TP53*	H193R	80	Y	100
023	*TP53* *TP53*	L264Vfs*83 I251N	38 34	N Y	50
024	*FLT3* *DNMT3A* *IDH1* *NPM1*	ITD c.2409-1G>A splice R132H c.863_864insTGCC	AR 0.2 45 44 43	N N Y N	33
025	*DNMT3A* *RUNX1* *SF3B1* *TET2*	I780T A142Gfs*4 K666W K546Rfs*15	43 36 43 23	N Y N N	50
026	*TP53* *NRAS*	R175H Q61H	75 12	No response	

027	*FLT3* *FLT3* *RUNX1* *ASXL1* *SRSF2* *STAG2*	D835Y ITD R201Q L775X P95H L629X	54 AR 2.0 46 44 43 80	No response	
028	*FLT3* *NPM1* *DNMT3A*	ITD Type A Y528_Q534dup	AR 1.1 45 43	N Y N	50
029	*DNMT3A* *NRAS* *U2AF1* *KMT2A*	W297Gfs*19 G12A R156H Q1357E	46 37 7 47	No response	
030	No mutations				
031	*TP53*	c.376-1G>A splice site	9	Y	100
032	*NPM1* *DNMT3A* *SMC1A*	Type A R882H D1159dup	32 41 26	Y N N	50
033	*IDH2* *SRSF2* *CEBPA* *ASXL1*	R140Q P95H A40Rfs*120 G646Wfs*12	47 44 87 33	Y Y N N	66
034	*DNMT3A* *DNMT3A* *NPM1* *ETV6* *KRAS* *GATA2*	G550R c.1279+1G>A splice type A R103_Y104delinsH G13D R343G	40 18 22 15 14 35	No response	
035	*KIT* *NRAS* *NOTCH1*	N822Y G12D G1751S	8 13 13	Y Y N	66
036	*TP53* *TP53*	E258G Y205F	48 45	Y Y	100
037	*DNMT3A* *RUNX1* *U2AF1* *BCOR* *NRAS*	R882H R201Q R156H Y660Tfs*9 G13C	38 40 37 42 36	N Y Y N Y	75
038	*TP53* *TP53* *GATA2* *ASXL1*	M246V C124X W10C K9E	32 24 53 10	No response	
039	*TP53*	Y220H	6	No response	

040	*TP53*	V173L	52	No response	
041	*FLT3*	D839G	10	Y	100
	NPM1	Type A	42	Y	
	DNMT3A	Q231X	93	N	
	SF3B1	G740E	43	Y	
042	*TP53*	V216M	52	No response	
	TP53	C176T	48		
	SETBP1	G870R	49		

AR=allele ratio, ddPCR=digital droplet PCR; DTA=mutations in *DNMT3A, TET2 and ASXL1*, MRD=measurable residual disease

Supplemental Table 3: Measurable residual disease as a predictor of outcomes stratified and non-stratified by allogeneic stem cell transplantation status

	Unadjusted Analysis Hazard Ratio (95% Confidence Interval), p-value	**Transplant-Stratified Analysis** Hazard Ratio (95% Confidence Interval), p-value
Duration of Response		
Full MRD Negativity	0.85 (0.33, 2.16), 0.7325	Transplant (N=9): 2.46 (0.25, 23.75), 0.4370
		No Transplant (N=17): 0.51 (0.17, 1.48), 0.2135
MRD Negative by ddPCR	1.24 (0.51, 3.00), 0.6379	Transplant (N = 10): 3.47 (0.31, 38.39), 0.3106
		No Transplant (N = 18): 0.71 (0.26, 1.98), 0.5138
MRD Negative by Flow Cytometry	0.41 (0.18, 0.97), 0.0424	Transplant (N = 10): 0.55 (0.12, 2.48), 0.4335
		No Transplant (N = 18): 0.20 (0.06, 0.67), 0.0092
Event Free Survival		
Full MRD Negativity	0.84 (0.33, 2.15), 0.7214	Transplant (N=9): 2.46 (0.25, 23.75), 0.4370
		No Transplant (N=17): 0.50 (0.17, 1.46), 0.2056
MRD Negative by ddPCR	1.27 (0.52, 3.08), 0.5992	Transplant (N = 10): 3.47 (0.31, 38.39), 0.3106
		No Transplant (N = 18): 0.71 (0.26, 1.98), 0.5138
MRD Negative by Flow Cytometry	0.43 (0.19, 1.01), 0.0536	Transplant (N = 10): 0.63 (0.14, 2.84), 0.5455
		No Transplant (N = 18): 0.20 (0.06, 0.67), 0.0088
Overall Survival		
Full MRD Negativity	0.89 (0.32, 2.44), 0.8137	Transplant (N=9): 3.97 (0.36, 43.93), 0.2606 No Transplant (N=17): 0.42 (0.13, 1.34), 0.1418
MRD Negative by ddPCR	1.24 (0.48, 3.16), 0.6596	Transplant (N = 10): 3.47 (0.31, 38.39), 0.3106
		No Transplant (N = 18): 0.64 (0.22, 1.87), 0.4165
MRD Negative by Flow Cytometry	0.53 (0.22, 1.31), 0.1715	Transplant (N = 10): 1.03 (0.17, 6.25), 0.9731
		No Transplant (N = 18): 0.23 (0.07, 0.71), 0.0113

MRD=measurable residual disease

Supplemental Table 4: Clonal evolution at progression defined as new cytogenetic abnormality or new gene mutations from baseline testing.

Patient	**New Cytogenetic Clone at Progression**	**New Gene Mutation at Progression**	**Stopped Azacitdine**
1	90,slx2[3]	N	Y
2	N	*FLT3* ITD	N
5	N	Not Performed	Y
6	N	*WT1*	N
13	45,sl,der(6)add(6)(p21)add(6)(q13),-7[3]/45,sl,del(3)(q12),der(3)del(3)(p21p21)add(3)(q21),-7,-10,+r1[2]/44,sl,-4,-17,-18,add(21)(q22),+mar1[4]/44,sdl3,-mar1,+r2[2]/56,sl,+Y,+1,+del(5)(q13q31),+8,+11,+13,+14,del(17)(p11.2),+21,+22,+22[5]/56,sdl5,add(2)(q31),-11,der(19)t(11;19)(q13;q13.3),+der(?)t(?;11)[4]	*TP53*	Y
16	N	*GATA2, NRAS, RAD21, JAK2*	N
21	N	*KRAS*	N
25	der(19)t(1;19)(q12;q13.1),add(21)(p11.2)[7]	N	Y
32	N	Not Performed	Y
36	+19	Not Performed	N
41	N	*BCOR*	N

Supplemental Table 5: Outcomes for patients who stopped azacitidine, with and without measurable residual disease guidance

	Duration of Response, months (95% Confidence Interval)	Event Free Survival, months (95% Confidence Interval)	Overall Survival, months (95% Confidence Interval)
Discontinued Azacitidine After Achieving Measurable Residual Disease Negativity (N=7)	16.0 (7.1, Not Reached)	17.4 (8.5, Not Reached)	17.4 (8.5, Not Reached)
Discontinued Azacitidine Without Achieving Measurable Residual Disease Negativity (N=4)	12.9 (5.5, Not Reached)	14.3 (6.6, Not Reached)	18.8 (6.6, Not Reached)

Supplemental Table 6: Variables associated with response to therapy

Variable	**Odds Ratio (95% CI)**	**p-value**
Sex (Ref = Male)	5.67 (1.41, 22.76)	0.0145
Age	1.01 (0.92, 1.11)	0.8705
Baseline Blast %	1.02 (0.99, 1.05)	0.2380
Known Prior MDS (Ref = N)	1.03 (0.21, 5.06)	0.9693
Treatment Related AML (Ref = N)	0.57 (0.10, 3.22)	0.5202
Secondary AML (Ref = N)	0.74 (0.20, 2.75)	0.6536
FAB M0/M1 (Ref = N)	2.50 (0.56, 11.21)	0.2315
FAB M5 (Ref = N)	0.81 (0.64, 1.03)	0.0488
FLT3 (Ref = N)	2.73 (0.28, 26.86)	0.3900
FLT3 ITD (Ref = N)	0.91 (0.13, 6.16)	0.9256
NPM1 (Ref = N)	7.94 (0.90, 70.22)	0.0624
ASXL1 (Ref = N)	0.39 (0.08, 2.04)	0.2656
TP53 (Ref = N)	0.14 (0.03, 0.61)	0.0083
RAS pathway (Ref = N)	0.40 (0.09, 1.79)	0.2315
IDH (Ref = N)	5.53 (0.61, 49.97)	0.1281
RUNX1 (Ref = N)	4.50 (0.49, 41.43)	0.1843
ELN Risk Group		
Favorable vs. Adverse	8.00 (0.89, 72.10)	0.9759
Adverse vs. Intermediate	Cannot be estimated	Cannot be estimated
Need hydroxyurea (Ref = N)	1.60 (0.35, 7.34)	0.5478
No blast clearance on Cycle 1 Day 8	1.56 (0.36, 6.66)	0.5162

MDS=myelodysplastic syndromes; FAB=French American British; ITD=internal tandem duplication

Supplemental Table 7: Variables associated with duration of response, event free survival and overall survival

	Duration of Response		Event Free Survival		Overall Survival	
Variables	p-value	Hazard Ratio (95% CI)	p-value	Hazard Ratio (95% CI)	p-value	Hazard Ratio (95% CI)
Sex (Ref = M)	0.4404	1.40 (0.60, 3.26)	0.5997	0.84 (0.44, 1.61)	0.6351	0.85 (0.43, 1.67)
Age	0.4614	1.02 (0.97, 1.08)	0.7038	1.01 (0.96, 1.06)	0.6969	1.01 (0.96, 1.06)
Baseline Blast %	0.6157	1.00 (0.99, 1.02)	0.9108	0.999 (0.99, 1.01)	0.6539	0.997 (0.98, 1.01)
Known Prior MDS (Ref = N)	0.5709	1.34 (0.49, 3.63)	0.5123	1.30 (0.53, 2.86)	0.5226	1.31 (0.57, 3.03)
Treatment-Related AML (Ref = N)	0.8865	1.09 (0.32, 3.76)	0.4902	1.37 (0.56, 3.32)	0.3061	1.60 (0.65, 3.92)
Secondary AML (Ref = N)	0.5630	1.30 (0.54, 3.12)	0.2973	1.43 (0.73, 2.81)	0.2055	1.58 (0.78, 3.19)
FAB M0/M1 (Ref = N)	0.7685	0.83 (0.25, 2.83)	0.2254	0.61 (0.28, 1.36)	0.6942	0.85 (0.37, 1.95)
FAB M5 (Ref = N)	Cannot be estimated	Cannot be estimated	0.0110	5.64 (1.49, 21.39)	0.0031	7.51 (1.97, 28.59)
FLT3 (Ref = N)	0.0098	5.07 (1.48, 17.37)	0.1631	2.02 (0.75, 5.42)	0.0531	2.72 (0.99, 7.45)
FLT3 ITD (Ref = N)	0.0031	6.41 (1.87, 21.95)	0.1085	2.25 (0.84, 6.06)	0.0287	3.10 (1.13, 8.53)
NPM1 (Ref = N)	0.2823	1.61 (0.68, 3.84)	0.9018	0.95 (0.45, 2.03)	0.6752	1.18 (0.55, 2.54)
ASXL1 (Ref = N)	0.1171	0.20 (0.03, 1.50)	0.5251	0.74 (0.28, 1.90)	0.7940	0.88 (0.34, 2.28)
TP53 (Ref = N)	0.3791	1.58 (0.57, 4.41)	0.0243	2.24 (1.11, 4.52)	0.0152	2.40 (1.18, 4.87)
RAS pathway (Ref = N)	0.0581	0.14 (0.02, 1.07)	0.4164	0.69 (0.29, 1.68)	0.6668	0.82 (0.34, 2.00)
IDH1/2 (Ref = N)	0.0404	0.32 (0.11, 0.95)	0.0211	0.32 (0.12, 0.84)	0.0301	0.31 (0.11, 0.90)
RUNX1 (Ref = N)	0.5055	0.71 (0.26, 1.94)	0.2722	0.61 (0.25, 1.48)	0.3323	0.62 (0.24, 1.62)
ELN Risk Group						
Favorable vs. Adverse	0.9632	0.98 (0.37, 2.56)	0.3096	0.65 (0.28, 1.50)	0.6095	0.80 (0.34, 1.87)
Adverse vs. Intermediate	0.0420	4.04 (1.05, 15.50)	0.5931	1.40 (0.41, 4.73)	0.5428	1.46 (0.43, 4.93)
Need hydroxyurea (Ref = N)	0.0156	3.17 (1.24, 8.06)	0.1047	1.87 (0.88, 3.97)	0.4004	1.39 (0.64, 3.02)
No Blast Clearance on Cycle 1 D8	0.0401	2.58 (1.04, 6.40)	0.2542	1.53 (0.74, 3.16)	0.5983	1.23 (0.57, 2.66)
Allogeneic Stem Cell Transplantation (Ref = N)	0.1165	0.49 (0.20, 1.20)	0.0093	0.33 (0.14, 0.76)	0.0033	0.24 (0.09, 0.62)
MRD Negative by Flow Cytometry (Ref = N)	0.0424	0.41 (0.18, 0.97)	0.0536	0.43 (0.19, 1.01)	0.1715	0.53 (0.22, 1.31)
MRD Negative by ddPCR (Ref = N)	0.6379	1.24 (0.51, 3.00)	0.5992	1.27 (0.52, 3.08)	0.6596	1.24 (0.48, 3.16)
Full MRD Negative (Ref = N)	0.7325	0.85 (0.33, 2.16)	0.7214	0.84 (0.33, 2.15)	0.8137	0.89 (0.32, 2.44)

MDS=myelodysplastic syndromes; FAB=French American British; ITD=internal tandem duplication; MRD=measurable residual disease; ddPCR=digital droplet PCR