SUPPLEMENTAL METHODS

Immunophenotyping of Lymphocytes and Blasts from BM Aspirates and Peripheral Blood

PBMCs or BMAs from patients were collected in Vacutainer or Cell Preparation Tubes (CPT) containing sodium heparin (BD Vacutainer, Franklin Lakes, NJ). Mononuclear cells (MC) were isolated from PB or BMA by centrifuging the cell preparation tubes (CPT) at 2000rpm (863g) for 15 minutes at room temperature. Samples were diluted sample 1:5 with Phosphate Buffered Salie (PBS) and layered over 10ml of Ficoll. The mixture was centrifuged at 2000rpm (863g) for 20 minutes at room temperature with no brakes. The interface cells were harvested and washed twice with PBS containing 10% fetal calf serum at 500g and 450g for 10 minutes respectively. MCs were then re-suspended in PBS and used for further analysis. 17-color multi-parameter flow-cytometry was performed on BMA cells using fluorescence-conjugated monoclonal antibodies or mass cytometry (CyTOF) using metal tagged antibodies either purchased pre-conjugated from Fluidigm or purchased purified and conjugated in house using MaxPar X8 Polymer kits (Fluidigm) according to the manufacturer's instructions. Cells for flow cytometry were acquired using Fortessa (BD Biosciences, Heidelberg, Germany) and analysis was performed using FlowJo software (Tree Star, Ashland, OR, USA) and samples for CyTOF were acquired on a Helios mass cytometer (Fluidigm) and analyzed by viSNE using Matlab.

Immunohistochemistry Processing and Staining of BM clots and slides

BM clots and biopsies were fixed in 10% formalin, embedded in paraffin, and transversely sliced into 4 μm sections. Slides were stained with mouse anti-human monoclonal antibodies against CD3 (Dako, Cat#A0452, 1:100) and CD8 (Thermo Scientific, MS-457-S, 1:25). For IHC quantification, H&E and IHC slides were scanned and digitalized using the ScanscopeXT system from Aperio/Leica Technologies.

Mutational Analysis by Next Generation Sequencing

We performed mutation analysis using a 28-gene panel as previously described [4]. Briefly,

genomic DNA was extracted from BM aspirates or peripheral blood. Amplicon-based next-generation sequencing (NGS) targeting the entire coding regions of a panel of 28 genes associated with myeloid neoplasms was performed using a MiSeq platform (Illumina, San Diego, CA). The genes analyzed included are noted in Supplemental Table 3. A sequencing library was prepared using 250 ng of DNA template. Equal quantities of DNA from purified sequencing libraries were used for TruSeq paired-end sequencing on the MiSeq sequencer using the MiSeq Reagent Kit v2 (500 cycles). Variant calling was performed with Illumina MiSeq Reporter Software using human genome build 19 (hg 19) as a reference. For clinical reporting, a minimum sequencing coverage of 3250 (bidirectional true paired-end sequencing) was required. The analytical sensitivity was established at 5% mutant reads in a background of wild-type reads. CEBPA was performed using PCR followed by Sanger sequencing. Internal tandem duplications of FLT3 gene and NPM1 mutation (exon12) were assessed using PCR followed by capillary electrophoresis (all of these methods have been previously described).

Supplemental Table 1: Multivariate analysis for significant univariate factors for response to therapy

(N=40; ORR = 19)	Odds Ratio	95%CI for OR		p
Prior HMA				
Yes				
No	0.22	0.05	1.06	0.059
ASXL1				
Negative				
Positive	6.37	0.97	41.69	0.053
BM BL>/=20				
No				
Yes	1.25	0.24	6.42	0.788
WBC>10				
No				
Yes	1.00			
Pretherapy BM CD3	1.03	1.00	1.07	0.065

Supplemental Table 2: Median overall survival by pretherapy characteristics

	Survival (months)	p-value
Response		**<0.001**
CR, CRi, PR, HI	15.9	
SD	16.2	
NR	4.1	
Age		0.78
<60	5.95	
>/=60	6.60	
Age		0.95
<70	5.98	
>/=70	6.60	
Salvage status		**0.003**
S1	10.55	
>S1	5.68	
Salvage status		0.39
S1/S2	5.68	
>S2	6.27	
Diagnosis		0.46
AML - de novo	7.59	
Secondary AML	5.98	
Prior ASCT		0.55
Yes	6.8	
No	6.4	
Prior HMA		0.35
Yes	6.30	
No	8.18	
Cytogenetic		0.06
Diploid	11.2	
Miscellaneous	3.8	
-5/-7/complex	6.8	
TP53		0.65
Negative	6.60	
Positive	5.98	
IDH1		0.44
Negative	6.6	
Positive	5.94	
IDH2		0.16
Negative	5.97	
Positive	12.6	
RAS		0.83
Negative	6.40	
Positive	9.60	
ASXL1		**0.04**
Negative	5.7	
Positive	15.2	
BM BL>/=20		0.29
No	9.6	
Yes	5.9	
BM BL>/=10		0.27
No	11.2	
Yes	6.4	
WBC>10		0.08
No	7.58	
Yes	3.48	
PLT>50		0.36
No	5.58	
Yes	7.58	
Pretherapy PB CD3 >20.5		0.10
No	5.29	
Yes	11.89	
Pretherapy BM CD3 >13.2		0.09
No	5.68	
Yes	12.45	

Supplemental Table 3: Historical HMA-based clinical trials at MDACC between 2005-2017.

1- HMA + Targeted or chemo based therapy (NCT03404193, NCT03132454, NCT02400281, NCT02257138, NCT03047993, NCT00741234, NCT02096055, NCT02141477, NCT00741234, NCT02003573, NCT00357708, NCT02190695, NCT01828346, NCT01926587, NCT01636609, NCT01202877, and NCT00569010).

2- HMA + Immunotherapy (NCT02953561, NCT02399917, NCT00968071, NCT01038635).

3- Single agent HMA (NCT02096055).

Protocol #	Regimen	
2016-0772	Palbociclib+Decitabine	NCT03132454
2014-0862	Azacitidine+Lirilumab	NCT02399917
2014-0344	Ruxolitinib+Decitabine	NCT02257138
2014-0152	Azacitidine+CB-839	NCT03047993
2013-0873	Azacitidine+Pracinostat	NCT00741234
2013-0843	SGI-110 5+Cladribine	NCT02096055
2013-0843	SGI-110 10	NCT02096055
2013-0812	Decitabine+Omacetaxine	NCT02141477
2013-0596	Azacitidine+Pracinostat	NCT00741234
2013-0583	Decitabine+Volasertib	NCT02003573
2005-0723	SAHA+Decitabine	NCT00357708
2013-0543	Decitabine+Carboplatin	NCT02190695
	Decitabine	
	Decitabine+ Arsenic	
2013-0141	Azacitidine+Birinapant	NCT01828346
2013-0030	Azacitidine+Rigosertib	NCT01926587
2011-0188	Tosedostat+Aza	NCT01636609
2010-0374	Azacitidine+PKC412	NCT01202877
2009-0467	Azacitidine+Lenalidomide	NCT01038635
2008-0288	Decitabine+Mylotarg	NCT00968071
2005-0291	Azacitidine+Ara-C	NCT00569010

Supplemental Table 4

Supplemental Table 4A. T-tests comparison by ORR and OS>1 year							
	No			Yes			
	N	Mean	SD	N	Mean	SD	p-value
ORR							
cd3 onlive	23	17.56	19.77	19	32.47	26.20	**0.042**
Treg onlive	23	0.83	0.89	19	1.13	1.29	0.377
Teff onlive	23	8.97	11.20	19	15.61	12.71	**0.080**
cd8 onlive	23	6.93	8.69	19	13.12	14.43	0.094
cd8_treg	23	15.53	18.23	18	24.94	43.03	0.349
teff_treg	23	15.53	18.23	18	24.94	43.03	0.349
cd8 icos	23	15.04	10.30	19	11.21	11.14	0.255
Teff icos	23	19.20	22.13	19	7.18	7.68	**0.030**
cd8 pd1	23	36.98	18.74	19	33.71	14.80	0.540
Teff pd1	23	39.22	25.12	19	33.71	23.07	0.467
cd8 ctla4	23	12.74	18.35	19	6.48	7.39	0.171
Teff ctla4	23	10.48	10.60	19	9.23	10.54	0.707
OS>1 year							
cd3onlive	30	21.43	21.94	17	31.49	25.81	0.164
tregonlive	30	0.92	1.02	17	1.19	1.23	0.424
teffonlive	30	11.00	12.45	17	14.89	12.02	0.304
cd8onlive	30	8.12	9.88	17	13.08	13.92	0.161
cd8_treg	30	16.13	18.69	16	25.32	45.52	0.337
teff_treg	30	16.13	18.69	16	25.32	45.52	0.337
cd8icos	30	13.46	10.09	17	12.49	11.23	0.763
tefficos	30	14.96	15.35	17	9.86	20.26	0.336
cd8pd1	30	37.46	19.11	17	31.53	15.54	0.281
teffpd1	30	38.25	25.78	17	33.54	19.88	0.519
cd8ctla4	30	9.31	15.18	17	8.63	12.26	0.876
teffctla4	30	10.19	10.98	17	8.18	8.53	0.519

Supplemental Table 4B Empirical estimation of cut-points by Yeomans index (Bone marrow)					
Baseline measurements	Cut-point	Sensitivity	Specificity	AUC	p-value
ORR					
cd3onlive	13.20	0.74	0.65	0.69	**0.029**
tregonlive	2.18	0.26	0.91	0.59	0.284
teffonlive	11.60	0.58	0.78	0.68	**0.041**
cd8onlive	4.01	0.74	0.61	0.67	**0.054**
tregctla4	26.90	0.58	0.48	0.53	0.929
tregicos	1.91	0.95	0.09	0.52	0.890
tregpd1	62.50	0.16	0.96	0.56	0.486
teffctla4	39.10	0.05	1.00	0.53	0.890
tefficos	1.22	0.84	0.26	0.55	0.626
teffpd1	62.60	0.21	0.87	0.54	0.764
cd8ctla4	2.62	0.68	0.39	0.54	0.827
cd8icos	30.10	0.16	0.91	0.54	0.800
cd8pd1	17.80	0.79	0.30	0.55	0.703
cd8_treg	1.79	0.94	0.22	0.58	0.176
teff_treg	1.79	0.94	0.22	0.58	0.176
OS>1year					
cd3onlive	9.12	0.82	0.47	0.65	0.084
tregonlive	1.85	0.35	0.83	0.59	0.253
teffonlive	5.12	0.82	0.50	0.66	0.055
cd8onlive	3.31	0.82	0.53	0.68	0.036
tregctla4	12.80	0.94	0.13	0.54	0.631
tregicos	1.91	0.94	0.07	0.50	0.681
tregpd1	17.80	0.76	0.33	0.55	0.634
teffctla4	1.73	0.82	0.27	0.55	0.648
tefficos	55.00	0.06	1.00	0.53	0.681
teffpd1	20.80	0.76	0.40	0.58	0.357
cd8ctla4	7.48	0.41	0.77	0.59	0.308
cd8icos	21.30	0.29	0.80	0.55	0.647
cd8pd1	17.80	0.76	0.30	0.53	0.813

Supplemental Table 4C. Empirical estimation of cut-points by Yeomans index (peripheral blood)							
	Cutpoint	Sensitivity	Specificity	AUC	p-value		
ORR							
CD3	20.50	0.72	0.68	0.70	0.028		
CR/CRi/PR							
CD3	57.60	0.46	0.93	0.69	0.020		
OS>1year							
CD3	33.30	0.71	0.69	0.70	0.031		
T-tests comparison by ORR and OS>1 year							
	No			Yes			
	N	Mean	SD	N	Mean	SD	p-value
ORR							
CD3	22	21.83	21.70	18	45.07	26.99	0.004
OS>1year							
CD3	26	25.52	24.06	14	44.87	27.36	0.026

	Definite/Possible/Probable						Unlikely/Unrelated					
Adverse event	G1	G2	G3	G4	G5	Total	G1	G2	G3	G4	G5	Total
Alanine/aspartate transaminase elevation			2 (3)			2 (3)			3 (4)			3 (4)
Abdominal pain							4 (6)	2 (3)	3 (4)			10 (14)
Abdominal distension							1 (1)					1 (1)
Acidosis									1 (1)			1 (1)
Anorexia							5 (7)					5 (7)
Arthralgia		1 (1)				1 (1)		2 (3)				2 (3)
Atrial fibrillation								2 (3)	3 (4)			5 (7)
Autoimmune disorder		1 (1)				1 (1)			1 (1)			1 (1)
Elevated bilirubin		1 (1)				1 (1)						
Back pain							3 (4)	1 (1)	2 (3)			6 (9)
Blurred vision							1 (1)					1 (1)
Bone pain		2 (3)				2 (3)						
Cardiac arrest							1 (1)					1 (1)
Catheter related infection									1 (1)			1 (1)
Chest pain - cardiac	1 (1)					1 (1)			1 (1)			1 (1)
Chills								2 (3)				2 (3)
Confusion	1 (1)					1 (1)	4 (6)					5 (7)
Constipation	15 (21)	3 (4)				18 (26)	7 (10)					7 (10)
Colitis			1 (1)			1 (1)			2 (3)			2 (3)
Cough	1 (1)	1 (1)				2 (3)	6 (9)		1 (1)			7 (11)
Creatinine increased	2 (3)	1 (1)				3 (4)			3 (4)	1 (1)		4 (6)
Cytokine release syndrome		1 (1)	1 (1)			2 (3)						
Delirium							2 (3)					2 (3)
Depression							1 (1)	2 (3)				3 (4)
Diarrhoea	14 (20)					14 (20)	10 (14)	3 (4)	1 (1)			14 (20)
Dizziness	1 (1)					1 (1)		1 (1)				1 (1)
Dry eye							1 (1)					1 (1)
Dry skin	3 (4)					3 (4)	1 (1)					1 (1)
Dyspepsia							1 (1)					1 (1)
Dysphagia	1 (1)					1 (1)						
Dyspnoea	2 (3)					2 (3)		1 (1)	4 (6)			5 (7)
Enema of limbs							5 (7)	3 (4)	2 (3)			10 (14)
Enterocolitis		1 (1)				1 (1)						
Epistaxis								1 (1)	1 (1)			2 (3)
Erectile dysfunction							1 (1)					1 (1)
Erythema multiforme			1 (1)			1 (1)	1 (1)					1 (1)
Eye disorders			1 (1)			1 (1)						
Falls							2 (3)	2 (3)	2 (3)			6 (9)
Fatigue		1 (1)	1 (1)			2 (3)	1 (1)	1 (1)				2 (3)
Febrile neutropenia			4 (6)			4 (6)			38 (54)			38 (54)
Fever							2 (3)	3 (4)	8 (11)			13 (18)
Gastrointestinal disorders		1 (1)				1 (1)	3 (4)	3 (4)	2 (3)			8 (11)
Generalized muscle weakness		2 (3)				2 (3)			1 (1)			1 (1)
Gingival pain							1 (1)					1 (1)
Headache							4 (6)					4 (6)
Heart failure											1 (1)	1 (1)
Haematuria							1 (1)		1 (1)			2 (3)
Hepatic failure										1 (1)		1 (1)
Hypo/Hypercalcemia									1 (1)			1 (1)
Hypo/Hyperkalaemia									10 (14)			10 (14)
Hyponatremia									9 (12)			9 (12)
Hyperglycaemia									4 (6)	2 (3)		6 (9)
Hypophosphatemia									3 (4)			3 (4)
Hypotension							1 (1)	1 (1)	7 (10)	2 (3)		11 (16)
Hypoxia								1 (1)	2 (3)			3 (4)
Ileus								2 (3)				2 (3)
Immune system disorders		1 (1)				1 (1)						
Infections and infestations							3 (4)	7 (11)	13 (18)		1 (1)	24 (34)
Insomnia	1 (1)					1 (1)		1 (1)				1 (1)
Intracranial haemorrhage										1 (1)		1 (1)
Laryngeal haemorrhage							2 (3)		1 (1)			3 (4)
Lower GI haemorrhage									2 (3)			2 (3)
Lung infection			5 (7)		2 (3)	7 (11)			31 (44)	1 (1)	3 (4)	35 (50)
Mucositis oral	1 (1)					1 (1)	1 (1)					1 (1)
Multiorgan failure										1 (1)	3 (4)	4 (5)
Musculoskeletal & connective tissue							1 (1)	2 (3)	1 (1)			4 (5)
Myalgia							2 (3)					2 (3)
Myocardial infarction									1 (1)			1 (1)
Myositis		1 (1)				1 (1)						
Nasal congestion							1 (1)	2 (3)				3 (4)

Nausea	8 (11)					8 (11)	9 (13)	1 (1)				10 (14)
Neck pain							3 (4)					3 (4)
Non-cardiac chest pain							3 (4)					3 (4)
Pain							3 (4)	2 (3)	7 (10)			12 (17)
Pancreatitis								1 (1)				1 (1)
Pharyngitis								1 (1)				1 (1)
Pleural effusion								1 (1)				1 (1)
Pneumonitis		8 (11)	1 (1)			9 (13)						
Pruritus			2 (3)			2 (3)						
Rash, acneiform	1 (1)					1 (1)						
Rash, maculo-papular	1 (1)	4 (6)				5 (8)	1 (1)					1 (1)
Renal and urinary disorders							2 (3)					2 (3)
Respiratory failure										3 (4)	4 (6)	7 (11)
Respiratory, thoracic and mediastinal disorders								3 (4)	1 (1)			4 (6)
Sepsis										3 (4)	8 (12)	11 (16)
Skin and subcutaneous tissue disorders	2 (3)	1 (1)	1 (1)			4 (6)	7 (10)	4 (6)				11 (16)
Sinus bradycardia	4 (6)	1 (1)				5 (8)						
Sinus tachycardia							5 (8)	2 (3)	2 (3)			9 (13)
Sinusitis									2 (3)			2 (3)
Skin infection									9 (13)			9 (13)
Sore throat	1 (1)					1 (1)	2 (3)	1 (1)	2 (3)			5 (7)
Stroke										1 (1)		1 (1)
Thromboembolic event								1 (1)	1 (1)			2 (3)
Toxic epidermal necrolysis										1 (1)		1 (1)
Tremor							1 (1)					1 (1)
Tumor lysis syndrome									3 (4)			3 (4)
Upper respiratory tract infection									3 (4)			3 (4)
Urinary tract infection									3 (4)			3 (4)
Vertigo							1 (1)					1 (1)
Vomiting	6 (9)					6 (9)	5 (7)	1 (1)	1 (1)			7 (11)
Weight loss							1 (1)	1 (1)				2 (3)
Total	**62 (89)**	**29 (41)**	**15 (21)**	**0 (0)**	**1 (1)**	**107**	**123 (176)**	**67 (94)**	**200 (290)**	**17 (24)**	**20 (29)**	**428**

Supplemental Table 5: All adverse Events irrespective of attribution (related and un-related)

Supplemental Table 6: Dose levels of 5-azacitidine and nivolumab during the lead-in phase and for drug-related AE management

Dose level	5-azacitidine (mg/m^2/day, Days 1-7)	Nivolumab (mg/kg, Days 1 and 14)
-4	25	0.1
-3	50	0.1
-2	50	0.3
-1	75	1.0
0	**75**	**3.0 (starting dose and established RP2D)**

Supplemental Table 7: Dose adjustments for non-hematologic drug-related AEs

Grade	Occurrence	Dose modification
1 or 2	Any time	No dose reduction
3 or 4 (Persistent grade 2: Consider similar dose adjustments if persistent and not responding to optimal management in the opinion of PI and treating physician)	1st and 2nd time	Hold nivolumab and 5-azacitidine. Resume nivolumab and 5-azacitidine at prior dose if recovery to ≤ Grade 1 occurs within 14 days. If toxicity persists for 15-28 days, hold therapy and resume nivolumab at prior dose and 5-azacitidine at prior dose if recovery to ≤ Grade 1 OR resume nivolumab at prior dose and 5-azacitidine at ONE dose level below current dose if recovery to Grade 2. Dose re-escalation to prior dose of 5-azacitidine is permitted in accordance with the dose-escalation guidelines guidelines in section 5.3.6.
	3rd and 4th time	Hold nivolumab and 5-azacitidine. Follow until toxicity ≤ Grade 2. Resume nivolumab at prior dose and 5-azacitidine at TWO dose level below current dose.
	5th time	permitted in Take patient off the study.

Supplemental Table 8: Coverage by genes and codons tested for adequate amplicons

Gene	Exons (codons) tested
ABL1 (NM_005157)	1-11 (1-1004), 11 (1079-1150)
ASXL1 (NM_015338)	2-12 (20-1542)
BRAF (NM_004333)	1 (1-23), 1-18 (34-717), 18 (725-767)
DNMT3A (NM_022552)	2-23 (1-913)
EGFR (NM_005228)	1 (1-24), 2-20 (30-786), 20-23 (792-927), 23-26 (935-1043), 26-27 (1047-1061), 27-28 (1067-1211)
EZH2 (NM_004456)	2-5 (3-162), 6-7 (171-217), 8-20 (243-752)
FLT3 (NM_004119)	2-24 (15-994)
GATA1 (NM_002049)	2-5 (1-287), 6 (291-405), 6 (407-414)
GATA2 (NM_032638)	2-6 (1-481)
HRAS (NM_005343)	2-5 (1-190)
IDH1 (NM_005896)	3-10 (1-415)
IDH2 (NM_002168)	1 (1-14), 1-11 (26-453)
IKZF2 (NM_016260)	2-8 (1-527)
JAK2 (NM_004972)	3-18 (1-806), 19-25 (812-1133)
KIT (NM_000222)	1-6 (1-334), 6-21 (367-977)
KRAS (NM_004985)	2-5 (1-189)
MDM2 (NM_002392)	1-11 (1-498)
MLL (NM_005933)	1-5 (103-1190), 6-36 (1207-3970)
MPL (NM_005373)	1-9 (1-488), 10-12 (490-636)
MYD88 (NM_002468)	1 (1-6), 1-5 (15-310)
NOTCH1 (NM_017617)	2-31 (21-1893), 31-34 (1902-2286), 34 (2289-2552)
NPM1 (NM_002520)	1-7 (1-181), 7-11 (187-295)
NRAS (NM_002524)	2-5 (1-190)
PTPN11 (NM_002834)	1-4 (1-157), 5-15 (176-594)
RUNX1 (NM_001754)	2-4 (1-70), 4-9 (73-437)
TET2 (NM_001127208)	3 (1-854), 3-11 (866-2003)
TP53 (NM_000546)	2-11 (1-394)
WT1 (NM_024426)	1 (1-59, 72-105, 122-216), 2-10 (216-518)

*Sequencing coverage of the genes: The above table describes adequacy of coverage in our assay across the full set of covered genes, exons, and codons. Adequately covered amplicons are defined as those having total coverage depth of greater than or equal to 250 reads, or for which an orthogonal mutation analysis testing has been performed. Presence of mutations outside the tested regions listed below cannot be ruled out. Due to space limitations, only certain genes may be listed. A full list of covered genes & codons for the specific test results on this sample is available upon request.

Supplemental Table 9A: Flow-cytometry antibody list.

Lymphocyte panel

Vendor	Antibody	Clone
BD Biosciences	**CD134/OX40**	ACT35
BD Biosciences	**CD8**	RPA-T8
BD Biosciences	**CD3**	SK7
eBioscience	**CD357/GITR**	eBioAITR
BD Biosciences	**CD13**	WM15
Biolegend	**CD137/4-1BB**	4B4-1
Life Technologi	**Yellow Live/Dead**	
Biolegend	**TIM-3**	F38-2E2
BD Biosciences	**CD279/PD-1**	EH12.1
BD Biosciences	**CD127/IL-7Ra**	HIL-7R-M21
eBioscience	**CD45**	HI30
eBioscience	**CD223/LAG-3**	3DS223H
BD Biosciences	**CD33**	WM53
BD Biosciences	**CD34**	581
eBioscience	**CD4**	SK3
eBioscience	**CD278/ICOS**	ISA-3
BD Biosciences	**CD152/CTLA-4**	BNI3
eBioscience	**FoxP3**	PCH101

Lineage Cocktail

Vendor	Antibody	Clone
BD Biosciences	CD3	UCHT1
BD Biosciences	CD14	MϕP9
BD Biosciences	CD16	3G8
BD Biosciences	CD56	NCAM16.2

Ligand panel

Vendor	Antibody	Clone
eBioscience	**CD275/ICOSL**	MIH12
BD Biosciences	**CD80/B7-1**	L307.4
BD Biosciences	**HLA-DR**	G46-6
Biolegend	**CD34**	561
Life Technologi	**Yellow Live/Dead**	
BD Biosciences	**CD38**	HB7
BD Biosciences	**CD273/PD-L2**	MIH18
BD Biosciences	**CD86/B7-2**	2331 FUN-1
BD Biosciences	**CD274/PD-L1**	MIH1
eBioscience	**CD45**	HI30
BD Biosciences	**CD137L/41-BB**	C65-485
BD Biosciences	**CD33**	WM53
BD Biosciences	**CD13**	WM15
Biolegend	**Galectin-9**	9M1-3
BD Biosciences	**CD252/OX40L**	ik-1

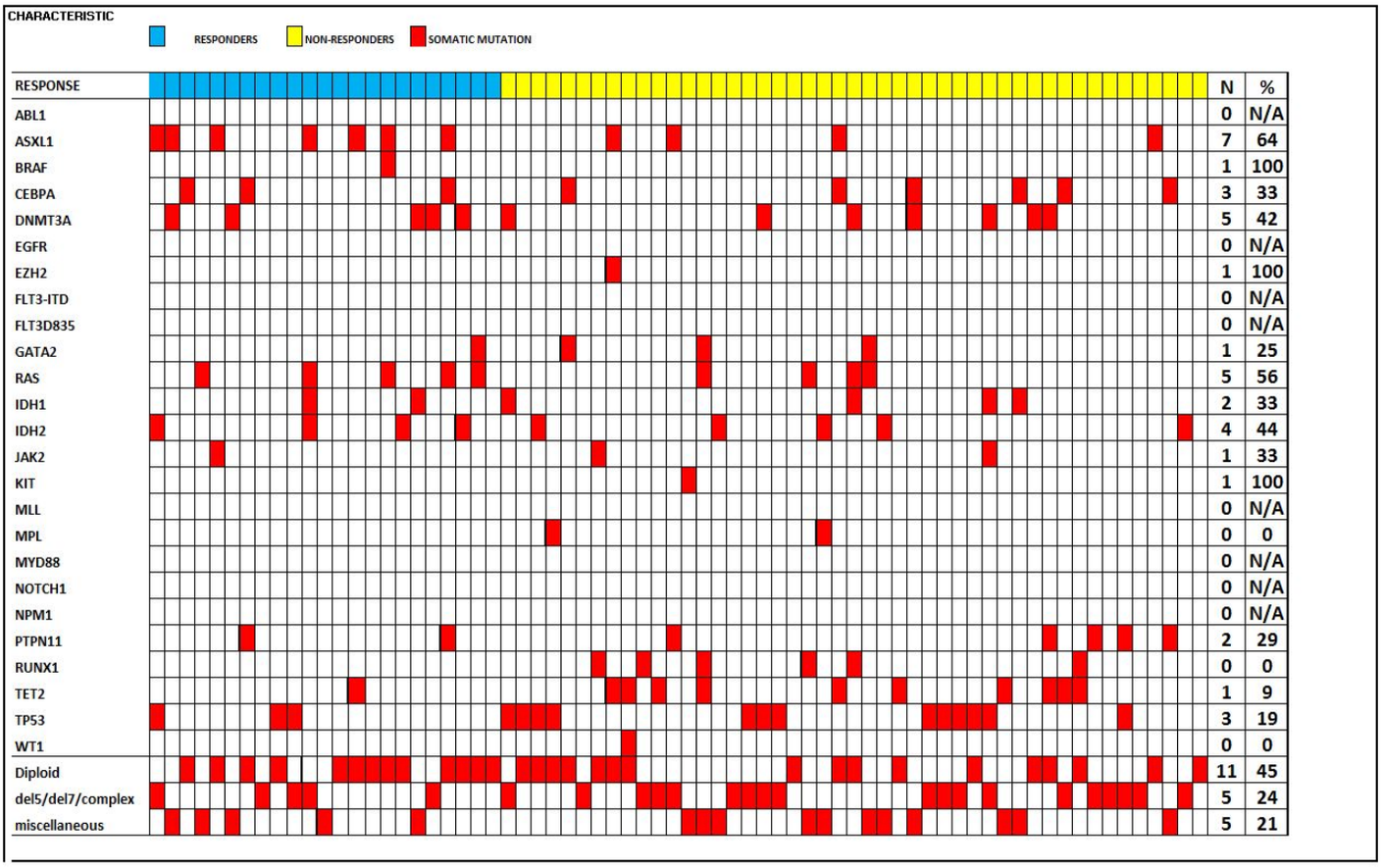
Supplemental Table 9B: Mass Cytometry (CYTOF) antibody list

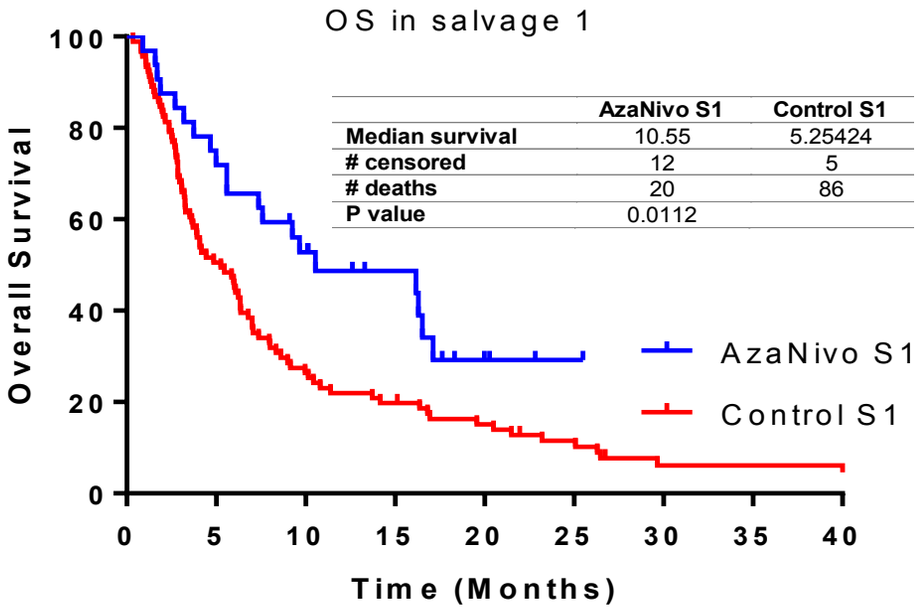
Marker	Clone
CD45	HI30
CD8a	RPA-T8
TIGIT	MBSA43
CD68	Y1/82A
CD34	581
CD4	RPA-T4
ICOSL	2D3
ICOS	ISA-3
OX40	ACT35
LAG-3	874501
PD-L1	MIH1
CD3	UCTH1
Tbet	4B10
TIM-3	F38-2E2
HVEM	ANC3B7
CD45RA	HI100
CD86	IT2.2
PD-1	EH12.2H7
VISTA	730804
CD19	HIB19
CCR7	G043H7
FoxP3	PCH101
BTLA	J168-540
RORgt	AFKJS-9
KI67	B56
CD28	CD28.2
CXCR3	G025H7
B7-H4	9M1-3
CD13, CD33	WM15, WM53
CTLA-4	14D3
GITR	621
PD-L2	24F.10C12
Eomes	WD1928
CD45RO	UCHL1
4-1BB	4B4-1
CCR6	G034E3
HLA-DR	L243

Supplemental Table 9C: Time points of bone marrow aspirate and peripheral blood collection for immune monitoring by flow cytometry or CYTOF

PA13 ACC#	Pre-Therapy		EOC1		EOC2		EOC4	
	BMA	PBMC	BMA	PBMC	BMA	PBMC	BMA	PBMC
638	x	X	x					
766	x	X	x		x		x	
854	x	X	x	X	x	X		
864	x	X	x	X	x	X		
871	x	X	x	X	x	X		
916	x	X	x	X	x	X	x	
917	x	X	x	X	x	X		
946	x	X			x	X		
1012	x	X	x	X	x	X		X
1051	x	X	x	X	x	X		
1063	x	X	x	X				
1065	x	X	x	X	x	X	x	
1074	x	X	x	X		X	x	X
1112	x	X	x	X	x	X		X
1127	x	X	x	X	x	X		
1215	x	X	x	X	x	X	x	X
1295	x	X	x	X	x	X	x	X
1310	x	X	x	X	x	X		
1342	x	X	x	X	x	X	x	X
1387	x	X	x	X	x	X	x	X
1523	x	X	x	X	x			X
1539	x	X	x	X	x	X		
1552	x	X	x	X		X		
1556	x	X	x	X	x	X		
1633	x	X	x		x			
1655	x	X	x	X	x	X		
1673	x	X	x	X	x	X	x	
1721	x	X	x	X	x	X		
1753	x	X	x	X	x	X		
1795	x	X	x	X	x			
1817	x	X	x		x			
1857	x	X	x	X			X	X
1993	x	X		X	x	X		X
2016	x	X	x	X				
2050	x	X	x	X	x		x	X
2148	x	X	x	X	x			X
2180	x	X	x	X			x	X
2254	x		x					
2259	x	X	x	X				X
2281	x	X	x	X	x			
2359	x	X	x	X	x	X	x	
2384	x	X	x	X				

Supplemental Figure 1: Correlation of responses (CR, CRi, PR, HI) to molecular and cytogenetic features





	AzaNivo S1	Control S1
Median survival	10.55	5.25424
# censored	12	5
# deaths	20	86
P value	0.0112	

Supplemental Figure 2A: Comparison of OS in azacitidine with nivolumab versus other HMA-based clinical trials in salvage 1 setting with median OS of 10.6 and 5.25 months, respectively (p value=0.0112)

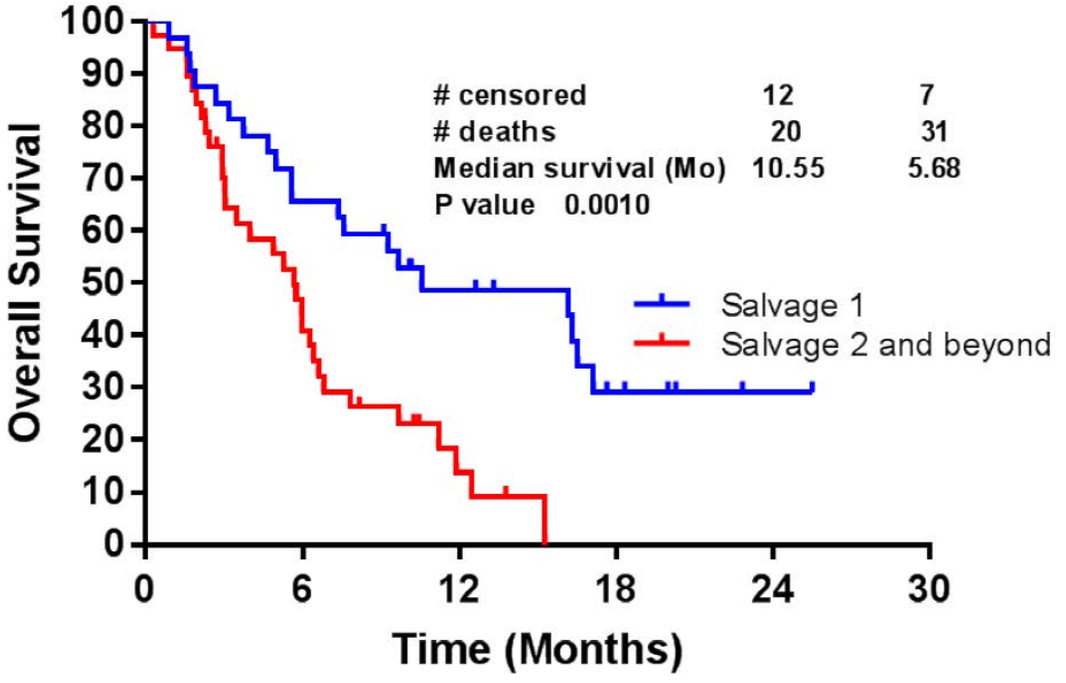
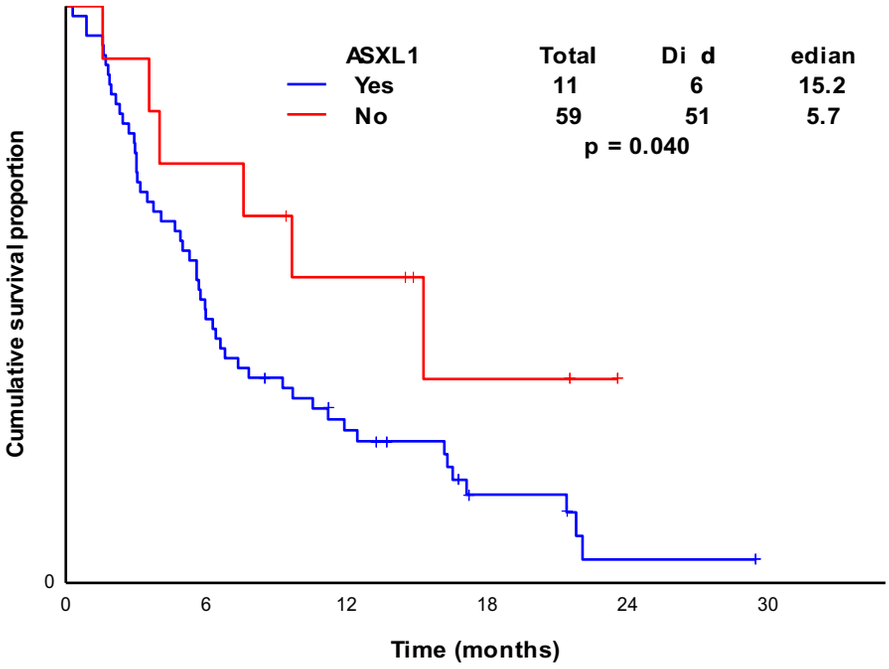
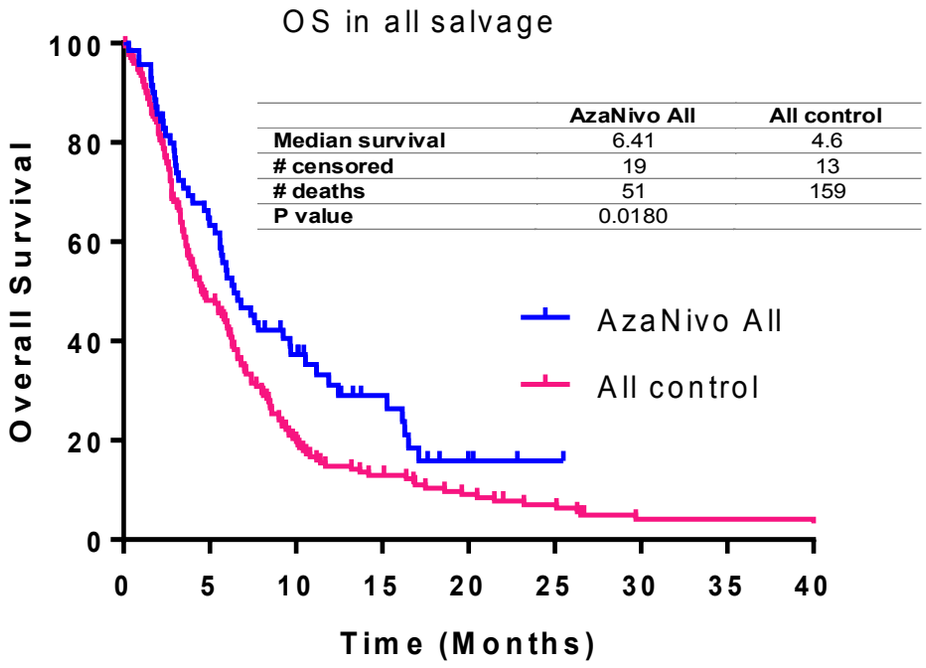


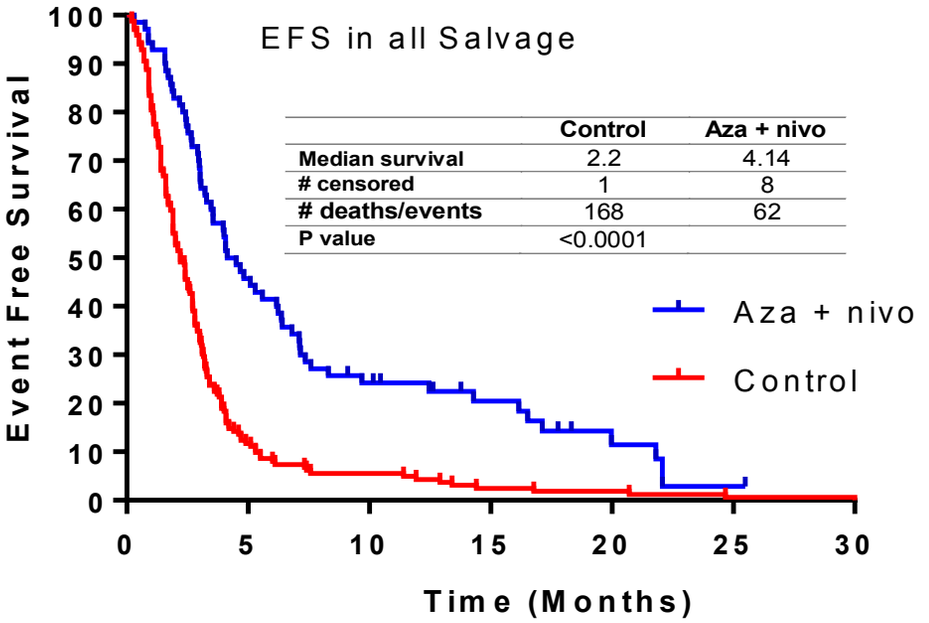
Figure 2B. Overall survival in patients who received azacitidine and nivolumab in first salvage (N=32) versus patients who received this in salvage 2 or beyond (N=38) (P value=0.001).



Supplemental Figure 2C: Overall survival with azacitidine and nivolumab in patients harboring an ASXL1 mutation versus those who do not have an ASXL1 mutation

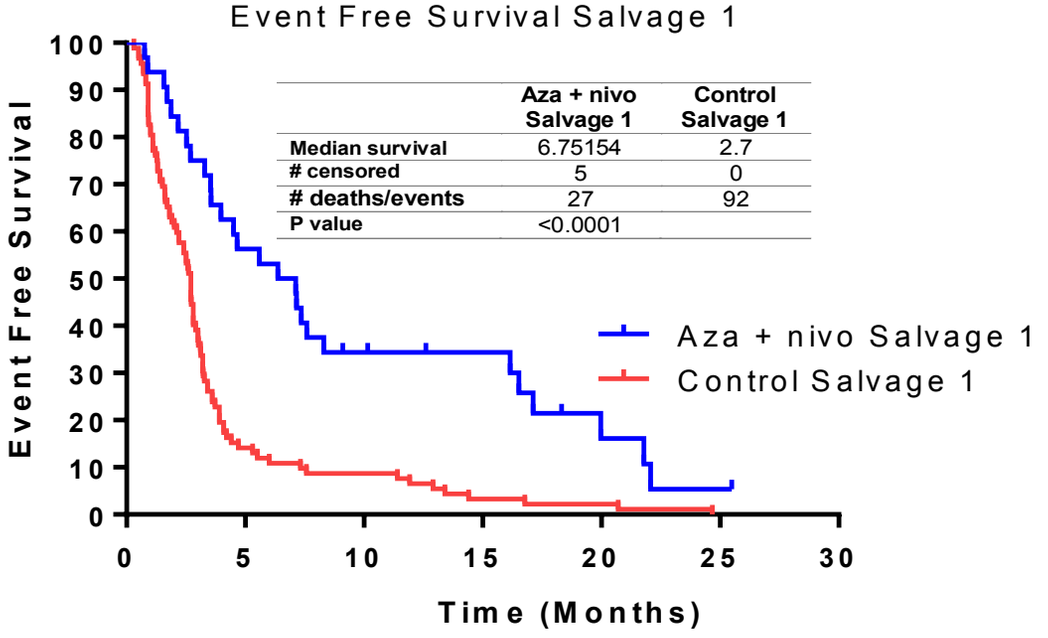


Supplemental Figure 2D: Comparison of OS in azacitidine with nivolumab versus other HMA-based clinical trials in all salvage setting with median OS of 6.3 and 4.6 months, respectively (p value=0.018)



	Control	Aza + nivo
Median survival	2.2	4.14
# censored	1	8
# deaths/events	168	62
P value	<0.0001	

Supplemental Figure 3A: Event Free Survival with azacitidine with nivolumab versus other HMA-based clinical trials in all salvage setting. The median EFS is 4.2 vs 2.2, respectively (P value= <0.0001).

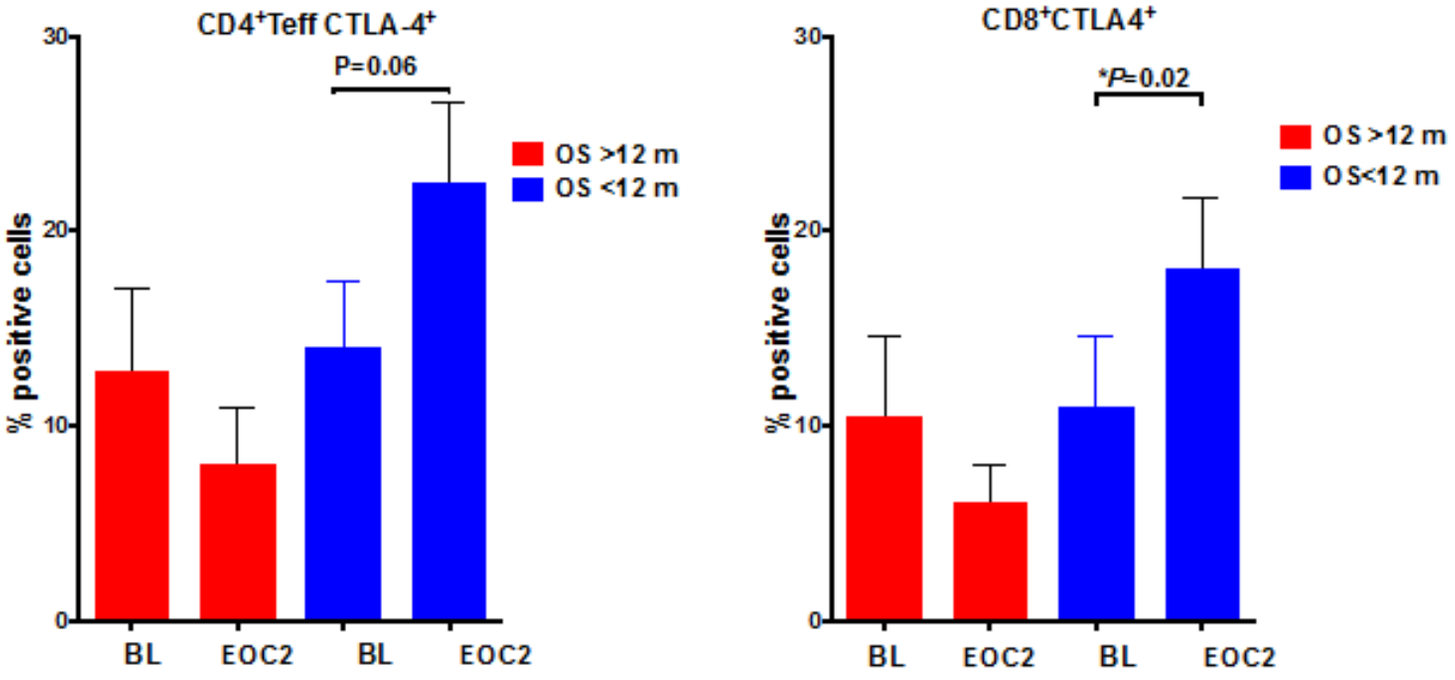


	Aza + nivo Salvage 1	Control Salvage 1
Median survival	6.75154	2.7
# censored	5	0
# deaths/events	27	92
P value	<0.0001	

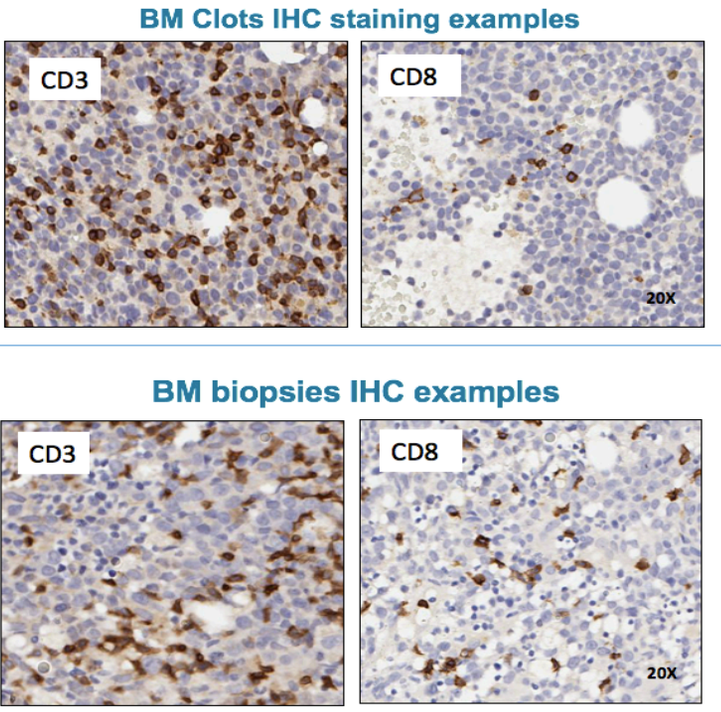
Supplemental Figure 3B: Event Free Survival with azacitidine with nivolumab versus other HMA-based clinical trials in the first salvage setting. The median EFS is 6.8 vs 2.7, respectively (P value= <0.0001)

Supplemental Figure 4

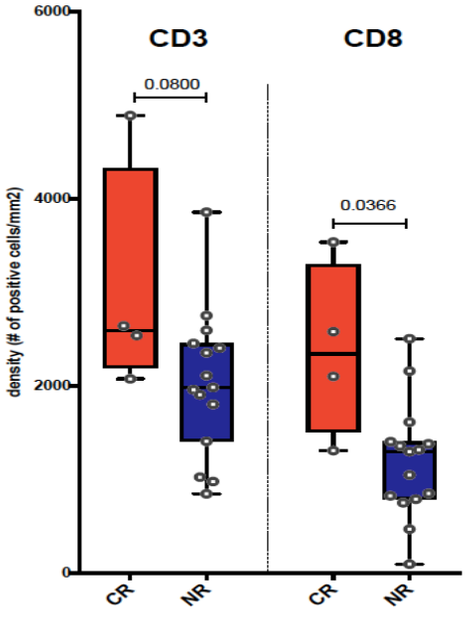
Increase in the frequency of CD4+Teff CTLA-4+ and CD8+CTLA4+ cells in BMAs following 4 doses of nivolumab (EOC2) was seen in patients with survival <1 year (n=30) but not in patients with survival >1 year (n=12), by 17-color flow cytometry.



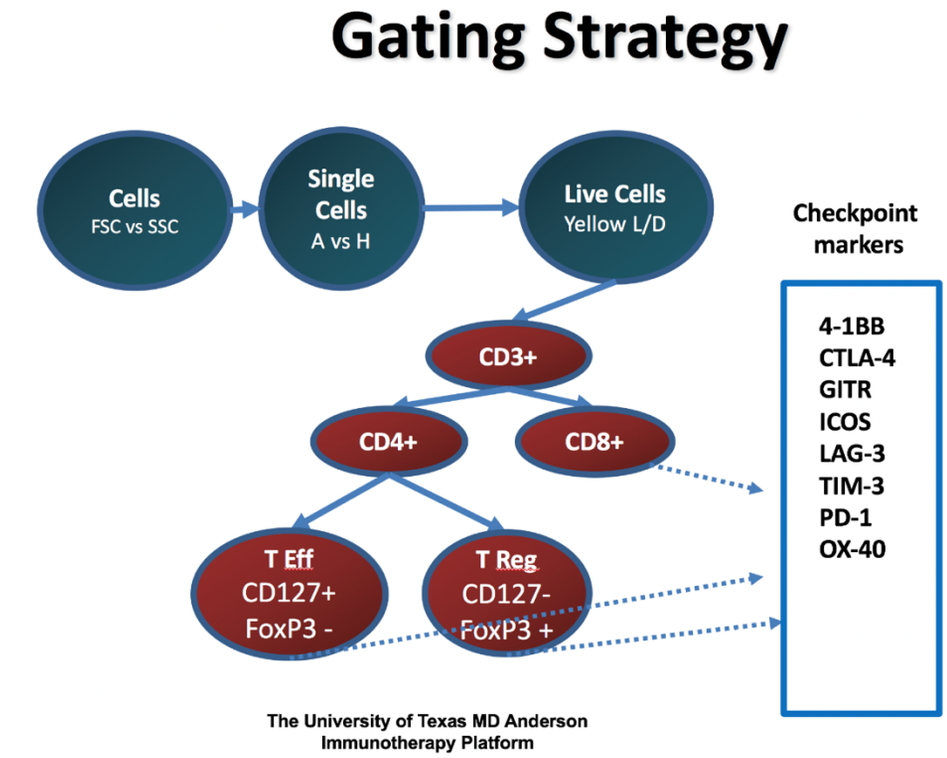
Supplemental Figure 5A: Representative samples of CD3 and CD8 staining by immunohistochemistry on bone marrow clots and bone marrow biopsies



Supplemental Figure 5B: Pretherapy bone marrow clot T cell infiltration in responders versus non-responders
Pretherapy $CD3^+$ and $CD8^+$ cell density was higher in the bone marrow clots of patients with CR/CRi/PR versus non-responders, by immunohistochemistry.
Abbreviations; CR: CR/CRi/PR; NR: Non-responder



Supplemental Figure 6A: Flow-cytometry gating strategy for T cells from bone marrow and peripheral blood



Supplemental Figure 6B: Flow-cytometry gating strategy for AML blasts from bone marrow and peripheral blood