Supplemental Appendix

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Methods

Detailed CTEP 10026 Trial Protocol

Eligibility

Eligible patients were aged ≥18 years with WHO diagnosis of AML or MDS with morphologic relapsed, refractory or secondary disease. Prior HMA therapy was allowed (protocol was amended after 3 patients progressed during lead-in decitabine cycle 0 to exclude patients who had evidence of overt disease progression on HMA within 12 weeks prior to study enrollment (stable disease allowed)). Patients with prior immune checkpoint inhibitor therapy or history of autoimmune disease were ineligible. Patients who were post HSCT were required to be >3 months from donor cell infusion, >8 weeks from donor lymphocyte infusion, have no history of serious (grade III-IV) acute GVHD, have ≥20% donor T-cell chimerism and be >14-days off systemic immunosuppressive agents (prednisone 5 mg/day and topicals allowed). Patients who underwent prior HSCT were enrolled into Arm A and those who were transplant naïve were enrolled into Arm B.

Study design and treatment

We conducted an open-label, investigator-initiated, Cancer Therapy Evaluation Program (CTEP)-sponsored phase 1 multi-center trial of ipilimumab combined with decitabine (NCT02890329; CTEP10026). The trial was approved by the central institutional review board (IRB) and the IRB at each participating institution and performed in accordance with the principles of the Declaration of Helsinki. All patients provided written informed consent. All authors had access to the primary clinical trial data. This trial was conducted at eight U.S. sites between 09/05/2017-08/02/2021. These sites included: Dana-Farber

Cancer Institute, University of Virginia, Case Comprehensive Cancer Center, Beth Israel Deaconess Medical Center, City of Hope, Massachusetts General Hospital, the Blood and Marrow Transplant Program at Northside Hospital, and the University of California-Davis.

Protocol treatment was the same for patients in Arm A (prior HSCT) and Arm B (transplant naïve), and consisted of a single lead-in cycle of decitabine monotherapy, followed by 'Ipilimumab Induction Phase' with combination of decitabine and ipilimumab (IPI+DEC) (cycles 1-4), and then 'Ipilimumab Maintenance Phase' with IPI+DEC (cycles 5-12). Cycles were every 28 days. DEC was 20 mg/m^2 by intravenous (IV) infusion on days 1-5 each cycle. In the absence of GVHD or disease progression during DEC lead-in cycle, patients proceeded to the Ipilimumab Induction Phase. Patients received IPI by IV infusion at 3 mg/kg (starting dose level, DL0), 5 mg/kg (DL1), or 10 mg/kg (DL2) on day 1 of each (cycles 1-4) or every other cycle (cycles 5-12). No intra-patient dose-escalation of IPI was allowed. DEC cycles could continue if IPI was held or discontinued for immune-related adverse events (irAEs). IPI was resumed once off systemic corticosteroids and resolution of irAE within 8 weeks, or permanently discontinued for any grade III or higher acute GVHD events. Protocol treatment was planned for up to 1 year with no more than 8 total doses of IPI. Upon completion of study treatment decitabine could continue off-study at the investigator's discretion.

The primary endpoint was to separately determine the maximum tolerated dose (MTD) or recommended phase 2 dose (RP2D) of combination IPI+DEC in prior HSCT and

transplant naïve patients. Secondary endpoints were to assess treatment efficacy and safety profile in each arm.

Safety and response assessments

All patients who received study treatment were evaluable for toxicity. Adverse events (AE) were coded according to the Common Terminology Criteria for Adverse Events, version 5.0. For immune monitoring, screening echocardiogram and EKG were required. At screening and every 12 weeks gamma-GT, direct bilirubin, LDH, TSH, and lipase were required. The incidence of acute and chronic GVHD was captured per established criteria.[1,2] Treatment response was determined per ELN for AML and IWG for MDS.[3,4] Response assessments were performed at the end of combination cycles 1, 2, 4, and every 3 cycles thereafter until off treatment. Donor chimerism was used for post-treatment assessments for PFS calculations. Additional assessments were required for those with myeloid sarcoma including PET/CT every 12 weeks or when clinically indicated. Overall response rate (ORR) as defined per protocol included complete remission (CR) and CR with incomplete count recovery (CRi) for those with AML and CR and marrow CR (mCR) with or without hematologic improvement (HI) for those with MDS. Marrow CR without HI has since been associated with outcomes similar to stable disease[5] and thus in additional studies including uni/multivariate analysis and comparison to historical cohort, responses were restricted to CR/CRi for AML and CR/marrow CR with HI for MDS for all patients included in the analysis.

Duration of remission is measured from the time of CR, CRi or marrow CR with HI (whichever is first recorded) until the first date of recurrent or progressive disease and censored at the time of analysis for administrative purposes.

Historical cohorts

We identified two historical AML cohorts who were treated with single-agent decitabine with response data at the Dana-Farber Cancer Institute (2016-2022). This includes 46 AML patients with morphologic relapse after transplant, including 33 with adverse risk and 5 with intermediate risk cytogenetics; 7/46 had missing cytogenetic data. Fifteen of these 46 patients received HMA prior to transplant. The transplant-naïve cohort included 44 older untreated AML patients, including 23 adverse risk, 15 intermediate risk, and 1 favorable risk based on available baseline characteristics. Only 1 untreated AML patient had prior HMA for MDS indication. A relapse cohort that received single-agent decitabine with response data could not be readily identified.

Correlative laboratory studies

Serial correlative samples were obtained from blood and bone marrow at pre-determined time points before, during and after treatment. Screening aspirate samples were submitted for targeted sequencing of commonly mutated myeloid genes.[6] Flow cytometry was performed on fresh whole blood samples before and after treatment with previously described staining, acquisition, and analysis methods.[7] A panel of directly conjugated monoclonal antibodies was used to define functionally distinct immune cell subsets. After staining, cells were acquired on a Fortessa LSR flow cytometer (BD) and analyzed using

FACSDiva software (BD). Multiplexed immunofluorescence (MIF) with a panel of antibodies against CD34, CD3, CD4, CD8, and granzyme B (GZMB), was applied to pre- and post-treatment bone marrows.

Statistical analysis

Dose escalation was determined using a standard 3+3 design with a 30% target dose-limiting toxicity (DLT) rate. DLTs were assessed during the first 8 weeks from time of first IPI dose administration and defined as any treatment-related death (not due to disease or intercurrent illness), acute GVHD overall grade III or higher, ≥ grade 3 non-hematologic toxicity, and grade 4 hematologic toxicity that did not recover by end of DLT period. This lengthy DLT observation was incorporated for potential delayed irAE. Grade 3 irAE that responded to corticosteroids and improved to ≤ grade 1 within 4 weeks were not considered as a DLT. Expansion cohorts (at least 6 additional patients per arm) were implemented to confirm safety and tolerability at MTD/RP2D. Descriptive summaries were provided for patient demographics, baseline characteristics, patient disposition, toxicity and objective responses. The Kaplan-Meier method was used to assess progression-free survival (PFS) and OS. Time-to-event variables were calculated from study entry. Methods for statistical comparisons are noted in figure legends. All comparisons used 2-sided $p<0.05$ for significance. All analyses were based on the 17 February 2022 data cut-off date.

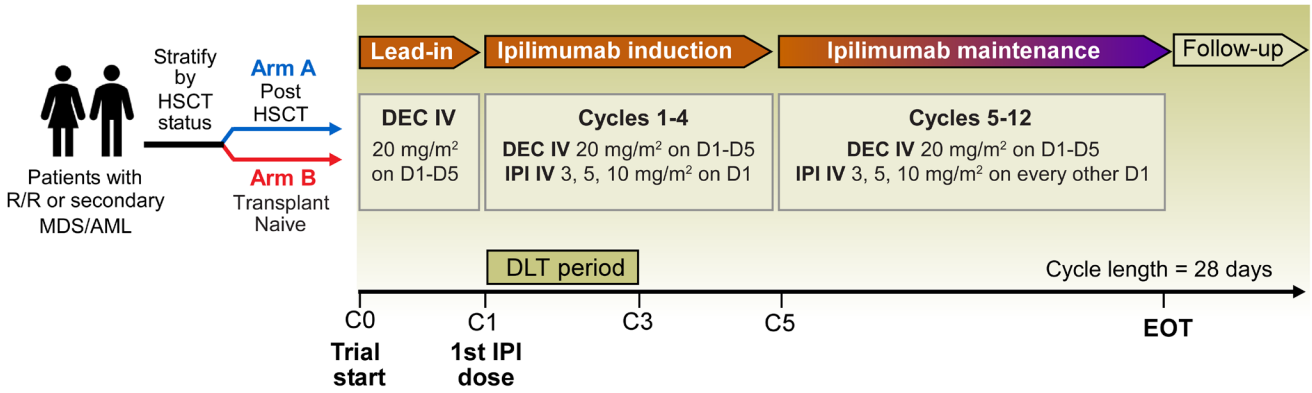
Multiplexed immunofluorescence

Staining was performed on the BOND RX fully automated stainers (Leica Biosystems). Tissue sections of 5-µm thick FFPE were incubated for 3 hours at 60°C before loading into the BOND RX. Slides were deparaffinized (BOND DeWax Solution, Leica Biosystems, Cat. AR9590) and rehydrated with series of graded ethanol to deionized water. Antigen retrieval was performed in BOND Epitope Retrieval Solution 1 (pH 6) (ER1, Leica Biosystems, Cat. AR9961) at 95°C. Deparaffinization, rehydration and antigen retrieval were pre-programmed and executed by the BOND RX. Next, slides were serially stained with primary antibodies with an incubation time of 40 minutes per antibody. As an additional step for mouse antibodies, rabbit anti-mouse IgG (Post Primary, BOND Polymer Refine Detection Kit, Leica Biosystems, Cat. DS9800) was applied for 15 minutes. Signal for antibody complexes was labeled and visualized by their corresponding Opal Fluorophore Reagents (Akoya) by incubating the slides for 5 minutes. Slides were then manually counterstained with DAPI (NucBlue Fixed Cell ReadyProbes Reagent, Invitrogen, Cat. R37606), washed with deionized water, air dried, and mounted with ProLong Diamond Anti-fade Mountant (Life Technologies, Cat. P36965). Slides were stored in a light-proof box at 4℃ prior to imaging. The target antigens, antibody clones, dilutions, and antigen retrieval conditions are listed in **Supplementary Table 5**.

Image acquisition was performed using the Mantra Quantitative Pathology Workstation (Akoya Biosciences). Areas with non-tumor or residual normal tissue were excluded from the analysis. Representative regions of interest were chosen by the pathologist, and 1-6 fields of view (FOV) were acquired at 20x resolution. Once the FOV were spectrally

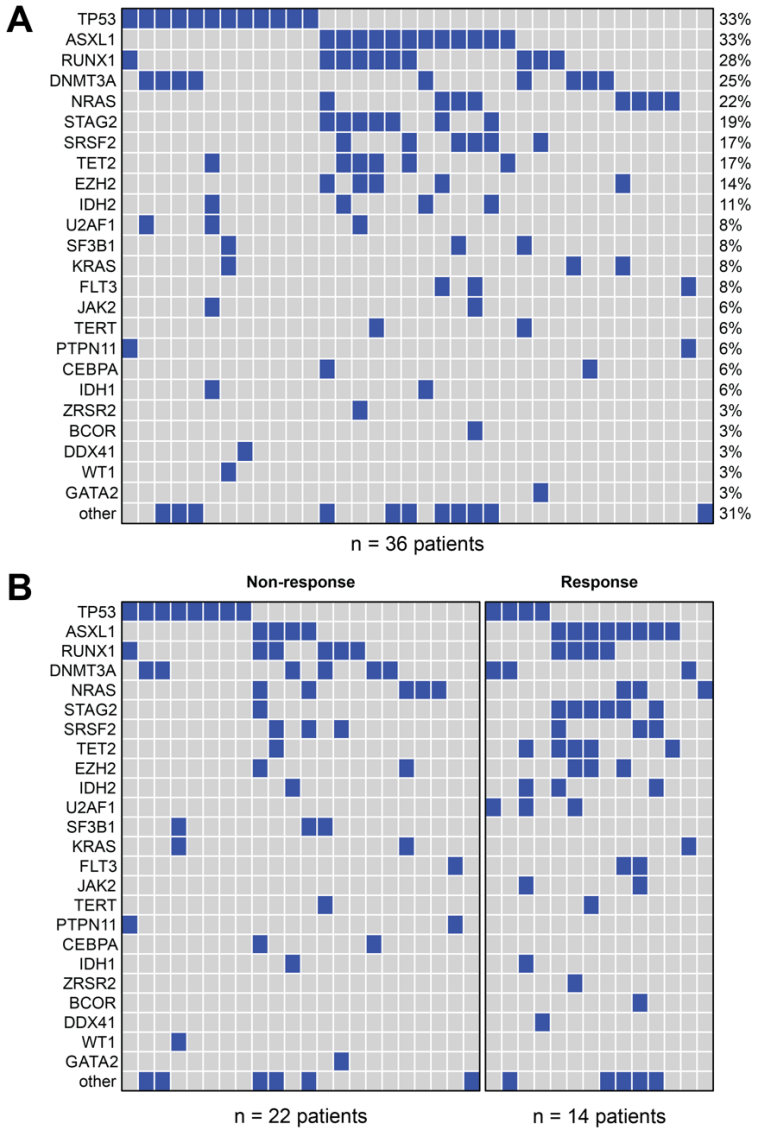
unmixed, cell identification was performed using supervised machine learning algorithms within Inform 2.4 (Akoya). This image analysis software assigns phenotypes to all cells in the image, based on a combination of immunofluorescence characteristics associated with segmented nuclei (DAPI signal). Each cell-phenotype specific algorithm is based upon an iterative training / test process, whereby a small number of cells (training phase, typically 15-20 cells) are manually selected as being most representative of each phenotype of interest and the algorithm then predicts the phenotype for all remaining cells (testing phase). The software's predictions can be over-ruled to improve accuracy until phenotyping is optimized. Thresholds for "positive" staining and the accuracy of phenotypic algorithms were optimized and confirmed under pathologist supervision for each case.

Supplemental Figures

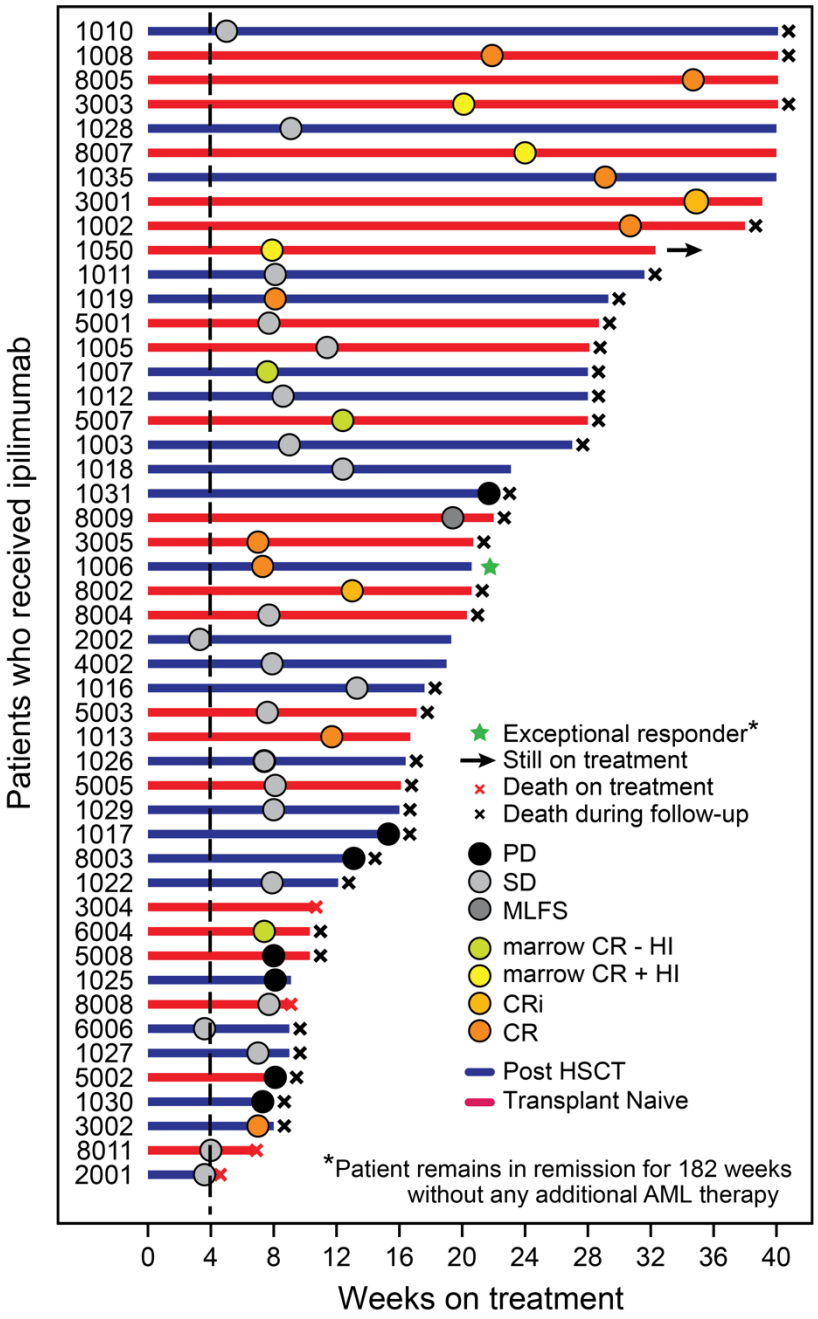


Supplemental Figure 1: CTEP 10026 study design.

Trial design with lead-in decitabine cycle, ipilimumab induction and ipilimumab maintenance phases of treatment with combination therapy and dosing schedule. IPI, ipilimumab; HSCT, allogeneic hematopoietic cell transplant; R/R, relapsed/refractory; MDS, myelodysplastic syndromes; AML, acute myeloid leukemia; DEC, decitabine; EOT, end of treatment; DLT, dose-limiting toxicity



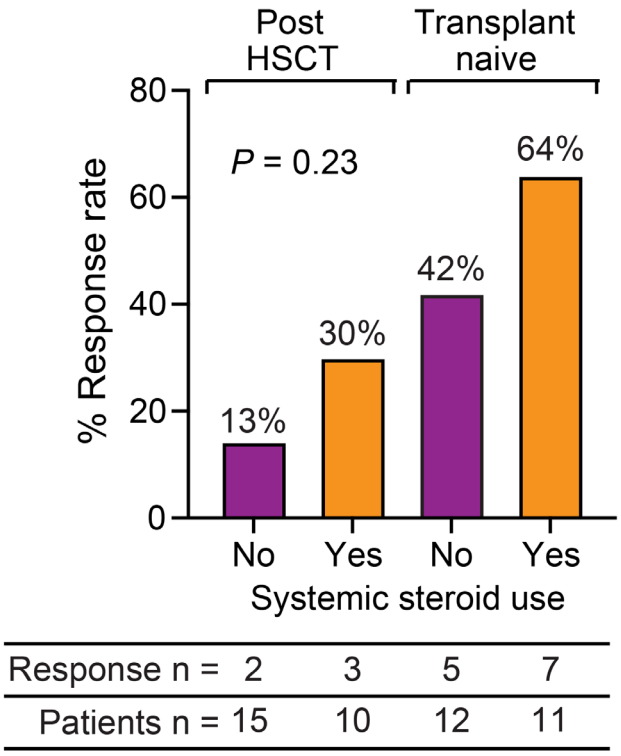
Supplemental Figure 2: Baseline co-mutation plot by next-generation sequencing. Among 41 patients treated with IPI+DEC with evaluable next-generation sequencing (NGS) baseline testing, 5 had no pathogenic variant detected. Genes with genomic alterations are listed in descending order of frequency and each column represents an individual patient. Blue indicates that alteration was detectable on a clinical NGS panel. Other mutations present at low frequency: CBL, RIT1, SETBP1, CUX1, GNB1, NF1, ETV6, ATRX and NFE2. All 36 evaluable patients ordered by frequency of mutated genes **(A)** and grouped by achievement of objective response on study treatment **(B)**.



Supplemental Figure 3: Swimmer Plot Showing Duration of Study Treatment.

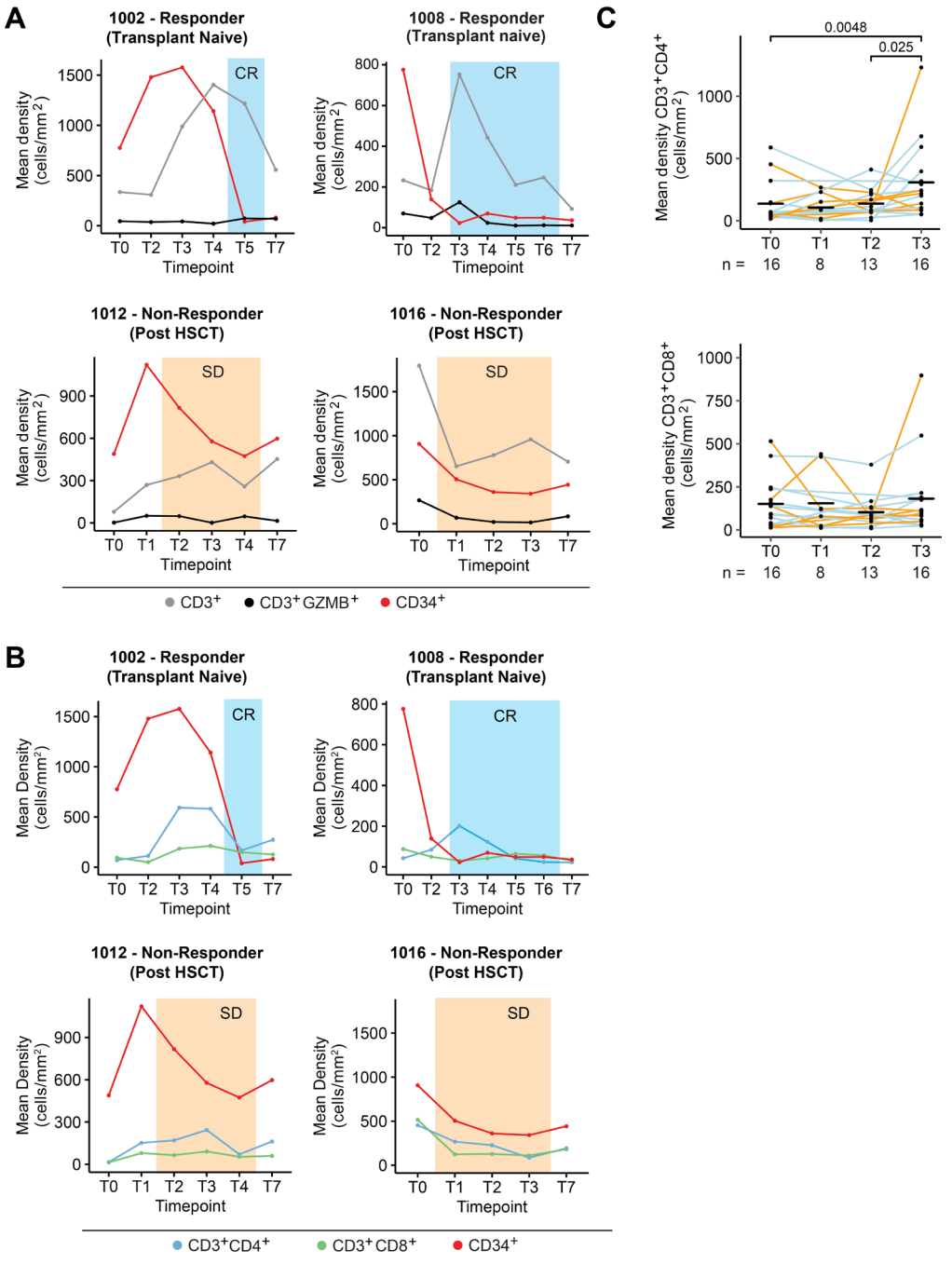
Swimmer plot demonstrates time to best response for individual patients among those who received ipilimumab in Arm A, post HSCT (blue) and in Arm B, transplant naive (red). Key indicates response achieved on study and whether patients continued after 40 weeks on treatment. Dashed line indicates when first dose of ipilimumab was received. *Green star denotes an exceptional post HSCT responder (patient 1006) who rapidly achieved

CR after one combination cycle of IPI+DEC and developed concomitant GVHD. Subject remains in CR >3.5 years without requiring any additional therapy.



Supplemental Figure 4. Response and immune toxicity after IPI+DEC.

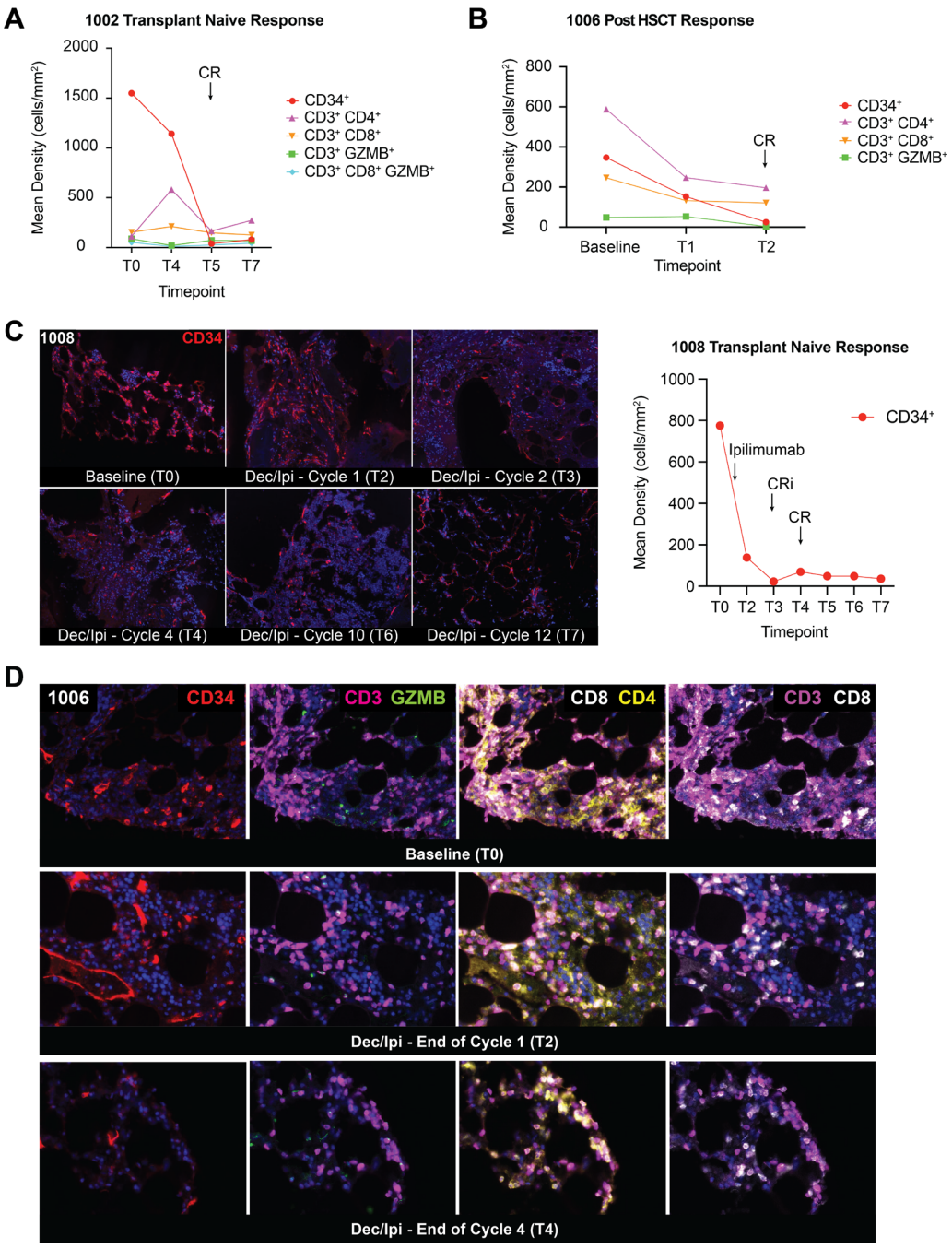
The proportion of patients who achieved an objective response (complete remission (CR), CR with incomplete count recovery (CRi), or marrow CR with or without hematologic improvement) among those who received IPI+DEC with concomitant irAE requiring systemic steroid use by treatment arm. Note, patients with irAE requiring topical steroids only were not included in this analysis (n=2). Among those with immune toxicity requiring systemic steroid use, 4 of the responders had a response observed prior to an immune toxicity (1 in post-HSCT (Arm A), 3 in transplant-naïve (Arm B)), and 6 afterwards (2 in post-HSCT (Arm A), 4 in transplant-naïve (Arm B)).



Supplemental Figure 5. Longitudinal dynamics of T cell subsets by multiplex immunofluorescence.

Multiplex immunofluorescence of bone marrow biopsies obtained serially from patients before and after combination decitabine and ipilimumab therapy in representative cases

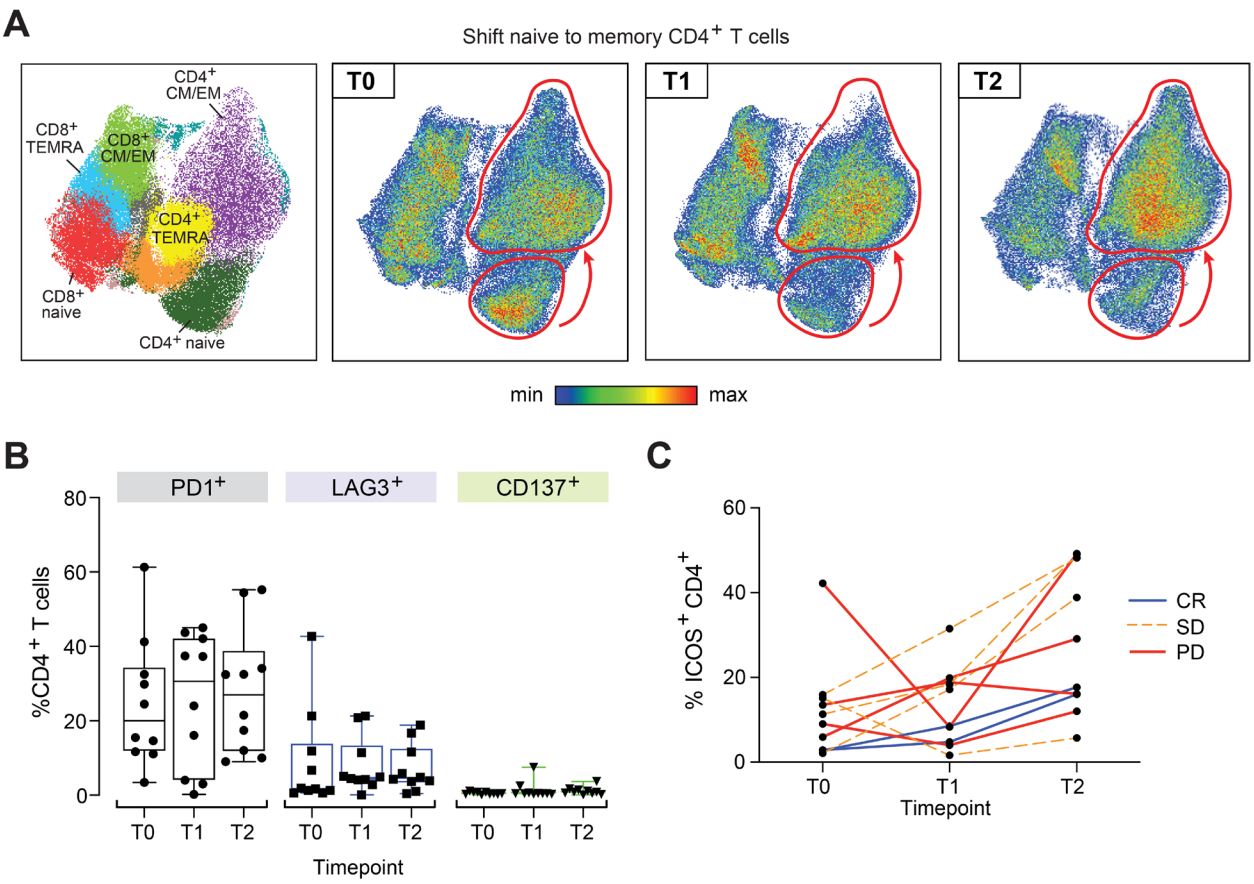
and pooled data from paired samples. Immune histochemistry (IHC) staining density was semi-quantified by Inform software. **(A)** Longitudinal measurements of mean density of CD3+, CD3+ GZMB+, and CD34+ cells at each time point and time of response achievement (shaded blue) in two responders (1002 and 1008 are complete remission (CR) cases) and non-responders (1012 and 1016 are stable disease cases). **(B)** Longitudinal measurements of mean density CD3+CD4+ and CD3+CD8+ T cell subsets and CD34+ in transplant-naïve responders (patient 1002 and patient 1008) and two post-transplant non-responders (patients 1012 and 1016). **(C)** Dynamic changes in T cell subsets including $CD3^{+}CD4^{+}$ and $CD3^{+}CD8^{+}$ among 16 available paired samples before and after IPI+DEC treatment. Statistical testing using a Wilcoxon signed-rank test for paired samples and Wilcoxon rank-sum for unpaired samples. T0, screening; T1, end of lead-in decitabine; T2, end of combination cycle 1; T3, end of combination cycle 2; T4, end of combination cycle 4; T5, end of combination cycle 7; T6, end of combination cycle 10; and T7, end of treatment or relapse.



Supplemental Figure 6. Longitudinal evaluation of tumor immune infiltrate among responder cases.

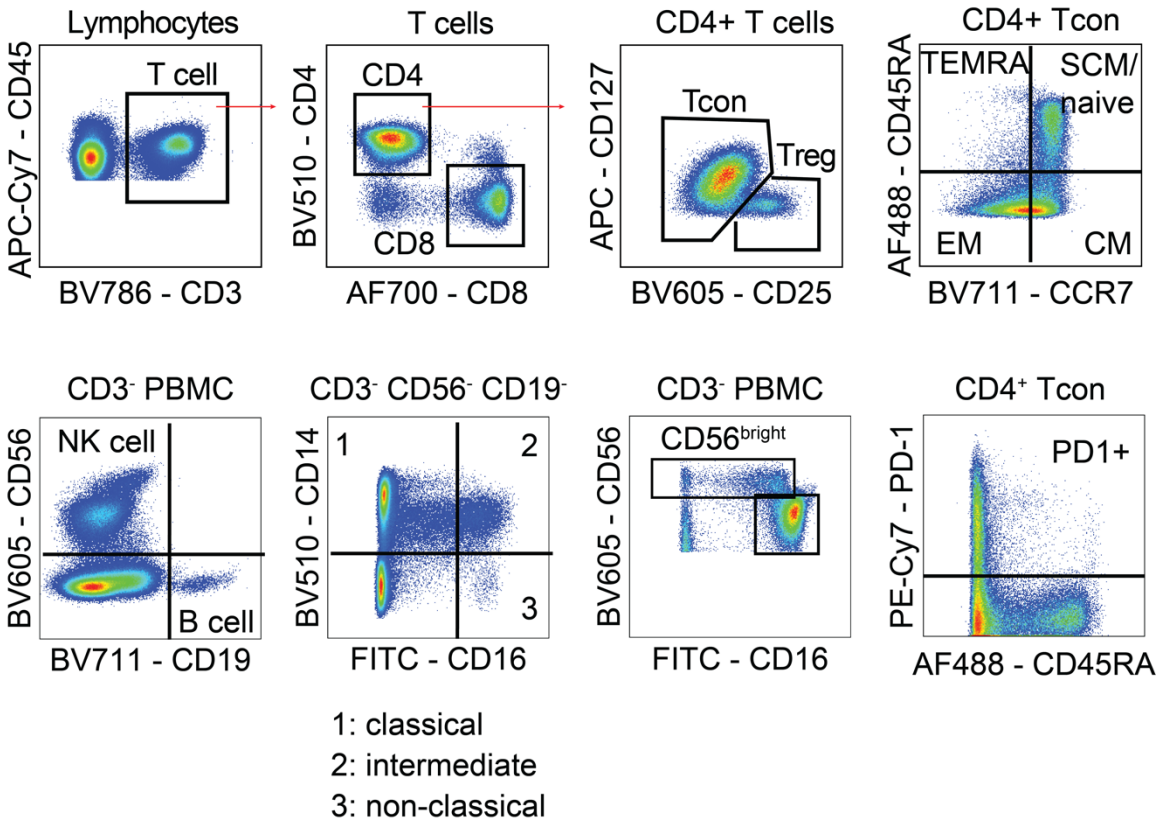
Multiplexed immunofluorescence (MIF) analysis of bone marrow biopsies obtained serially from patients before and after combination decitabine and ipilimumab therapy in representative cases. Immune histochemistry (IHC) staining density was semi-quantified

by Inform software. Shown are measurements of mean density of $CD34^+$, $CD3^+$ $CD4^+$, $CD3^+$ $CD8^+$, CD3+GZMB+, and CD3+CD8+GZMB+ cells. **(A)** In transplant-naïve patient 1002 (responder) scatter plot shows mean density of $CD34^+$, $CD3^+$ $CD4^+$, $CD3^+$ $CD8^+$, $CD3^+$ $GZMB^+$, $CD3^+$ $CD8^+$ $GZMB^+$ cells at baseline, right before response (T4), at remission (black arrow, T5) and early relapse two cycles later (T7). **(B)** Scatter plot from patient 1006 (Arm A/prior HSCT), who was an exceptional responder to study treatment. Mean density of $CD34^+$, $CD3^+$ $GZMB^+$, $CD3^+$ $CD4^+$ and $CD3^+$ $CD8^+$ cells at each time point and time of complete remission (CR) achievement (black arrow). **(C)** *Left*, MIF images showing decreasing leukemia burden in response to DEC+IPI in patient 1008 (Arm B/transplant naïve) who achieved CR with incomplete count recovery (CRi) at the end of combination cycle 2 (T3) and CR at end of combination cycle 4 (T4). *Right,* scatterplot shows the mean density $CD34^+$ cells over time with arrows indicating IPI initiation and CR/CRi achievement. (**D)** Serial MIF images with CD34 (red), CD3 (purple), CD4 (yellow), GZMB (green) and CD8 (white) staining from responder patient 1006 (Arm A/prior HSCT).



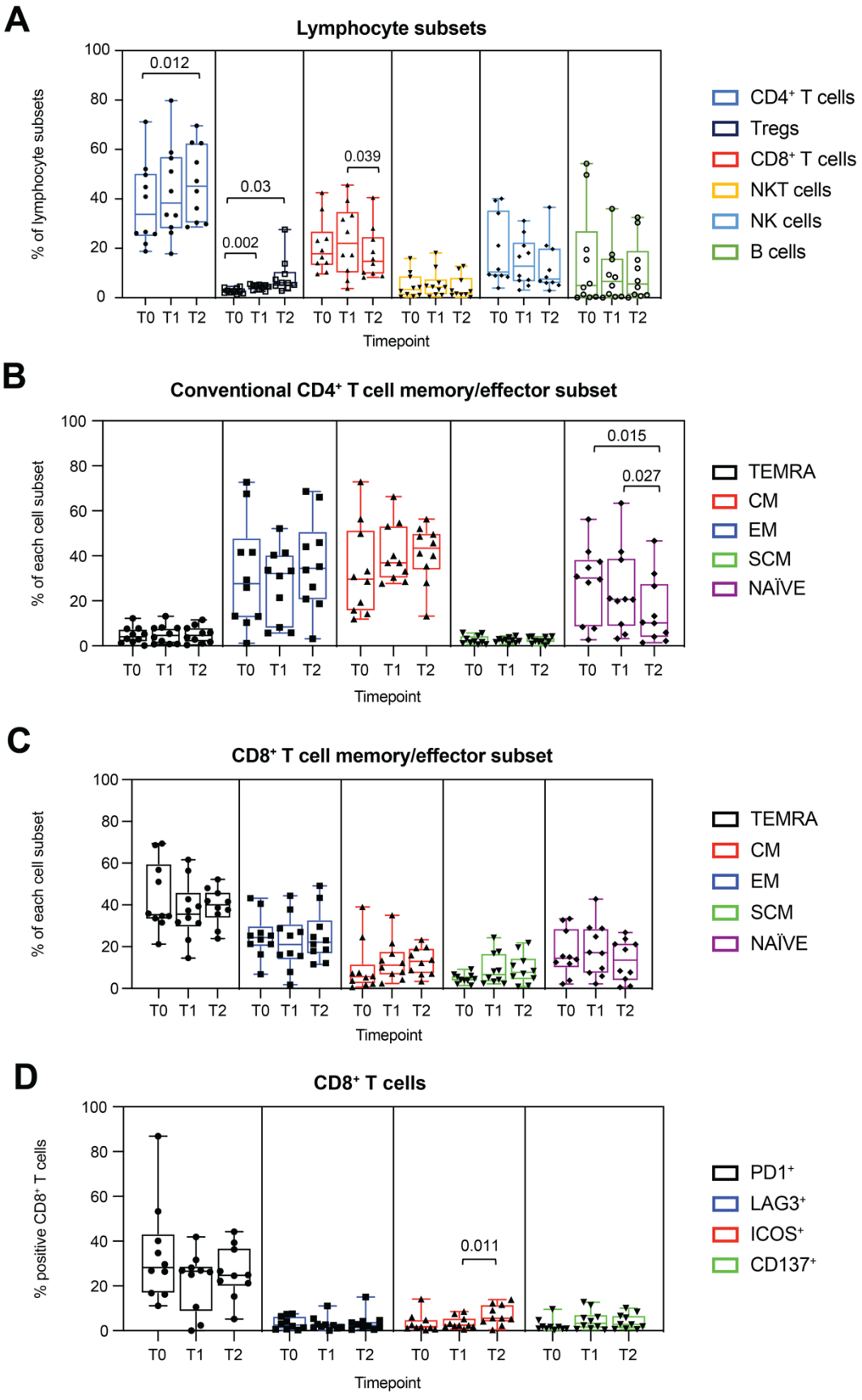
Supplemental Figure 7. Serial immunophenotyping before and after decitabine and ipilimumab therapy.

(A) UMAP plot with cells colored according to the memory/effector T cell subpopulations obtained from FlowSOM (left panel). Generated UMAPs were stratified by each timepoint T0 (screening), T1 (after 1 cycle of decitabine priming) and T2 (after 1 cycle of combination decitabine and ipilimumab) (right panels). **(B)** Differences in expression of PD1, LAG3, ICOS and CD137 positive cells in $CD4^+$ T-cells. **(C)** $ICOS^+$ cells were increased regardless of clinical response. Box plots indicate median, Q1 and Q3 and min and max. *P*-values were determined with the two-sided, paired t-test. T0, screening; T1, end of lead-in decitabine; T2, end of combination IPI+DEC cycle 1.



Supplemental Figure 8. Gating strategy used for flow cytometry analysis of peripheral blood.

Lymphocytes were identified by their low side scatter, low forward scatter and CD45 expression. Blood cell subsets were quantified by using the expression of the 9-10 surface markers, SSC and FSC signals. 10,000 cells/sample were randomly selected and used from all available raw fcs data files.


A
Lymphocyte subsets
% of lymphocyte subsets
100
80
60
40
20
0
0.012
0.03
0.002
0.039
T0 T1 T2
Timepoint
CD4⁺ T cells
Tregs
CD8⁺ T cells
NKT cells
NK cells
B cells
B
Conventional CD4⁺ T cell memory/effector subset
% of each cell subset
0.015
0.027
TEMRA
CM
EM
SCM
NAÏVE
C
CD8⁺ T cell memory/effector subset
D
CD8⁺ T cells
% positive CD8⁺ T cells
0.011
PD1⁺
LAG3⁺
ICOS⁺
CD137⁺

Supplemental Figure 9. Characterization of lymphocyte subsets before and after decitabine/ipilimumab.

(A) Comparison of lymphocyte subsets between each timepoint. Flow cytometric quantification of conventional $CD4^+$ T cells (dark blue), regulatory T cells (black), $CD8^+$ T cells (red), NKT cells (yellow), NK cells (light blue) and B cells (green). (**B-C**) The differences in the percentages of each T cell memory/effector cell subset in conventional $CD4^+$ T cells and $CD8^+$ T cells. TEMRA (effector memory T cells re-expressing CD45RA; $CD45RA^+/CCR7^-$, black), EM (effector memory; $CD45RA^-/CCR7^-$, blue), CM (central memory; $CD45RA^-/CCR7^+$, red), SCM (stem cell memory; $CD45RA^+/CCR7^+/CD95^+$, green) and NAÏVE ($CD45RA^+/CCR7^+/CD95^-$, purple). **(D)** PD1 (black), LAG3 (blue), ICOS (red) and CD137 (green) positive cells in $CD8^+$ T cells. Box plots indicate median, Q1 and Q3 and min and max. *P*-values were determined with the two-sided, paired t-test. T0, screening; T1, after decitabine lead-in cycle, T2 at the end of combination IPI+DEC cycle 1.

Supplemental tables

Supplemental Table 1: Demographic and clinical characteristics of entire study cohort, stratified by treatment arm assignment

Characteristic	Treatment Arm		*P*
	Arm A N=27	Arm B N=27	
Median age (range), y	66 (29-79)	75 (34-85)	0.006
Sex, n (%)			
Male	10 (37)	20 (74)	0.006
Female	17 (63)	7 (26)	
ECOG PS, n (%)			
0	6 (22)	3 (11)	0.59
1	20 (74)	22 (82)	
2	1 (4)	2 (7)	
Histology, n (%)			0.10
MDS	2 (20)	8 (80)	
untreated t-MDS[a]	0 (0)	1 (13)	>0.99
R/R	2 (100)	7 (88)	
AML	25 (93)	19 (70)	
untreated sAML[b]	0 (0)	3 (16)	0.07
R/R	25 (100)	16 (84)	
Myeloid sarcoma without marrow involvement, n (%)	5 (19)	1 (4)	0.19
Number of prior therapies, median (range)	2 (1-6)	1 (0-3)	0.005
Prior HMA exposure, n (%)	18 (67)	17 (63)	0.78
Number of prior HMA cycles, median (range)	3 (1-35)	7 (1-70)	0.42
***TP53* mutation, n (%)**			0.34
No	20 (74)	14 (52)	
Yes	5 (19)	7 (26)	
Unknown	2 (7)	6 (22)	
Days from allo-HSCT, median (range)	308 (99-3070)	n/a	-
History of GVHD, n (%)	10 (37)	n/a	-
HLA matching, n (%)		n/a	-
Full HLA-MRD	6 (22)	n/a	-
Full HLA-MUD	12 (44)	n/a	-
9/10 mismatched	5 (19)	n/a	-
HLA-haploidentical donor	4 (15)	n/a	-
Donor source, n (%)		n/a	-
Peripheral-blood stem cells	23 (85)	n/a	-
Marrow	4 (15)	n/a	-

HSCT, allogeneic hematopoietic stem cell transplant; HLA, human leukocyte antigen; MRD, matched related donor; MUD, matched unrelated donor
[a]Subject received prior growth factor and luspatercept.
[b]Includes 1 case of AML arising out of MDS (previously treated with growth factor only) and 2 cases of AML arising out of MPN.

Supplemental Table 2A. Patient disposition on trial.

Patient Disposition	Treatment Arm		Total N
	Arm A Post HSCT	Arm B No Prior HSCT	
Number of total enrolled patients	27	27	54
Discontinued study treatment after lead-in decitabine cycle	2 (7)	4 (15)	6
Disease progression[a], n (%)	2 (7)	1 (4)	3
Withdrawal[b], n (%)	-	3 (11)	3
Number that received Ipilimumab on study	25	23	48
DL0	7	4	11
DL1	3	3	6
DL2 (escalation + expansion)	15	16	31
Median number of Ipilimumab doses (range)	3 (1-7)	3 (1-8)	3 (1-8)
Death within 30 days of enrollment[c], n (%)	2 (7)	2 (7)	4
Death within 60 days of enrollment[d], n (%)	4 (15)	3 (11)	7

[a]Progression in patients who progressed on HMA immediately prior to study entry; trial amended to exclude patients who progress on HMA within 12 weeks of study entry
[b]Withdrawal reasons: patient preference
[c]None considered DLT events (Ipilimumab was not received)
[d]Includes those that died within 30 days of study. None considered DLT events. Four did not receive IPI+DEC. Reasons for death include: 3 disease-related complications; 1 bronchopulmonary hemorrhage; 1 respiratory failure, 1 stroke from uncontrolled atrial fibrillation, and 1 neutropenic fever.

Supplemental Table 2B. Number of Treatment Cycles Received

		Cycle											
	Decitabine Lead-in, Cycle 0	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**
# patients	54	48	36	33	24	17	11	9	7	6	6	5	4

Supplemental Table 3. Clinical Annotation of Individual Study Patients

Study ID	Arm	Dose Level	MDS or AML	Sole EM AML	EM Organ(s) Involved	Disease Status at Study Entry	Prior HMA (Y/N)	Screening Cytogenetics	Screening Mutations	Screen Aspirate Blast (%)	Screen Core Blast (%)	Hx of DLI?	Baseline Chimerism	Received IPI on Trial
6001	A	3	AML	No	n/a	relapse	No	Complex	n/a	24	51	No	100	No
1003	A	3	AML	No	skin	refractory	Yes	Normal	CEBPA, DNTM3A	0	5 to 10	No	77	Yes
1006	A	3	AML	No	n/a	relapse	Yes	Normal	U2AF1	11	5 to 10	No	92	Yes
1007	A	3	MDS	No	n/a	relapse	Yes	Normal	IDH2, ASXL1, RUNX1, SRSF2, STAG2, TET2	13	5 to 10	No	59	Yes
1010	A	3	AML	No	n/a	relapse	Yes	Normal	NRAS	44	30	No	99	Yes
1011	A	3	AML	No	n/a	relapse	No	Normal	DNMT3A	28	50	No	20	Yes
1012	A	3	AML	No	n/a	refractory	Yes	Complex	TP53	32	50	No	80	Yes
2001	A	3	AML	No	n/a	refractory	Yes	Complex Monosomal	TP53	78	80	No	85	Yes
1015	A	5	AML	No	n/a	refractory	No	Complex Monosomal	DNMT3A, TP53	15	20 to 30	Yes	100	No
1016	A	5	AML	No	n/a	relapse	No	n/a	None Detected	0	30 to 40	No	100	Yes
1017	A	5	AML	Yes	pleural effusion, 5th left lateral rib, soft tissue masses in mediastinum and along thoracic vertebrae	relapse	No	n/a	None Detected	1	<5	No	99	Yes
8003	A	5	AML	No	n/a	refractory	Yes	46,XX,t(6;11)(q27;q23)[20]	NRAS	30	70	No	23	Yes
1018	A	10	AML	Yes	R femur	relapse	No	Normal	None Detected	0	<5	Yes	100	Yes
1019	A	10	AML	No	n/a	relapse	Yes	47,XY,+8[4]/ 47,XY,+21[3]/ 46,XY[8]	ASXL1, RUNX1, STAG2	22	60	Yes	99	Yes

1022	A	10	AML	No	n/a	refractory	Yes	Complex Monosomal	DNMT3A, RUNX1, SF3B1, TERT	13	50	No	64	Yes
1025	A	10	AML	Yes	L2 soft tissue mass	refractory	Yes	Normal	None Detected	3	<5	No	100	Yes
1026	A	10	AML	No	n/a	refractory	Yes	46,XX,t(9;12)(q22;q13)c[cp20]	ASXL1, GNB1, NRAS, SF3B1, SRSF2	26	5	No	62	Yes
1027	A	10	AML	No	n/a	relapse	Yes	Complex Monosomal	DNMT3A, NF1, TP53	21	60	No	70	Yes
1028	A	10	MDS	No	n/a	relapse	Yes	47,XX,+8[3]/47,idem,t(12;15)(p13;q15)[17]	GATA2, RUNX1, SRSF2	11	10 to 15	No	96	Yes
1029	A	10	AML	No	n/a	refractory	Yes	46, XY, +1, der(1;12)(q10;q10[1]/46,XY, dup(1)(q21q32)[18]/46,XY[1]	ASXL1, CBL, CEBPA, EZH2, NRAS, RUNX1, STAG2	2	<5	No	98	Yes
1030	A	10	AML	No	n/a	relapse	No	Normal	DNMT3A, IDH1, IDH2, NPM1, PTPN11	39	60	No	97	Yes
1031	A	10	AML	No	Epidural masses and R femoral neck	relapse	No	Normal	TP53, U2AF1	27	80	No	100	Yes
1035	A	10	AML	Yes	Breast	refractory	No	Normal	KRAS, WT1	2	<5	No	99	Yes
2002	A	10	AML	No	n/a	relapse	No	47,XY,+13,del(15)(q11.2q22)[10]/46,XY[10]	SETD2, RUNX1, RUNX1	51	40	No	94	Yes
3002	A	10	AML	Yes	Skin only	relapse	Yes	n/a	n/a	-	-	No	97	Yes
4002	A	10	AML	No	n/a	relapse	No	del(9q)[18]	KRAS, NRAS, EZH2	40	40	No	100	Yes
6006	A	10	AML	No	n/a	refractory	Yes	Monosomy 17	BCOR, DNMT3A, EGFL7, IDH1, RUNX1, TP53, URBR5	48	48	No	98	Yes

1002	B	3	AML	No	n/a	untreated sAML	No	Complex	IDH1, IDH2, JAK2, TET2, TP53, TP53, U2AF1	56	60	n/a	n/a	Yes
7001	B	3	AML	No	n/a	refractory	Yes	Complex Monosomal	n/a	22	30	n/a	n/a	No
1001	B	3	AML	No	n/a	untreated sAML	No	45,XX,der(7;8)(q10;q10)[8]/46,XX[12]	IDH1, NPM1	27	20 to 30	n/a	n/a	No
5001	B	3	AML	No	n/a	refractory	Yes	Monosomy 17	TP53, SF3B1, KRAS, WT1	31.5	30	n/a	n/a	Yes
5002	B	3	MDS	No	n/a	refractory	Yes	del 5q; del 7q	n/a	5	5	n/a	n/a	Yes
5003	B	3	AML	No	n/a	relapse	No	46,XY,del(7)(q21)[10]/47,XY,+8[5]/46,XY[5]	RUNX1 and KRAS	7	n/a	n/a	n/a	Yes
1005	B	5	AML	No	n/a	refractory	Yes	Normal	DNMT3A, ASXL1, IDH1, IDH2	16	20	yes	n/a	Yes
1008	B	5	AML	No	n/a	untreated sAML	No	Normal	PHF6, RUNX1, SF3B1, TET2	39	30 to 40	n/a	n/a	Yes
6002	B	5	AML	No	n/a	refractory	Yes	46,XY,del(6)(p22.2)[7]	ASXL1	80	n/a	n/a	n/a	No
8001	B	5	AML	No	n/a	refractory	Yes	Normal	ASXL1, SF3B1	46	n/a	n/a	n/a	No
8002	B	5	AML	No	n/a	refractory	Yes	Normal	ASXL1, IDH2, SRSF2, STAG2	12	n/a	n/a	n/a	Yes
1013	B	10	AML	Yes	skin only	relapse	No	Normal	DNMT3A, KRAS	0	<5	n/a	n/a	Yes
1050	B	10	MDS	No	n/a	refractory	No	Complex	ASXL1, STAG2, EZH2, NRAS, FLT3-TKD	5	5	no	n/a	Yes
3001	B	10	AML	No	n/a	untreated sAML	No	Normal	SRSF2, ASXL1, BCOR, JAK2, NRAS, FLT3-TKD	35	20 to 30	n/a	n/a	Yes

3003	B	10	MDS	No	n/a	refractory	Yes	Normal	U2AF1, ZRSR2, STAG2, EZH2, RUNX1, TET2	14	17	n/a	n/a	Yes
3004	B	10	AML	No	n/a	refractory	Yes	Complex Monosomal	TP53, DNMT3A	30	n/a	n/a	n/a	Yes
3005	B	10	AML	No	n/a	relapse	No	45,XX,dic(5;17)(q11.2;p11.2)	TP53, U2AF1, DNMT3A	29	5 to 10	n/a	n/a	Yes
5005	B	10	MDS	No	n/a	refractory	Yes	Complex	TP53	7	n/a	n/a	n/a	Yes
5007	B	10	MDS	No	n/a	refractory	Yes	Complex	ASXL1, TET2	3.5	5 to 9	n/a	n/a	Yes
5008	B	10	MDS	No	n/a	refractory	Yes	Complex Monosomal	n/a	3 to 5	n/a	n/a	n/a	Yes
6004	B	10	MDS	No	n/a	refractory	Yes	Normal	ASXL1, STAG2, EZH2, RUNX1, TERT, TET2	5 to 9	n/a	n/a	n/a	Yes
8004	B	10	AML	No	n/a	refractory	Yes	45,XX,-7[13]/46,XX[7]	PTPN11 and FLT53	67	80	n/a	n/a	Yes
8005	B	10	AML	No	n/a	refractory	Yes	Normal	TP53, DDX41	20	25	n/a	n/a	Yes
8007	B	10	MDS	No	n/a	refractory	Yes	Complex Monosomal	TP53, DNMT3A	12	10 to 15	n/a	n/a	Yes
8008	B	10	AML	No	n/a	refractory	No	Complex	TP53, RUNX1, PTPN11	6	10	n/a	n/a	Yes
8009	B	10	AML	no	n/a	relapse	Yes	47,XY,del(20)(q11.2),+21[18]/46,XY[3]	SRSF2, ASXL1, RUNX1, TET2	12	15	n/a	n/a	Yes
8011	B	10	AML	no	n/a	relapse	Yes	Complex Monosomal	None Detected	2	10	n/a	n/a	Yes

Supplemental Table 4A: Treatment-emergent grade ≥3 adverse events regardless of attribution in patients that received decitabine/ipilimumab*

AE	Arm A N=25						Arm B N=23					
	Grade 3		Grade 4		Grade 5		Grade 3		Grade 4		Grade 5	
System	**n**	**%**	**n**	**%**	**n**	**%**	**n**	**%**	**n**	**%**	**n**	**%**
Blood/lymph												
Anemia	5	20	-	-	-	-	12	52	-	-	-	-
Febrile neutropenia	9	36	-	-	-	-	14	61	-	-	-	-
Cardiac disorders												
Heart failure	-	-	-	-	-	-	3	13	-	-	-	-
Pericardial effusion	-	-	2	8	-	-	-	-	-	-	-	-
GI disorders												
Colitis	2	8	-	-	-	-	-	-	-	-	-	-
Diverticulitis	-	-	-	-	-	-	2	9	-	-	-	-
Enterocolitis	-	-	-	-	-	-	2	9	-	-	-	-
General disorders												
Fatigue	-	-	-	-	-	-	4	17	-	-	-	-
Infections												
Lung infection	-	-	-	-	-	-	7	30	-	-	-	-
Sepsis	-	-	-	-	-	-	-	-	2	9	-	-
Skin infection	-	-	-	-	-	-	2	9	-	-	-	-
Investigations												
ALT increased	3	12	-	-	-	-	-	-	-	-	-	-
AST increased	3	12	-	-	-	-	-	-	-	-	-	-
Blood bilirubin increased	-	-	-	-	-	-	2	9	-	-	-	-
Lymphocyte count decreased	2	8	-	-	-	-	4	17	4	17	-	-
Neutrophil count decreased	-	-	8	32	-	-	2	9	9	39	-	-
Platelet count decreased	-	-	7	28	-	-	3	13	8	35	-	-
White blood cell decreased	3	12	4	16	-	-	3	13	5	22	-	-
Metabolism and nutrition disorders												
Anorexia	-	-	-	-	-	-	2	9	-	-	-	-
Hyperglycemia	-	-	-	-	-	-	3	13	-	-	-	-
Hypokalemia	-	-	-	-	-	-	3	13	-	-	-	-
Hyponatremia	-	-	-	-	-	-	3	13	-	-	-	-
Musculoskeletal disorders												
Generalized muscle weakness	-	-	-	-	-	-	3	13	-	-	-	-
Nervous system disorders												
Syncope	-	-	-	-	-	-	2	9	-	-	-	-
Renal disorders												
Acute kidney injury	2	8	-	-	-	-	4	17	-	-	-	-
Respiratory disorders												
Dyspnea	-	-	-	-	-	-	4	17	-	-	-	-

Hypoxia	-	-	-	-	-	-	4	17	-	-	-	-
Pneumonitis	3	12	-	-	-	-	-	-	-	-	-	-
Pulmonary edema	-	-	-	-	-	-	2	9	-	-	-	-
Skin disorders												
Rash maculo-papular	-	-	-	-	-	-	2	9	-	-	-	-
Vascular disorders												
Hypertension	-	-	-	-	-	-	3	13	-	-	-	-

Any AE occurring in 2 or more patients are reported.

*Four grade 5 events are not shown in Table 4A as these occurred in <2 patients. Two DLTs occurred at IPI 10 mg/kg (both grade 5), including one patient with acute grade III GVHD (GI and liver) with septic shock two months later and one patient with grade 3 pneumonitis complicated by concurrent disease progression and infection with grade 5 respiratory failure. Two additional grade 5 events that were not considered DLTs occurred, including one patient with hemorrhagic stroke from uncontrolled atrial fibrillation (dosed at IPI 3 mg/kg) and one patient with neutropenic fever with active disease (dosed at IPI 10 mg/kg).

Table 4B. Immune-related adverse events of all grades by dose level and arm in patients who received ipilimumab*

	Arm A, Post-HSCT N=25				Arm B, Transplant Naive N=23			
	n (%)				n (%)			
AE	**DL0**	**DL1**	**DL2**	**Total**	**DL0**	**DL1**	**DL2**	**Total**
Arthritis							1 (4)	1 (4)
Arthritis/hypophysitis	-	-	-	-	-	-	1 (4)	1 (4)
Colitis**	-	1 (4)	2 (8)	3 (12)	1 (4)	-	-	1 (4)
Colitis/dermatitis	-	-	-	-	-	-	2 (9)	2 (9)
Dermatitis	-	-	-	-	1 (4)	-	2 (9)	3 (13)
Dermatitis/ITP	-	-	-	-	-	-	1 (4)	1 (4)
Hepatitis	-	-	-	-	-	-	1 (4)	1 (4)
Pneumonitis**	-	-	1 (4)	1 (4)	-	-	1 (4)	1 (4)
Acute GVHD								
overall grade I	-	-	1 (4)	1 (4)				
overall grade II	-	-	1 (4)	1 (4)				
overall grade III	-	-	1 (4)	1 (4)				
Chronic GVHD								
mild	-	-	-	-				
moderate	1 (4)	1 (4)	1 (4)	3 (12)				
severe	1 (4)	-	-	1 (4)				

IRAE reporting was based on clinical history, labs and examinations. We required baseline echocardiogram and EKG. At screen and every 12 weeks we required gamma-GT, direct bilirubin, LDH, TSH, and lipase.

*Any Gr 3 or higher immune-related AE occurring in 2 or more patients are also represented in **Table 4A**. Detailed individual events associated with GVHD are shown in Supplemental **Table 4C**.

**In these 4 irAEs which were identified among post HSCT patients, there were no concomitant concerns for clinical/laboratory GVHD in other organs and pathology of affected organ was available which demonstrated evidence of immune checkpoint inhibitor toxicity but no overlapping known or definitive GVHD pathologic features.

Supplemental Table 4C: Details of individual case descriptions of irAE reported in post-HSCT setting (Arm A)

IPI Dose (mg/ kg)	History of Prior GVHD	Organ(s) Involved	Max Grade/ Stage	Additional Clinical Details and Available Pathology	ORR	DLT
3	No	eyes, skin, esophagus	Severe Chronic GVHD	At end of cycle 1 of IPI+DEC, subject developed dysphagia with suspected esophageal web by upper GI series and lichenoid dermatitis with superficial dermal sclerosis consistent with lichen planus-like manifestation of epidermal-type chronic GVHD. Treated with prednisone with resolution of symptoms after prolonged course.	CR	No
3	Yes	skin	Moderate Chronic GVHD	Developed skin GVHD during combination cycle 7. Skin biopsy shows atypical endophytic squamous proliferation in association with epidermal lymphocytic satellitosis with apoptotic keratinocytes. Treated with prednisone with resolution of symptoms.	SD	No
5	Yes	skin, eyes, gastric	Moderate Chronic GVHD	Subject entered study on prednisone 5 mg daily for history of skin and gastric GVHD. During combination cycle 3, worse (known) skin GVHD. Treated with topical steroids with resolution.	PD	No
5	Yes	colon	Grade 2 colitis	History of oral and eye GVHD prior to trial. Developed new onset colitis after combination cycle 1 without clinical worsening of eye/oral GVHD. GI biopsy demonstrated gastritis and reactive gastropathy. Colonic mucosa with chronic inflammation and reactive changes. Treated with prednisone with resolution of symptoms.	SD	No
10	Yes	skin	Acute Grade I GVHD	History of oral and skin GVHD prior to trial. Developed skin rash. Treated with topical steroids with resolution.	SD	No
In 10	Yes	GI, liver	Acute Grade III GVHD	Subject experienced acute grade III GVHD of colon/liver after combination cycle 1 of IPI+DEC. Treatment with systemic corticosteroids, mycophenolate mofetil, budesonide, and ruxolitinib were implemented and controlled the GVHD. However, after a two-month prolonged course in the hospital, subject ultimately succumbed to sepsis in setting of immunosuppression.	CR	Yes in dose-esc
10	No	Colon	Grade 3 colitis	Subject was in combination cycle 4 when there was grade 3 diarrhea. Flexible sigmoidoscopy was performed on 12/24/19, with pathology consistent with moderately active colitis consistent with immune colitis. Treated with corticosteroids with resolution of symptoms.	SD	No
10	Yes	skin	Acute Grade II GVHD	History of skin GVHD prior to trial. Developed rash consistent with prior GVHD at end of combination cycle 1. Treated with topical steroids with resolution.	PD	No
10	No	Colon	Grade 3 colitis	After combination cycle 1, subject developed grade 3 steroid-refractory colitis (patchy active colitis with Paneth cell metaplasia) that responded to infliximab.	SD	No
10	No	Lungs	Grade 3 pneumonitis	Subject has no history of GVHD who developed pneumonitis requiring corticosteroids. Course was complicated by orbital cellulitis, sepsis	PD	Yes in dose-exp

				and disease progression while on immune suppression. Transitioned to comfort measures.		
10	Yes	skin, oral, GI	Moderate Chronic GVHD	History of oral/skin GVHD prior to trial. After combination cycle 2 subject had poor oral intake and dysphagia and diarrhea. Duodenal biopsy demonstrated mucosa with moderately increased crypt epithelial apoptosis and reactive changes. Treated with corticosteroids with resolution.	SD	No

Acute GVHD staged according to 1994 consensus conference criteria. Chronic GVHD graded according to NIH consensus criteria.

Supplemental Table 5. Multiplex Immunofluorescence Antibodies

Antibody	Clone	Company	Catalog #	Antibody Dilution	Opal Fluor	Fluor Dilution	Antigen Retrieval, Time (min)
CD4	4B12	Dako	M7310	1:250	520	1:100	ER1, 10
CD8	C8/144 B	Dako	M7103	1:5000	540	1:100	ER1, 10
CD34	QBend10	Beckman Coulter	IM125OU	1:150	570	1:200	ER1, 10
GZMB	GrB-7	Dako	M7235	1:100	620	1:200	ER1, 10
CD3	Polyclonal	Dako	A0452	1:1000	650	1:100	ER1, 10

Supplemental Table 6: MIF Analysis

MIF analysis, evaluable patients who received combination Ipilimumab + Decitabine

	Responders Mean (N)	Non-Responders Mean (N)	P-value*
T0			
CD3+CD4+	155.5 (13)	184.9 (20)	0.69
CD3+CD8+	137.9 (13)	178.4 (20)	0.51
(CD3+CD8+)/CD34+	2.78 (13)	0.69 (18)	0.17
T2/T0			
CD3+CD4+	0.95 (4)	3.13 (6)	0.25
CD3+CD8+	0.44 (4)	1.65 (6)	0.17
CD3+CD8+GzB+	0.65 (4)	6.99 (5)	0.18
(CD3+CD4+)/CD34+	3.41 (4)	2.60 (5)	0.78
(CD3+CD8+)/CD34+	1.30 (4)	1.37 (5)	0.93
(CD3+CD8+GzB+)/CD34+	2.06 (4)	6.01 (4)	0.39
T4/T0			
CD3+CD4+	4.40 (4)	2.04 (5)	0.33
CD3+CD8+	1.27 (4)	1.91 (5)	0.59
CD3+CD8+GzB+	1.65 (4)	9.08 (4)	0.32
(CD3+CD4+)/CD34+	14.02 (4)	2.76 (4)	0.16
(CD3+CD8+)/CD34+	5.12 (4)	2.19 (4)	0.22
(CD3+CD8+GzB+)/CD34+	2.78 (4)	7.92 (3)	0.27

*Using Welch's t-test

Supplemental Table 7. Immunophenotyping Panels

Panel 1

Antibody	Fluorochrome	Clone	Vendor	Catalog#
CD16	FITC	3G8	BD	555406
PD-1	PeCy 7	J105	eBioscience	25-2799-42
CD19	BV711	HIB19	BioLegend	302246
CD45	APC Cy 7	2D1	BD	560178
CD8	Alexa 700	RPA-T8	BioLegend	301027
ICOS	BV421	C398.4A	BioLegend	313524
CD3	BV786	UCHT1	BioLegend	300472
CD4	BV510	RPA-T4	BioLegend	300545
CD56	BV605	NCAM	BioLegend	318333
CD14	BV650	M5E2	BioLegend	301836
CD137	APC	4B4-1	BioLegend	309810
7-AAD	PE-Cy5		BD	559925

Panel 2

Antibody	Fluorochrome	Clone	Vendor	Catalog#
CD45RA	Alexa 488	HL100	BioLegend	304114
PD-1	PE-Cy 7	J105	eBioscience	25-2799-42
CD127	APC	eBioRDR5	eBioscience	17-1278-42
CD8	Alexa 700	RPA-T8	BioLegend	301027
CD45	APC-Cy7	2D1	BD	560178
CD95	BV 421	DX2	BioLegend	305624
CD4	BV 510	RPA-T4	BioLegend	300545
CD25	BV 605	M-A251	BioLegend	356142
LAG-3	BV 650	11C3C65	BioLegend	369316
CCR7	BV 711	G043-H7	BioLegend	353227
CD3	BV 786	UCHT1	BioLegend	300472
7-AAD	PE-Cy5		BD	559925

References

1. Przepiorka D, Weisdorf D, Martin P, et al. 1994 Consensus Conference on Acute GVHD Grading. *Bone Marrow Transplant*. 1995;15(6):825-828.
2. Jagasia MH, Greinix HT, Arora M, et al. National Institutes of Health Consensus Development Project on Criteria for Clinical Trials in Chronic Graft-versus-Host Disease: I. The 2014 Diagnosis and Staging Working Group report. *Biol Blood Marrow Transplant*. 2015;21(3):389-401 e381.
3. Cheson BD, Greenberg PL, Bennett JM, et al. Clinical application and proposal for modification of the International Working Group (IWG) response criteria in myelodysplasia. *Blood*. 2006;108(2):419-425.
4. 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.
5. Komrokji RS, Al Ali NH, Sallman D, et al. Validation of International Working Group response criteria in higher-risk myelodysplastic syndromes: A report on behalf of the MDS Clinical Research Consortium. *Cancer Med*. 2021;10(2):447-453.
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. Rambaldi B, Kim HT, Reynolds C, et al. Impaired T- and NK-cell reconstitution after haploidentical HCT with posttransplant cyclophosphamide. *Blood Adv*. 2021;5(2):352-364.