Supplementary data:

Table 1: Inclusion and exclusion criteria for the phase I Lenzilumab CMML clinical trial

Subject Population Subjects with CMML who have relapsed, or were refractory and/or intolerant to prior treatment with an HMA, HU, other standard of care treatment, or investigational agent for the management of CMML. The diagnosis of CMML must be confirmed according to WHO criteria.

Inclusion Criteria

1. Confirmed diagnosis of CMML using the WHO criteria.
2. Subjects with CMML who have relapsed, or were refractory and/or intolerant to prior treatment with an HMA, HU, other standard of care treatment, or to an investigational agent used for the management of CMML (front line or salvage setting). Patients who were deemed to be ineligible for therapy with HMA and/or HU were also allowed enrollment.
3. Age ≥18 years.
4. Eastern Cooperative Oncology Group (ECOG) performance status of ≤2.
5. Must be able and willing to provide adequate bone marrow aspirate and biopsy specimens suitable for histopathological analysis and standard cytogenetic analysis.

- If prior bone marrow sample was obtained <90 days from Cycle 1 Day 1 and patient has not had therapy directed for CMML, with the exception of HU, within this time period, a repeat bone marrow sample at screening may not be required after discussion with the Sponsor's medical monitor

6. Must have the following local laboratory results:

- Platelet count ≥20,000/µL
- Absolute neutrophil count (ANC) ≥500/µL
- Liver function (total bilirubin ≤1.5* x upper limit of normal [ULN], aspartate aminotransferase [AST] and/or alanine aminotransferase [ALT] ≤3 x ULN)
  – For subjects with hepatomegaly due to extramedullary hematopoiesis, AST and/or ALT < 5 x ULN
  – *For subjects with total bilirubin >1.5 x ULN, direct bilirubin ≤ ULN
- Kidney function (serum creatinine < 2 mg/dL or creatinine clearance >30 mL/min)

7. Male subjects and female subjects of childbearing potential must agree to use adequate contraception (hormonal or barrier method of birth control, abstinence) prior to study entry and for 8 weeks following their last dose of study drug. A negative serum beta human chorionic gonadotropin (β-hCG) is required for all female subjects of childbearing potential within 1 week prior to receiving first dose of study drug.
8. Ability to understand the requirements of the study and informed consent. A signed informed consent by the subject or a legally authorized representative is required prior to enrollment in this study.

Exclusion Criteria

1. MDS/MPN unclassifiable syndromes other than CMML, as assessed by the investigator.
2. Known or suspected hypersensitivity to KB003 or components of the KB003 formulation.

3. Use of approved or experimental agents for the treatment of CMML (with the exception of HU) or radiotherapy within approximately 14 days (or within 5 half-lives of agent, whichever is longer) prior to Cycle 1 Day 1. Subjects must have recovered from the toxic effects of that therapy to ≤ Grade 1 or baseline grade. HU may be given during the first 14 days of Cycle 1 only; longer usage of HU during Cycle 1 must be approved by KaloBios.
4. Concurrent use of administered hGM-CSF (sargramostim) or GCSF administration within 7 days of screening hematology laboratory tests.
5. Pregnant or breastfeeding female subjects.
6. Known HIV infection (screening test not required).
7. History of other malignancy within the last 2 years. Basal or squamous cell skin carcinomas adequately treated are allowed. Subjects with a history of low-grade localized prostate cancer will be eligible even if diagnosed <2 years previously.
9. Uncontrolled inter-current illness including but not limited to ongoing or active infection, symptomatic congestive heart failure, unstable angina pectoris, cardiac arrhythmia, or psychiatric illness/social situations that would limit compliance with study requirements.
10. Pulmonary alveolar proteinosis (PAP) or history of PAP (see Section 8.5).
11. Hypoxemia at screening (oxygen saturation at room air <92%).
12. Inability to give consent or unwillingness or inability to comply with study procedures.
13. Any other reason that in the opinion of the Investigator would prevent the subject from completing participation or following the study schedule.

Table 2- Baseline Patient Characteristics

Baseline Characteristics	**Phase 1 (n=15)**
Median Age (range)	74 (52-85)
Male (%)	12 (80%)
FAB Classification	
MPN-CMML (%)	10 (66%)
MDS-CMML (%)	5 (33%)
WHO Classification	
CMML-0 (%)	9 (60%)
CMML-1 (%)	3 (20%)
CMML-2 (%)	3 (20%)
Cytogenetics	
Normal or -Y (%)	11 (73%)
High (%)	23 (27%)
Prior Treatment overall	12 (80%)
HMA	9 (60%)
Hydroxyurea	3 (20%)
Palpable Splenomegaly (%)	8 (53%)
Median WBC x 10^3/dL (range)	10 (3-59.8)
Median AMC x10^3/dL (range)	4.5 (1.3-10)
Median HGB g/dL (range)	9.7 (7.6-14)
Median PLT x10^3/dL (range)	147 (16-942)
Median Bone Marrow Percent Blasts (range)	5.1 (0-17)

Table 3. Table showing clinical and genetic information chronic myelonocytic leukemia patients enrolled in the KB003 clinical trial.

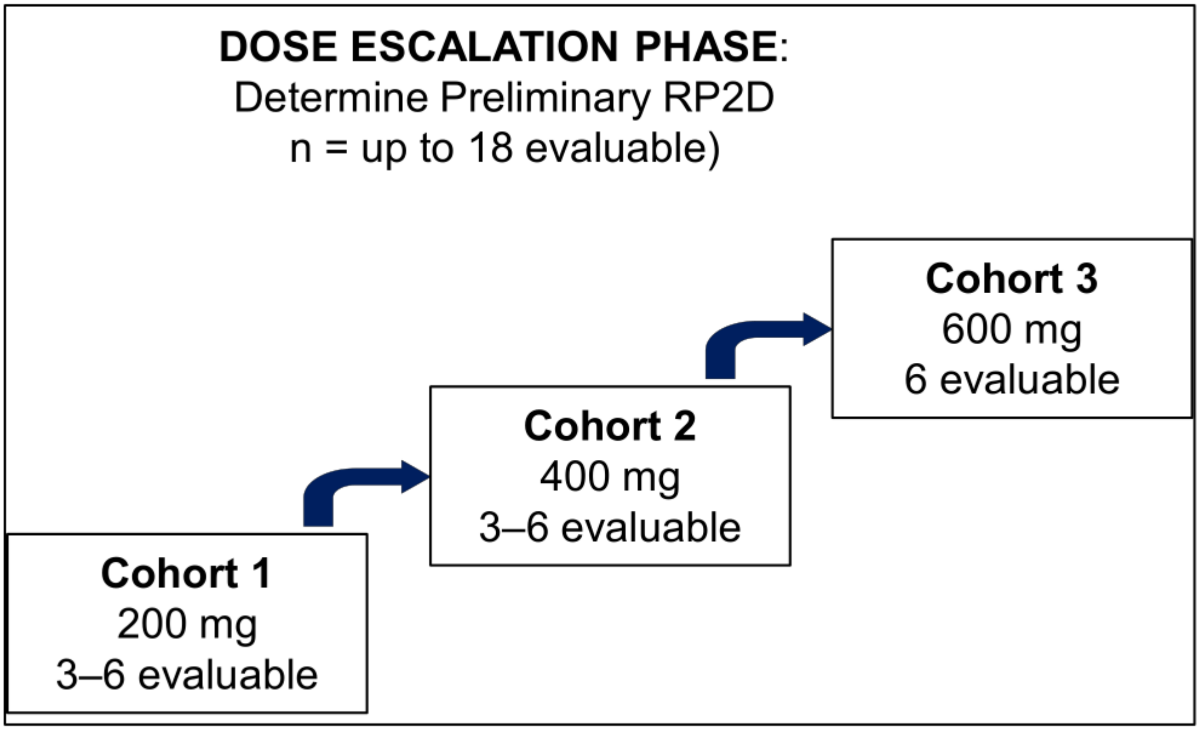
Pt. No.	*Hb (gm/dL)*	*WBC (x 10^9/L)*	*AMC (x 10^9/L)*	*Platelet count (x 10^9/L)*	*PB blast%*	*BM blast %*	*LDH (U/L)*	*Splenomegaly (yes or no)*	*ECOG PS*
1	9.4	18.5	4.4	191	3	6	155	Yes	1
2	8.2	25.2	18.6	249	0	0	133	No	1
3	10	5.4	1.9	62	0	4	245	No	1
4	9.2	18.6	1.3	45	0	3	214	No	1
5	10.9	24.1	6.8	77	0	1	167	Yes	1
6	8.9	3	0.4	16	0	1	118	No	0
7	9	55.8	10	133	0	1	193	No	1
8	11.9	55	8.3	48	1	3	696	Yes	1
9	8.8	43.1	9.9	25	14	15	346	Yes	2
10	10.2	11.8	2.7	50	8	17	178	Yes	2
11	14	59.8	9	66	0	6	317	No	0
12	8.7	9.1	12	218	0	0.2	381	Yes	1
13	7.6	17.2	4.8	53	5	10	104	Yes	1
14	10.3	2.9	1.3	35	0	6	327	No	1
15	9	20	2.2	942	0	3	226	Yes	1

***Abbreviations*: Hb=Hemoglobin; WBC=White blood cell count; AMC=absolute monocyte count; PB=peripheral blood; BM=bone marrow; LDH=lactate dehydrogenase; ECOG PS=Eastern cooperative group performance status.**

Table 4. Summary of best responses

Best Response	Phase 1 (n=15)
Complete Response	0
Partial Response	0
Optimal Marrow Response	0
Partial Marrow Response	1
Clinical Benefit (total patients)	**4**
Erythroid	0
Platelet	3
Spleen	0
Neutrophil	1
Stable Disease	5
Progressive Disease	5
Overall Response Rate	5 (33.33%)

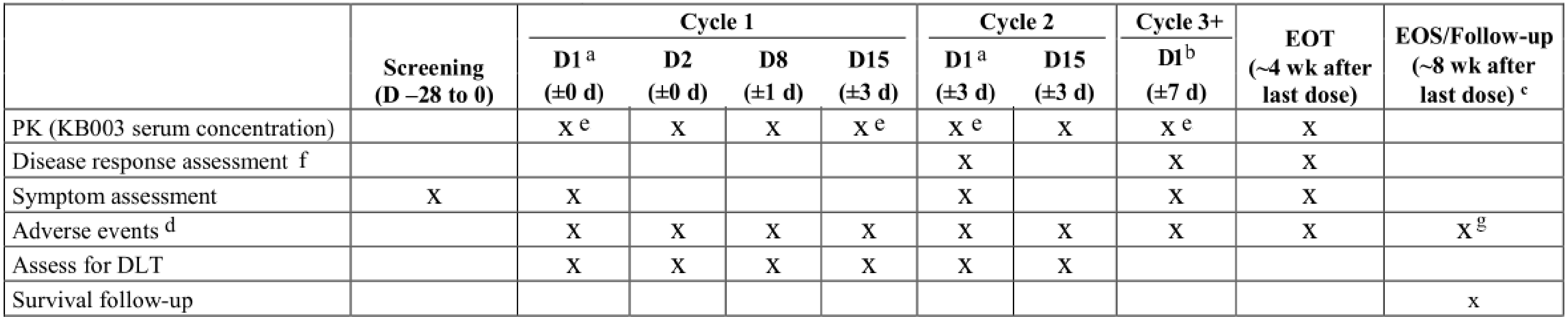
Figure 1- Phase 1 study of lenzilumab in CMML patients: clinical trial schema



Notes:

- KB003 will be administered by IV infusion as part of a 28-day dosing cycle, with an additional (loading) dose on Day15 of Cycle 1.
- In each cohort, safety will be assessed at the end of Cycle 1 before enrolling the next cohort

Study Schedule of Events



		Cycle 1				Cycle 2		Cycle 3+	EOT (~4 wk after last dose)	EOS/Follow-up (~8 wk after last dose) [c]
	Screening (D –28 to 0)	D1 [a] (±0 d)	D2 (±0 d)	D8 (±1 d)	D15 (±3 d)	D1 [a] (±3 d)	D15 (±3 d)	D1 [b] (±7 d)		
PK (KB003 serum concentration)		X [e]	X	X	X [e]	X [e]	X	X [e]	X	
Disease response assessment [f]						X		X	X	
Symptom assessment	X	X				X		X	X	
Adverse events [d]		X	X	X	X	X	X	X	X	X [g]
Assess for DLT		X	X	X	X	X	X			
Survival follow-up										x

Footnotes

a **Cycle 1 Day 1 column: (C1D1)**: is defined as the day of the first dose of study drug.

b **C3D1 column:** Procedures noted in this column to be performed on C3D1 and D1 of every cycle thereafter (C4D1, C5D1, etc.) unless footnoted otherwise.

c **Survival:** Patients will be followed for survival for duration of the study.

d **Medical history:** Events or conditions prior to infusion on C1D1 are considered medical history unless the reportable event is the result of a protocol-mandated study procedure. Events or conditions resulting from protocol-mandated study procedures should be recorded as AEs.

e **PK:** Samples collected preinfusion, end of infusion, and approximately 1 hour following the end of infusion on C1D1, then preinfusion only at subsequent visits. An unscheduled sample may be taken in the event of suspected KB003-related toxicity.

f **Disease response assessment:** Assessment includes results from peripheral blood, bone marrow, and cytogenetic findings; spleen assessment; TI; and symptoms.

g **Follow-up AEs/SAEs:** Follow-up for related AEs/SAEs only and for survival information up to 8 weeks after the last dose of study drug or until the start of new treatment, whichever comes first. Report events during the follow-up period as AEs/SAEs only if deemed related to KB003.

Figure 2: Violin plots demonstrating pSTAT5 by flow cytometry in CMML patients enrolled in the lenzolumab clinical trial, with samples being obtained at screening and at cycle 3 of therapy, showing no significant differences between responders and non-responders.

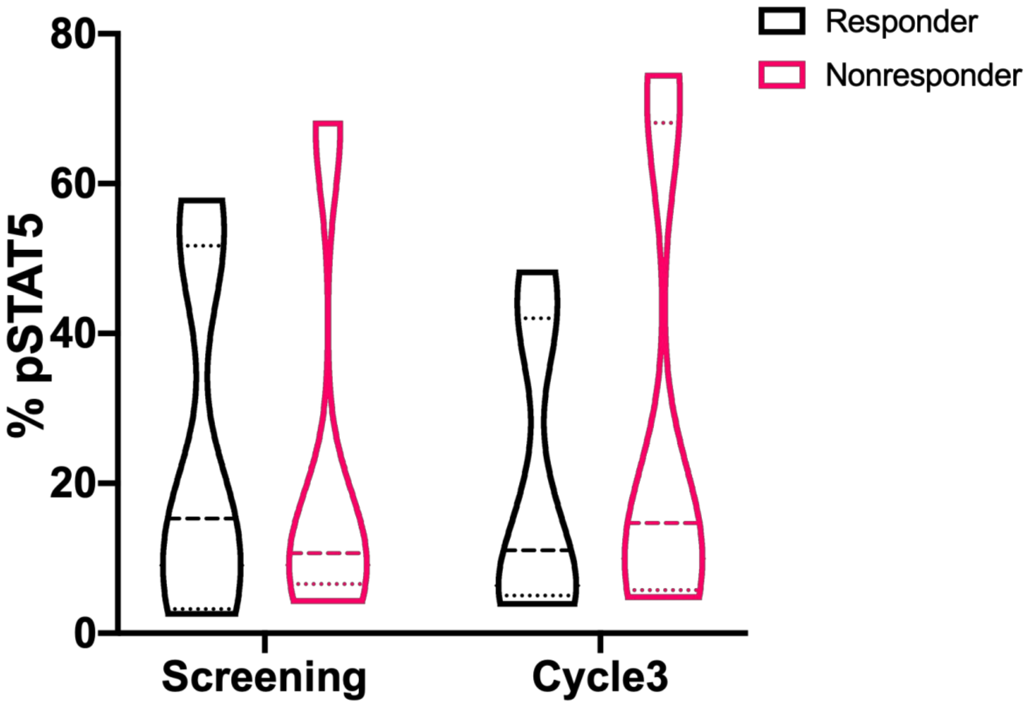
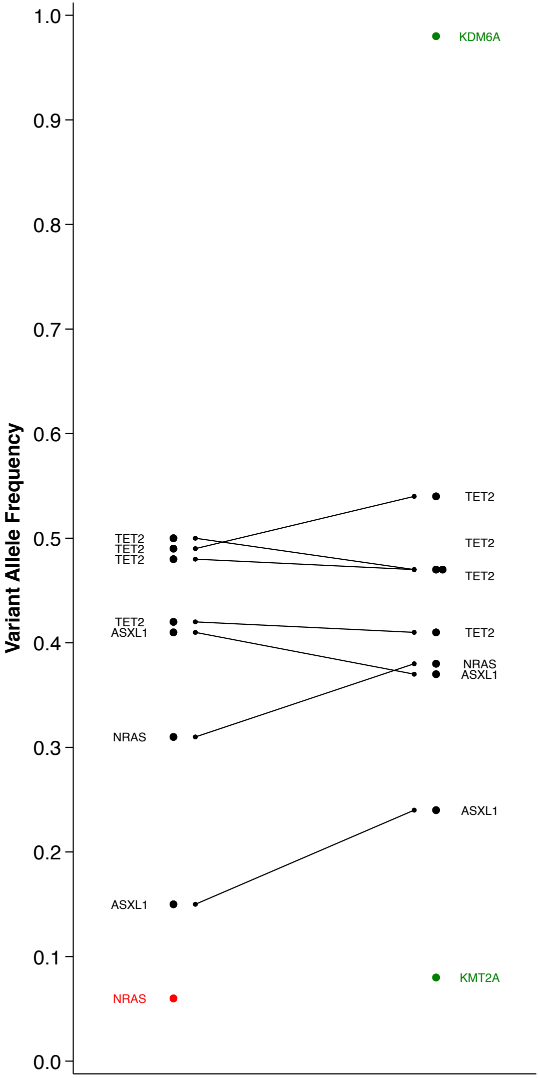


Figure 3 - Variant allele frequencies of individual patients. (pages 10-37)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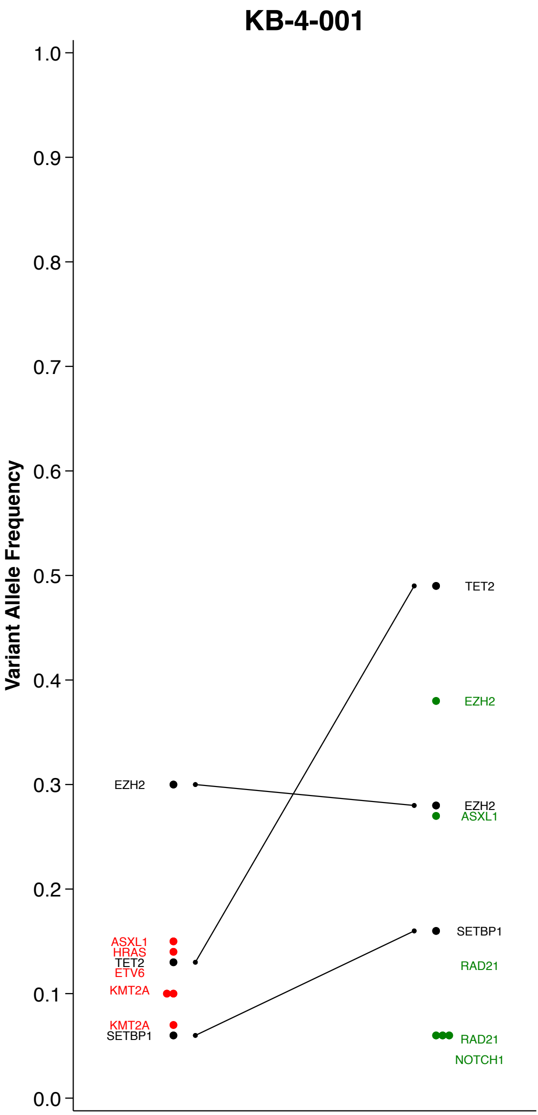
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0
TET2
TET2
TET2
TET2
ASXL1
NRAS
ASXL1
NRAS
KDM6A
TET2
TET2
TET2
TET2
NRAS
ASXL1
ASXL1
KMT2A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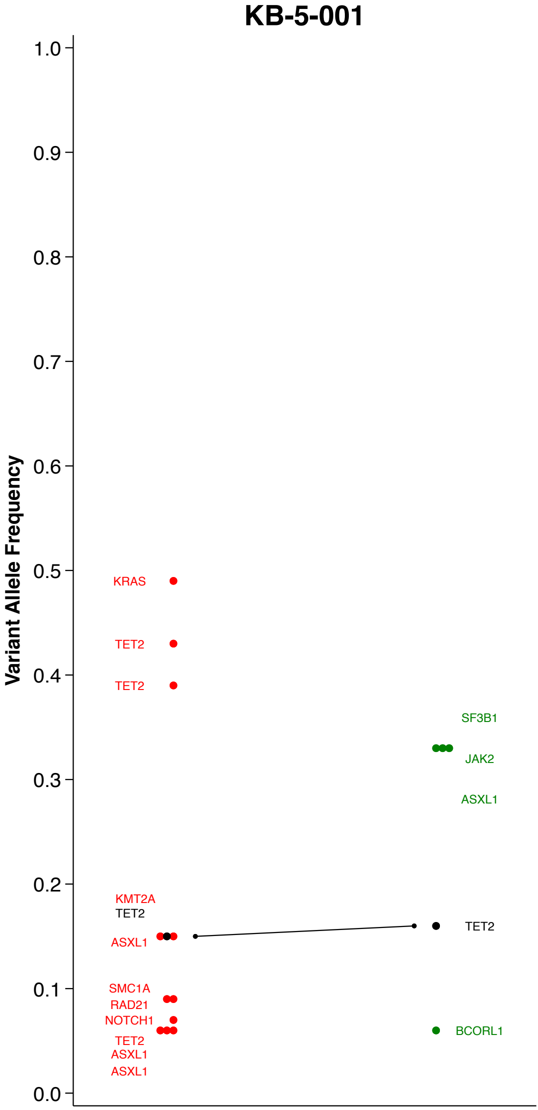
0.6
0.5
0.4
0.3
0.2
0.1
0.0
EZH2
ASXL1
HRAS
TET2
ETV6
KMT2A
KMT2A
SETBP1
TET2
EZH2
EZH2
ASXL1
SETBP1
RAD21
RAD21
NOTCH1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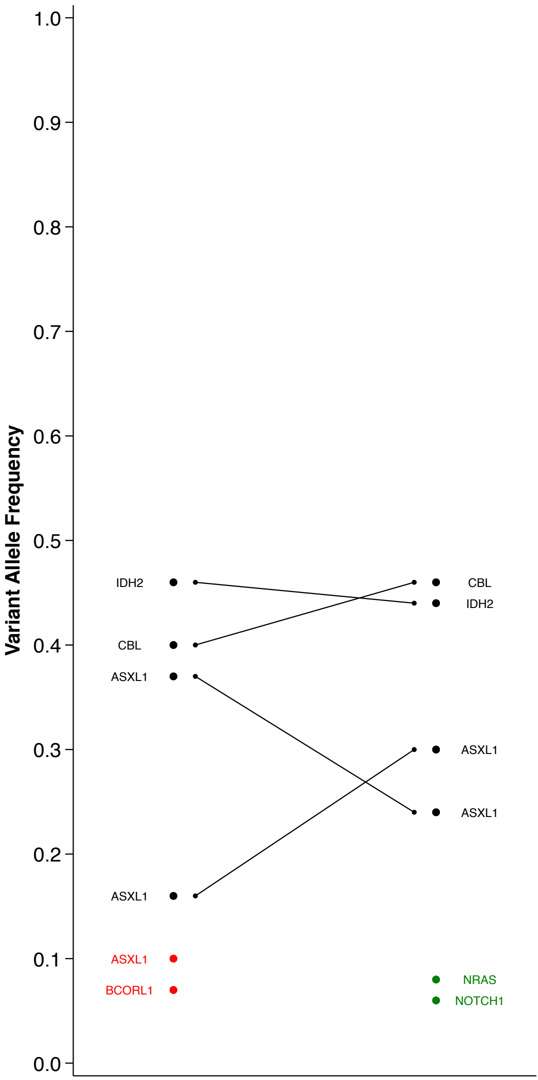
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0
KRAS
TET2
TET2
KMT2A
TET2
ASXL1
SMC1A
RAD21
NOTCH1
TET2
ASXL1
ASXL1
SF3B1
JAK2
ASXL1
TET2
BCORL1

KB-6-001


Variant Allele Frequency
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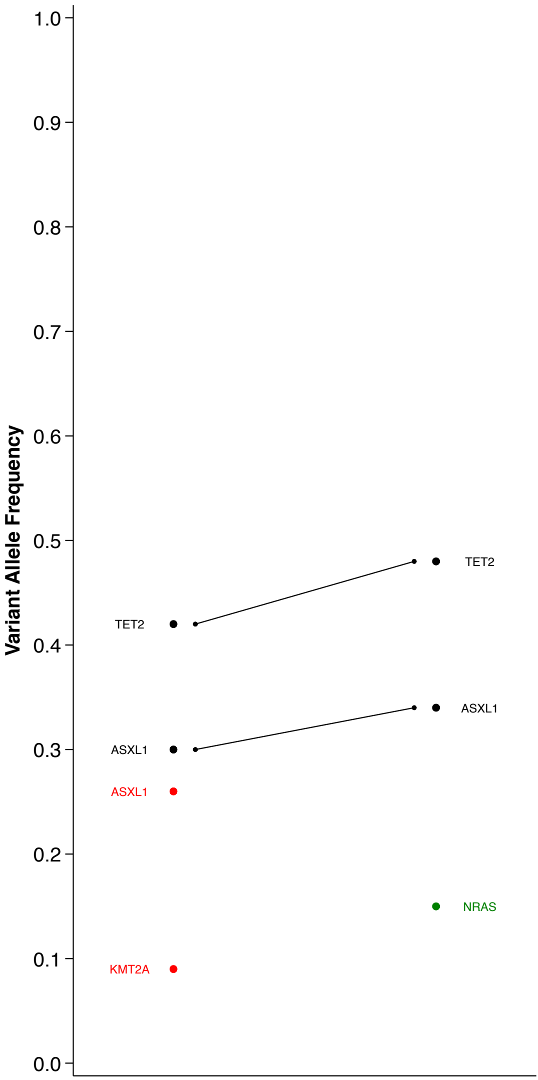
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0
IDH2
CBL
ASXL1
ASXL1
ASXL1
BCORL1
CBL
IDH2
ASXL1
ASXL1
NRAS
NOTCH1

KB-7-001


Variant Allele Frequency
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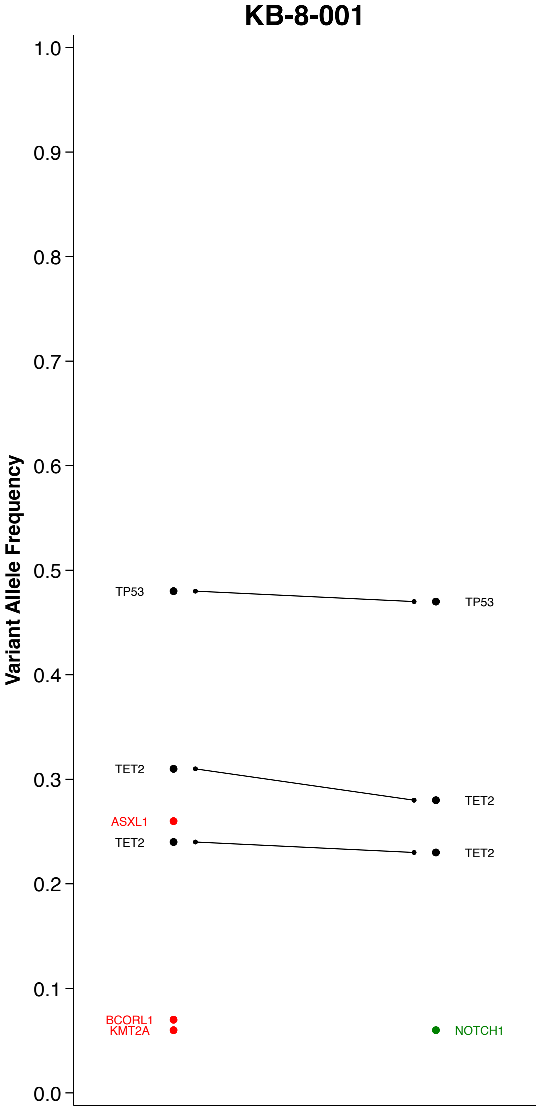
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0
TET2
ASXL1
ASXL1
KMT2A
TET2
ASXL1
NRAS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Variant Allele Frequency
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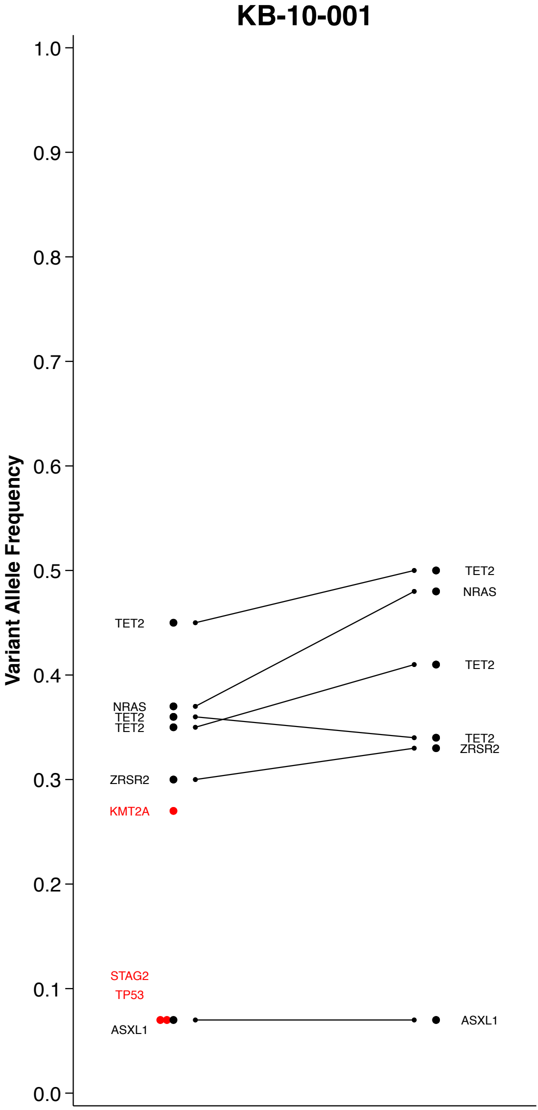
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0
TP53
TP53
TET2
TET2
ASXL1
TET2
TET2
BCORL1
KMT2A
NOTCH1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Variant Allele Frequency
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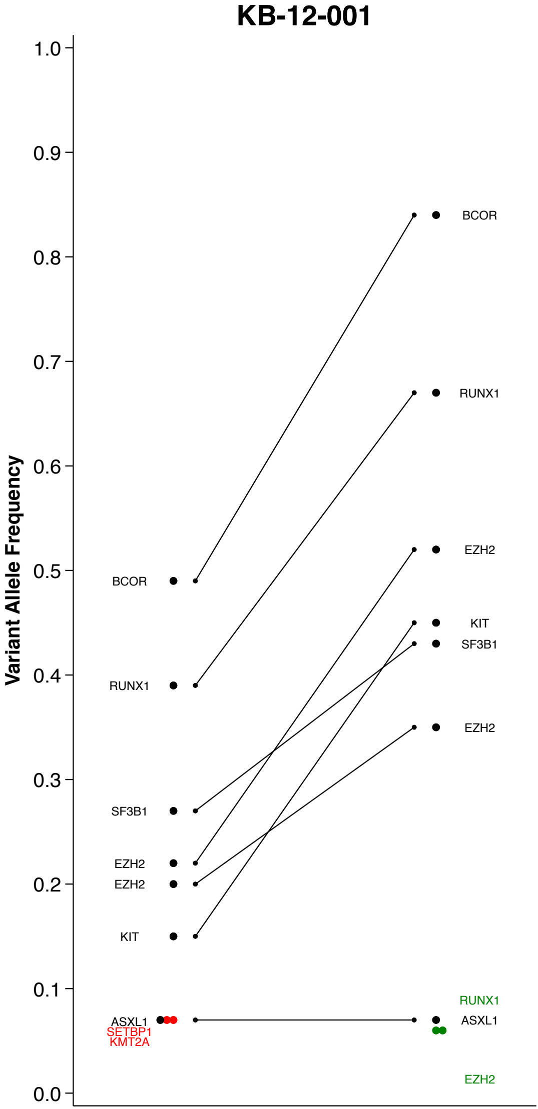
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0
TET2
NRAS
TET2
TET2
ZRSR2
KMT2A
STAG2
TP53
ASXL1
TET2
NRAS
TET2
TET2
ZRSR2
ASXL1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Variant Allele Frequency
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0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0
BCOR
RUNX1
SF3B1
EZH2
EZH2
KIT
ASXL1
SETBP1
KMT2A
BCOR
RUNX1
EZH2
KIT
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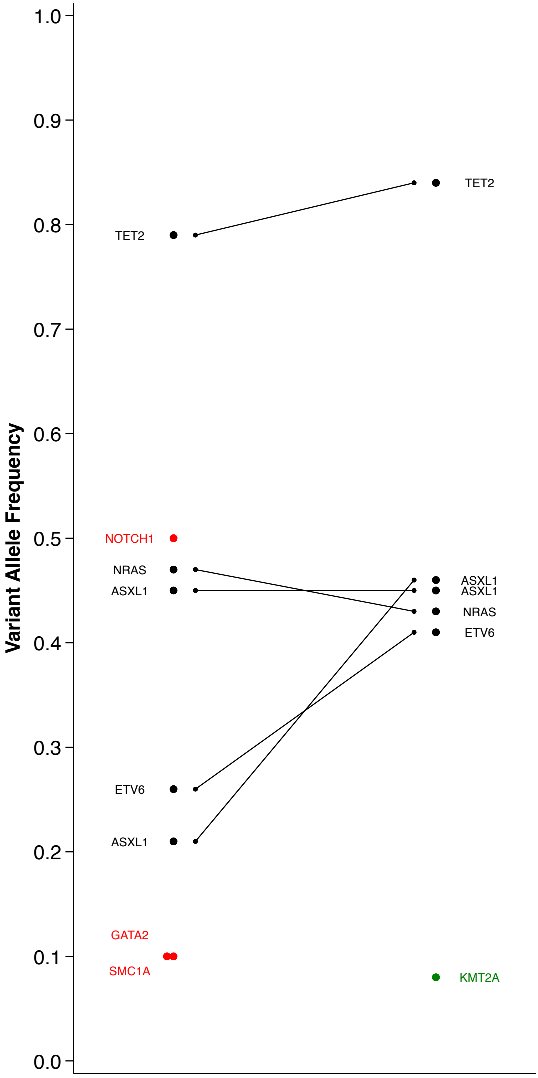
EZH2
RUNX1
ASXL1
EZH2



KB-13-001

Variant Allele Frequency
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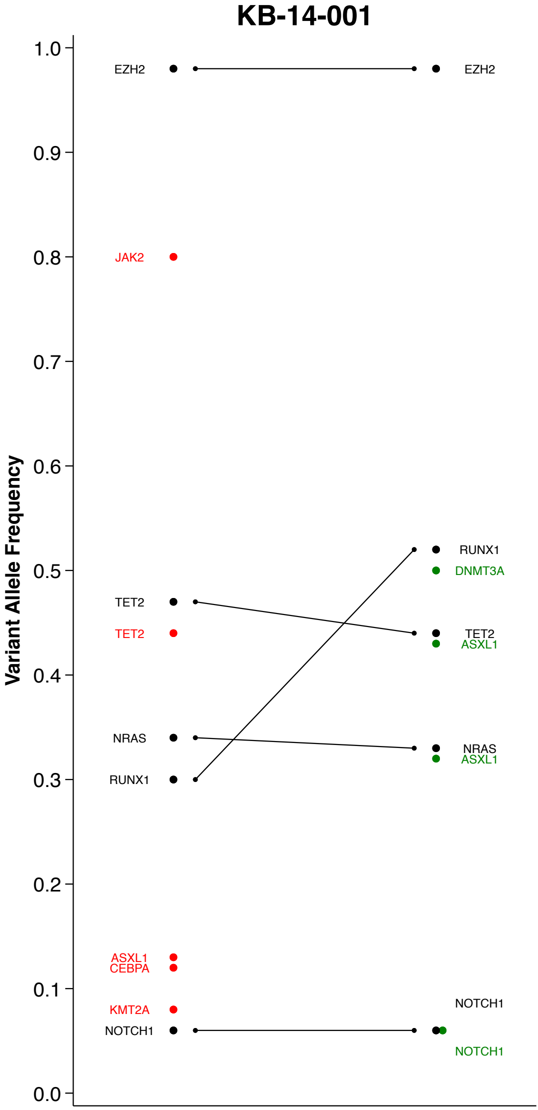
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0
TET2
NOTCH1
NRAS
ASXL1
ETV6
ASXL1
GATA2
SMC1A
TET2
ASXL1
ASXL1
NRAS
ETV6
KMT2A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Variant Allele Frequency
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0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0
EZH2
JAK2
TET2
TET2
NRAS
RUNX1
ASXL1
CEBPA
KMT2A
NOTCH1
EZH2
RUNX1
DNMT3A
TET2
ASXL1
NRAS
ASXL1
NOTCH1
NOTCH1

Raindance Paired VAF Analysis

Orange samples in paired patient samples indicate variants found both in screening and post-treatment samples.

PAIRED TIMEPOINT SAMPLES

NON-RESPONDERS:

KB-6-001 (103-001)

Screening ("RainC")

GENE	AF	TYPE	CHR	START	END
CBL	0.40	nonsynonymous SNV	11	119278535	119278535
IDH2	0.46	nonsynonymous SNV	15	90088702	90088702
ASXL1	0.16	frameshift insertion	20	32434640	32434640
ASXL1	0.37	nonsynonymous SNV	20	32435156	32435156
ASXL1	0.10	frameshift deletion	20	32435578	32435581
BCORL1	0.07	nonsynonymous SNV	X	130014637	130014637

C3D1 Treated ("Rain5")

GENE	AF	TYPE	Chr	Start	End
NRAS	0.08	nonsynonymous SNV	1	114716127	114716127
CBL	0.46	nonsynonymous SNV	11	119278535	119278535
IDH2	0.44	nonsynonymous SNV	15	90088702	90088702
ASXL1	0.30	frameshift insertion	20	32434640	32434640
ASXL1	0.24	nonsynonymous SNV	20	32435156	32435156
NOTCH1	0.06	nonsynonymous SNV	9	136496655	136496655

Changes in AF of mutations in both samples:
CBL: increase AF by 0.06 after treatment
IDH2: decrease AF by 0.02 after treatment
ASXL1 (FSI): increase AF by 0.14 after treatment
ASXL1 (NS): decrease AF by 0.13 after treatment

Differences:
Lost ASXL1 frameshift deletion and BCORLI nonsynonymous SNV mutations after treatment.
Gain NRAS and NOTCH1 nonsynonymous SNV mutations after treatment.

Raindance Paired VAF Analysis

KB-7-001 (103-003)

Screening ("RainE")

GENE	AF	TYPE	CHR	START	END
KMT2A	0.09	nonsynonymous SNV	11	118472267	118472267
ASXL1	0.30	frameshift insertion	20	32434639	32434639
ASXL1	0.26	nonsynonymous SNV	20	32435156	32435156
TET2	0.42	frameshift deletion	4	105236290	105236290

C3D1 Treated ("Rain6")

GENE	AF	TYPE	CHR	START	END
NRAS	0.15	nonsynonymous SNV	1	114716126	114716126
ASXL1	0.34	frameshift insertion	20	32434639	32434639
TET2	0.48	frameshift deletion	4	105236290	105236290

Changes in AF of mutations in both samples:
ASXL1 (FSI): increase AF by 0.04 after treatment
TET2 (FSD): increase AF by 0.06 after treatment

Differences:
Lost KMT2A and ASXL1 nonsynonymous SNV mutations after treatment.
Gain KRAS nonsynonymous SNV mutation after treatment.

Raindance Paired VAF Analysis

KB-10-001 (103-007)

Screening ("RainG")

GENE	AF	TYPE	CHR	START	END
NRAS	0.37	nonsynonymous SNV	1	114716126	114716126
KMT2A	0.27	nonsynonymous SNV	11	118472267	118472267
TP53	0.07	nonsynonymous SNV	17	7676193	7676193
ASXL1	0.07	stopgain	20	32434789	32434789
TET2	0.36	frameshift insertion	4	105235432	105235432
TET2	0.45	frameshift deletion	4	105237004	105237004
TET2	0.35	nonsynonymous SNV	4	105275613	105275613
ZRSR2	0.30	nonframeshift insertion	X	15823113	15823113
STAG2	0.07	nonsynonymous SNV	X	124051210	124051210

C3D1 Treated ("Rain9")

GENE	AF	TYPE	CHR	START	END
NRAS	0.48	nonsynonymous SNV	1	114716126	114716126
ASXL1	0.07	stopgain	20	32434789	32434789
TET2	0.34	frameshift insertion	4	105235432	105235432
TET2	0.50	frameshift deletion	4	105237004	105237004
TET2	0.41	nonsynonymous SNV	4	105275613	105275613
ZRSR2	0.33	nonframeshift insertion	X	15823113	15823113

Changes in AF of mutations in both samples:
NRAS: increase AF by 0.11 after treatment
ASXL1: no change in AF after treatment
TET2 (FSI): increase AF by 0.02 after treatment
TET2 (FSD): increase AF by 0.05 after treatment
TET2 (NS): increase AF by 0.06 after treatment
ZRSR2: increase AF by 0.03 after treatment

Differences:
Lost KMT2A, TP53, and STAG2 nonsynonymous SNV mutations after treatment.

Raindance Paired VAF Analysis

KB-12-001 (103-009)

Screening ("RainI")

GENE	AF	TYPE	CHR	START	END
KMT2A	0.07	nonsynonymous SNV	11	118472267	118472267
SETBP1	0.07	nonsynonymous SNV	18	44951793	44951793
SF3B1	0.27	nonsynonymous SNV	2	197402110	197402110
ASXL1	0.07	frameshift deletion	20	32435305	32435305
RUNX1	0.39	nonsynonymous SNV	21	34834505	34834505
KIT	0.15	nonsynonymous SNV	4	54733155	54733155
EZH2	0.22	frameshift insertion	7	148815519	148815519
EZH2	0.20	frameshift deletion	7	148816719	148816720
BCOR	0.49	frameshift insertion	X	40062943	40062943

C3D1 Treated ("Rain10")

GENE	AF	TYPE	CHR	START	END
SF3B1	0.43	nonsynonymous SNV	2	197402110	197402110
ASXL1	0.07	frameshift deletion	20	32435305	32435305
RUNX1	0.67	nonsynonymous SNV	21	34834505	34834505
RUNX1	0.06	nonsynonymous SNV	21	34886895	34886895
KIT	0.45	nonsynonymous SNV	4	54733155	54733155
EZH2	0.52	frameshift insertion	7	148815519	148815519
EZH2	0.35	frameshift deletion	7	148816719	148816720
EZH2	0.06	nonsynonymous SNV	7	148817266	148817266
BCOR	0.84	frameshift insertion	X	40062943	40062943

Changes in AF of mutations in both samples:
SF3B1: increase AF by 0.16 after treatment
ASXL1: no change in AF after treatment
RUNX1: increase AF by 0.28 after treatment
KIT: increase AF by 0.30 after treatment
EZH2 (FSI): increase AF by 0.30 after treatment
EZH2 (FSD): increase AF by 0.15 after treatment
BCOR: increase AF by 0.35 after treatment

Differences:
Lost KMT2A and SETBP1 nonsynonymous SNV mutations after treatment.
Gain of RUNX1 and EZH2 nonsynonymous SNV mutations after treatment.

Raindance Paired VAF Analysis

RESPONDERS:

KB-1-001 (101-001)

Screening ("RainA")

GENE	AF	TYPE	CHR	START	END
NRAS	0.31	nonsynonymous SNV	1	114716126	114716126
NRAS	0.06	nonsynonymous SNV	1	114716127	114716127
ASXL1	0.41	nonsynonymous SNV	20	32435156	32435156
ASXL1	0.15	frameshift deletion	20	32436270	32436270
TET2	0.49	nonsynonymous SNV	4	105234686	105234686
TET2	0.48	nonsynonymous SNV	4	105235227	105235227
TET2	0.50	frameshift deletion	4	105243683	105243683
TET2	0.42	frameshift deletion	4	105259654	105259654

C3D1 Treated ("Rain1")

GENE	AF	TYPE	CHR	START	END
NRAS	0.38	nonsynonymous SNV	1	114716126	114716126
KMT2A	0.08	nonsynonymous SNV	11	118472267	118472267
ASXL1	0.37	nonsynonymous SNV	20	32435156	32435156
ASXL1	0.24	frameshift deletion	20	32436270	32436270
TET2	0.54	nonsynonymous SNV	4	105234686	105234686
TET2	0.47	nonsynonymous SNV	4	105235227	105235227
TET2	0.47	frameshift deletion	4	105243683	105243683
TET2	0.41	frameshift deletion	4	105259654	105259654
KDM6A	0.98	nonsynonymous SNV	X	45061396	45061396

Changes in AF of mutations in both samples:
NRAS: increase AF by 0.07 after treatment
ASXL1 (NS): decrease AF by 0.04 after treatment
ASXL1 (FSD): increase AF by 0.09 after treatment
TET2 (NS [I]): increase AF by 0.05 after treatment
TET2 (NS [II]): decrease AF by 0.01 after treatment
TET2 (FSD [I]): decrease AF by 0.03 after treatment
TET2 (FSD [II]): decrease AF by 0.01 after treatment

Differences:
Lost NRAS nonsynonymous SNV mutation after treatment.
Gain KMT2A and KDM6A nonsynonymous SNV mutations after treatment.

Raindance Paired VAF Analysis

KB-4-001 (101-004)

Screening ("10")

GENE	AF	TYPE	CHR	START	END
HRAS	0.14	nonsynonymous SNV	11	533568	533568
KMT2A	0.07	nonsynonymous SNV	11	118472267	118472267
KMT2A	0.10	nonsynonymous SNV	11	118503264	118503264
ETV6	0.10	nonsynonymous SNV	12	11869562	11869562
SETBP1	0.06	nonsynonymous SNV	18	44951942	44951942
ASXL1	0.15	nonsynonymous SNV	20	32435156	32435156
TET2	0.13	nonsynonymous SNV	4	105237193	105237193
EZH2	0.30	nonsynonymous SNV	7	148814090	148814090

C3D1 Treated ("Rain3")

GENE	AF	TYPE	CHR	START	END
SETBP1	0.16	nonsynonymous SNV	18	44951942	44951942
ASXL1	0.27	frameshift insertion	20	32434639	32434639
TET2	0.49	nonsynonymous SNV	4	105237193	105237193
EZH2	0.38	nonsynonymous SNV	7	148809351	148809351
EZH2	0.28	nonsynonymous SNV	7	148814090	148814090
RAD21	0.06	frameshift deletion	8	116847604	116847604
RAD21	0.06	frameshift deletion	8	116847606	116847606
NOTCH1	0.06	nonsynonymous SNV	9	136496655	136496655

Changes in AF of mutations in both samples:
SETBP1: increase AF by 0.10 after treatment
TET2: increase AF by 0.36 after treatment
EZH2: decrease AF by 0.02 after treatment

Differences:
Lost HRAS, KMT2A (x2), ETV6, and ASXL1 nonsynonymous SNV mutations after treatment. Gain ASXL1 frameshift insertion, EZH2 and NOTCH1 nonsynonymous SNV mutations, and two RAD21 frameshift deletions after treatment.

Raindance Paired VAF Analysis

KB-5-001 (101-005)

Screening ("12")

GENE	AF	TYPE	CHR	START	END
KMT2A	0.15	nonsynonymous SNV	11	118472267	118472267
KRAS	0.49	nonsynonymous SNV	12	25245332	25245332
ASXL1	0.06	nonsynonymous SNV	20	32433455	32433455
ASXL1	0.06	nonsynonymous SNV	20	32433457	32433457
ASXL1	0.15	frameshift insertion	20	32434639	32434639
TET2	0.06	nonsynonymous SNV	4	105236698	105236698
TET2	0.15	nonsynonymous SNV	4	105243612	105243612
TET2	0.43	frameshift deletion	4	105243778	105243781
TET2	0.39	nonsynonymous SNV	4	105275613	105275613
RAD21	0.09	nonsynonymous SNV	8	116857362	116857362
NOTCH1	0.07	nonsynonymous SNV	9	136496655	136496655
SMC1A	0.09	nonsynonymous SNV	X	53413242	53413242

C3D1 Treated ("Rain4")

GENE	AF	TYPE	CHR	START	END
SF3B1	0.33	nonsynonymous SNV	2	197402649	197402649
ASXL1	0.33	nonsynonymous SNV	20	32435156	32435156
TET2	0.16	nonsynonymous SNV	4	105243612	105243612
JAK2	0.33	nonsynonymous SNV	9	5073770	5073770
BCORL1	0.06	nonsynonymous SNV	X	130014637	130014637

Changes in AF of mutations in both samples:
TET2: increase AF by 0.01 after treatment

Differences:
Lost KMT2A, KRAS, ASXL1 (x3), TET2 (x3), RAD21, NOTCH1, and SMC1A mutations after treatment.
Gain SF3B1, ASXL1, JAK2, and BCORL1 nonsynonymous SNV mutations after treatment.

Raindance Paired VAF Analysis

KB-8-001 (103-004)

Screening ("RainF")

GENE	AF	TYPE	CHR	START	END
KMT2A	0.06	nonsynonymous SNV	11	118472267	118472267
TP53	0.48	nonsynonymous SNV	17	7676225	7676225
ASXL1	0.26	nonsynonymous SNV	20	32435156	32435156
TET2	0.24	frameshift deletion	4	105236165	105236165
TET2	0.31	frameshift deletion	4	105243705	105243706
BCORL1	0.07	nonsynonymous SNV	X	130014637	130014637

C3D1 Treated ("Rain7")

GENE	AF	TYPE	CHR	START	END
TP53	0.47	nonsynonymous SNV	17	7676225	7676225
TET2	0.23	frameshift deletion	4	105236165	105236165
TET2	0.28	frameshift deletion	4	105243705	105243706
NOTCH1	0.06	nonsynonymous SNV	9	136496655	136496655

Changes in AF of mutations in both samples:
TP53: decrease AF by 0.01 after treatment
TET2 (FSD [I]): decrease AF by 0.01 after treatment
TET2 (FSD [II]): decrease AF by 0.03 after treatment

Differences:
Lost KMT2A, ASXL1, and BCORL1 nonsynonymous SNV mutations after treatment.
Gain NOTCH1 nonsynonymous SNV mutation after treatment.

Raindance Paired VAF Analysis

KB-13-001 (103-010)

Screening ("3")

GENE	AF	TYPE	CHR	START	END
NRAS	0.47	nonsynonymous SNV	1	114716126	114716126
ETV6	0.26	nonsynonymous SNV	12	11869562	11869562
ASXL1	0.45	frameshift deletion	20	32434807	32434807
ASXL1	0.21	nonsynonymous SNV	20	32435156	32435156
GATA2	0.10	nonsynonymous SNV	3	128486911	128486911
TET2	0.79	stopgain	4	105275056	105275056
NOTCH1	0.50	nonsynonymous SNV	9	136502383	136502383
SMC1A	0.10	nonsynonymous SNV	X	53413242	53413242

C3D1 Treated ("Rain11")

GENE	AF	TYPE	CHR	START	END
NRAS	0.43	nonsynonymous SNV	1	114716126	114716126
KMT2A	0.08	nonsynonymous SNV	11	118472267	118472267
ETV6	0.41	nonsynonymous SNV	12	11869562	11869562
ASXL1	0.45	frameshift deletion	20	32434807	32434807
ASXL1	0.46	nonsynonymous SNV	20	32435156	32435156
TET2	0.84	stopgain	4	105275056	105275056

Changes in AF of mutations in both samples:
NRAS: decrease AF by 0.04 after treatment
ETV6: increase AF by 0.15 after treatment
ASXL1 (FSD): no change in AF after treatment
ASXL1 (NS): increase AF by 0.25 after treatment
TET2: increase AF by 0.05 after treatment

Differences:
Lost GATA2, NOTCH1, and SMC1A nonsynonymous SNV mutations after treatment.
Gain KMT2A nonsynonymous SNV mutation after treatment.

Raindance Paired VAF Analysis

KB-14-001 (103-011)

Screening ("11")

GENE	AF	TYPE	CHR	START	END
NRAS	0.34	nonsynonymous SNV	1	114716126	114716126
KMT2A	0.08	nonsynonymous SNV	11	118472267	118472267
CEBPA	0.12	nonframeshift deletion	19	33302102	33302104
ASXL1	0.13	frameshift deletion	20	32434600	32434622
RUNX1	0.30	frameshift insertion	21	34792405	34792405
TET2	0.47	nonsynonymous SNV	4	105241408	105241408
TET2	0.44	stopgain	4	105275312	105275312
EZH2	0.98	nonsynonymous SNV	7	148846579	148846579
JAK2	0.80	nonsynonymous SNV	9	5073770	5073770
NOTCH1	0.06	nonsynonymous SNV	9	136496655	136496655

C3D1 Treated ("Rain12")

GENE	AF	TYPE	CHR	START	END
NRAS	0.33	nonsynonymous SNV	1	114716126	114716126
DNMT3A	0.50	nonframeshift substitution	2	25246633	25246634
ASXL1	0.32	frameshift insertion	20	32434639	32434639
ASXL1	0.43	nonsynonymous SNV	20	32435156	32435156
RUNX1	0.52	frameshift insertion	21	34792405	34792405
TET2	0.44	nonsynonymous SNV	4	105241408	105241408
EZH2	0.98	nonsynonymous SNV	7	148846579	148846579
NOTCH1	0.06	nonsynonymous SNV	9	136496646	136496646
NOTCH1	0.06	nonsynonymous SNV	9	136496655	136496655

Changes in AF of mutations in both samples:
NRAS: decrease AF by 0.01 after treatment
RUNX1: increase AF by 0.22 after treatment
TET2: decrease AF by 0.03 after treatment
NOTCH1: no change after treatment

Differences:
Lost KMT2A, CEBPA, ASXL1, TET2, EZH2, and JAK2 mutations after treatment.
Gain DNMT3A, ASXL1 (x2), EZH2, and NOTCH1 mutations after treatment.

Raindance Paired VAF Analysis

SINGLE TIMEPOINT SAMPLES

SCREENING

KB-3-001 (101-003) – Non-Responder ("RainB")

GENE	AF	TYPE	CHR	START	END
TP53	0.08	nonsynonymous SNV	17	7676193	7676193
ASXL1	0.37	frameshift deletion	20	32434822	32434822

KB-11-001 (103-008) – Non-Responder ("RainH")

GENE	AF	TYPE	CHR	START	END
NRAS	0.06	nonsynonymous SNV	1	114716126	114716126
IDH2	0.47	nonsynonymous SNV	15	90088702	90088702
TP53	0.07	nonsynonymous SNV	17	7676193	7676193
GATA2	0.46	nonsynonymous SNV	3	128486117	128486117

KB-15-001 (103-012) – Non-Responder ("13")

GENE	AF	TYPE	CHR	START	END
CSF3R	0.44	nonsynonymous SNV	1	36466862	36466862
CBL	0.65	nonsynonymous SNV	11	119278541	119278541
SETBP1	0.48	nonsynonymous SNV	18	44951942	44951942
ASXL1	0.46	stopgain	20	32434485	32434485
ASXL1	0.15	frameshift deletion	20	32434600	32434622
ASXL1	0.20	nonsynonymous SNV	20	32435156	32435156
TET2	0.44	stopgain	4	105234282	105234282
TET2	0.21	frameshift insertion	4	105275524	105275524
NOTCH1	0.09	nonsynonymous SNV	9	136496655	136496655

KB-17-001 (103-002) – Non-Responder ("RainD")

GENE	AF	TYPE	CHR	START	END
IDH2	0.46	nonsynonymous SNV	15	90088702	90088702
ASXL1	0.35	nonsynonymous SNV	20	32435156	32435156
ASXL1	0.25	frameshift deletion	20	32435578	32435581
JAK2	0.18	nonsynonymous SNV	9	5073770	5073770
BCORL1	0.07	nonsynonymous SNV	X	130014637	130014637

Raindance Paired VAF Analysis

KB-16-001 (103-013) – Non-Responder (“14”)

GENE	AF	TYPE	CHR	START	END
KMT2A	0.10	nonsynonymous SNV	11	118472267	118472267
KMT2A	0.07	nonsynonymous SNV	11	118503264	118503264
CBL	0.51	nonsynonymous SNV	11	119278268	119278268
ASXL1	0.44	nonsynonymous SNV	20	32431326	32431326
ASXL1	0.40	frameshift insertion	20	32434639	32434639
ASXL1	0.14	nonsynonymous SNV	20	32435156	32435156
RUNX1	0.32	frameshift insertion	21	34792566	34792566
GATA2	0.39	nonsynonymous SNV	3	128486911	128486911
TET2	0.43	stopgain	4	105235468	105235468
TET2	0.24	nonsynonymous SNV	4	105237193	105237193
TET2	0.39	nonsynonymous SNV	4	105243631	105243631
EZH2	0.35	nonsynonymous SNV	7	148809351	148809351
BCORL1	0.13	nonsynonymous SNV	X	130014637	130014637

C3 TREATED

KB-2-003 (101-002) – Non-Responder (“Rain2”)

GENE	AF	TYPE	CHR	START	END
TET2	0.11	frameshift insertion	4	105242894	105242894
TET2	0.11	stopgain	4	105275464	105275464

KB-9-002 (103-005) – Non-Responder (“Rain8”)

GENE	AF	TYPE	CHR	START	END
TET2	0.51	frameshift insertion	4	105236000	105236000
TET2	0.45	stopgain	4	105243621	105243621

OTHER PATIENT SAMPLES

1-V-001 (“2”)

GENE	AF	TYPE	CHR	START	END
KMT2A	0.17	nonsynonymous SNV	11	118472267	118472267
CBL	0.52	nonsynonymous SNV	11	119278541	119278541
ASXL1	0.06	nonsynonymous SNV	20	32433455	32433455
ASXL1	0.06	nonsynonymous SNV	20	32433457	32433457
SMC1A	0.13	nonsynonymous SNV	X	53413242	53413242
BCORL1	0.98	nonsynonymous SNV	X	130015441	130015441

Raindance Paired VAF Analysis

2-N-001 (“4”)

GENE	AF	TYPE	CHR	START	END
KMT2A	0.14	nonsynonymous SNV	11	118472267	118472267
ETV6	0.17	frameshift deletion	12	11839271	11839272
ETV6	0.21	nonsynonymous SNV	12	11869562	11869562
ASXL1	0.09	stopgain	20	32433447	32433447
ASXL1	0.08	nonsynonymous SNV	20	32433455	32433455
ASXL1	0.08	nonsynonymous SNV	20	32433457	32433457
ASXL1	0.32	nonsynonymous SNV	20	32435156	32435156
TET2	0.47	nonsynonymous SNV	4	105236541	105236541
TET2	0.38	nonsynonymous SNV	4	105275677	105275677
JAK2	1.00	nonsynonymous SNV	9	5073770	5073770
SMC1A	0.39	nonsynonymous SNV	X	53422495	53422495

1-M-001 (“5”)

GENE	AF	TYPE	CHR	START	END
KMT2A	0.12	nonsynonymous SNV	11	118472267	118472267
KMT2A	0.08	nonsynonymous SNV	11	118503264	118503264
CEBPA	0.09	nonframeshift deletion	19	33302102	33302104
ASXL1	0.18	nonsynonymous SNV	20	32435156	32435156
TET2	0.27	frameshift deletion	4	105243705	105243706
TET2	0.33	stopgain	4	105259687	105259687

2-F-001 (“6”)

GENE	AF	TYPE	CHR	START	END
SMC3	0.34	nonsynonymous SNV	10	110602030	110602030
ETV6	0.15	frameshift deletion	12	11839271	11839272
ETV6	0.17	nonsynonymous SNV	12	11869562	11869562
MAP2K1	0.08	nonsynonymous SNV	15	66435113	66435113
ASXL1	0.10	nonsynonymous SNV	20	32433455	32433455
ASXL1	0.10	nonsynonymous SNV	20	32433457	32433457
ASXL1	0.11	nonsynonymous SNV	20	32435156	32435156
TET2	0.46	nonsynonymous SNV	4	105236541	105236541
TET2	0.48	frameshift insertion	4	105275086	105275086
TET2	0.36	nonsynonymous SNV	4	105275677	105275677
EZH2	0.07	nonsynonymous SNV	7	148817266	148817266
JAK2	1.00	nonsynonymous SNV	9	5073770	5073770
SMC1A	0.45	nonsynonymous SNV	X	53422495	53422495
BCORL1	0.06	nonsynonymous SNV	X	130014637	130014637
BCORL1	0.36	frameshift deletion	X	130056036	130056036

Raindance Paired VAF Analysis

1-N-001 ("7")

GENE	AF	TYPE	CHR	START	END
NRAS	0.13	nonsynonymous SNV	1	114716123	114716123
NRAS	0.17	nonsynonymous SNV	1	114716126	114716126
KMT2A	0.20	nonsynonymous SNV	11	118472267	118472267
KMT2A	0.06	frameshift deletion	11	118472296	118472296
SETBP1	0.50	nonsynonymous SNV	18	44951948	44951948
ASXL1	0.48	frameshift insertion	20	32434639	32434639

3-A-001 ("9")

GENE	AF	TYPE	CHR	START	END
KRAS	0.46	nonsynonymous SNV	12	25245350	25245350
SETBP1	0.44	nonsynonymous SNV	18	44951946	44951946
ASXL1	0.06	nonsynonymous SNV	20	32433455	32433455
ASXL1	0.06	nonsynonymous SNV	20	32433457	32433457
ASXL1	0.18	nonsynonymous SNV	20	32435156	32435156
ASXL1	0.09	frameshift deletion	20	32435315	32435315
TET2	0.15	frameshift deletion	4	105237059	105237059
TET2	0.21	frameshift insertion	4	105275524	105275524
SMC1A	0.10	nonsynonymous SNV	X	53413242	53413242

1-T-001 ("Rain13")

GENE	AF	TYPE	CHR	START	END
IDH2	0.40	nonsynonymous SNV	15	90088702	90088702
TET2	0.37	nonsynonymous SNV	4	105243759	105243759
JAK2	0.37	nonsynonymous SNV	9	5073770	5073770
NOTCH1	0.07	nonframeshift deletion	9	136496493	136496495
NOTCH1	0.09	nonsynonymous SNV	9	136496655	136496655

Raindance Paired VAF Analysis

OTHER SAMPLES (LISTED BY RAINDANCE ID)

Rain422

None meet Markus' cutoffs/filtering (however the "all" file contains more mutations)

Rain518

GENE	AF	TYPE	CHR	START	END
KRAS	0.12	nonsynonymous SNV	12	25245286	25245286
KRAS	0.34	nonsynonymous SNV	12	25245347	25245347
KDM6A	0.09	nonsynonymous SNV	X	45069826	45069826
KDM6A	0.07	nonsynonymous SNV	X	45069854	45069854
KDM6A	0.06	nonsynonymous SNV	X	45069915	45069915
KDM6A	0.06	nonsynonymous SNV	X	45069919	45069919

Rain520

GENE	AF	TYPE	CHR	START	END
KRAS	0.09	nonsynonymous SNV	12	25245286	25245286
KRAS	0.33	nonsynonymous SNV	12	25245347	25245347
SMC1A	0.06	nonsynonymous SNV	X	53413242	53413242
BCORL1	0.08	nonsynonymous SNV	X	130014637	130014637

Rain522

GENE	AF	TYPE	CHR	START	END
KMT2A	0.10	nonsynonymous SNV	11	118472267	118472267
KRAS	0.33	nonsynonymous SNV	12	25245347	25245347
CEBPA	0.08	nonframeshift deletion	19	33302102	33302104
ASXL1	0.39	nonsynonymous SNV	20	32435156	32435156
RUNX1	0.08	nonsynonymous SNV	21	34886895	34886895
BCORL1	0.08	nonsynonymous SNV	X	130014637	130014637

Human in Mouse Samples

16

GENE	AF	TYPE	CHR	START	END
DNMT3A	0.18	stopgain	2	25244203	25244203
BCORL1	0.13	nonsynonymous SNV	X	130014637	130014637

17

None meet Markus' cutoffs/filtering

Raindance Paired VAF Analysis

18

None meet Markus' cutoffs/filtering

19

GENE	AF	TYPE	CHR	START	END
RAD21	0.09	nonsynonymous SNV	8	116847685	116847685
NOTCH1	0.35	nonsynonymous SNV	9	136496655	136496655
BCOR	0.09	nonsynonymous SNV	X	40052348	40052348

20

GENE	AF	TYPE	CHR	START	END
MAP2K1	0.08	nonsynonymous SNV	15	66435113	66435113
ASXL1	0.06	nonsynonymous SNV	20	32433455	32433455
ASXL1	0.33	nonsynonymous SNV	20	32435156	32435156
TET2	0.10	frameshift deletion	4	105237120	105237120
NOTCH1	0.14	nonsynonymous SNV	9	136496655	136496655
SMC1A	0.10	nonsynonymous SNV	X	53413242	53413242

21

GENE	AF	TYPE	CHR	START	END
KRAS	0.18	nonsynonymous SNV	12	25245348	25245348
MAP2K1	0.08	nonsynonymous SNV	15	66435113	66435113
ASXL1	0.09	nonsynonymous SNV	20	32433455	32433455
ASXL1	0.09	nonsynonymous SNV	20	32433457	32433457
ASXL1	0.25	frameshift insertion	20	32434639	32434639
ASXL1	0.28	nonsynonymous SNV	20	32435156	32435156
NOTCH1	0.12	nonsynonymous SNV	9	136496655	136496655
SMC1A	0.10	nonsynonymous SNV	X	53413242	53413242
BCORL1	0.11	nonsynonymous SNV	X	130014637	130014637

22

GENE	AF	TYPE	CHR	START	END
MAP2K1	0.07	nonsynonymous SNV	15	66435113	66435113
ASXL1	0.09	nonsynonymous SNV	20	32433455	32433455
ASXL1	0.09	nonsynonymous SNV	20	32433457	32433457
ASXL1	0.20	nonsynonymous SNV	20	32435156	32435156
NOTCH1	0.07	nonframeshift deletion	9	136496493	136496495
SMC1A	0.09	nonframeshift substitution	X	53413242	53413243

Raindance Paired VAF Analysis

23

GENE	AF	TYPE	CHR	START	END
KMT2A	0.11	nonsynonymous SNV	11	118472267	118472267
ETV6	0.18	frameshift deletion	12	11839271	11839272
ETV6	0.14	nonsynonymous SNV	12	11869562	11869562
PTPN11	0.21	nonsynonymous SNV	12	112450393	112450393
ASXL1	0.46	frameshift insertion	20	32434639	32434639
ASXL1	0.32	nonsynonymous SNV	20	32435156	32435156
TET2	0.07	nonsynonymous SNV	4	105236698	105236698
TET2	0.13	nonsynonymous SNV	4	105275677	105275677
RAD21	0.39	stopgain	8	116866721	116866721
NOTCH1	0.08	nonsynonymous SNV	9	136496655	136496655

24

GENE	AF	TYPE	CHR	START	END
KMT2A	0.10	nonsynonymous SNV	11	118472267	118472267
ETV6	0.21	frameshift deletion	12	11839271	11839272
ETV6	0.23	nonsynonymous SNV	12	11869562	11869562
PTPN11	0.18	nonsynonymous SNV	12	112450393	112450393
SF3B1	0.32	nonsynonymous SNV	2	197402649	197402649
ASXL1	0.08	nonsynonymous SNV	20	32433455	32433455
ASXL1	0.08	nonsynonymous SNV	20	32433457	32433457
TET2	0.10	nonsynonymous SNV	4	105275677	105275677
JAK2	0.28	nonsynonymous SNV	9	5073770	5073770
NOTCH1	0.06	nonsynonymous SNV	9	136496655	136496655
SMC1A	0.06	nonsynonymous SNV	X	53413242	53413242
BCORL1	0.08	nonsynonymous SNV	X	130014637	130014637

Raindance Paired VAF Analysis

ADDITIONAL INFORMATION

Analysis files located in /share/lab_padron/Meghan/TargetedPanel/Hannah-Raindance-2019/results and each Raindance ID is a folder with all the analysis. Raindance IDs are matched to D-IDs and indicated in parentheses. Within a Raindance ID folder > GATK has the raw VCF file with the suffix "-haplo.vcf.gz". Filtered and annotated SNPs are identified in "final" files.

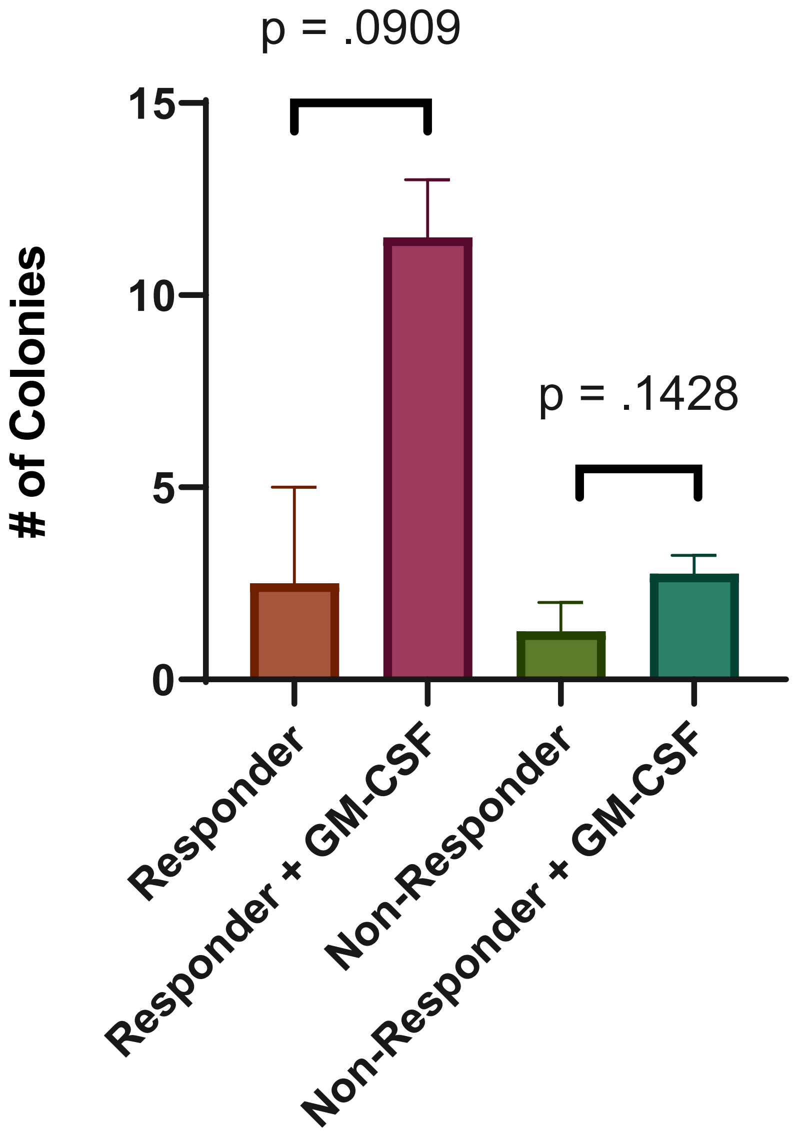
Filters applied to VCF:

depth > 100
SOR < 3
QUAL > 100
AF > 0.05

Suffix "att9" has only the first three filters (i.e. does not apply the allelic frequency cutoff) and "att10" has all the filters applied. Above compiled VAF information is given based on "att10" results. These filters were the same ones applied by Markus to Andrew Kuykendall's targeted panel.

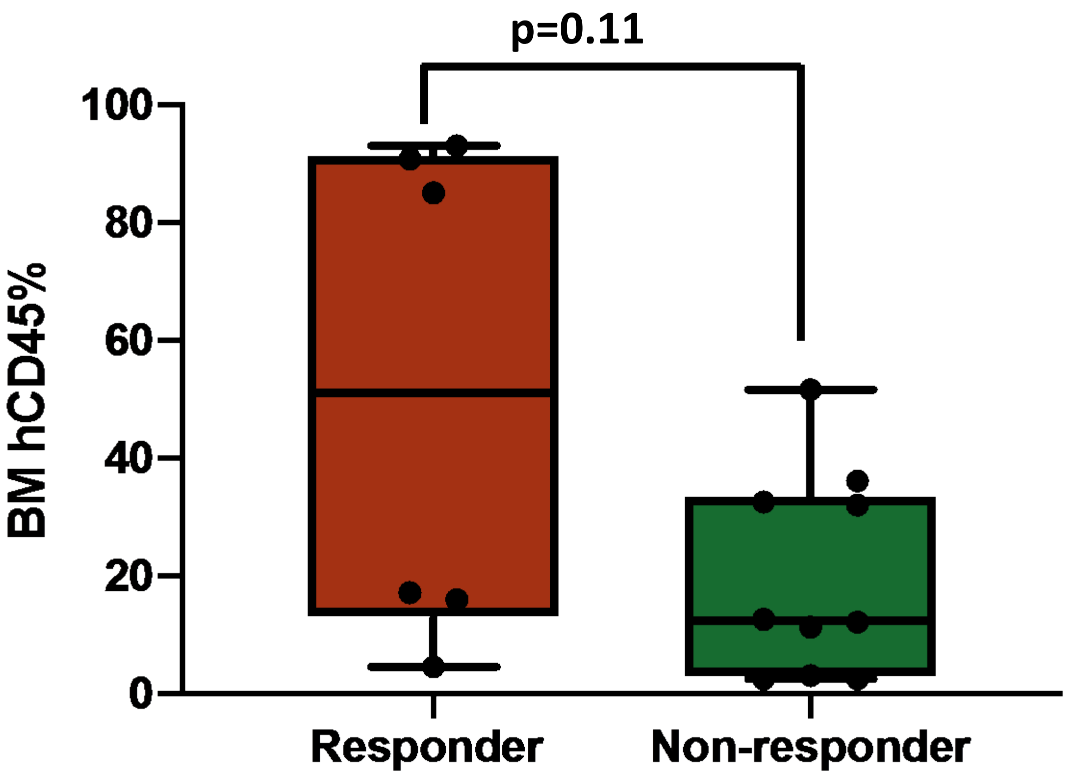
Supplemental Figure 4

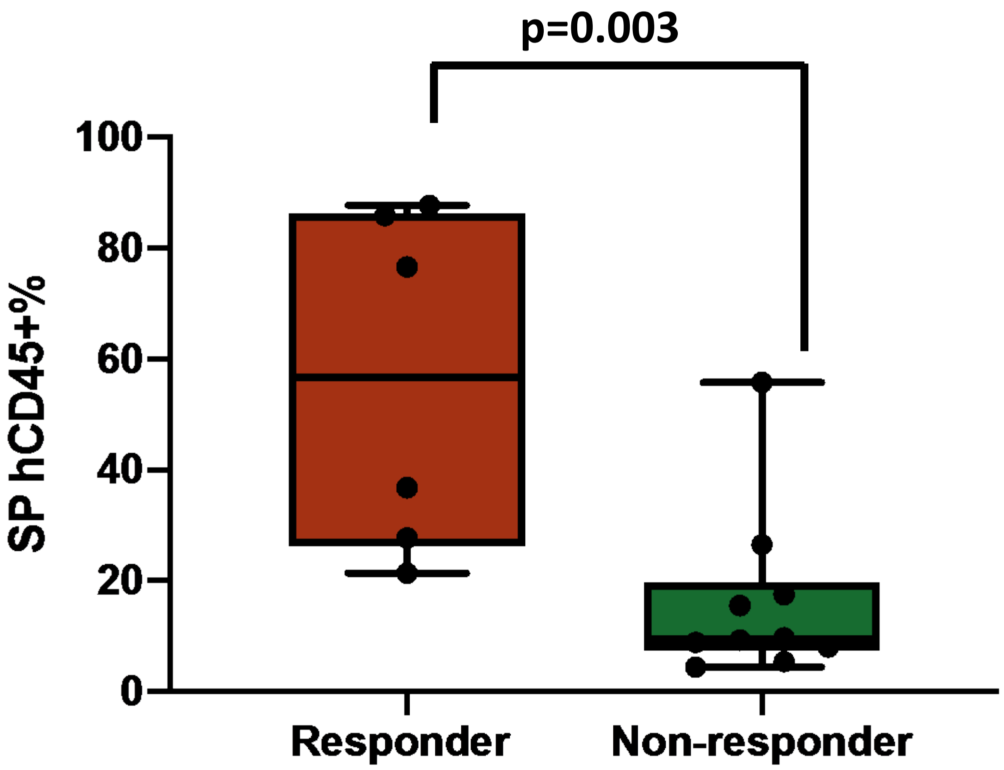
COLONY FORMATION ASSAYS FOR 1 RESPONDER AND 2 NONRESPONDERS IN DUPLICATE



Supplemental Figure 5a

BONE MARROW(BM) AND SPLEEN (SP) HUMAN CD45% ENGRAFTMENT OF KB-003 SCREENING PATIENT SAMPLES - MORIBUND





Supplemental Figure 5b

SPLEEN WEIGHTS OF KB-003 SCREENING PATIENT SAMPLES -MORIBUND