Supplementary Materials

Figure S1

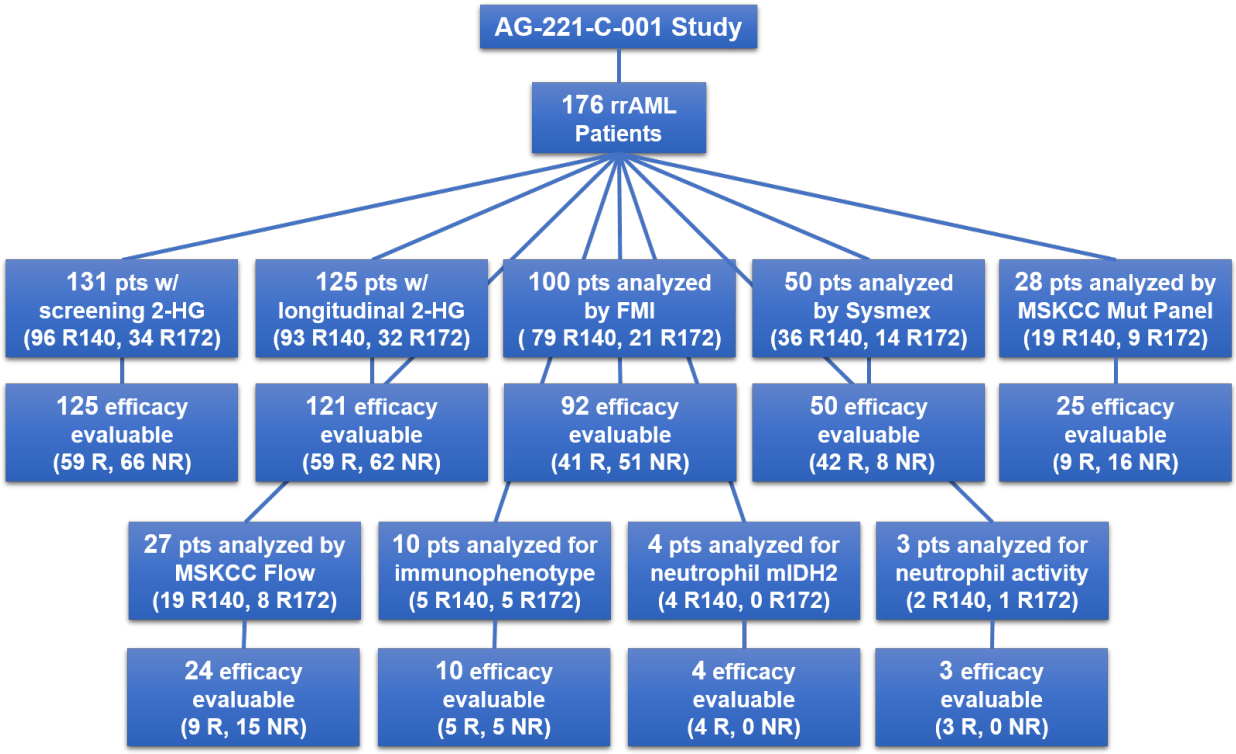

Figure S1: Patient Sample Availability and Analysis Disposition

Patient sample disposition indicating of 176 rrAML patients from the AG-221-C-001 study; i.e., the sample availability for each assay. Of available samples, only a subset had clinical efficacy data, defined as those patients who received enasidenib and an investigator-assessed clinical response was captured from at least one time-point during treatment. Number of samples from patients with R140 vs R172 m*IDH2* and from patients achieving a response (R) vs no response (NR) (as defined in Figure 1) are indicated. rrAML, relapsed/refractory acute myeloid leukemia, FMI, Foundation Medicine, Inc. FoundationOne® Heme Assay. MSKCC, Memorial Sloan Kettering Cancer Center.

Figure S2

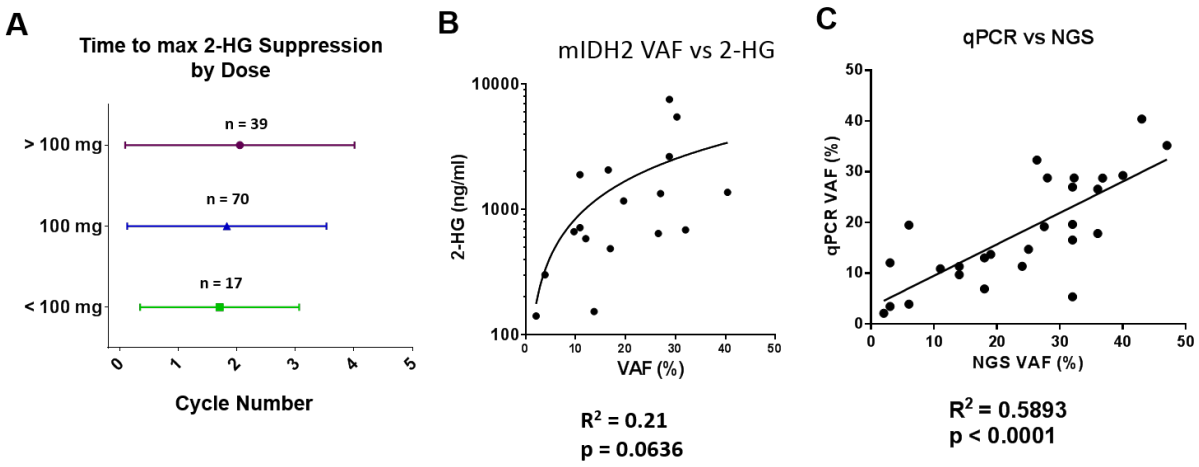

Figure S2. Analysis of 2-HG and mIDH2 Variant Allele Frequency

A. Whisker plot indicating mean and standard deviation of cycle to maximum 2-HG suppression (Max 2-HG) stratified by patients dosed with <100mg, 100mg, or >100mg enasidenib daily.

B. Scatter plot and regression analysis of m*IDH2* variant allele frequency (VAF) in patient samples at screening analyzed by Sysmex OncoBeam digital PCR assay with levels of 2-HG in plasma in the same patient (n=17).

C. Scatter plot and regression analysis of m*IDH2* VAF in patient samples when VAF was measured in the same patient at the same time point by both an NGS panel (either FoundationOne® Heme, n=26, or MSKCC panel, n=3, as discussed in Methods) and Sysmex OncoBeam digital PCR assay.

Figure S3

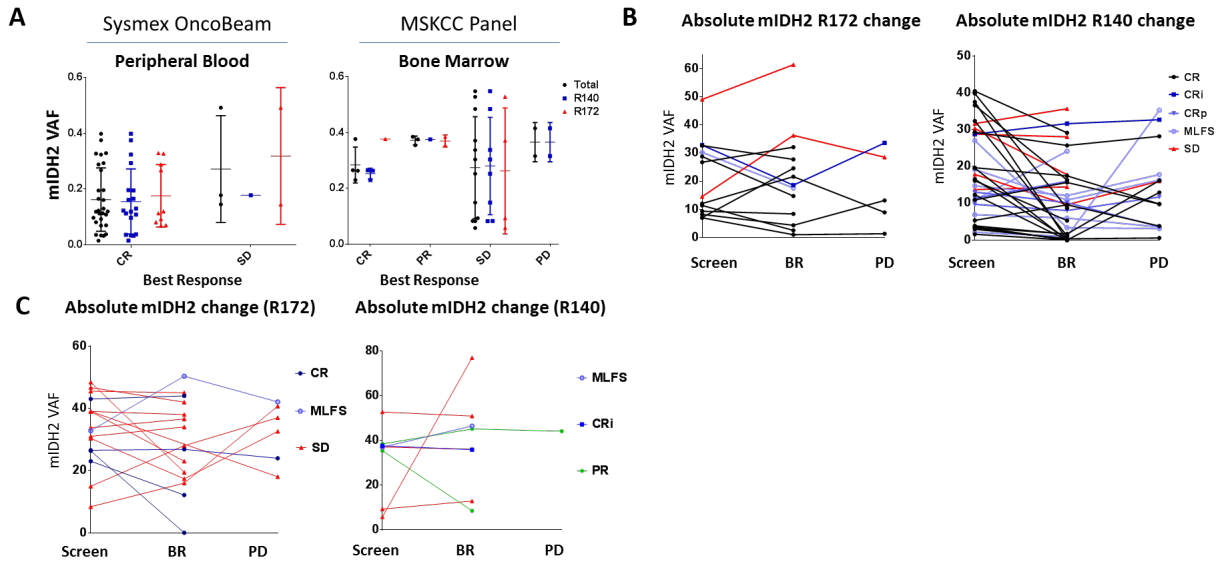

Figure S3. mIDH2 Variant Allele Frequency and Response

A. Dot plot of m*IDH2* VAF in patient samples measured by Sysmex digital PCR (left) or Memorial Sloan Kettering Cancer Center (MSKCC) mutational panel (right) at screening. Measurements were separated by best response (BR) achieved by the patient (CR, CRp, CRi, or MLFS) and m*IDH2* status (red = R140, blue = R172, black = combined).

B. Scatter plots of m*IDH2* VAF level measured by Sysmex digital PCR in R172 (left) and R140 (right) m*IDH2* patients. Lines indicate changes in m*IDH2* levels in individual patients from screening to first achievement of BR to disease progression (PD, if available). Black lines indicate patients' BR of CR, blue lines indicate BR of CRi, CRp, or MLFS, and red lines indicate BR of SD (SD patients' samples were analyzed at Cycle 3 Day 1).

C. Scatter plots of mIDH2 VAF level measured by NGS panels in mIDH2 R172 (left) and R140 (right) patients. Lines indicate changes in m*IDH2* levels in individual patients from screening to first achievement of BR and then to PD (if available). Black lines indicate patients' BR of CR, blue lines indicate BR of CRi or MLFS, green lines indicate BR of PR, and red lines indicate BR of SD (SD patients' samples were analyzed at Cycle 3 Day 1).

Figure S4

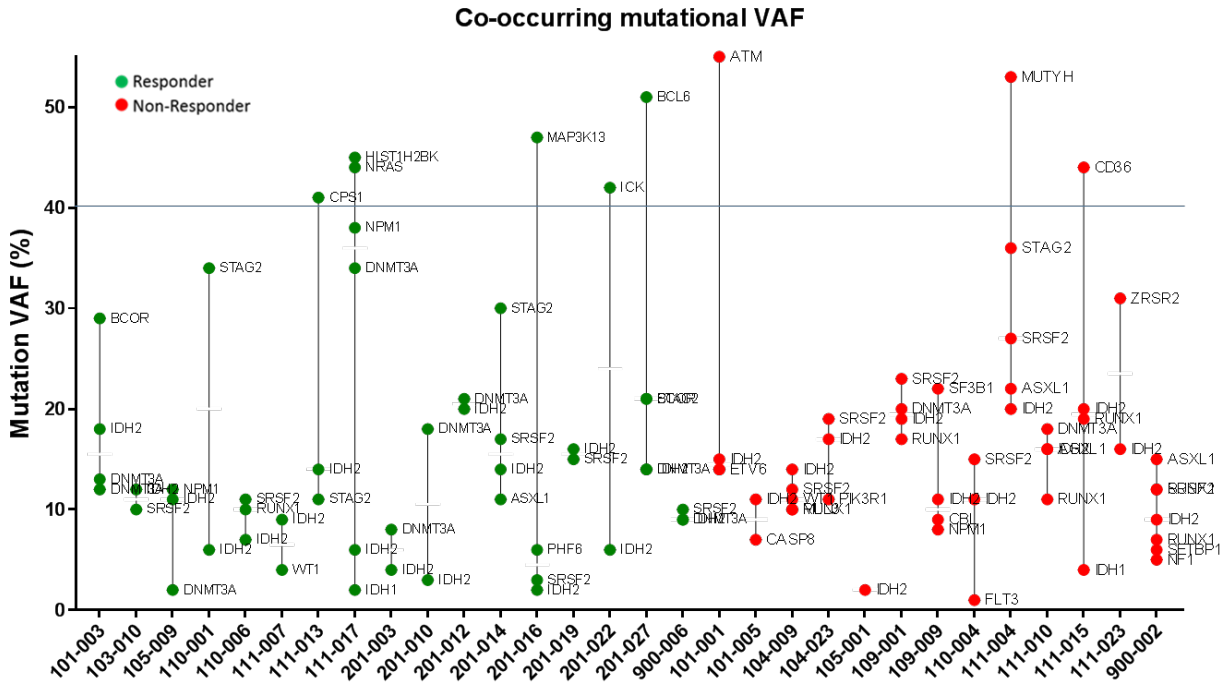

Figure S4: VAF of co-occurring mutations in patients with low mIDH2

Co-mutational variant allele frequency (VAF) measured by FoundationOne® Heme panel in individual patients with subclonal mIDH2 (VAF <20%). Responding patients (CR, CRi, CRp, MLFS, or PR) in green, non-responding patients (SD or PD) in red.

Figure S5

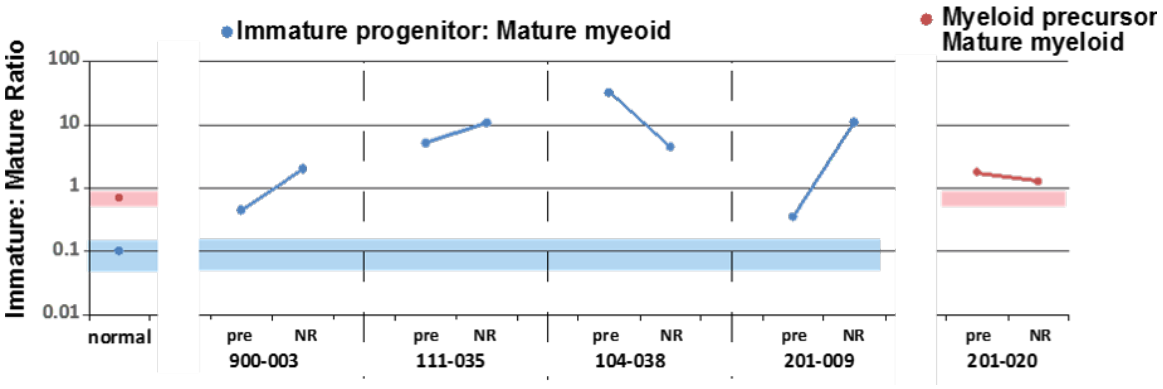

Figure S5. Mature and Immature Cell Populations from Bone Marrow in Non-Responding Patients

Graph showing ratio of immature to mature cell populations from bone marrow: The average ratios of myeloid progenitor or myeloid precursors to mature myeloid cells in bone marrow from normal donors (n=12) are shown. Colored bars represent the 95% confidence interval. The same analysis was applied to samples from four non-responding patients with expanded leukemic myeloid progenitors and one patient (201-020) who had expanded leukemic myeloid precursors.

Figure S6

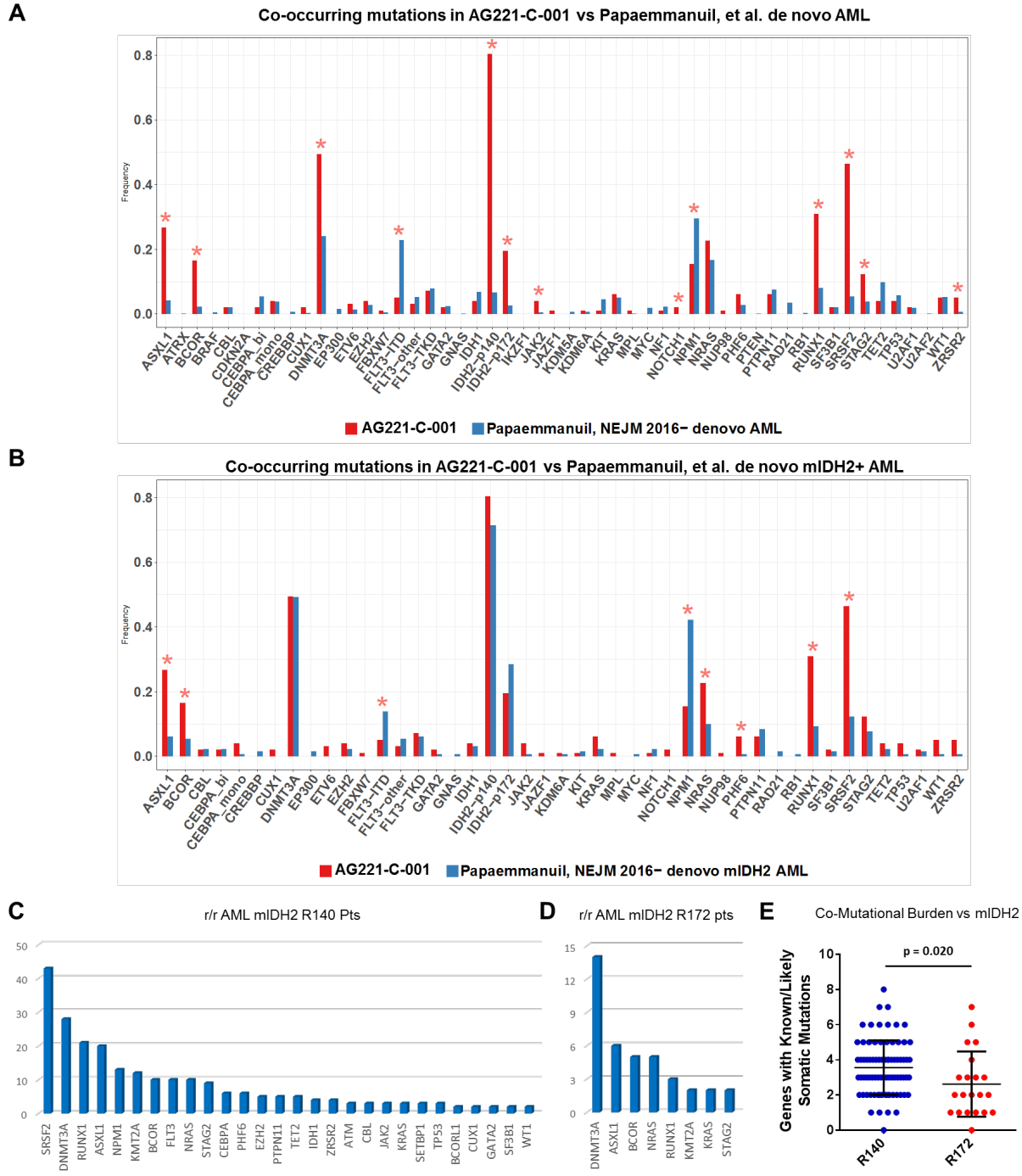

Figure S6: Co-occurring Mutation Frequency vs Response

A. Comparison of co-occurring mutation frequency in AG-221-C-001 (red) vs the same mutations in all 1376 cases of de novo AML from Papaemmanuil, et al (blue).[13] Genes

that were not part of the Papaemmanuil, et al. dataset or FoundationOne® Heme panel were removed. Fisher Exact Test was used to compare gene counts between datasets. * indicates significant enrichment of gene in either data set (p-value ≤ 0.05).

B. Comparison of co-occurring mutation frequency in AG-221-C-001 (red) vs same co-occurring mutations in all 130 cases of de novo AML from Papaemmanuil, et al that were mIDH2 positive (blue).[13] Genes that were not part of the ULM dataset or FoundationOne® Heme panel were removed. Fisher Exact Test was used to compare gene counts between datasets. * indicates significant enrichment of gene in either data set (p-value ≤ 0.05).

C. Histogram of mutation frequency in genes found by FoundationOne® Heme panel in R140 m*IDH2* patients.

D. Histogram of mutation frequency in genes found by FoundationOne® Heme panel in R172 m*IDH2* patients.

E. Number of mutations found per patient separated by patients with m*IDH2* R140 vs R172. Hashes represent mean with standard deviation, p-value from student's t-test.

Table S1. Patient baseline characteristics in all relapsed/refractory AML patients from the AG221-C-001 trial and patient subgroup analyzed.

	FoundationOne® Patients n=100	Sysmex mIDH2 VAF Patients n=50	2-HG Patients N= 125	All R/R AML Patients n=176
ORR (%)	41.0	84.0	47.2	40.3
CR (%)	19.0	60.0	20.8	19.3
Median Age, years	65.7	67.6	65.2	65.2
Sex M/F (%)	51.0/49.0	36.0/64.0	52.0/48.0	51.1/48.9
Gene Mutation (%): R140/R172	79.0/21.0	74.0/26.0	74.4/26.6	73.9/25.6
No. of Prior Anticancer therapies (%): 1/2/3/4/>=5	46.0/23.0/16.0/ 11.0/4.0	56.0/26.0/6.0/ 10.0/2.0	50.4/22.4/12.8/ 10.4/4.0	46.6/26.1/14.2/ 8.5/4.5
Prior Transplant (%)	14.0	16.0	14.4	13.6
Mean Bone Marrow Blast (%)	49.9	36.4	47.1	49.4
Mean ANC ($x10^9$/L)	1.5	1.6	1.3	1.3
Prior MDS (%)	16.0	10.0	16.0	17.0

Overall Response Rate (ORR) and Complete Remission (CR) rate and baseline characteristics, including age, sex (male/female), *IDH2* mutation subtype, number of prior anticancer therapies, patients who have received a transplant, baseline bone marrow blast percentage, mean absolute neutrophil count (ANC) and patients with prior diagnosis of MDS in the rrAML cohort in the AG-221-C-001 and in patient subgroups analyzed by FoundationOne® Heme mutation panel, Sysmex mIDH2 VAF digital PCR, and 2-HG assay.

Table S2. Multi-parameter Flow Cytometry (MFC) Ten-"Color" Panel.

Myeloid tube 1	Myeloid tube 2	Myeloid tube 3
CD15 FITC	CD64 FITC	CD7 BB515
CD33 PE	CD123 PE	CD56 PE
CD117 PC5	CD14 PC5	CD5 PerCP-Cy5.5
CD13 PE-CY7	CD13 PE-CY7	CD33 PE-CY7
CD34 APC	CD34 APC	CD34 APC
CD71 APC ALEXA700	CD16 APC ALEXA700	CD4 APC-ALEXA700
CD38 APC ALEXA 750	CD38 APC ALEXA750	CD38 APC-ALEXA750
HLA-DR PAC BLUE	HLA-DR PAC BLUE	CD2 BV421
CD45 V500C	CD45 V500C	CD45 V500c
CD19 BV605	CD11b BV605	CD25 BV605

Table S3. Antibody List Used for Hematopoietic Stem, Progenitor, and Mature Cell Population Immunophenotyping

Antigen	Clone	Fluorochrome	Source
CD10	eBIOCB-CALLA	None	Ebioscience, UK
CD117	104D2	PE	Biolegend, UK
CD11b	ICRF44	APC	Ebioscience, UK
CD19	HIB19	none	Ebioscience, UK
CD2	RPA-2.10	none	Ebioscience, UK
CD20	2H7	none	Ebioscience, UK
CD235a	HIR2	none	Ebioscience, UK
CD3	HIT3a	none	Ebioscience, UK
CD34	561	BV421	Biolegend, UK
CD4	RPA-T4	none	Ebioscience, UK
CD8a	RPA-T8	none	Ebioscience, UK
goat F(ab')2 anti-mouse secondary		QDOT605	Invitrogen
Streptavidin		APC EFluor780	Ebioscience, UK

Table S4. **Detection of *IDH2* Mutation in Peripheral Blood Neutrophils and Monocytes in Four Patients Treated with Enasidenib**

Patient	**Time point**	**Response**	**Mature cells analysed**	**m*IDH2* detected**
201-001	C1D8	NA	Neutrophil-enriched	yes
201-001	C2D1	MLFS	Neutrophil-enriched	yes
201-001	C3D1	CRi	Neutrophil-enriched	yes
201-001	C4D1	CRi	Neutrophil-enriched	yes
201-001	C4D8	NA	Neutrophil-enriched	yes
-	-	-	-	-
201-002	pre-treatment	NA	Neutrophil-enriched	yes
201-002	C1D8	NA	Neutrophil-enriched	yes
201-002	C1D15	CR	Neutrophil-enriched	yes
201-002	C1D18	CR	Neutrophil-enriched	yes
201-002	C1D25	CR	Neutrophil-enriched	yes
201-002	C2D1	CR	Neutrophil-enriched	yes
201-002	C2D15	CR	Neutrophil-enriched	yes
201-002	C2D22	CR	Neutrophil-enriched	yes
201-002	C3D1	CR	Neutrophil-enriched	yes
201-002	C3D4	CR	Neutrophil-enriched	yes
201-002	C3D8	CR	Neutrophil-enriched	yes

201-002	C3D22	CR	Neutrophil-enriched	yes
201-002	C4D1	CR	Neutrophil-enriched	yes
-	-	-	-	-
201-006	pre-treatment	NA	Neutrophil-enriched	yes
201-006	C1D15	CR	Neutrophil-enriched	yes
-	-	-	-	-
201-023	C1D8	NA	CD14+ monocytes	yes
201-023	C1D8	NA	CD16+ neutrophils	yes
201-023	C6D1	NA	CD14+ monocytes	yes
201-023	C6D1	NA	CD16+ neutrophils	yes
201-023	C8D15	CR	CD14+ monocytes	Yes

ANC = absolute neutrophil count; CR = complete remission; m*IDH2* = mutant *isocitrate dehydrogenase 2*

Mature neutrophils and monocytes isolated from longitudinal peripheral blood samples of enasidenib-treated patients were analyzed for the presence of *IDH2* mutation (R140Q or in sample 201-013: R172K) by TaqMan SNP genotyping. Samples were CD14+ monocytes and CD16+ neutrophils. C*n*D*n* denotes the cycle number and the day of the cycle the sample was obtained. Response assessments were made on D1 of each cycle, except for an additional early assessment at C1D15. Blood samples taken between response assessments are assigned the response at the previous cycle. NA denotes time points when response assessment was not available.

Table S5: Association of Known Somatic Mutations Identified by FoundationOne® Heme Panel with Response to Enasidenib.

Gene	R140 (n)	R172 (n)	ORR	ORR, OR	ORR, p-value	CR	CR, OR	CR, p-value
NRAS	12	5	0.188	0.275	0.0604	0.063	0.102	0.0114
PTPN11	5	1	0.000	0.114	0.0708	0.000	0.151	0.1598
RUNX1	21	3	0.261	0.418	0.1110	0.217	0.439	0.1609
JAK2	3	1	0.000	0.141	0.1351	0.000	0.186	0.2976
FLT3	11	1	0.200	0.312	0.1901	0.200	0.418	0.3300
SRSF2	45	0	0.341	0.610	0.2067	0.293	0.655	0.3498
ASXL1	21	6	0.385	0.799	0.6703	0.269	0.598	0.3750
CEBPA	6	0	0.167	0.254	0.2378	0.167	0.339	0.4181
STAG2	10	2	0.455	1.111	0.9999	0.455	1.500	0.5307
GATA2	2	0	0.000	0.507	0.2587	0.000	0.342	0.5344
BCORL1	2	0	0.000	0.574	0.5067	0.000	0.342	0.5344
TP53	3	0	0.000	0.259	0.5067	0.000	0.342	0.5344
ETV6	1	1	0.500	1.329	0.9999	0.000	0.342	0.5344
NPM1	15	0	0.417	0.942	0.9999	0.250	0.561	0.5388
SETBP1	3	0	0.000	0.183	0.2601	0.000	0.242	0.5544
SF3B1	2	1	0.000	0.183	0.2601	0.000	0.242	0.5544
EZH2	5	0	0.200	0.321	0.3920	0.200	0.542	0.6539
KRAS	3	2	0.400	0.879	0.9999	0.200	0.428	0.6539
KMT2A	12	2	0.357	0.716	0.7789	0.286	0.679	0.7724

U2AF1	1	1	1.000	6.799	0.1837	0.500	1.763	0.9999
DNMT3A	28	14	0.513	1.548	0.2689	0.359	0.974	0.9999
ATM	3	0	0.500	1.164	0.9999	0.500	1.763	0.9999
BCOR	10	5	0.429	0.996	0.9999	0.357	0.970	0.9999
CBL	3	0	0.333	0.657	0.9999	0.333	0.873	0.9999
CUX1	2	0	0.500	1.329	0.9999	0.500	1.763	0.9999
IDH1	4	0	0.500	1.333	0.9999	0.250	0.576	0.9999
PHF6	6	0	0.500	1.338	0.9999	0.333	0.871	0.9999
TET2	5	0	0.333	0.657	0.9999	0.333	0.873	0.9999
WT1	2	1	0.333	0.657	0.9999	0.333	0.873	0.9999
ZRSR2	4	1	0.400	0.879	0.9999	0.400	1.172	0.9999
CR = complete remission; ORR = overall response rate; OR = odds ratio								

Genes with known and likely somatic mutations identified by FoundationOne® Heme panel found in 2 or more patient samples sorted by p-value (Fisher's Exact test on contingency table). Overall response rates include CR, CRi, CRp, MLFS, or PR. CR rates include CR, CRi, CRp and MLFS.