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## **Enasidenib Induces Acute Myeloid Leukemia Cell Differentiation to Promote Clinical Response**

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**Key Points:**

- Enasidenib inhibits mIDH2 leading to leukemic cell differentiation with emergence of functional mIDH2 neutrophils in rrAML patients.
- RAS pathway mutations, and increased mutational burden overall, are associated with a decreased response rate to mIDH2 inhibition.

**Abstract:** Recurrent mutations at R140 and R172 in *isocitrate dehydrogenase 2 (IDH2)* occur in many cancers, including ~12% of acute myeloid leukemia (AML). In preclinical models these mutations cause accumulation of the oncogenic metabolite R-2-hydroxyglutarate (2-HG) and induce hematopoietic differentiation block. Single-agent enasidenib (AG-221/CC-90007), a selective mutant IDH2 (mIDH2) inhibitor, produced an overall response rate of 40.3% in relapsed/refractory AML patients with *mIDH2* in a phase 1 trial. However, its mechanism of action and biomarkers associated with response remain unclear. Here, we measured 2-HG, *mIDH2* allele burden, and co-occurring somatic mutations in sequential patient samples from the clinical trial and correlated these with clinical response. Furthermore, we used flow cytometry to assess inhibition of mIDH2 on hematopoietic differentiation. We observed potent 2-HG suppression in both R140 and R172 *mIDH2* AML subtypes, with different kinetics, which preceded clinical response. Suppression of 2-HG alone did not predict response, as most non-responding patients also exhibited 2-HG suppression. Complete remission (CR) with persistence of *mIDH2* and normalization of hematopoietic stem and progenitor compartments with emergence of functional *mIDH2* neutrophils was observed. In a subset of CR patients, *mIDH2* allele burden was reduced and remained undetectable with response. Co-occurring mutations in *NRAS* and other MAPK pathway effectors were enriched in non-responding patients, consistent with RAS signaling contributing to primary therapeutic resistance. Together, these data support differentiation as the main mechanism of enasidenib efficacy in relapsed/refractory AML patients and provide insights into resistance mechanisms to inform future mechanism-based combination treatment studies.

## Introduction

Somatic mutations in the *isocitrate dehydrogenase 2 (IDH2)* gene occur at conserved arginine residues (R140 and R172). These mutant proteins possess neomorphic enzymatic activity resulting in R-2-hydroxyglutarate accumulation.<sup>1-4</sup> R-2-HG competitively inhibits a set of alpha-ketoglutarate-dependent enzymes including the TET family of 5-methylcytosine (5mC) hydroxylases and the Jumonji-C domain histone demethylases.<sup>5,6</sup> This inhibition leads to DNA hypermethylation,<sup>7</sup> increased repressive histone methylation,<sup>6</sup> and impaired hematopoietic differentiation. Accordingly, inhibition of mutant IDH2 (mIDH2) reduces 2-HG levels and restores hematopoietic differentiation *in vitro*.<sup>6,8-10</sup>

Although both mutations are characterized by neomorphic enzymatic activity, myeloid malignancies with R140 and R172 IDH2 mutations are distinct with respect to clinical outcome, co-mutational profile, and molecular classification.<sup>11-13</sup> In preclinical studies, enasidenib (AG-221/CC-90007), a small-molecule inhibitor of mIDH2, reduced serum 2-HG, DNA hypermethylation, and repressive histone marks, and promoted hematopoietic differentiation in R140 and R172 mIDH2 models.<sup>14-17</sup> In a phase 1/2 clinical trial, enasidenib demonstrated clinical activity in patients with both R140 and R172 mIDH2 relapsed/refractory AML (rrAML) with an overall response rate (ORR) of 40.3%.<sup>18</sup> Here, we analyzed patient samples from this study to elucidate the mechanisms of action of enasidenib in R140 and R172 mIDH2 rrAML and to identify response biomarkers to targeted mIDH2 therapy.

## Materials and Methods

### *Study Participants and Treatment*

Analyses were performed on rrAML patient samples collected from the AG-221-C-001 study with informed consent. Patients were included in the AG-221-C-001 trial and in the translational studies here based on *IDH2* mutations detected by local testing. A retrospective central in vitro diagnostic test confirmed mutation status with a concordance rate above 95%. Enasidenib was administered to patients as described in the approved study protocol. Patient sample disposition indicating samples analyzed in each assay is supplied in **Figure S1**. Patient baseline characteristics and clinical responses of subpopulations analyzed vs the rrAML cohort as a whole are supplied in **Table S1**. Outcome data reflect an April 15<sup>th</sup> 2016 study cut-off date.

### *Measurement and Analysis of 2-hydroxyglutarate (2-HG)*

Serum samples were collected from patients at screening within 28 days before the first dose of enasidenib and/or pre-dose on day 1 of each treatment cycle. 2-HG concentration was determined using liquid chromatography tandem mass spectrometry by Covance, Inc (formerly Tandem Labs) according to an analytically validated method. Baseline total 2-HG was determined to be either the average of the screening sample and the pre-dose cycle 1 sample, or either sample if both were not available. Maximum suppression of 2-HG levels was determined by comparing the lowest level of 2-HG observed on-treatment to baseline 2-HG level. Time to maximum suppression was the first time point where 2-HG level was within 5% of maximum suppression for that patient.

### *Sysmex OncoBeam<sup>TM</sup> Digital Polymerase Chain Reaction*

Bone marrow and/or peripheral blood samples were collected at screening 28 days before the first dose of enasidenib and during treatment and processed to peripheral blood mononuclear cells (PBMC) and bone marrow mononuclear cells (BMMCs). Measurement of mutant and wild

type *IDH2* was determined using Sysmex BEAMing technology. Briefly, DNA was extracted from samples, pre-amplified in a multiplex PCR reaction and amplified with nested primers in an emulsion PCR reaction on the surface of magnetic beads in water–in-oil emulsions. Fluorescently labeled probes specific to the *IDH2* mutation and to the wild-type sequence were hybridized to the uncovered DNA fragments on the bead surface. Fluorescently labeled beads were quantified using flow cytometry.

#### *Blast Percentage Determination by Flow and mIDH2 Assessment*

Multi-parameter flow cytometry was performed on bone marrow aspirates at diagnosis and at day 1 of each treatment cycle. Abnormal populations were identified by antigen expression as described previously.<sup>19,20</sup> Briefly, up to 1.5 million cells from freshly drawn bone marrow aspirate were stained with three ten-“color” panels (**Table S2**), washed and acquired on a FACS-Canto-10 cytometer (BD Biosciences, San Jose CA). The results were analyzed using custom Woodlist software (generous gift of Wood BL, University of Washington). Following flow assessment, samples were lysed and assessed for *mIDH2* as part a 28-gene amplicon capture-based next generation sequencing (NGS) assay at Memorial Sloan Kettering Cancer Center.

#### *Hematopoietic Immunophenotyping and mIDH2 Assessment in Flow-Sorted Cells*

Viably-frozen BMMCs from normal donors (N=12) or refractory/relapsed rAML patients (N=9) were thawed, stained with antibodies listed in **Table S3** and sorted on either a BD LSR Fortessa or BD FACSAria Fusion (Becton Dickinson, Oxford UK). Sorted fractions (> 95% purity) of lin-ve CD34-CD117- cells, which represent mature myelomonocytic cells, and unsorted mononuclear cells were processed for genomic DNA (gDNA). Whole genome amplification (WGA, RepliG, Qiagen UK) was carried out in samples with less than 10<sup>4</sup> cells or where extracted gDNA was inadequate. Analyses of *mIDH2* at R140 and R172 codons were completed by PCR of exon 4 of the *IDH2* gene followed by NGS using a MiSeq (Illumina, UK).

### *Phagocytosis Assay*

Neutrophils were collected from fresh citrated blood by centrifugation, red blood cell sedimentation, and Percoll gradient cell separation. Purified neutrophils ( $1 \times 10^6$ ) were incubated with 3  $\mu$ L fluorescent green latex beads (Sigma, France) for 15 minutes at 37°C in 1 mL RPMI medium supplemented with 10% heat-inactivated fetal bovine serum (Sigma). Cells were washed, fixed in 3.3% paraformaldehyde, stained with DAPI and imaged with a laser-scanning TCS SP5 confocal microscope (Leica, France). The percentage of neutrophils containing latex beads was calculated by scoring 5 different fields of view for each sample. *IDH2* mutations were confirmed in neutrophils with a TaqMan® SNP Genotyping Assay (Life Technologies, Carlsbad CA).

### *FoundationOne Heme Panel*

FoundationOne® Heme analysis was conducted in a clinical laboratory improvement amendments (CLIA)-certified lab by Foundation Medicine, Inc. Briefly, fresh bone marrow and/or peripheral blood samples were collected from patients and DNA and RNA were extracted. Nucleic acid libraries were prepared, captured using custom bait-sets and sequenced to high depth using Illumina HiSeq for 405 cancer-related genes by DNA-sequencing (DNA-seq) and 265 frequently rearranged genes by RNA-sequencing (RNA-seq). Only known or likely gene mutations that are the targets of therapies, either approved or in clinical trials, or are otherwise known drivers of oncogenesis published in the literature were included in this analysis.<sup>21</sup> Co-mutational burden was calculated as the total sum of all unique known and likely somatic mutations, other than *IDH2*, identified in each patient.

### *Calculation of Variant Allele Frequency (VAF)*

In each assay VAF was calculated as measurement of mutated allele (i.e., *mIDH2*) over measurement of mutated allele + wild type allele (i.e., *mIDH2* + wild type *IDH2*) in each sample.



### *Statistical Analysis*

Statistical analyses were performed with GraphPad Prism software using methods noted in figure and table legends. Mutational associations with either overall response rate (ORR) or complete remission (CR) rate were assessed through a two-tailed Fisher's Exact test on a 2 x 2 contingency table analyzing the sum of patients achieving a response or not (ORR  $\geq$  partial remission [PR]; and CR  $\geq$  morphologic leukemia-free state [MLFS]) vs presence or absence of gene mutation identified by FoundationOne® Heme. Associations between prognostic risk groups and response were assessed using a two-tailed Chi-square test for trend on a 3 x 2 contingency table analyzing the sum of patients achieving a response or not (ORR  $\geq$  PR; CR = CR) vs risk classification as favorable, intermediate or adverse as stated in figure legends.

## Results

### *mIDH2 Inhibition Is Associated with Potent Reduction of 2-HG in mIDH2 AML*

Total 2-HG measurements in blood correlate with R-2-HG levels, tumor mass, clinical response to cytotoxic therapy and are a proposed biomarker of IDH2 mutations.<sup>4</sup> Therefore, we assessed total 2-HG levels in 125 rrAML patients with available samples prior to enasidenib treatment and every 28 days during therapy. Median 2-HG suppression (defined as the maximum extent of suppression compared to pre-therapy) was 90.6%, consistent with potent target inhibition. 2-HG suppression in patients with R172 mIDH2 was less than in patients with R140 mIDH2 rrAML (median suppression of 70.9% and 94.9%, respectively [ $p < 0.001$ ]); consistent with preclinical data and an interim analysis of samples from study AG-221-C-001 (**Figure 1A**).<sup>14-16,22-24</sup> Of note, 5 patients during treatment had an increase in 2-HG. Two of these patients achieved a best response of PR despite never having 2-HG levels below baseline in multiple samples analyzed. None of these patients were observed to have co-occurring mutations in *IDH1*.

We next assessed 2-HG suppression in patients dosed with <100mg, 100mg, or >100mg enasidenib daily. Although we observed a trend towards greater 2-HG suppression at higher doses in R140 mIDH2 patients, there were no statistical differences in maximal 2-HG suppression among the three dosing groups ( $p = 0.054$  for <100mg vs 100mg and  $p = 0.094$  for 100mg vs >100mg) (**Figure 1B**). In R172 mIDH2 patients, 2-HG suppression was more variable across dosing groups (95% CI: 43.9%-102.9%, 10.7%-61.0% and 62.5%- 82.7% in the <100mg, 100mg, and >100mg dosing groups, respectively). A statistical difference in 2-HG suppression was observed between 100mg and >100mg dosing cohorts. However, the 100mg group was confounded by 4 patients whose 2-HG levels increased and no statistical difference was found in 2-HG suppression between <100mg vs >100mg dosing groups ( $p = 0.152$  for <100mg vs 100mg,  $p = 0.022$  for 100mg vs >100mg and  $p = 0.955$  for <100mg vs >100mg). Furthermore, response rates for R172 mIDH2 patients were not statistically different between the 100mg and >100mg

dose groups analyzed (ORR 44.0% and 58.8%, respectively,  $p = 0.530$ , Fisher's exact test), suggesting no additional clinical benefit for R172 mIDH2 patients in dosing above 100mg. Importantly, no difference in time to maximum 2-HG suppression between patients dosed with <100mg, 100mg, or >100mg enasidenib daily was observed either (**Figure S2A**). Together, 2-HG suppression and efficacy data indicate that the 100mg enasidenib dose is biologically and therapeutically active in rrAML patients with mIDH2, regardless of the specific mutation.

We next assessed the relationship between pre-enasidenib therapy 2-HG levels and clinical response. We observed no significant difference in baseline 2-HG levels between patients achieving a CR, patients obtaining any response (R = CR with incomplete hematologic recovery [CRi], CR with incomplete platelet count recovery [CRp], MLFS, or PR) and patients who did not respond (NR = stable disease or progressive disease) (**Figure 1C**). We assessed whether timing of pharmacodynamic response, defined as maximal 2-HG suppression, correlated to best clinical response. Although the mean cycle number to maximal 2-HG suppression, best response (BR), and CR was ~1 treatment cycle later in R172 vs R140 patients (3 vs 2 cycles for maximal 2-HG suppression and BR, and 6 vs 5 cycles for CR) (**Figure 1D**), the ORR and CR rates for patients with R172 and R140 mutations were not statistically different.<sup>18</sup> These data demonstrate the kinetics of target 2-HG inhibition parallel the kinetics of clinical response without effect on response attainment.

#### *Clinical Responses to mIDH2 Inhibition Do Not Correlate with mIDH2 Allele Burden*

We evaluated whether mIDH2 allele burden at baseline or changes on-therapy correlated with response to enasidenib by quantification of mIDH2 VAF on unsorted samples using digital PCR and NGS. We observed a significant correlation between digital PCR and NGS for 45 matched patient samples run on both assays ( $R^2 = 0.59$ ,  $p < 0.0001$ ) (**Figure S2B**). A positive correlation trend was also observed between mIDH2 VAF and 2-HG levels at screening in 17 patients with available samples, however, it was not statistically significant ( $R^2 = 0.21$ ,  $p = 0.0636$ ) (Figure S2C).

Notably, *mIDH2* allele burden was highly heterogeneous at screening among patients, ranging from low-level mutant positivity to fully clonal (~50%) (**Figure 2A and Figure S3A**). No association between *mIDH2* VAF at screening and clinical response was observed, and patients achieving CR had both low and high *mIDH2* burden. To assess the possibility that ancestral (clonal) and non-ancestral (subclonal) *mIDH2* disease exhibit different responses, we analyzed the VAF of co-occurring mutations in the 30 patients with low *mIDH2* VAF (< 0.2). Eight of these patients, 5 responders and 3 non-responders, had co-occurring mutations with VAF > 0.4, consistent with *mIDH2* occurring as a non-ancestral event (**Figure S4**). Taken together with data showing responses in both clonal and subclonal *mIDH2*, these data suggest that patients with ancestral or non-ancestral *mIDH2* clones can respond to enasidenib.

Next, we analyzed changes in absolute *mIDH2* VAF from screening to best response. A decrease in *mIDH2* VAF was more commonly observed in responding patients than an increase, however, only one-half of the patients showed a VAF change of more than 5% points (**Figure 2B and Figure S3B-C**). Similar to recent results reported for a subset of *mIDH1* AML patients treated with an *mIDH1* inhibitor, longitudinal analysis by digital PCR in our cohort identified 9/29 CR patients for whom *mIDH2* became undetectable with enasidenib treatment.<sup>25</sup> Interestingly, all 9 patients had R140 *mIDH2* rrAML (50% of the 18 R140 CR patients) and loss of both a minor *mIDH2* subclone and a more substantive *mIDH2* clone (~40%) were observed. In 8 of these patients, *mIDH2* remained undetectable with continued treatment, consistent with persistent molecular remission (**Figure 2C**). However, no significant difference was observed in an initial analysis of event-free survival between patients achieving molecular remission vs patients achieving CR without molecular remission (295.9 vs 259.9 days, respectively,  $p = 0.784$ ; event-free survival was defined according to protocol as the interval from date of first dose to date of documented relapse, progression, or death due to any cause, whichever occurred first). Finally, we assessed the relationship between two measures of leukemic burden: bone marrow blast count (flow

cytometry) and *mIDH2* VAF (genetic) in 9 additional patients who responded to enasidenib (**Figure 2D**). In 6 of these patients, we observed marked blast count decreases to near 0% in aspirates with concomitant *mIDH2* VAF above 10%. This data demonstrates that *mIDH2* cells persist in most patients achieving CR and a reduction in *mIDH2* allele burden during treatment is neither necessary nor sufficient for clinical response to enasidenib.

#### *Clinical Response to mIDH2 Inhibition Is Associated with Induction of Myeloid Differentiation*

Given that a reduction in *mIDH2* VAF was not required for CR, we hypothesized that enasidenib might induce clinical response by promoting leukemic cell differentiation. We measured the magnitude of different immunophenotypic compartments in the hematopoietic hierarchy, before and during treatment, in 5 *mIDH2* rrAML patients who achieved CR or PR (**Figure 3A-B**). Prior to treatment, all 5 patients had expanded myeloid leukemic progenitor or precursor populations. Enasidenib treatment resulted in near normalization of the immature-to-mature cell population ratio at CR and PR in patients with both R140 (201-023 and 203-002) and R172 (104-036, 201-010 and 201-011) *mIDH2*. In contrast, no improvement of immature-to-mature ratio was observed in 5 non-responding patients (**Figure S5**). In the responding patients, we assayed *mIDH2* VAF by NGS in bulk BMMC and flow-sorted mature myeloid cells (**Figure 3B**). In 4 of 5 patients achieving CR or PR, *mIDH2* VAF remained stable (201-010, 201-011, 104-036) or increased (201-023) in both cell populations.

We extended these findings by measuring *mIDH2* VAF in peripheral blood neutrophils prior to enasidenib treatment and at the time of CR in 7 additional patients who achieved CR (**Figure 3C**, top panel). In 6 of 7 cases, *mIDH2* VAF remained constant between pre-therapy leukemic cells and neutrophils at CR, consistent with differentiation of *mIDH2* leukemia cells into mature neutrophils. Furthermore, in patient 104-018, the VAF of additional co-associated AML mutations remained unchanged in neutrophils at CR, consistent with enasidenib-mediated differentiation of a transformed leukemic clone (**Figure 3C**, middle panel). In contrast, in the 1 patient whose

mIDH2 VAF dropped to 0% in mature neutrophils at CR (104-010), the VAF of other AML-associated mutations showed heterogeneous changes consistent with clonal selection rather than loss of all clonally derived leukemic cells (**Figure 3C**, top and bottom panels). Next, we investigated the functional status of differentiated leukemic neutrophils with mIDH2 in 3 patients who achieved CR (**Table S4**). In each case, mutant neutrophils demonstrated intact phagocytic activity consistent with restoration of normal granulocyte function (**Figure 3D**).

#### *Genomic Predictors of Response to mIDH2 Inhibition*

We assessed whether additional somatic mutations were associated with differential response by performing capture-based NGS with FoundationOne® Heme mutational panel in 100 patients at screening. No overt biases in response, age, sex, prior treatments, bone marrow blast percentage, absolute neutrophil counts, or prior myelodysplastic syndrome diagnosis were observed between these patients and the 176 rrAML patients from the phase 1 portion of study AG-221-C-001 (**Table S1**). Ninety-eight percent of samples contained mutations other than mIDH2 and 17 co-occurring mutated genes were identified in  $\geq 5\%$  of patients (**Figure 4A-B**). The most frequent co-occurring mutations were in *SRSF2* (45%), *DNMT3A* (42%), *ASXL1* (27%), *RUNX1* (24%), *NRAS* (17%), and *BCOR* (15%). Notably, the prevalence of mutations in this cohort differed from an analysis of 1376 de novo AML samples from Papaemmanuil, et al (**Figure S6A-B**).<sup>13</sup> Our cohort included a significant enrichment of adverse risk mutations (*DNMT3A*, *ASXL1*, *RUNX1*). Statistical significance of *ASXL1* and *RUNX1* enrichment was also upheld when restricted to the 130 mIDH2 positive patients in the Papaemmanuil dataset.<sup>13</sup> A significantly lower level of favorable prognosis mutations (*NPM1*), as defined by *Grimwade, et al*, were also observed.<sup>26</sup> Differences in co-occurring mutations in patients with R140 mIDH2 AML, including increased co-mutational heterogeneity (60 different mutated genes vs 24 in R172 mIDH2 patients) and number of co-occurring mutations per patient (3.6 vs 2.6 mutations per patient in R172 mIDH2,  $p = 0.020$ ) were observed (**Figure 4A and Figure S6C-E**). Additionally, some mutated

genes were either exclusively observed (*SRSF2*, n = 45) or more prevalent in R140 *mIDH2* rrAML (*RUNX1*, 27.3% vs 14.3% in R172 *mIDH2* rrAML) or more prevalent in R172 *mIDH2* rrAML (*DNMT3A*, 66.7% vs 36.4% in R140 *mIDH2*). This data extends work on *de novo* AML by showing that R140 and R172 *mIDH2* rrAML are genetically distinct leukemia subtypes.<sup>13</sup>

Additionally, cytogenetics were overlaid with mutational data to characterize the cohort by four different risk classifications of *de novo* AML.<sup>13,26-28</sup> Using the European LeukemiaNet classification from 2010, 14% of patients were classified as favorable risk and 59% were classified as intermediate risk (**Figure 4C**).<sup>27,29</sup> Using the recently revised ELN classification from 2017, which includes *ASXL1* and *RUNX1* mutations in the adverse risk group, a disproportionately large group of patients (56%) were classified as adverse risk.<sup>28</sup> Furthermore a classification scheme by Grimwade et al, which includes *DNMT3A* mutations in the adverse risk group, showed an even greater enrichment of adverse risk patients (73%).<sup>26</sup> Finally, a classifier developed on the *de novo* AML cohort<sup>13</sup> revealed an enrichment of patients with mutations in splicing/chromatin associated genes (43%), and with *TP53* mutations or aneuploidy (29%). Only 14% of patients were categorized as *NPM1* mutation-associated AML, which has been reported as the largest AML subset (**Figure 4D**). Importantly, clinical responses to enasidenib were observed across the risk spectrum in the 72 efficacy-evaluable patients with full genomic/cytogenetic data (**Table 1**).

Finally, we investigated whether the number of co-occurring mutations or specific mutant alleles correlated with enasidenib response. Patients who achieved a response ( $\geq$ PR or CR) had significantly fewer co-occurring mutations than non-responders ( $p < 0.001$ , **Figure 5A**). Segregating patients in three tertiles ( $\leq 3$ , 3 to 6, and  $\geq 6$  co-mutations) revealed a significant difference in ORR for patients with the most vs least co-occurring mutations (ORR 21.9% vs 70.4%, respectively,  $p < 0.001$ ) (**Figure 5B**). Analysis of co-mutated genes with response indicated a lower, but statistically non-significant, ORR with co-occurring *SRSF2* (34%), *ASXL1* (39%), *RUNX1* (26%), and *NRAS* (19%) mutations (**Table S5**). However, significantly fewer

patients with co-occurring *NRAS* mutations achieved CR ( $p = 0.0114$ ; **Table S5**). When the most common mutations known to activate *NRAS* signaling (G12, G13, or Q61) were analyzed vs ORR, the observed decrease in response rate was statistically significant ( $p = 0.002$ , **Figure 5C**). Notably, overall mutational burden was significantly higher ( $p < 0.001$ ) in patients with m*NRAS* (G12, G13, or Q61) and mutations in *NRAS* were frequently subclonal (**Figure 5D-E**). Analysis of other gene mutations involved in activating MAPK signaling revealed that no patients with m*PTPN11* responded and m*KRAS* was not associated with response (**Table S5**). Together these data suggest that some RAS pathway mutations, either in the dominant or minor subclone, may directly or indirectly attenuate responses to mIDH2 inhibition.



## Discussion

Our study of *mIDH2* rrAML patient samples from the phase 1 trial of enasidenib indicates that this cohort is enriched for mutations more commonly seen in adverse-risk or secondary AML (e.g., *SRSF2*, *RUNX1*, and *ASXL1*). Additionally, these studies extend previous observations in *de novo* R140 and R172 *mIDH2* AML, confirming that the two *mIDH2* subtypes are genetically distinct. Despite these observations, enasidenib exhibited potent target inhibition in both subtypes and an ORR of ~40% with no statistical difference between R140 and R172 *mIDH2* rrAML.<sup>18</sup> Additionally, our data confirms the preclinical mechanism of action of *mIDH2* inhibition by enasidenib and provides the first insight into the genetic basis of primary resistance.<sup>10,14-16</sup>

Evidence suggests that human AML is composed of a hierarchy of both tumor-propagating leukemic stem cells (LSCs) arrested at a progenitor or precursor stage of hemopoiesis and more mature non-tumor propagating leukemic cells.<sup>30,31</sup> Our observations demonstrate enasidenib promotes terminal differentiation of *mIDH2* leukemic cells of granulocytic lineage in patients who achieve CR or PR. Furthermore, we observed *ex-vivo* phagocytic function in differentiated *mIDH2*-containing neutrophils. These observations are clinically important and may explain the lower frequency of infections in patients achieving CR with enasidenib treatment.<sup>32</sup> Our studies also demonstrate decreases in *mIDH2* below a detectable limit in a subgroup of patients. Both observations support differentiation as the mechanism of action of enasidenib monotherapy. Where *mIDH2* cells persist, it's most plausible that *mIDH2* LSCs are not eradicated but differentiate to give rise to *mIDH2*-containing functional neutrophils. When molecular CR is achieved, *mIDH2* inhibition may result in terminal or near terminal exhaustion through differentiation of the *mIDH2* clone. The differential effects may be due to the specific cellular and genetic contexts of the *IDH2* mutation and additional work will be required to dissect the mechanisms accounting for these observations. It is also intriguing and unclear how CR is

achieved in the context of subclonal *mIDH2*; this requires further mechanistic studies of cell autonomous and cell non-autonomous effects of *IDH2* mutations in AML.

In this study, we measured 2-HG, which includes both L-2-HG and R-2-HG, whereas only R-2-HG is produced by neomorphic IDH mutations. It has been shown that total 2-HG levels correlate with R-2-HG, *mIDH2* allelic burden, tumor mass and clinical status (i.e., CR vs absence of CR), consistent with the majority of 2-HG being derived from *mIDH2* production of R-2-HG.<sup>4</sup> When 2-HG levels are low, measurement of R-2-HG, rather than total 2-HG, may improve sensitivity, however, this would not have changed our observations relating to the consistent, dose-dependent suppression of 2-HG seen in nearly all patients. Previously, serum 2-HG levels have been suggested as a biomarker for chemotherapy response in *mIDH2* positive patients.<sup>1-4</sup> Our data demonstrates this is not the case in targeted therapy because enasidenib is able to inhibit the *mIDH2* enzyme and suppress 2-HG regardless of clinical response. Consistent with this hypothesis the level of suppression of 2-HG is not prognostic of response. We also observed that in R172 *mIDH2* patients, though the extent of 2-HG suppression is more variable and maximal suppression takes longer to achieve, clinical responses were equivalent to the R140 *mIDH2* subtype. Paradoxically, in 4 R172 patients, 2-HG levels rose on enasidenib therapy. This anomaly could arise from differential production of 2-HG by different cell populations during enasidenib-induced differentiation, specific co-mutational patterns, or alterations in AML cell metabolism in these patients not seen in the larger cohort. Taken together, these data demonstrate enasidenib potently suppresses 2-HG, the extent of 2-HG suppression does not predict clinical response, and primary resistance to enasidenib is not due to an inability to suppress *mIDH2* enzyme activity. These data also suggest other factors determine clinical response to enasidenib. Patients with a higher mutational burden and/or co-occurring mutations in the RAS pathway were observed to be less likely to respond to *mIDH2* inhibition. The observation that increasing number of driver mutations is associated with poorer outcome with *mIDH2* inhibitor therapy mirrors similar

observations in newly diagnosed AML patients treated with chemotherapy.<sup>13</sup> From this data, it is unclear if constitutive activation of the RAS pathway imposes a 2-HG-independent differentiation block or whether mutations in *RAS* and other signaling pathways are a marker of overall higher mutational burden and other mechanisms of 2-HG-independent differentiation arrest. Notably, *NRAS* mutations were frequently present in a minor subclone and this intriguing observation requires further investigation.

Although enasidenib responses are clinically durable, the genetic heterogeneity observed in our patients suggests combination with other therapies may be required to achieve long-term disease remission in more patients. This is reminiscent of the impressive, but not durable, activity of ATRA monotherapy in acute promyelocytic leukemia.<sup>33</sup> Our data now suggests that targeted therapies may optimally be delivered in combination with other therapies. Current clinical studies combining enasidenib with combination chemotherapy or azacitidine, (NCT02677922 and NCT02632708) and future orthogonal targeted therapies will address this question. While this is only a subgroup analysis of a large single-arm experience, taken together, the clinical response and translational data demonstrate that single-agent mIDH2 inhibition by enasidenib in rrAML represents a critical and novel differentiation therapy. It also provides the platform for future combination therapy regimens to optimize clinical response and further improve outcomes in mIDH2 AML.

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## Tables

**Table 1:** Analysis of Response in Patients with Favorable, Intermediate, and Adverse-Risk rrAML

| <b>Risk Assessment</b>          | <b>ORR / CR, Favorable Risk Profile<br/>%, (n)</b> | <b>ORR / CR, Intermediate Risk (I+II) Profile<br/>%, (n)</b> | <b>ORR / CR, Adverse Risk Profile<br/>%, (n)</b> | <b>P-value (ORR / CR)</b> |
|---------------------------------|--|--|--|---------------------------|
| <b>Risk Classification</b>      |  |  |  |                           |
| ELN 2010                        | 22.2% / 0%, (9)                                    | 55.8% / 32.6%, (43)  | 30.0% / 10.0%, (20)                              | 0.7322 / 0.8493           |
| ELN 2017                        | 37.5% / 0%, (8)                                    | 65.2% / 43.5%, (23)  | 34.1% / 14.6%, (41)                              | 0.2049 / 0.5816           |
| Grimwade                        | 33.3% / 0%, (6)                                    | 66.7% / 58.3%, (12)  | 40.7% / 16.7%, (54)                              | 0.6121 / 0.4487           |
| <b>Molecular Classification</b> |  |  |  |                           |
| Papaemmanuil*                   | 80.0% / 60.0%, (5)                                 | 36.4% / 9.0%, (11)   | 42.8% / 21.4%, (56)                              | 0.4631 / 0.2605           |

ORR (CR, CRi, CRp, MLFS, and PR) by risk assessment based on cytogenetic testing and mutations identified by FoundationOne® Heme panel according to ELN 2010<sup>29</sup>, ELN 2017<sup>28</sup>, Grimwade et al.<sup>26</sup>, and Papaemmanuil et al.<sup>13</sup> \*Papaemmanuil risk was inferred from survival analysis of molecular classifications where mCEBPA and mIDH2-R172 groups were considered favorable, mNPM1 and others were considered intermediate, and chromosome 3 inversion, Chromatin-Spliceosome, MLL fusions and mTP53-aneuploidy were considered adverse. P-value for contingency test for logical trend from favorable to adverse risk.

## Figure Legends

### Figure 1. mIDH2 Inhibition Is Associated with Potent Reduction of 2-HG in mIDH2 AML.

- (A) Dot plot with median and interquartile range showing maximum 2-HG suppression (% change from baseline) in blood observed in patients segregated by R140 and R172 mIDH2. Numbers indicate number of patients from each genotype graphed.
- (B) Dot plot with median and interquartile range showing maximum 2-HG suppression (% change from baseline) observed in patients segregated by total daily dose received (<100 mg in green, 100mg in blue, >100 mg in purple) and stratified by R140 and R172 mIDH2.
- (C) Dot plot with median and interquartile range showing blood plasma 2-HG (ng/ml) at screening in patients segregated by best response achieved and stratified by R172 (red) and R140 (blue) mIDH2. Response (R) is defined as either complete remission (CR), CR with incomplete count recovery (CRi), CR with incomplete platelet count recovery (CRp), morphologic leukemia-free state (MLFS) or partial remission (PR). No response (NR) is defined as stable disease (SD) or progressive disease (PD).
- (D) Whisker plot indicating mean and standard deviation of cycle to CR, Best Response (BR) or maximum 2-HG suppression (Max 2-HG) stratified by R172 (red) and R140 (blue) mIDH2.

### Figure 2. Clinical Responses to mIDH2 Inhibition Do Not Correlate with mIDH2 Allele Burden

- (A) Dot plot of mIDH2 variant allele frequency (VAF) (R140 mIDH2 in blue and R172 mIDH2 in red) in patient samples measured at screening in either peripheral blood (PB) or bone marrow (BM) by FoundationOne® Heme panel. Measurements separated by the best response achieved by patients, as defined in Figure 1. Numbers indicate the number of patient samples in the graph.
- (B) Waterfall plot indicating absolute change in mIDH2 VAF from screening to achievement of best response measured by Sysmex OncoBeam digital PCR. Responders in green and non-responders in red. Patients achieving CR are outlined in black. The dotted line indicates the largest VAF decrease observed in a non-responder.
- (C) Line graph of mIDH VAF over time (days of treatment) in 9 patients achieving molecular remission (undetectable mIDH2) for at least one time point during treatment.
- (D) Scatter plot of bone marrow mIDH2 VAF vs blast percentage measured by flow cytometry in nine responsive patients in samples taken pre-treatment (pre-Rx: red) and at response (CR, CRi, CRp or MLFS; green). Blue line indicates expected ratio (2:1) between blast percent:mIDH2 VAF in clonal mIDH2 disease. Three data points (in green) are superimposed with values close to or at 0.

### Figure 3. Clinical Response to mIDH2 Inhibition Is Associated with Induction of Myeloid Differentiation



- (A) Representative immunophenotypic analyses by flow cytometry on sequential bone marrow samples. Cell-surface markers studied are shown. Left, data from a responding patient (pre-treatment [Pre] to complete remission (CR) to relapse). Right, data from a non-responding patient (pre-treatment to progressive disease [PD]) who remained in stable disease during treatment. Numbers in FACS plots refer to size of the population as a percentage of lineage-negative bone marrow mononuclear cells. For normal bone marrow (n=12) standard deviation of  $\pm 2.7\%$  for immature progenitor,  $\pm 9.6\%$  for immature precursors, and  $\pm 9.7\%$  for mature myeloid cells.
- (B) Top, Graph showing ratio of immature to mature cell populations by flow cytometry from bone marrow over time: The average ratios of myeloid progenitor or myeloid precursors to mature myeloid cells in bone marrow from normal donors (n=12) and 5 patients who either had a CR or PR with enasidenib are shown. In patient 201-010, the changing size of myeloid precursor (red) cell populations relative to mature myeloid cells shown. In the remaining 3 patients, the changing size of myeloid progenitor (blue) populations to mature cells is shown. Colored bars represent the 95% confidence interval in normal controls. Bottom, the *mIDH2* VAF in each patient at different time points in all bone marrow mononuclear cells (VAF total) and in FACS-sorted mature myeloid cells (CD34<sup>+</sup>CD117<sup>+</sup>).
- (C) Top, *mIDH2* VAF in bone marrow mononuclear cells prior to treatment (Pre, blue) and in sorted peripheral blood neutrophils at time of best response (Post, red) in seven patients achieving CR. Middle and Bottom, VAF of indicated mutation in bone marrow mononuclear cells prior to treatment and in sorted peripheral blood neutrophils at time of best response in two patients achieving CR.
- (D) Histogram of the percentages of functional neutrophils observed in *ex-vivo* enasidenib-treated patient samples (left) and representative images (right) assessed by phagocytic assay quantifying neutrophils (blue) that contained latex beads (green). The percentage of neutrophils containing beads was measured by scoring five different fields of view per sample.

**Figure 4. Association of Co-occurring Mutations with Clinical Response and Classification of Patients from Cytogenetic and Molecular Abnormalities**

- (A) Tile plot showing the number of co-occurring somatic mutations by gene identified in FoundationOne® Heme panel from efficacy-evaluable patients separated by R140 and R172 *mIDH2*. Only mutated genes occurring in two or more patients are shown. Mutations associated with higher risk are in red and mutations associated with lower risk are in green, as defined by Grimwade, et al.<sup>26</sup>
- (B) Histogram of the number of mutations identified in each gene from all 100 patient samples analyzed. Number of mutations identified in responding patients in blue, number of mutations identified in non-responding patients in red, and number of mutations in patients who were not efficacy evaluable in grey.
- (C) Pie charts of proportions of patients in various risk categories according to European LeukemiaNet (ELN) 2010 AML risk stratification,<sup>29</sup> ELN 2017 AML risk stratification,<sup>28</sup> and

Grimwade et al.,<sup>26</sup> based on cytogenetic testing completed before start of Cycle 2 and mutations identified at screening.

- (D) Pie chart of proportions of patients in various genomic classifications according to Papaemmanuil et al.,<sup>13</sup> based on cytogenetic testing completed before start of Cycle 2 and mutations identified at screening.

**Figure 5. Co-mutational Burden and *NRAS* Mutations Are Associated with Lack of Response**

- (A) Scatter plot showing mean and standard deviation of number of mutations found per patient, separated by response. P-value < 0.001 comparing difference between non-responders (NR) and either responders (R: CRi, CRp, MLFS, or PR) or patients achieving a CR.
- (B) Pie charts of response assessment and overall response rate (ORR; patients achieving CR, CRi, CRp, MLFS or PR) in patients with the lowest third number of co-mutations ( $\leq 3$  mutations) and highest third ( $\geq 6$  mutations).
- (C) Pie chart indicating proportion of responders and non-responders in the 14 efficacy evaluable patients with *NRAS* co-mutations (m*NRAS*), specifically at G12, G13, and Q61.
- (D) Number of mutations found per patient separated by presence of G12, G13, or Q61 mutant m*NRAS* indicating patients with m*NRAS* at G12, G13, or Q61 have increased mutational burden in this cohort. The only m*NRAS*+ patient to achieve a CR is highlighted in green.
- (E) Dot plot of m*IDH2* (blue) and m*NRAS* (red) VAF in same patient in the 14 efficacy evaluable patients with *NRAS* co-mutations specifically at G12, G13, and Q61. R = responder, NR = non-responder.

Figure 1.

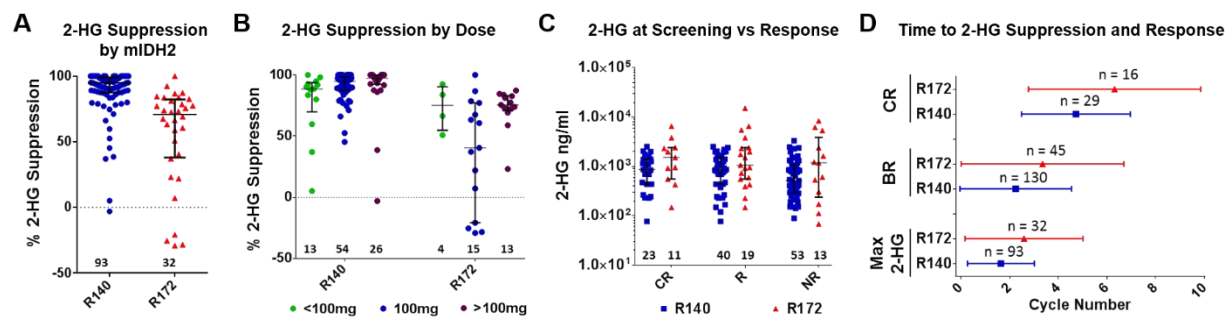


Figure 2.

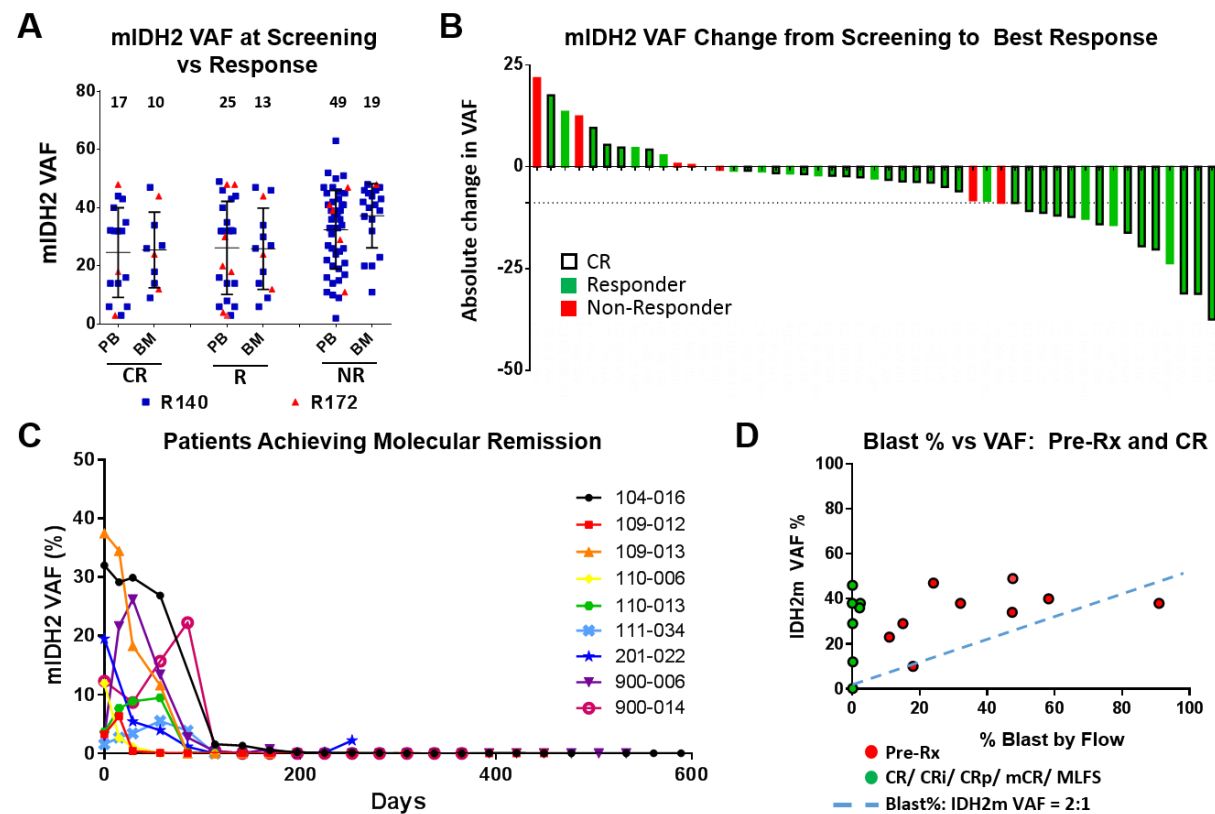


Figure 3.

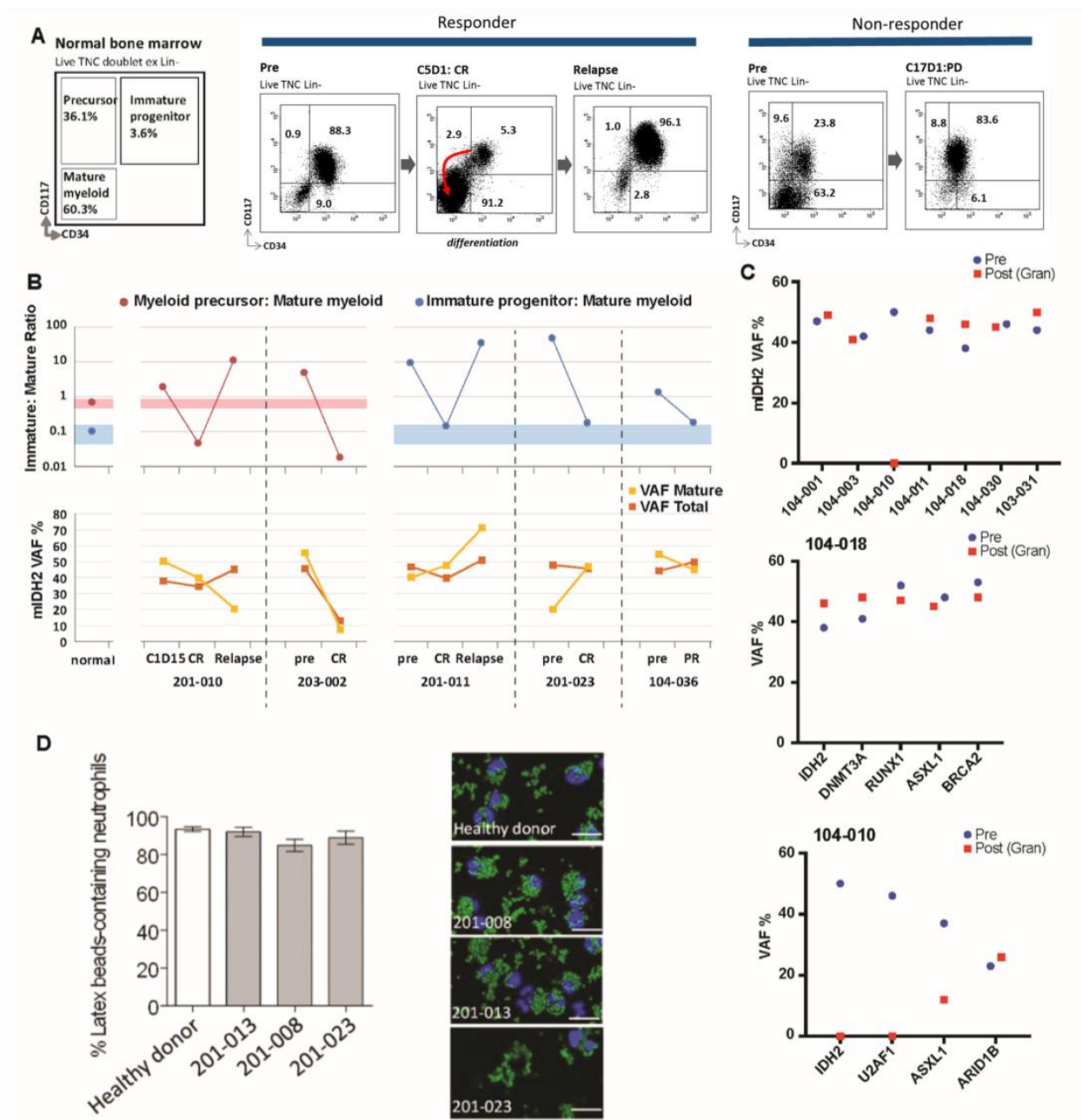
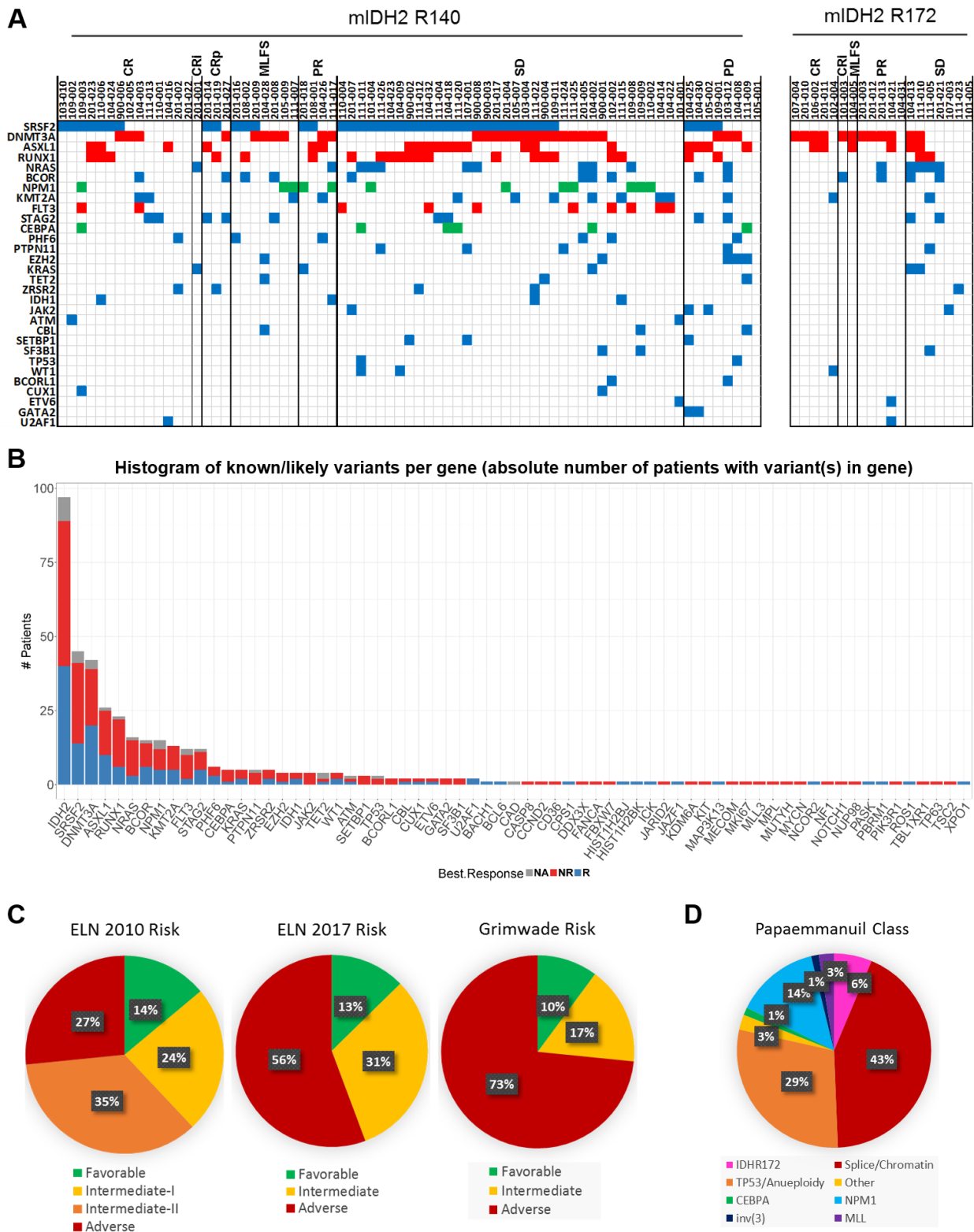
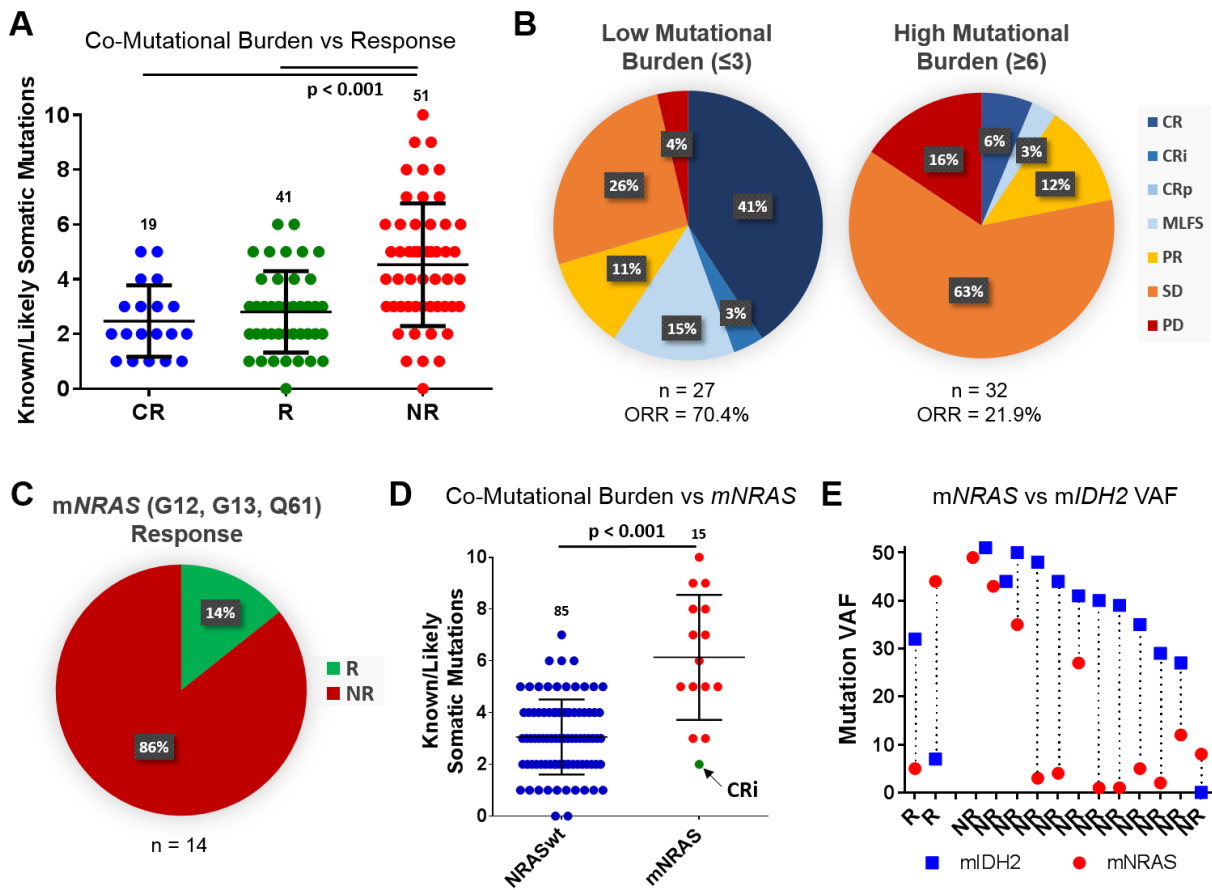


Figure 4.



**Figure 5.**



## **Supplementary Materials**

Figure S1. Patient Sample Availability and Analysis Disposition

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

Figure S3. mIDH2 Variant Allele Frequency and Response

Figure S4. VAF of Co-occurring Mutations in Patients with Low mIDH2

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

Figure S6. Co-occurring Mutation Frequency and Clonality vs Response

Table S1. Patient Baseline Characteristics in all rrAML Patients from Phase 1 Portion of Study AG-221-C-001 and Patient Subgroups Analyzed.

Table S2. Multi-parameter Flow Cytometry (MFC) Ten-“color” Panel.

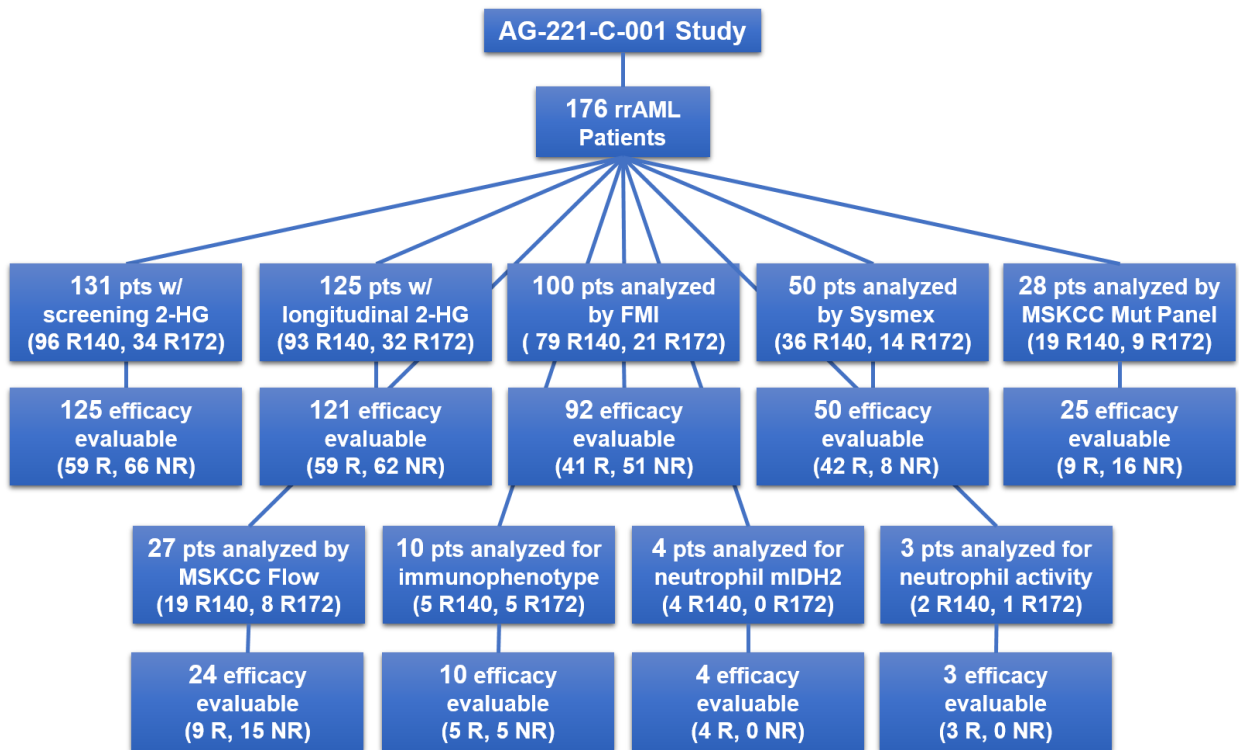
Table S3. Antibody List used for Hematopoietic Stem, Progenitor, and Mature Cell Population Immunophenotyping

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

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



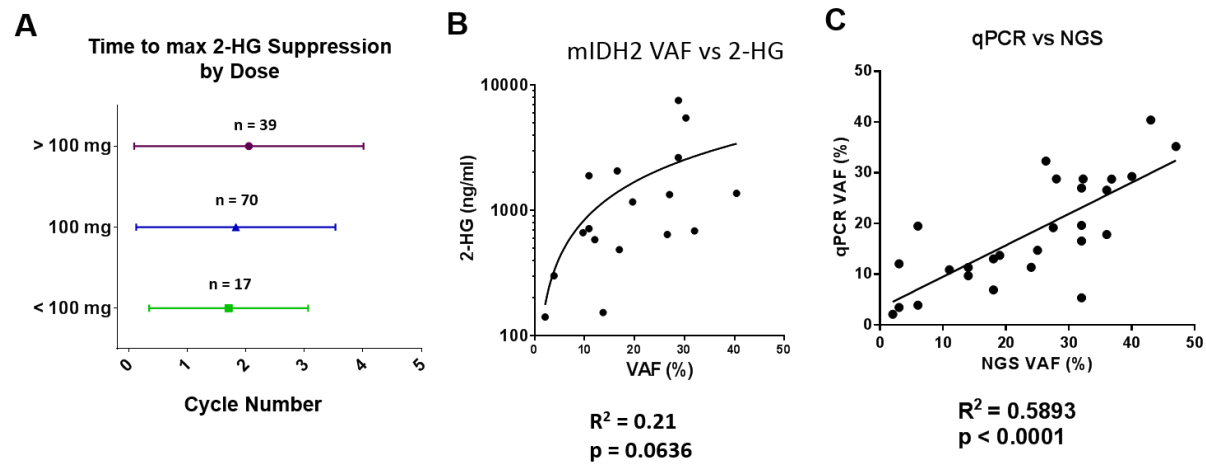
**Figure S1**



**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 *mIDH2* 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.

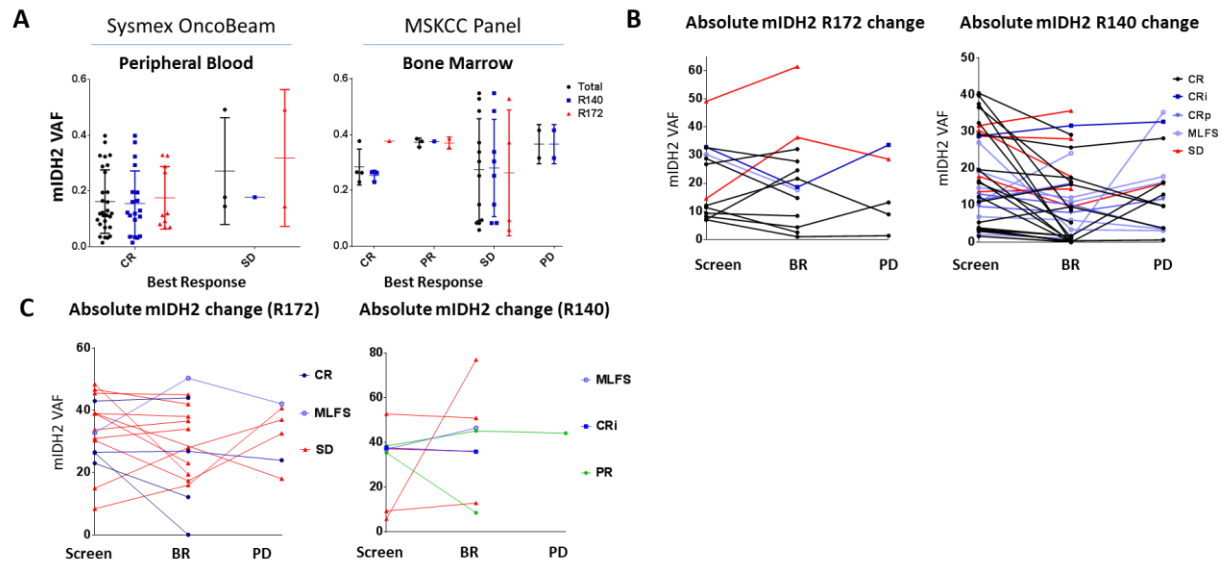
**Figure S2**



**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 mIDH2 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 mIDH2 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.

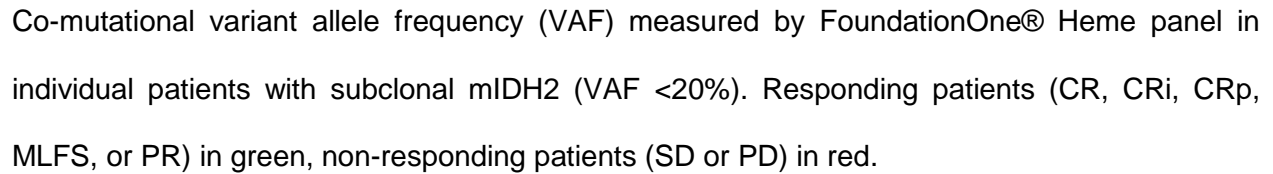
**Figure S3**



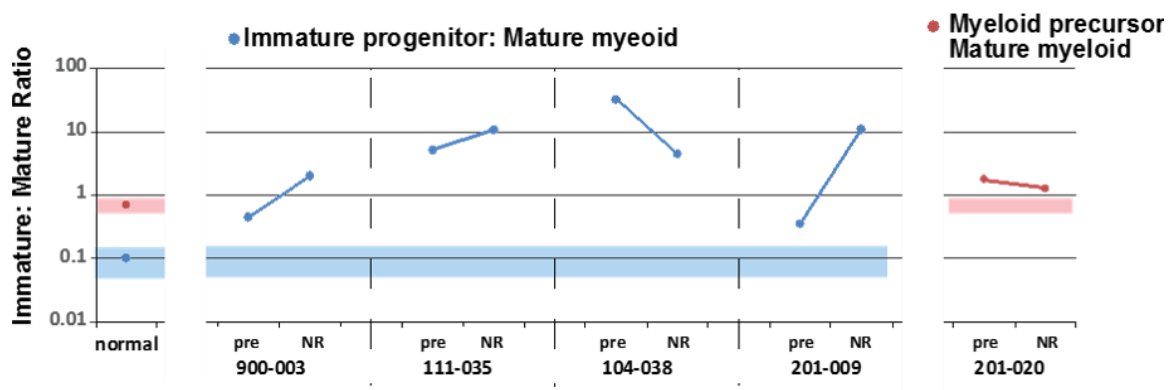
**Figure S3. mIDH2 Variant Allele Frequency and Response**

- Dot plot of mIDH2 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 mIDH2 status (red = R140, blue = R172, black = combined).
- Scatter plots of mIDH2 VAF level measured by Sysmex digital PCR in R172 (left) and R140 (right) mIDH2 patients. Lines indicate changes in mIDH2 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).
- Scatter plots of mIDH2 VAF level measured by NGS panels in mIDH2 R172 (left) and R140 (right) patients. Lines indicate changes in mIDH2 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: VAF of co-occurring mutations in patients with low mIDH2**



**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

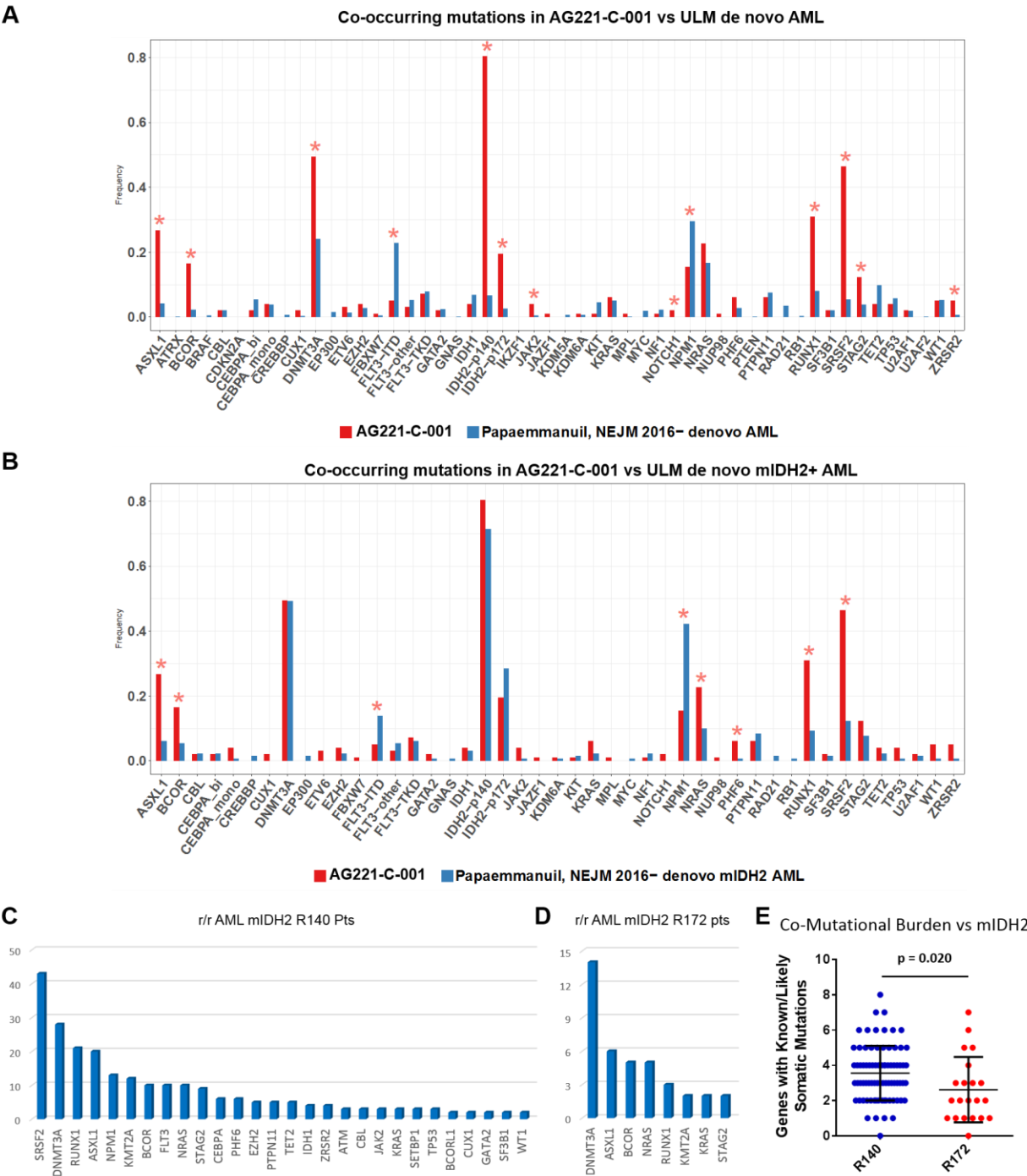


Figure S6: Co-occurring Mutation Frequency vs Response

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).<sup>13</sup> 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 ( $p\text{-value} \leq 0.05$ ).

- 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).<sup>13</sup> 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 ( $p\text{-value} \leq 0.05$ ).
- C. Histogram of mutation frequency in genes found by FoundationOne® Heme panel in R140 mIDH2 patients.
- D. Histogram of mutation frequency in genes found by FoundationOne® Heme panel in R172 mIDH2 patients.
- E. Number of mutations found per patient separated by patients with mIDH2 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®<br>Patients<br>n=100 | Sysmex mIDH2<br>VAF Patients<br>n=50 | 2-HG Patients<br>N= 125     | All R/R AML<br>Patients<br>n=176 |
|--|-------------------------------------|--------------------------------------|-----------------------------|----------------------------------|
| <b>ORR (%)</b>   | 41.0                                | 84.0                                 | 47.2                        | 40.3                             |
| <b>CR (%)</b>  | 19.0                                | 60.0                                 | 20.8                        | 19.3                             |
| <b>Median Age,<br/>years</b>   | 65.7                                | 67.6                                 | 65.2                        | 65.2                             |
| <b>Sex M/F (%)</b>   | 51.0/49.0                           | 36.0/64.0                            | 52.0/48.0                   | 51.1/48.9                        |
| <b>Gene Mutation<br/>(%):<br/>R140/R172</b>                              | 79.0/21.0                           | 74.0/26.0                            | 74.4/26.6                   | 73.9/25.6                        |
| <b>No. of Prior<br/>Anticancer<br/>therapies (%):<br/>1/2/3/4/&gt;=5</b> | 46.0/23.0/16.0/<br>11.0/4.0         | 56.0/26.0/6.0/<br>10.0/2.0           | 50.4/22.4/12.8/<br>10.4/4.0 | 46.6/26.1/14.2/<br>8.5/4.5       |
| <b>Prior<br/>Transplant (%)</b>  | 14.0                                | 16.0                                 | 14.4                        | 13.6                             |
| <b>Mean Bone<br/>Marrow Blast<br/>(%)</b>                                | 49.9                                | 36.4                                 | 47.1                        | 49.4                             |
| <b>Mean ANC<br/>(x10<sup>9</sup>/L)</b>                                  | 1.5                                 | 1.6                                  | 1.3                         | 1.3                              |
| <b>Prior MDS (%)</b>   | 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') <sub>2</sub> 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**

| <b>Patient</b> | <b>Time point</b> | <b>Response</b> | <b>Mature cells analysed</b> | <b>m<i>IDH2</i> detected</b> |
|----------------|-------------------|-----------------|------------------------------|------------------------------|
| 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 <i>IDH2</i> = mutant <i>isocitrate dehydrogenase 2</i> |               |    |                     |     |

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. *CnDn* 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<br>(n) | R172<br>(n) | ORR   | ORR,<br>OR | ORR,<br>p-value | CR    | CR,<br>OR | CR,<br>p-<br>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.