Title: Serum 2-hydroxyglutarate levels predict isocitrate dehydrogenase mutations and clinical outcome in acute myeloid leukemia.

Running Head: 2HG predicts IDH mutations and clinical outcome in AML.

Authors: Courtney D DiNardo¹, Kathleen J Propert¹, Alison W Loren¹, Elisabeth Paietta³, Zhuoxin Sun⁴, Ross L Levine², Kimberly S Straley⁵, Katharine Yen⁵, Jay P Patel², Samuel Agresta⁵, Omar Abdel-Wahab², Alexander E Perl¹, Mark R Litzow⁶, Jacob M Rowe⁷, Hillard M Lazarus⁸, Hugo F Fernandez⁹, David J Margolis¹, Martin S Tallman², Selina M Luger¹, Martin Carroll¹

¹ University of Pennsylvania, Philadelphia, Pennsylvania
² Memorial Sloan Kettering Cancer Center, New York, New York
³ Montefiore Medical Center North Div, Albert Einstein College of Medicine, Bronx, New York
⁴ Dana Farber Cancer Institute, Boston, Massachusetts
⁵ Agios, Cambridge, Massachusetts
⁶ the Mayo Clinic, Rochester, Minnesota
⁷ The Rambam Medical Center, Haifa, Israel
⁸ University Hospitals Case Medical Center, Cleveland, Ohio
⁹ H. Lee Moffitt Cancer Institute, Tampa, Florida

Corresponding Author:
Martin Carroll, MD
Division of Hematology/Oncology
Hospital of the University of Pennsylvania
Rm 708 BRB II/III
Philadelphia, PA 19146
carroll2@mail.med.upenn.edu
215) 573-5217 (Office)
215) 573-7049 (Fax)
Key Points:

- Serum 2HG analysis by GC-MS can accurately identify AML patients with and without IDH mutations.
- Oncometabolite testing of serum 2HG is indicated as a diagnostic, prognostic and therapeutic monitoring tool in AML.

Abstract:

Cancer-associated IDH mutations produce the metabolite 2-hydroxyglutarate (2HG), but the clinical utility of serum 2HG measurements is not established. We studied whether 2HG measurements in AML patients correlate with IDH mutations, and whether diagnostic or remission 2HG measurements predict survival. Serum from 223 adults with de novo AML (62 IDH-mutated, 161 wild-type) were analyzed for 2HG concentration by reverse-phase liquid chromatography-mass spectrometry (LC-MS). Pretreatment 2HG levels ranged from 10 to 30,000ng/ml and were elevated in IDH-mutants (median 3004ng/ml), compared to the wild-type cohort (median 61ng/ml) ($p<0.0005$). 2HG levels did not differ among IDH1 or IDH2 allelic variants. In receiver operating curve (ROC) analysis, a discriminatory level of 700ng/ml segregated patients with and without IDH mutations with 86.9% sensitivity and 90.7% specificity. On repeat mutational analysis of 13 IDH wild-type samples with 2HG levels $>700$ng/ml, IDH mutations were identified in nine samples. IDH-mutant patients with 2HG levels $>200$ng/ml at complete remission experienced shorter overall survival compared to those with 2HG$<200$ng/ml (HR 3.9, $p=0.02$). We establish a firm association between IDH mutations and elevated serum 2HG concentration in AML. These data confirm that serum measurement of an oncometabolite provides useful diagnostic and prognostic information, and can improve patient selection for IDH-targeted therapies.
Introduction:

Recurrent somatic mutations in isocitrate dehydrogenase (IDH) enzymes IDH1 and IDH2 have been identified in patients with acute myeloid leukemia (AML) and other myeloid malignancies, with an estimated prevalence of 5-30%.\(^1\)-\(^9\) IDH1 and IDH2 mutations occur more frequently in patients with normal karyotype and older age,\(^3\),\(^6\),\(^8\),\(^10\) and are associated with co-occurring NPM1 mutations.\(^2\)-\(^4\),\(^11\),\(^12\)

IDH enzymes function within the citric acid cycle, catalyzing the oxidative decarboxylation of isocitrate to \(\alpha\)-ketoglutarate (\(\alpha\)KG) while reducing NADP+ to NADPH. Somatic mutations within the IDH1 and IDH2 enzyme active sites, specifically at codon R132 of IDH1 and codons R140 or R172 of IDH2, lead to a loss of function for the above described reaction and yield a reverse reaction that reduces \(\alpha\)KG to 2-hydroxyglutarate (2HG) via the conversion of NAPDH to NADP+.\(^13\)-\(^17\) 2HG can be measured in patient serum, and 2HG levels correlate with the presence of IDH mutations in small AML patient cohorts.\(^13\),\(^18\),\(^19\)

Despite recent insights into the distinctive pathophysiology of IDH mutations, the role of 2HG elevations within neoplastic cells has not been firmly established. Recent studies suggest increased 2HG functions through competitive inhibition of \(\alpha\)KG-dependent enzymatic activities, including TET2-dependent DNA hydroxymethylation as well as histone demethylation, leading to dysregulated epigenetic programming.\(^20\),\(^21\) Activation of the HIF-1\(\alpha\) pathway via inhibition of prolyl hydroxylases has also been implicated.\(^15\),\(^22\),\(^23\).

The prognostic significance of IDH mutations remains controversial, perhaps confounded by a differing prognosis conferred by the different allelic mutations and by
different therapeutic regimens in different patient cohorts.\textsuperscript{24-27} IDH2 R140 mutations appear to confer a more favorable outcome, and all IDH mutations were associated with favorable outcome when present in conjunction with NPM1 mutations.\textsuperscript{11,27} However, definitive information on prognostic importance is lacking. Improved scientific discernment of this pathway is important, especially given the ongoing development of targeted IDH modulators for clinical use.\textsuperscript{28,29} Moreover, a specific role for epigenetically-targeted therapies, including DNA methyltransferase inhibitors and histone deacetylase inhibitors, may be particularly efficacious in AML subsets characterized by IDH mutations and by mutations in other genes implicated in epigenetic regulation.\textsuperscript{30,31}

Preliminary research has suggested a screening and/or diagnostic role of serum 2HG analysis in myeloid neoplasms.\textsuperscript{14,32} However, a systematic analysis of 2HG as a predictor of IDH mutation status or to assess clinical response has not been performed. We therefore measured 2HG levels in serum from patients with \textit{de novo} AML, treated on the Eastern Cooperative Oncology Group (ECOG) E1900 trial.

\textbf{Methods:}

\textit{Patients and Treatment:}

Pretreatment peripheral blood (PB) sera from 223 E1900 patients were analyzed for 2HG levels, in addition to 14 PB serum samples from healthy adult volunteers (>18 years old). All subjects provided written informed consent for research. Detailed inclusion and exclusion criteria for E1900 have been previously published.\textsuperscript{33} Median follow-up of subjects in our cohort was 21 months (range 0.4 – 81 months).
Serum was obtained from 62 E1900 patients with IDH mutations\textsuperscript{11}, identified by mutational analysis of coding exons with known somatic mutations using PCR amplification and bidirectional Sanger sequencing as previously described.\textsuperscript{13} The majority (87\%) had intermediate or indeterminate cytogenetics as determined by ECOG/SWOG classification system.\textsuperscript{34} In 29 of those patients, serum collected within 2 weeks of morphologic complete remission (CR) at post-induction or post-consolidation (including autologous transplantation) time-points were also available. Additionally, 161 control samples, selected based on intermediate-risk cytogenetics and IDH wild-type status from the primary mutational studies\textsuperscript{11} were analyzed. Analysts were blinded to the IDH mutational status. Over 90\% of the patients with intermediate risk cytogenetics had a normal diploid karyotype.

Additional covariates analyzed within the E1900 cohort included the patient-related variables age and sex, disease-related variables such as white blood cell (WBC) count, platelet count, BM blast percentage (\%) and circulating blast percentage, and treatment covariates including the treating institution and randomized E1900 treatment allocation. Less than 5\% of patients received an allogeneic stem cell transplant during first CR in our E1900 study cohort. The study was approved by the University of Pennsylvania Institutional Review Board. All samples were obtained after informed consent in accordance with the Declaration of Helsinki.

\textit{2HG Analysis:}

Serum 2HG levels were measured by reverse-phase liquid chromatography coupled to mass spectrometry (LC-MS) by Agios Pharmaceuticals (Cambridge, MA).
Integrated elution peaks were compared to metabolite standard curves for absolute quantification, using established methods. A 2HG level <10ng/ml was defined as below the level of detection using the LC-MS assay (i.e. below the quantitation limit, BQL) and was analyzed as 10ng/ml. Similarly, 2HG levels >30,000ng/ml were considered above the quantitation limit (AQL), and were analyzed as 30,000ng/ml, as values above this level were extrapolated.

2HG analysis was repeated for 21 selected samples, and demonstrated reproducible 2HG levels with a mean intra-sample coefficient of variation of 24.8%. The intra-sample variation was most pronounced in samples with markedly elevated 2HG levels, and did not affect the interpretation of the IDH mutational status.

Statistical Analyses:

Non-parametric analyses were performed due to the non-Gaussian distribution of 2HG levels. Categorical variables were compared using $\chi^2$ or Fisher’s exact test, and continuous variables using the Wilcoxon rank-sum test. 2HG was evaluated as both a continuous and a categorical variable. Discriminatory cut-off values for 2HG were evaluated to maximize sensitivity, specificity, and create an optimal cut-point for clinical purposes through receiver operating curve (ROC) analysis.

OS was measured as the time from AML diagnosis to death or date of last follow-up (censored). For the remission cohort analysis (n=29), OS was measured from time of complete remission (CR). Leukemia-free survival (LFS) was defined as the time from CR to treatment failure including relapse, death, or date at last follow-up (censored).
Kaplan-Meier analysis was used to construct OS and LFS curves, and curves were compared by log-rank test.

Due to the presence of 2HG values above or below the limits of accurate LC-MS detection, a censored regression model (Tobit) was employed which estimates linear relationships between variables when there is either left- or right- censoring of the dependent variable. Adjusted Cox proportional hazards models were constructed to examine the effect of potential confounders. All analyses were two-sided, with statistical significance established as $p<0.05$ using STATA software, version 12 (College Station, TX).

**Results:**

*Patient Characteristics:*

Clinicopathologic characteristics of our E1900 cohort are displayed in Table 1 and Table S1, including patient age, sex, treatment arm, diagnostic laboratory values, cytogenetic risk group, and presence of additional somatic mutations. Given the favorable prognosis of IDH2-R140 mutations, characteristics for this subgroup are additionally detailed. No statistically significant differences in age, sex, or treatment group were observed between patients with and without IDH mutations, or by treating institution. IDH-mutant patients had a higher platelet count ($p=0.002$), and a higher BM blast percentage at diagnosis ($p=0.009$) compared to IDH wild-type patients. The presence of an IDH mutation correlated with DNMT3A mutations ($p=0.022$) and was inversely correlated with FLT3 mutations ($p<0.0005$) and CEPBA mutations ($p=0.023$).
While the incidence of TET2 mutations was low overall, no patient with an identified IDH mutation had a co-occurring TET2 mutation, as has been previously reported.\textsuperscript{11,20}

\textit{Elevated 2HG levels in AML are associated with IDH mutations:}

Pre-treatment serum 2HG levels ranged from 10ng/ml to 30,000ng/ml, with a median value of 101ng/ml for all patients tested. In IDH wild-type AML patients, 2HG ranged from 10ng/ml to 14,181ng/ml (median 61ng/ml). Serum 2HG in IDH-mutants ranged from 10ng/ml to 30,000ng/ml (median 3,004ng/ml). 2HG could be detected in 159 (71\%) of all samples, including in 60/62 (96.8\%) samples with IDH mutations, and 99/161 (61.5\%) of wild-type IDH subjects. 2HG levels were significantly higher in IDH-mutants, as compared to IDH wild-type AML patients ($p<0.0005$) [Figure 1]. Serum 2HG levels in volunteers were also obtained (see “Serum 2HG levels in normal healthy volunteers”), and ranged from 33ng/ml to 176ng/ml (median 48ng/ml).

On univariate Tobit regression analysis, an elevated serum 2HG level at diagnosis was associated with an elevated platelet count ($p<0.0005$), and an elevated BM blast percentage at diagnosis ($p=0.027$). We observed an association between an elevated WBC count and serum 2HG level of borderline significance ($p=0.057$); when analyzing only IDH-mutants a strong association between WBC count and level of serum 2HG elevation was identified ($p=0.007$) [Figure 2]. There was no statistically significant association between 2HG level and patient age, gender, treating institution, or study arm treatment.

We next evaluated serum 2HG levels in IDH-mutants, based on the specific IDH1 or IDH2 mutation present (Figure 3). No significant difference in 2HG levels among
IDH-mutants was identified based on the specific allelic mutation. Of note, 2HG levels in IDH2-R140 mutants did not differ significantly from the other IDH mutants, at baseline or after adjustment for WBC count, BM or circulating blast percentage.

**Serum 2HG levels can identify AML patients with IDH mutations:**

We next asked whether a “threshold” level of serum 2HG could serve as a useful screening test to segregate AML patients with or without IDH mutations. Using ROC analysis, a strong correlation between serum 2HG level and IDH mutations was identified, with an area under the ROC curve (AUC) between wild-type IDH and IDH mutants of 0.918 [Figure 4], and an optimal cut-point of 700ng/ml. When applying this discriminatory 700ng/ml value, diagnostic sensitivity and specificity of serum 2HG level for predicting IDH mutations was 86.9% and 90.7%, respectively, with 89.6% of patients correctly classified.

There were 15 IDH wild-type E1900 samples with pretreatment 2HG levels measuring >700ng/ml. Of those, 13 samples were reanalyzed for IDH1 and IDH2 mutations using identical mutation detection methodology, at the same institution as the initial mutation analysis, with manual inspection of all traces. On repeat testing, 9 of the 13 samples were discovered to have an IDH mutation (2 samples with an IDH1-R132 mutation, 6 with an IDH2-R140Q mutation, and 1 with an IDH2-R172K mutation). In most cases, the mutant allele burden was less than 20%, such that it fell below the level of detection of the analysis pipeline, which employed Mutation Surveyor as primary screen. Four samples were again found to be without identifiable IDH1 or IDH2 mutations detectable by Sanger sequencing. Interestingly, however, two of these four
samples had sequencing traces with a potential low abundance mutant IDH allele. For all further statistical analyses, the nine IDH wild-type samples with identified IDH mutations on re-sequencing were analyzed primarily within the IDH wild-type cohort (akin to an intention-to-treat analysis). Exploratory analyses were conducted in which these nine samples were dropped from analysis, or analyzed in the IDH mutant cohort (see Supplementary Text).

**Serum 2HG levels in normal healthy volunteers:**

To further investigate the potential of serum 2HG measurements as a diagnostic test, we studied 2HG levels in the serum of 14 healthy volunteers. In these volunteers, the median 2HG level was 48ng/ml, with a range of 33ng/ml to 176ng/ml. There was no association between 2HG level with age or gender. Of note, there was no significant difference between the median 2HG level in volunteers (48ng/ml) with the median value of IDH wild-type AML samples (61ng/ml; or 50ng/ml if the 9 samples with identified IDH mutations were excluded). All 14 healthy volunteers had detectable serum 2HG levels by LC-MS.

**2HG levels at remission of IDH-mutant patients:**

Sera from 29 of the IDH-mutant E1900 subjects at the time of CR (see Supplemental Text) revealed a median 2HG level of 95ng/ml (range 28–3,149ng/ml), compared to a median 2HG of 3,234ng/ml (range 10-13,638ng/ml) in these 29 subjects at AML diagnosis. The median change in 2HG from diagnosis to remission was 3,088ng/ml (range +43 to -13,363ng/ml). 2HG levels decreased in all but one patient
(this patient had a diagnosis 2HG level of BQL and 53ng/ml at CR). Neither the absolute difference in 2HG nor the percent change in 2HG levels, from diagnosis to CR, was associated with survival.

We next assessed whether achieving a “normal level” of serum 2HG at CR provided prognostic value. Given that 2HG levels of healthy volunteers ranged from 33ng/ml to 176ng/ml, we defined a normal 2HG level as <200ng/ml. Of the 29 IDH-mutant remission samples, 20 (69%) achieved a remission 2HG level <200ng/ml (Figure 5a). Patients who did not achieve a 2HG level <200ng/ml at CR experienced inferior OS (HR 3.9, 95% CI 1.2–12.7, p=0.02) and LFS (HR 3.6, 95% CI 1.1 – 11.9, p=0.046), which retained significance on multivariable analysis (Table S2) with a median OS that was 23.5 months in the 2HG >200ng/ml group, and unreached in the <200ng/ml group (Figure 5b). Since mutational analysis for IDH at the time of CR was not performed, we cannot compare the sensitivity of serum 2HG to molecular tests for MRD.

Effect of 2HG levels on outcome:

There was no impact of elevated serum 2HG level on attainment of CR (p=0.24). There was a suggestion of improved survival in patients with an elevated pretreatment 2HG level, although this was not statistically significant for OS (HR 0.72, 95% CI 0.49–1.06, p=0.09) or LFS (HR 0.66, 95% CI 0.41–1.06, p=0.08).

Interactions were identified between an elevated serum 2HG (>700ng/ml) and mutational status of co-occurring mutations including NPM1 and CEPBA. This implies that other genetic abnormalities may alter the magnitude of the relationship between 2HG level and clinical outcome, and is consistent with recent data suggesting that
NPM1/IDH double-mutants have favorable outcome. When evaluating pretreatment 2HG levels using our threshold of 700ng/ml on multivariable analysis (including allocated treatment group), an elevated 2HG level was associated with improved OS in patients with NPM1 mutations (n=106) (HR 0.42, 95% CI 0.22–0.77, p=0.004), and in those without DNMT3A mutations (n=144) (HR 0.48, 95% CI 0.26-0.84, p=0.01) or CEPBA mutations (n=198) (HR 0.63, 95% CI 0.43-0.94, p=0.02) [see Supplementary Text, Tables and Figures].

Finally, the level of serum 2HG was analyzed separately within the three IDH allelic mutant cohorts and split by the median 2HG level observed in IDH mutants (3004ng/ml). Despite small cohort size, there was a suggestion of decreased overall survival in patients with a more elevated 2HG level and IDH2-R172 mutations (HR 7.29, 95% CI 0.79–67.1, p=0.08). Overall survival in IDH1-R132 mutants or IDH2-R140 mutants did not appear to be affected by level of serum 2HG elevation at diagnosis (HR 1.01, 95% CI 0.34-2.96, p=0.98) and (HR 1.8, 95% CI 0.59-5.57, p=0.28), respectively.

Discussion:

Discovery of neomorphic 2HG production as a consequence of recurrent IDH mutations in AML and in other malignancies has strengthened the proposed link between abnormal cellular metabolism, epigenetic re-programming, and oncogenic transformation. Elevated serum 2HG levels in both IDH1 and IDH2 mutant patients have been previously described in several small AML cohorts, however the relationship between serum 2HG levels, IDH mutations, and patient outcome has not been previously defined. Our study is the first to fully assess pretreatment serum 2HG
levels in a cohort of AML patients treated on a uniform protocol, and to investigate the utility of 2HG as a prognostic biomarker.

Our analysis confirms a clear relationship between the presence of an IDH mutation (IDH1-R132, IDH2-R172 or IDH2-R140) and an elevated serum 2HG level, with 97% of IDH-mutants having a detectable 2HG level at the time of AML diagnosis, and a median 2HG concentration nearly fifty-fold higher than that of IDH wild-type subjects. An association between diagnostic WBC count and serum 2HG levels was identified in IDH-mutants, suggesting that circulating WBC count as a measure of “tumor burden” may relate to the level of serum 2HG in these patients. We also found low but detectable levels of serum 2HG in the majority of wild-type IDH AML patients, as well as normal volunteers. These data suggest that the LC-MS technique can detect minimal and non-pathologic levels of this metabolite in subjects without IDH mutations.

We found that pretreatment serum 2HG levels >700ng/ml best predict IDH mutational status. Notably, repeat mutation testing in 13 patients initially characterized as IDH wild-type and with diagnostic 2HG levels >700ng/ml reclassified 9 subjects as having IDH mutations, albeit at low allelic burden. The predictive utility of this value is notable, but will require validation in an independent dataset. Given that AML is a heterogeneous disease involving distinctive subclones even within the same patient, the ability to detect small IDH-mutant clones undetectable by current molecular techniques yet leading to 2HG accumulation must be considered. Whether such clones are of biologic or clinical relevance, or represent tractable therapeutic targets, is unknown. Additional sequencing and analysis of the four samples with 2HG levels >700ng/ml and without identified IDH mutations are ongoing. It is possible that mutations in the non-
coding regions of IDH1 or IDH2, or alterations in other enzymes involved in α-ketoglutarate metabolism occur in patients without canonical IDH1 or IDH2 mutations.

Our data suggest that diagnostic serum 2HG measurements may allow for rapid, accurate identification of AML patients with IDH mutations. 2HG testing in patients with glioma, cholangiocarcinoma, and chondrosarcoma may provide similarly useful diagnostic and/or prognostic information. Importantly, our data demonstrates the technical feasibility of this approach. As improved sequencing techniques increase the sensitivity of genetic tests, future studies will be required to compare the sensitivity of 2HG analysis to next-generation testing methodologies.

Drugs targeting mutant IDH are currently being tested in the preclinical setting with promising results, thus increasing the significance of serum 2HG for the selection of patients for such treatment. Serial 2HG measurements may emerge as an important pharmacodynamics marker of IDH modulating agents and other anti-leukemic therapies. Nevertheless, analytical validation of the LC-MS technique is warranted before this test can be used for clinical decision-making.

Our analysis suggests the level of serum 2HG alone does not explain the improved survival seen in the IDH2-R140 cohort compared to patients with other IDH1 or IDH2 mutations. Recent data demonstrates IDH+/NPM1+ mutations confer an especially favorable prognosis (9), although the frequency of co-occurring NPM1 mutations within our IDH2-R140 subgroup was not significantly different from the IDH-mutants overall. It is important to consider that serum 2HG is only a surrogate measurement for intracellular levels of 2HG in malignant cells, and the true biologic interpretation of serum 2HG levels is not clear at this time. The relationship between
intracellular and extracellular 2HG levels, the half-life of serum 2HG, and mechanisms of normal 2HG break-down and elimination will require further clarification.

In IDH-mutated patients, a decrease of serum 2HG to <200ng/ml at CR was predictive of improved OS ($p=0.02$). This suggests that 2HG testing may represent a sensitive means of assessing residual leukemic cells after induction chemotherapy, and serve as a marker of MRD with which to guide subsequent therapeutic decisions. Prospective studies are needed to confirm this observation and to inform the use of 2HG measurement as a quantitative tool in comparison to other markers of MRD such as flow cytometry and genetic analysis.

Taken together, our data provides a rationale for serum 2HG measurement as a diagnostic, prognostic, and monitoring tool in AML, and may establish a role for oncometabolite testing in the clinical setting to improve upon therapeutic decisions.

Research Support:
C.D.D. acknowledges support from the NIH Cancer Clinical Epidemiology Training Grant (T32 CA 009679). MC is supported by RO1 CA149566 and by VA Merit Award 1I01BX000918.

Authorship:
C.D.D. designed the research, performed the research, analyzed the data, performed statistical analysis, and wrote the paper. K.J.P. analyzed the data, performed statistical analysis, and wrote the paper. A.W.L. designed the research, analyzed the data, and wrote the paper. E.P. designed the research, contributed vital analytical tools, interpreted the data, and wrote the paper. Z.S. analyzed the data, performed statistical
analysis, and wrote the paper. R.L.L. designed the research, contributed vital analytical tools, and wrote the paper. K.S.S. performed the research, and contributed vital analytical tools. K.Y. performed the research and contributed vital analytical tools. J.P.P. performed the research, contributed analytical tools, and wrote the paper. S.A. performed the research and contributed vital analytical tools. O.A-W. performed the research, contributed vital analytical tools, and wrote the paper. A.E.P. analyzed the data and wrote the paper. M.R.L. analyzed the data and wrote the paper. J.M.R. analyzed the data and wrote the paper. H.M.L. analyzed the data and wrote the paper. H.F.F. analyzed the data and wrote the paper. D.J.M. designed the research, analyzed the data, and wrote the paper. M.S.T. designed the research, analyzed the data, and wrote the paper. S.M.L. designed the research, analyzed the data, and wrote the paper. M.C. designed the research, performed the research, analyzed the data and wrote the paper.

K.S.S., K.Y., and S.A. are employed by Agios Pharmaceuticals. R.L.L. has consulted for Agios Pharmaceuticals and received honoraria. The remaining authors declare no relevant financial conflict of interest.
References:


Table 1: Clinical characteristics and patient outcome of study cohort

IDH indicates isocitrate dehydrogenase; txmt, treatment; DNR, daunorubicin; WBC, white blood cell; dx, diagnosis; PLT, platelet; BM, bone marrow; PB, peripheral blood;

FLT3-ITD, FLT3 internal tandem duplication; FLT3-TKD, FLT3 tyrosine kinase domain
Table 1: Clinical characteristics and patient outcome of study cohort

<table>
<thead>
<tr>
<th></th>
<th>IDH Wild-type (n=161)</th>
<th>IDH Mutant (n=62)</th>
<th>P value</th>
<th>IDH2 R140Q Mutant (n=26)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Median age (range)</td>
<td>46 (18-60)</td>
<td>46 (18-60)</td>
<td>0.89</td>
<td>47 (27-59)</td>
</tr>
<tr>
<td>Male/Female Ratio</td>
<td>80/81 (1:1)</td>
<td>26/36 (1:1.4)</td>
<td>0.37</td>
<td>13/13 (1:1)</td>
</tr>
<tr>
<td>Txmt Group (%)</td>
<td></td>
<td></td>
<td>0.23</td>
<td></td>
</tr>
<tr>
<td>DNR 45mg/m2</td>
<td>83 (52%)</td>
<td>26 (42%)</td>
<td></td>
<td>8 (31%)</td>
</tr>
<tr>
<td>DNR 90mg/m2</td>
<td>78 (48%)</td>
<td>36 (58%)</td>
<td></td>
<td>18 (69%)</td>
</tr>
<tr>
<td>Median WBC count at dx (x10^9/L)</td>
<td>31.8 (0.6 – 212.8)</td>
<td>34.7 (1.0 – 191.8)</td>
<td>0.62</td>
<td>44.0 (1.7 – 191.8)</td>
</tr>
<tr>
<td>Median PLT count at dx (x10^9/L)</td>
<td>67.2 (3.9 – 452)</td>
<td>101.7 (6.2 – 650)</td>
<td>0.002</td>
<td>94.3 (17 – 304)</td>
</tr>
<tr>
<td>Median BM blast % at dx (range)</td>
<td>61.2 (8 – 99)</td>
<td>71.8 (22 – 100)</td>
<td>0.009</td>
<td>72.2 (25 – 100)</td>
</tr>
<tr>
<td>Median PB blast % at dx (range)</td>
<td>44.9 (0 – 98)</td>
<td>48.4 (0 – 97)</td>
<td>0.49</td>
<td>55.8 (0 – 97)</td>
</tr>
<tr>
<td>Cytogenetic Risk Group (%)</td>
<td></td>
<td></td>
<td>&lt;0.0005</td>
<td></td>
</tr>
<tr>
<td>Favorable</td>
<td>0 (0%)</td>
<td>1 (2%)</td>
<td></td>
<td>0 (0%)</td>
</tr>
<tr>
<td>Intermediate</td>
<td>146 (91%)</td>
<td>40 (65%)</td>
<td>16 (62%)</td>
<td></td>
</tr>
<tr>
<td>Unfavorable</td>
<td>1 (1%)</td>
<td>7 (11%)</td>
<td>4 (15%)</td>
<td></td>
</tr>
<tr>
<td>Indeterminate</td>
<td>14 (9%)</td>
<td>14 (23%)</td>
<td>6 (23%)</td>
<td></td>
</tr>
<tr>
<td>Mutations Present</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>FLT3-ITD</td>
<td>76 (48%)</td>
<td>12 (20%)</td>
<td>&lt;0.0005</td>
<td>6 (23%)</td>
</tr>
<tr>
<td>FLT3-TKD</td>
<td>12 (8%)</td>
<td>2 (3%)</td>
<td></td>
<td>2 (8%)</td>
</tr>
<tr>
<td>NPM1</td>
<td>74 (47%)</td>
<td>32 (53%)</td>
<td>0.45</td>
<td>16 (62%)</td>
</tr>
<tr>
<td>TET2</td>
<td>11 (7%)</td>
<td>0 (0%)</td>
<td>0.087</td>
<td>0 (0%)</td>
</tr>
<tr>
<td>DNMT3A</td>
<td>44 (28%)</td>
<td>20 (33%)</td>
<td>0.022</td>
<td>4 (15%)</td>
</tr>
<tr>
<td>CEBPA</td>
<td>20 (13%)</td>
<td>1 (2%)</td>
<td>0.023</td>
<td>0 (0%)</td>
</tr>
<tr>
<td>Achieved CR (%)</td>
<td>103 (64%)</td>
<td>40 (65%)</td>
<td>0.824</td>
<td>19 (73%)</td>
</tr>
<tr>
<td>Median Survival (mo)</td>
<td>18.2</td>
<td>45.3</td>
<td>0.022</td>
<td>Not Reached</td>
</tr>
</tbody>
</table>
Figure Legends:

Figure 1: Box plot of 2HG levels based on IDH mutation status

2HG indicates 2-hydroxyglutarate; IDH, isocitrate dehydrogenase; AML, acute myeloid leukemia; Dx, diagnosis

Figure 2: In IDH mutants, serum 2HG level relates to WBC count at AML diagnosis

IDH indicates isocitrate dehydrogenase; 2HG, 2-hydroxyglutarate; WBC, white blood cell count; AML, acute myeloid leukemia; Dx, diagnosis

Figure 3: Distribution of 2HG levels based on IDH allelic variant

2HG indicates 2-hydroxyglutarate; IDH, isocitrate dehydrogenase; AML, acute myeloid leukemia; Dx, diagnosis

Figure 4: ROC curve of IDH mutational status and 2HG level

ROC indicates receiver operating curve; IDH; isocitrate dehydrogenase, 2HG; 2-hydroxyglutarate

Figure 5a: 2HG levels at diagnosis and at CR in 29 IDH-mutant samples (dotted line at 200ng/ml)
Figure 5b: Kaplan-Meier survival curve of overall survival based on remission 2HG

2HG indicates 2-hydroxyglutarate; CR, complete remission; IDH, isocitrate dehydrogenase; AML, acute myeloid leukemia; Dx, diagnosis
Figure 1: Box plot of 2HG levels based on IDH mutation status

p < 0.0005
Figure 2: In IDH mutants, serum 2HG level relates to WBC count at AML diagnosis
Figure 3: Distribution of 2HG levels based on IDH allelic variant
Figure 4: ROC curve of IDH mutational status and 2HG level
Figure 5a: 2HG levels at diagnosis and at CR in 29 IDH-mutant samples (dotted line at 200ng/ml):
Figure 5b: Kaplan-Meier survival curve of overall survival based on remission 2HG level

OS of IDH mutants at CR based on remission 2HG level

log-rank p=0.02

Number at risk
2HG <=200ng/ml 20 15 8 4 0
2HG > 200ng/ml 9 5 1 1 0

2HG at CR <=200ng/ml 2HG at CR >200ng/ml
Serum 2-hydroxyglutarate levels predict isocitrate dehydrogenase mutations and clinical outcome in acute myeloid leukemia


Advance online articles have been peer reviewed and accepted for publication but have not yet appeared in the paper journal (edited, typeset versions may be posted when available prior to final publication). Advance online articles are citable and establish publication priority; they are indexed by PubMed from initial publication. Citations to Advance online articles must include digital object identifier (DOIs) and date of initial publication.