Exploratory Analyses:

Regarding IDH mutants and platelet counts at diagnosis: In our study sample, initial analysis using the primary IDH mutation status demonstrated that IDH-mutant patients had a higher overall platelet count (p=0.002), as detailed in the results and Table 1. We identified a correlation on univariate analysis between an elevated platelet count and elevated serum 2HG level (p<0.0005). Curiously, when we analyzed only the IDH mutants, the IDH-mutant cohort demonstrated a statistically non-significant correlation (p=0.08) between 2HG level and platelet count. This suggested that there may be a threshold level above which further increases in 2HG do not continue to associate with continually increasing platelets, although the loss of statistical significance may also have been related to the loss of sample size for this analysis, with a reduced power to detect these changes. On the other hand, in the IDH wild-type patients, 2HG levels were associated with higher platelet counts (p<0.0005). Importantly, when we repeat this analysis, but now allowing for re-classification of the 9 patients identified to have a low allele burden IDH mutation to the IDH mutant group (as an exploratory analysis), then statistical significance is established only for the IDH mutant group (p=0.03) and no longer for the wild-type group (p=0.64), indicating that indeed elevated 2HG levels and elevated platelet counts are significantly associated with the presence of IDH mutations. Overall, these data suggest an association between elevated serum 2HG and elevated platelet count and may represent a useful clinical finding, but this will require further confirmation in subsequent cohorts defined using sensitive methods of IDH mutation detection.

Regarding IDH mutants and WBC counts at diagnosis: Similarly, an overall association between an elevated WBC count at AML diagnosis and serum 2HG level of borderline significance (p=0.057) was observed. When analyzing by IDH mutant status, a strong association between elevated WBC count and serum 2HG level in the IDH mutant cohort was identified (p=0.007), and not for the IDH wild-type cohort (p=0.43). In an exploratory analysis where the 9 mutants identified from the secondary mutational analysis were categorized in the IDH mutant category, the relationship between 2HG level and WBC level became even more striking (p<0.0005). Consistent findings, albeit not statistically significant relationships, were found on the same exploratory analysis between serum 2HG level and either bone marrow blast (p=0.09) or peripheral blood blast (p=0.15) percentage within the IDH mutant cohort. This suggests that a lower WBC count, representing a rather blunt assessment of "leukemic tumor burden" may be related to the IDH mutant patients with low/normal serum 2HG levels (see Figure 2).

As visually depicted in Figure 2, there are two "outlier" patients with a white blood cell count > 100K and 2HG levels less than 700ng/ml. The IDH-mutant patient with the highest WBC count of 191.8K had a serum 2HG level of 201ng/ml. 2HG analysis was repeated and the repeat level was 182ng/ml. This patient was a 42yo M with unfavorable cytogenetic analysis with 46XY, t(6;11)(q27;q23), who also had a FLT3-ITD and MLL mutation identified in addition to an IDH2-R140 mutation. The remainder of his mutation profiling (including NPM1, TET2, DNMT3A and CEPBA) was negative. This patient obtained an initial CR but unfortunately relapsed at day 519 and OS was 578 days. The second patient, with a WBC count of 120.1K, had an undetectable serum 2HG level. On repeat analysis the 2HG level was 103ng/ml. This patient was a 27yo M, also with an IDH2-R140 mutation. He had a complex karyotype including del(1)(q32q34.3), add(10)(p11.2), add(11)(q13), del(12)(p11.2), add(16)(p11.2). His mutation profile was

negative in all mutations tested, and his OS was 31 days. While it is notable that both of these patients have unfavorable karyotypes in addition to the IDH2-R140 mutations present, the numbers are too small to make definitive conclusions based on these two patients. We did, however, evaluate all 7 patients with unfavorable cytogenetics in our entire IDH-mutant cohort, and in these 7 patients the median 2HG level was only 827ng/ml, which is only slightly higher than our identified threshold 2HG level of 700ng/ml. Whether this observation is maintained in future studies, and whether additional genetic or epigenetic abnormalities within the leukemia clone can interact within the αKG/2HG pathway to decrease 2HG levels in IDH-mutant patients will be a most interesting avenue of research.

Characteristics of the IDH-mutant remission samples (n=29):

Sera from the time of CR in 29 IDH-mutant E1900 subjects was available for analysis. Our (n=29) remission cohort did not differ significantly in regards to sex, IDH allelic mutation, or proliferative indices (white blood cell count, platelet count, bone marrow blast or peripheral blast percentage at diagnosis) from the remaining 33 IDH-mutant patients. Patients included in the remission cohort were more likely to be younger (median age 42.7 versus 50.2 years, $p = 0.008$), and more likely to have an NPM1 mutation (64.7% versus 38.5%, $p=0.043$). There was a trend towards an increased likelihood of having received high-dose anthracycline ("Arm B") treatment (68.6% vs 44.4%, $p=0.056$). These variables are known to have prognostic importance among AML patients, and so are expected differences when comparing patients who did and did not achieve a remission from induction chemotherapy.

Subgroup Mutational Analysis:

Analysis of our dataset identified than an elevated 2HG level at AML diagnosis was associated with an improved overall survival in patients with NPM1 mutations, which is consistent with published data that there is favorable prognosis in patients with the co-presence of IDH and NPM1 mutations. We also identified an improved overall survival in patients with an elevated 2HG and ***without*** DNMT3A mutations or ***without*** CEPBA mutations. This information along with the mutational complements are included in the Supplementary Figures S2-S6 and the multivariate analyses in Tables S2-S5. Given the small subset of patients with elevated 2HG and CEBPA mutations (n=1), this complementary graph was not included as the subset was too small to be appropriately interpreted.

Supplementary Table 1: Characteristics of 2HG test cohort compared with the entire E1900 cohort

	Test cohort (n=223)	Entire E1900 Cohort (n=657)
Median age (range)	46 (18-60)	48 (17-60)
Male/Female Ratio	105/116 (1:1.1)	335/322 (1:1)
Txmt Group (%)		
DNR 45mg/m2	108 (49%)	330 (50%)
DNR 90mg/m2	113 (51%)	327 (50%)
Median WBC count at dx (x10^9/L)	32.6 (0.6 – 212.8)	12.3 (0.6 – 366.0)
Median PLT count at dx (x10^9/L)	76.7 (3.9 – 650)	52.5 (1.0 – 995.0)
Median BM blast % at dx (range)	63.9 (8 – 100)	64.5 (3 – 100)
Median PB blast % at dx (range)	45.9 (0 – 98)	31.5 (0 – 99)
Cytogenetic Risk Group (%)		
Favorable	1 (0.5%)	89 (13.5%)
Intermediate	186 (83%)	267 (40.6%)
Unfavorable	8 (4%)	122 (18.6%)
Indeterminate	28 (13%)	176 (26.8%)
Mutations Present		
FLT3-ITD **FLT3-TKD**	88 (40%) 14 (6%)	n/a
NPM1	106 (48%)	n/a
TET2	11 (5%)	n/a
DNMT3A	64 (29%)	n/a
CEBPA	21 (10%)	n/a
Achieved Complete Remission (%)	64.1	63.9
Median Survival (mo)	23.8	20.7

Table S2: Multivariable Cox Regression Analysis in Remission Patients (n=29)

Variable	Estimated Coefficient	Standard Error	*p*-value	Estimated Hazard Ratio	95% CI
2HG at CR (>200ng/ml)	2.38	3.39	0.02	5.01	1.32 - 18.93
Cytogenetic Risk Group	1.66	0.98	0.09	1.15	0.97 - 1.36
Treatment Group	0.6	1.04	0.55	1.5	0.39 – 5.82

Table S3: Multivariable Cox Regression Analysis in NPM1-Mutant Patients (n=106)

Variable	Estimated Coefficient	Standard Error	*p*-value	Estimated Hazard Ratio	95% CI
2HG at Dx (>700ng/ml)	-2.49	0.14	<0.01	0.42	0.22-0.77
Age	1.58	0.16	0.11	1.02	0.99-1.06
Cytogenetic Risk Group	0.48	0.06	0.63	1.03	0.92-1.16
Treatment Group	-2.49	0.14	0.01	0.51	0.29-0.86

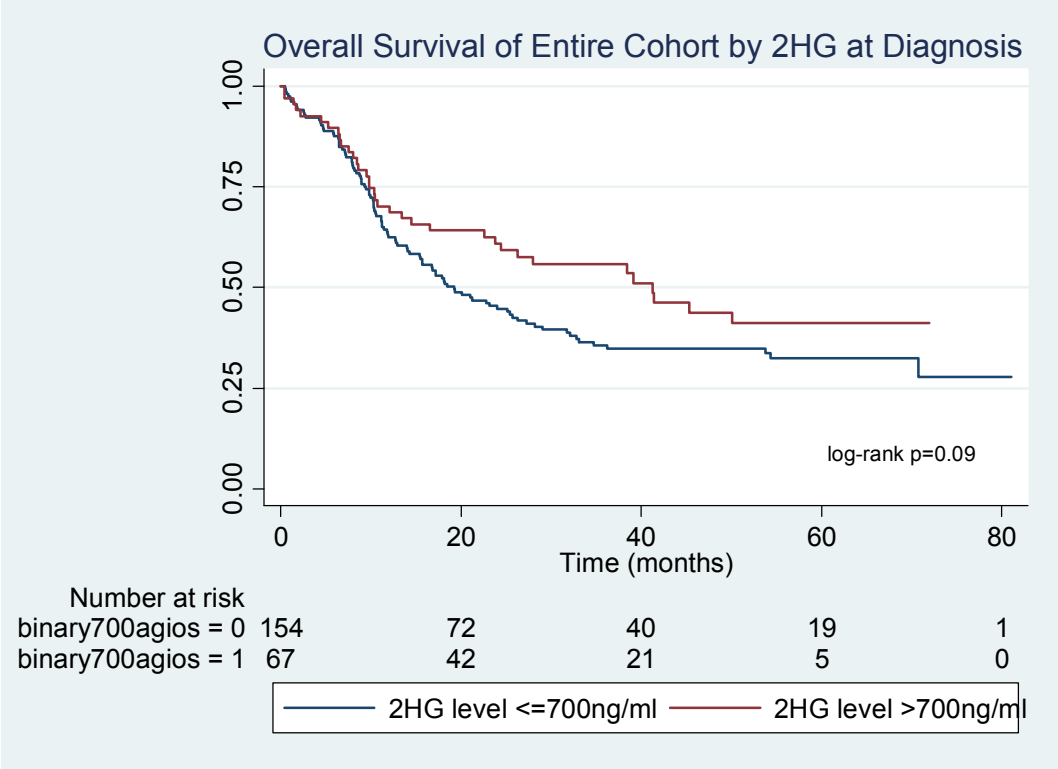
Table S4: Multivariable Cox Regression Analysis in DNMT3A wild-type Patients (n=144)

Variable	Estimated Coefficient	Standard Error	*p*-value	Estimated Hazard Ratio	95% CI
2HG at Dx (>700ng/ml)	-2.91	0.13	0.01	0.48	0.26-0.84
Age	1.04	0.01	0.29	1.01	0.99-1.03
Cytogenetic Risk Group	2.23	0.04	0.03	1.09	1.01-1.18
Treatment Group	-1.16	0.17	0.25	0.77	0.49-1.20

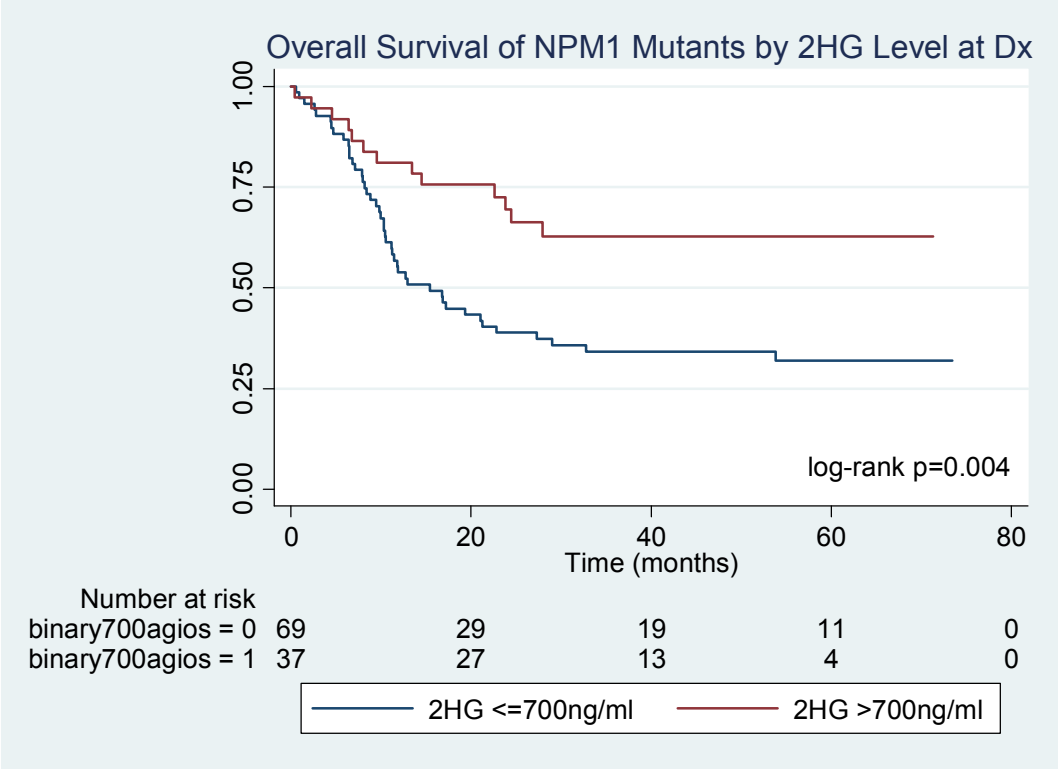
Table S5: Multivariable Cox Regression Analysis in CEBPA wild-type Patients (n-198)

Variable	Estimated Coefficient	Standard Error	*p*-value	Estimated Hazard Ratio	95% CI
2HG at Dx (>700ng/ml)	-2.57	0.12	0.02	0.63	0.43-0.94
Age	1.43	0.01	0.15	1.01	0.99-1.03
Cytogenetic Risk Group	1.28	0.03	0.20	1.04	0.98-1.11
Treatment Group	-3.13	0.10	<0.01	0.57	0.40-0.81

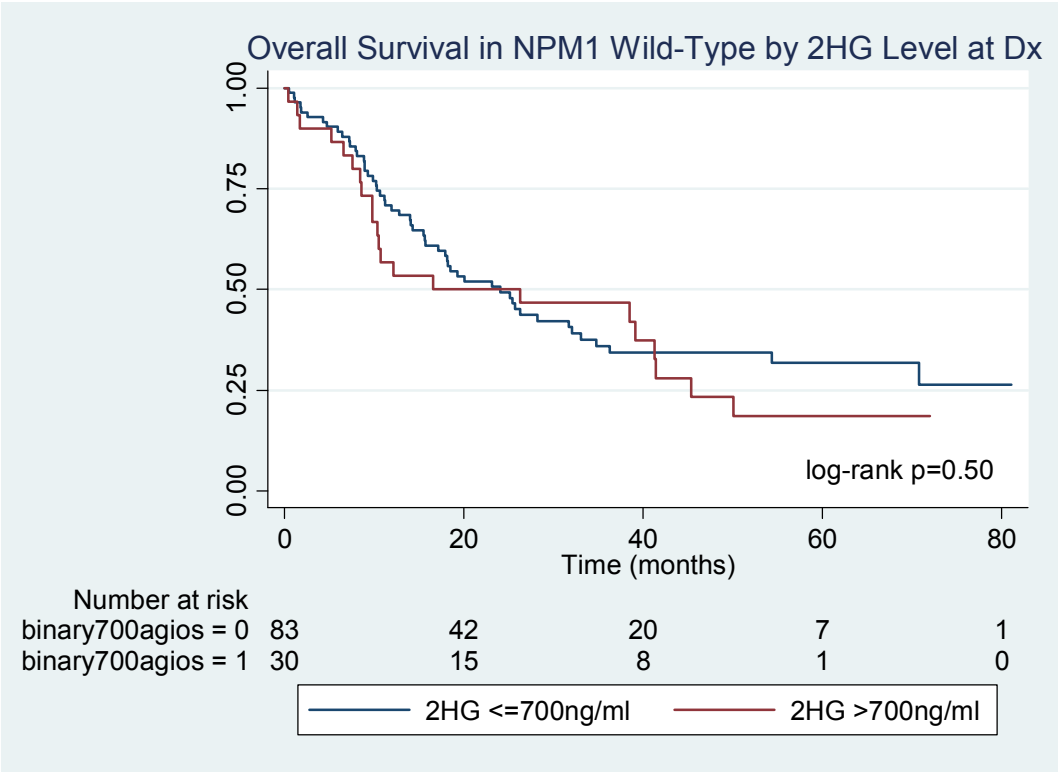
Supplementary Figure S1: Overall survival of entire 2HG test cohort by 2HG level at AML diagnosis



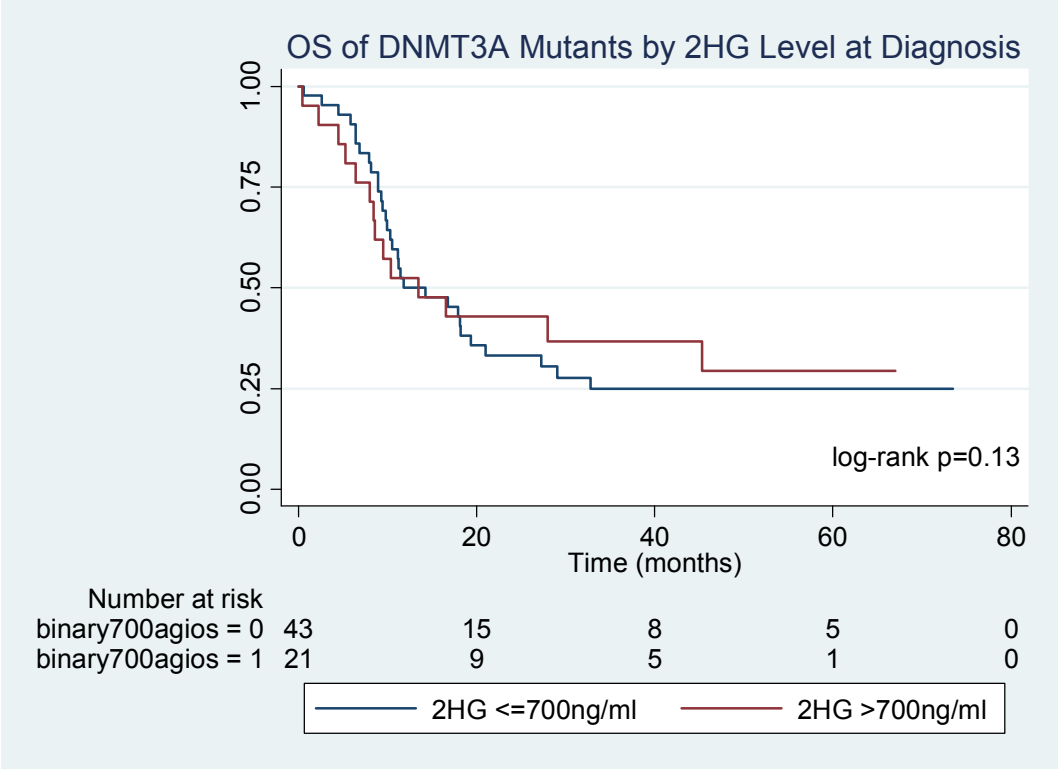
Supplementary Figure S2: Overall survival of NPM1 mutant patients by 2HG level at AML diagnosis



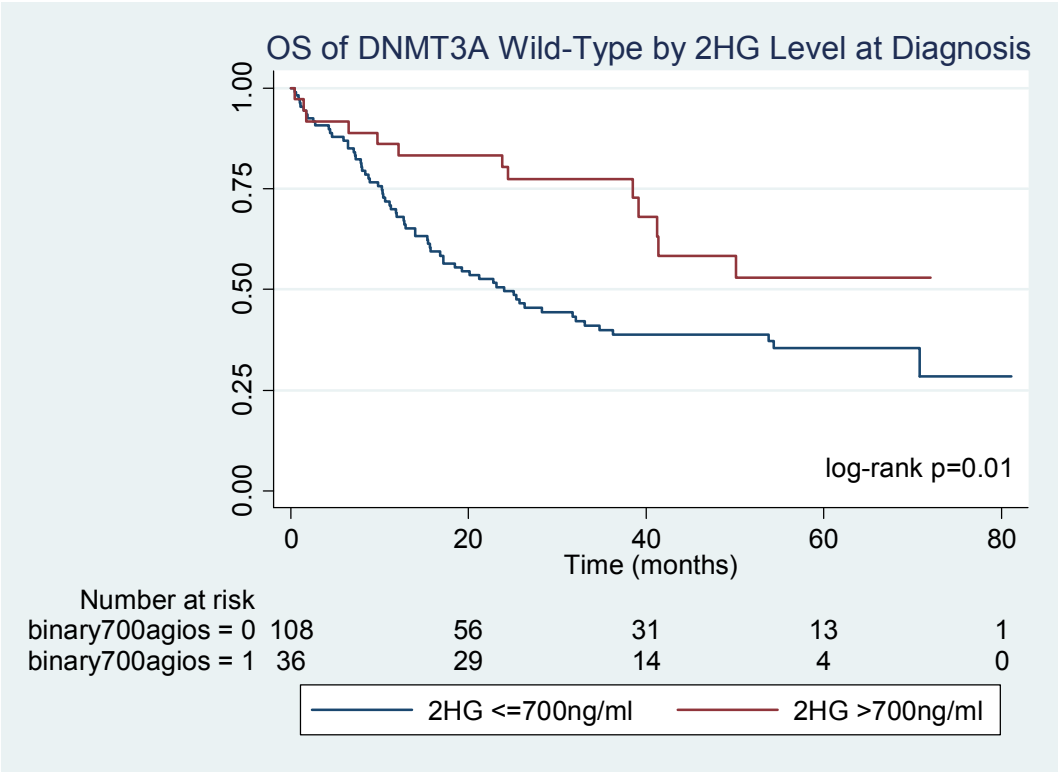
Supplementary Figure S3: Overall survival in NPM1 wild-type patients by 2HG level at AML diagnosis



Supplementary Figure S4: Overall survival of DNMT3A mutants by 2HG level at AML diagnosis



Supplementary Figure S5: Overall survival of DNMT3A wild-type patients by 2HG level at AML diagnosis



Supplementary Figure S6: Overall survival in CEBPA wild-type patients by 2HG level at AML diagnosis