**Supplemental Material**

**Genotype and intensive pre-treatment influence outcome of acute myeloid leukemia patients treated with venetoclax in combination with hypomethylating agents or low-dose cytarabine: “Real world” data from Germany**

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**Supplemental Methods**

Patient population and data collection

We reviewed medical charts of AML patients treated between 2017 and 2021 at two university hospital sites and two large outpatient clinics in Berlin and Munich (Germany) and identified n=73 patients who had received at least one venetoclax (VEN) dose in combination with hypomethylating agents (HMA) or low-dose cytarabine (LDAC). VEN was administered “off-label” in all cases due to pending EMA approval status. Baseline clinical characteristics, previous treatment lines, courses of VEN treatment as well as outcome data were extracted from medical charts and nursing records. Cytogenetics and molecular markers at the time of diagnosis were analyzed in local labs according to standard of practice. Information on cytogenetics, *NPM1* mutations and *FLT3*-ITD status was available for n=66 and n=65 patients, respectively, based on which patients were classified according to ELN2017 risk groups1. We further included *IDH1/2* mutational status in our analysis, which was available for n=57 patients. All patients gave written informed consent to analysis of their records for scientific purposes. Data analysis was granted within the ethical votes TUM 538/16S and EA1/152/10.

Definition of previous treatment lines

Due to the heterogeneity of pre-treatment regimens, we defined the following treatment groups: Intensive chemotherapy including allogeneic stem-cell transplantation (allo-HSCT) in first remission was considered as one treatment line; any salvage treatment due to relapsed/refractory (r/r) disease defined an independent treatment line. HMA or LDAC treatment was considered as an independent treatment line if >4 cycles were given before the addition of VEN. With respect to the heterogeneous time-points of VEN initiation, we distinguished whether VEN was started at day 1, during cycle 1, after cycle 1 or after >2 HMA/LDAC cycles due to disease progression. If VEN was delayed due to health insurance approval, patients with up to 2 cycles of previous HMA exposure and no signs of disease progression were considered as treated in first line.

Venetoclax administration

 Patients were hospitalized at cycle 1 for daily dose escalation of oral VEN up to a standard dose of 400 mg VEN per day. Azacitidine 75 mg/m² was administered subcutaneously from day 1 to day 7. Decitabine was given at a dosage of 20 mg/m² IV day 1 to day 5. LDAC at a dose of 20 mg/m² was applied subcutaneously from day 1 to day 7 or 10. Patients received IV hydration and were monitored for tumor lysis syndrome. After discharge, patients received VEN for durations between 7 to 28 days and were followed-up in outpatient clinics for subsequent treatment cycles. Discontinuation and dose reductions of VEN as well as the combination treatments occurred at the discretion of the treating physician.

Response assessment

 Bone marrow (BM) and peripheral blood (PB) response assessment was performed at the discretion of the treating physician and response based on blast counts was defined according to standard criteria1. Response evaluation was available for n=58 patients; all other patients had no documented response assessment available due to the retrospective nature of our study.

Statistical analysis

All statistical tests were performed using GraphPad Prism (Version 9.2.0) and SPSS (IBM SPSS Statistics 27). *P*-values <0.05 were considered statistically significant. Comparison of survival curves were performed using the Log-rank (Mantel-Cox) test and chi-squared test was used to compare quantitative data between two samples. Multivariate analyses were performed using Cox proportional hazards regression and logistic regression. Due to the retrospective analysis, sample size was chosen based on the number of available patients treated in the specified time frame.

**Supplemental References**

1. Döhner H, Estey E, Grimwade D, et al. Diagnosis and management of AML in adults: 2017 ELN recommendations from an international expert panel. *Blood*. Jan 26 2017;129(4):424-447. doi:10.1182/blood-2016-08-733196

**Supplemental Figures**

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**Supplemental Figure 1: Overall response rates:** Overall response rate (ORR) with respect to clinical parameters and pre-treatment regimens in n=58 patients with evaluable response. ORR was not significantly affected by age and prior treatment, but better in patients harboring a *NPM1* and/or *IDH1/2* mutations. (ns=not significant; \*\*\*p<0,01).

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**Supplemental Figure 2: Multivariate analysis of overall survival:** Forest plot of multivariate analysis showing hazard ratios (HR) and 95% confidence interval (CI) between overall survival (OS) and indicated variables on the left. Cox regression analysis shows a significantly better OS in patients <65 years, patients without previous allogeneic stem-cell transplantation (allo-HSCT) and *IDH1/2* and/or *NPM1* mutated patients. Previous treatment with hypomethylating agents (HMA) and more treatment lines had no significant impact on OS.



**Supplemental Figure 3: Survival curves:** Overall survival (OS) in univariate analysis was not influenced by (A) venetoclax combination partner, (B) previous therapy with hypomethylating agents (HMA) or (C) number of previous HMA cycles. OS was significantly influenced by (D) previous intensive treatment. (LDAC = low-dose cytarabin)