Supplemental appendix

Prognostic impact of *DDX41* germline mutations in intensively treated acute myeloid leukemia patients: an ALFA-FILO study

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Abbreviations

AML: acute myeloid leukemia
APL: acute promyelocytic leukemia
CBF: core binding factor
CR: complete remission
CRi: complete remission with incomplete recovery
CRp: complete remission with incomplete platelet recovery
HDAC: high-dose cytarabine
HSCT: hematopoietic stem cell transplantation
IDAC: intermediate-dose cytarabine
GO: gemtuzumab ozogamicin

Supplemental material: patient's eligibility criteria

ALFA0701 (NCT00927498):

- **Enrollment:** between January 2008 and November 2010.
- **Eligibility criteria:** patients aged 50 to 70 years-old with a previously untreated diagnosis of *de novo* AML (except APL).
- **Induction course:** patients were randomized to receive a "3+7" induction course with daunorubicin (60 mg/m^2/d on days 1 to 3) and cytarabine (200 mg/m^2/d on days 1-7) without (control group) or with GO (3 mg/m^2/d on days 1, 4 and 7).
- **Post remission therapy:** patients in CR/CRp received two consolidation courses of daunorubicin (60 mg/m^2 for 1 day [first course] or 2 days [second course]) in combination with cytarabine (1 g/m^2/12 h on days 1–4), with or without GO (3 mg/m^2 on day 1).
- Patients with > 10% bone marrow blasts at day 15 could receive a second induction course was given with daunorubicin (60 mg/m^2/d for 2 days) and cytarabine (1 g/m^2/12 h for 3 days) without GO.
- **HSCT eligibility:** patients in CR/CRp with intermediate- or adverse-risk AML according to the ELN-2010 classification were eligible for allogeneic HSCT if they had a compatible sibling or a 10/10 HLA-matched unrelated donor.

ALFA0702 (NCT00932412):

- **Enrollment:** between March 2009 and September 2013.
- **Eligibility criteria:** patients aged 18 to 59 years-old with a previously untreated diagnosis of *de novo* AML (except APL and CBF AML).
- **Induction course:** all patients received timed sequential induction chemotherapy with daunorubicin (60 mg/m^2/d on days 1 to 3 and 35 mg/m^2/d on days 8 and 9) and cytarabine (500 mg/m^2/d on days 1 to 3 and 1 g/m^2/12h on days 8 to 10).
- **Post remission therapy:** patients achieving CR/CRp with favorable-risk AML (*CEBPA* or *NPM1* mutation without *FLT3*-ITD) were planned to receive three HDAC courses. Patients with intermediate- or unfavorable-risk AML who were not eligible to HSCT were randomly assigned to either HDAC courses (3 g/m^2/12h on days 1, 3, and 5) or CLARA (clofarabine 30 mg/m^2/d on days 2 to 6 and cytarabine 1 g/m^2/d on days 1 to 5) consolidation cycles.
- **HSCT eligibility:** patients in CR/CRp with intermediate- or unfavorable-risk AML were eligible for allogeneic HSCT if they had a compatible sibling or a 10/10 HLA-matched

unrelated donor. Additionally, patients randomly assigned for HDAC/CLARA post-remission therapy with a late donor identification could receive HSCT in both arms.

ALFA1200 (NCT01966497):

- **Enrollment:** between September 2012 and June 2016.
- **Eligibility criteria:** patients aged 60 years or older with a previously untreated diagnosis of AML (except APL or AML evolving from myeloproliferative neoplasms).
- **Induction course:** all patients received a "3+7" induction course with idarubicin (12 mg/m^2/d on days 1-3) and cytarabine (200 mg/m^2/d on days 1-7).
- **Post remission therapy:** patients achieving CR/CRp received 2 IDAC courses (1.5 g/m^2/12h on days 1, 3, and 5; reduced to 1 g/m^2/12h for patients aged 70 years or older).
- Patients not achieving CR/CRp could receive the first IDAC course as a salvage therapy.
- **HSCT eligibility:** patients in CR/CRp with intermediate- or adverse-risk AML according to the ELN-2010 classification were eligible for allogeneic HSCT if they had a compatible sibling or a 9-10/10 HLA-matched unrelated donor.

ALFA1401 (NCT02473146):

- **Enrollment:** between January 2016 and March 2019.
- **Eligibility criteria:** patients aged 60 years or older with a previously untreated diagnosis of *de novo* AML (except APL) and a favorable or intermediate cytogenetic risk.
- **Induction course:** patients were randomized to receive an experimental GO-cytarabine combination or a standard anthracycline-cytarabine treatment. The standard arm consisted in a "3+7" induction course with idarubicin (12 mg/m^2/d on days 1-3) and cytarabine (200 mg/m^2/d on days 1-7). The GO arm consisted of two doses of GO (3 mg/m^2/d on days 1 and 4) and cytarabine (200 mg/m^2/d on days 1-7).
- **Post-remission therapy:** patients achieving CR/CRp/CRi received two courses of IDAC (1.5 g/m^2/12h on days 1, 3 and 5). In the GO arm, a third dose of GO (3 mg/m^2/d) was administered on day 1 of the first IDAC course.
- Patients not achieving CR/CRp/CRi could receive the first IDAC course as a salvage therapy.
- **HSCT eligibility:** the decision to perform allogeneic HSCT in first remission was left to the discretion of the physician.

LAM-SA 2007 (NCT00590837):

- **Enrollment:** between February 2008 and December 2011.
- **Eligibility criteria:** patients aged 60 years or older with a previously untreated diagnosis of *de novo* AML (except APL and isolated granulocytic sarcoma) and a favorable or intermediate cytogenetic risk. Patients who had previous chemotherapy or radiotherapy for another cancer were allowed.
- **Induction course:** patients were randomized to receive therapy with or without lomustine. Induction therapy consisted of idarubicin (8 mg/m^2/d on days 1 to 5) and cytarabine (100 mg/m^2/d on days 1 to 7) with or without lomustine (200 mg/m^2 on day 1).
- **Post-remission therapy:** patients achieving CR/CRi received a first consolidation with idarubicin (8 mg/m^2/d on days 1 to 3) and cytarabine (50 mg/m^2/12h on days 1 to 5) with or without lomustine (80 mg on day 1). In patients who were not eligible to HSCT, this was followed by 6 reinduction courses with reduced doses of idarubicin (8 mg/m^2 on day

1) and cytarabine (50 mg/m^2/12h on days 1 to 5) with or without lomustine (40 mg on day 1).

- Patients not achieving CR/CRi were not allowed to receive a second induction course and were treated at the discretion of local investigators.
- **HSCT eligibility:** patients in CR/CRi without a favorable molecular profile (according to *NPM1* and *FLT3*-ITD) were eligible for allogeneic HSCT if they had a compatible sibling or a 10/10 HLA-matched unrelated donor.

Supplemental material: 67-gene panel

Complete coding regions except otherwise stated: *ANKRD26* (5'UTR), *ASXL1* (e11-e12), *ASXL2* (e11-e12), *ATRX*, *BCOR*, *BCORL1*, *BRAF* (e11,e15), *CALR* (e09), *CBL* (e08-e09), *CEBPA*, *CRLF2* (e06), *CSF3R* (e14-e17), *CUX1*, *DDX41*, *DNMT3A*, *ETNK1*, *ETV6*, *EZH2*, *FLT3*, *GATA1* (e02-e03), *GATA2*, *GNAS* (e08-e09), *GNB1* (e05-e06), *HRAS* (e02-e04), *IDH1*, *IDH2*, *IKZF1*, *IL2RG* (e08), *IL7R* (e06), *JAK1*, *JAK2*, *JAK3*, *KDM6A*, *KIT* (e08-e11,e17), *KRAS* (e02-e04), *MPL*, *NF1*, *NFE2*, *NPM1* (e10-e11), *NRAS* (e02-e04), *PAX5*, *PHF6*, *PPM1D*, *PTPN11*, *RAD21*, *RIT1* (e05), *RUNX1*, *SAMD9*, *SAMD9L*, *SETBP1*, *SF3B1* (e13-e16), *SH2B3*, *SMC1A*, *SMC3*, *SRP72*, *SRSF2* (e01), *STAG2*, *STAT3*, *STAT5B*, *TERC*, *TERT*, *TET2*, *TP53*, *U2AF1* (e02,e06), *UBA1* (e03), *WT1* and *ZRSR2*.

Supplemental table 1. Application of the ACMG/AMP guidelines for *DDX41* variants interpretation.
See additional file.

Supplemental table 2. *DDX41* variants retained as Causal in the Clinical trial cohort.
See additional file.

Supplemental table 3. *DDX41* variants retained as VUS in the Clinical trial cohort.
See additional file.

Supplemental table 4. Interpretation of *DDX41* variants in the Real-life cohort.
See additional file.

Supplemental table 5. Bivariate analysis for response to induction.
See additional file.

Supplemental table 6. Multivariate analysis for response to induction.
See additional file.

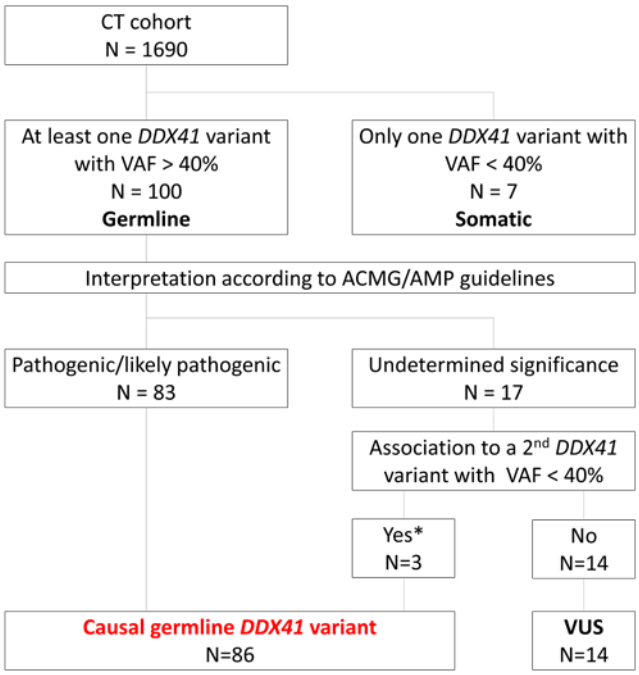
Supplemental table 7.Multivariate RMST analyses.
See additional file.

Supplemental Table 8. Subgroup RMST analyses.
See additional file.

Supplemental table 9. Characteristics of patients achieving HSCT in first CR.
See additional file.

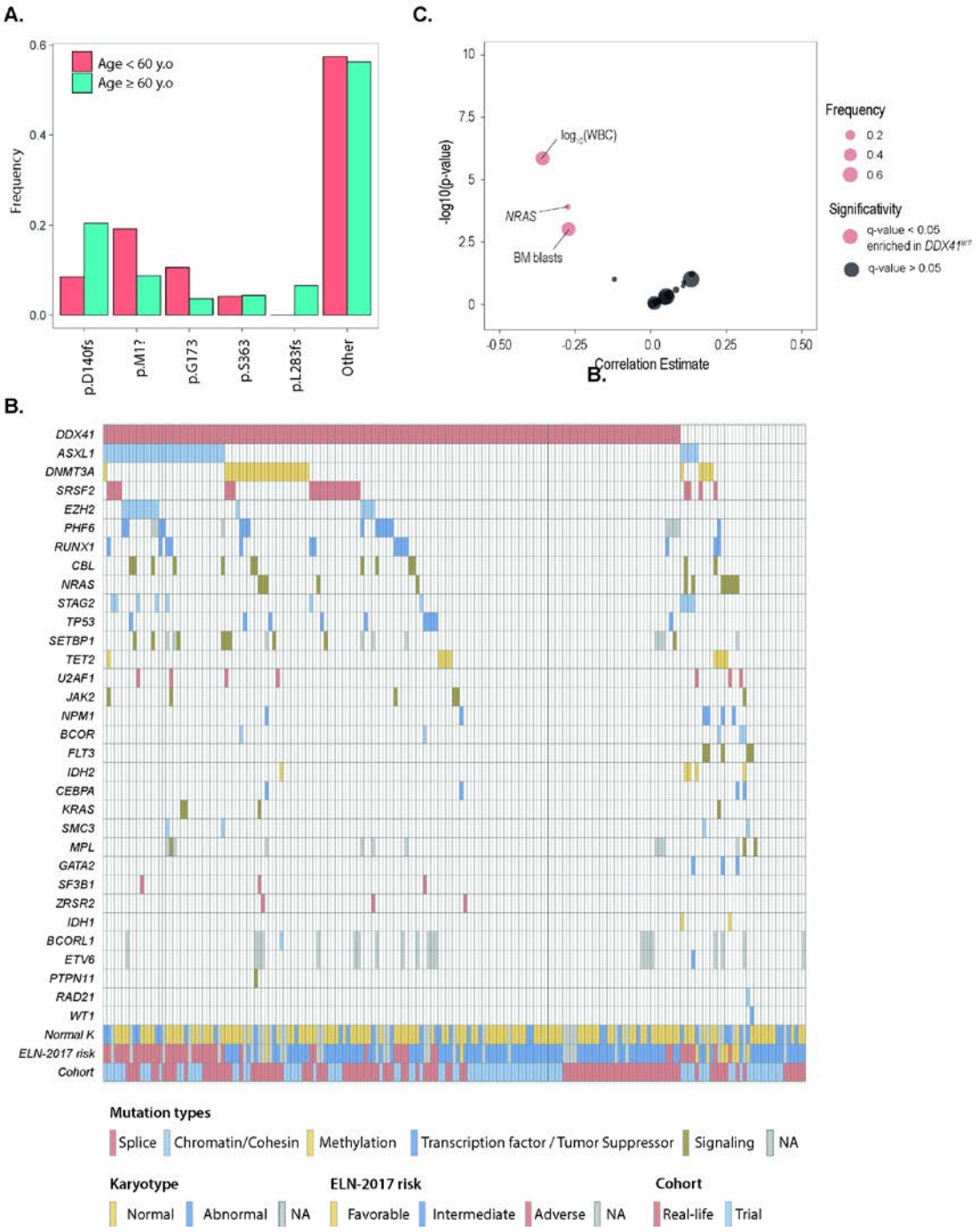
Supplemental table 10. AML with recurrent genetic abnormalities and *DDX41*^{MutGL} mutations.
See additional file.

Supplemental figure 1: Interpretation of *DDX41* variants in the CT cohort.

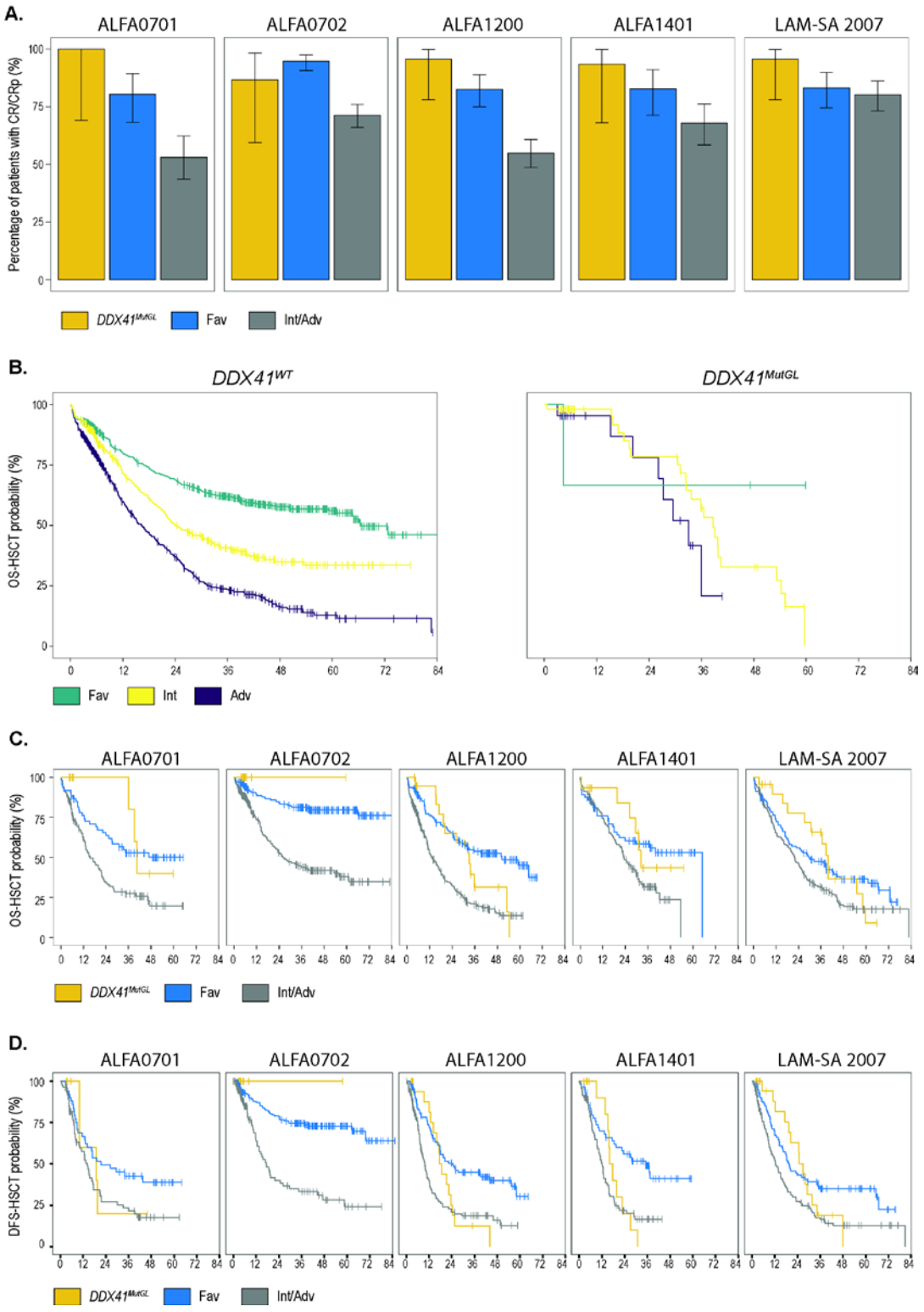


* All 3 patients harbored the same DDX41 p.G173R variant

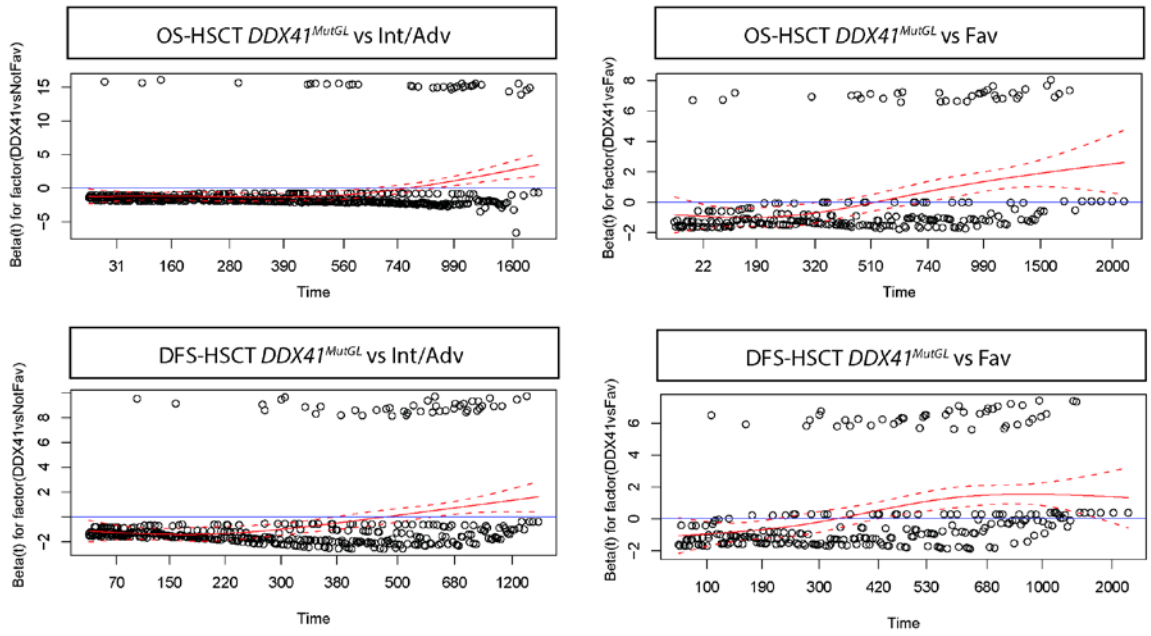
Supplemental figure 2: Genetic characteristics of *DDX41*^{MutGL} AML.
(A) Frequency of the most frequent germline *DDX41* variants between younger (< 60y) and older (≥ 60y) patients. (B) Molecular landscape of the 191 *DDX41*^{MutGL} AML at diagnosis. (C) Volcano plot representing the association between *DDX41*^{MutGL} variants and biological and mutational covariates (estimate of the point-biserial correlation [continuous variables] or F [dichotomous variables] on the x-axis) and the significance of the difference, expressed on an inverted logarithmic scale on the y-axis. The P values were calculated using the Mann-Whitney U test (continuous variables) or Fisher's exact (dichotomous) test. The size of the circle corresponds to the frequency of the variable in the cohort. For statistical power consideration we used only variables with frequency > 1% in the whole cohort (i.e. > 15 patients).



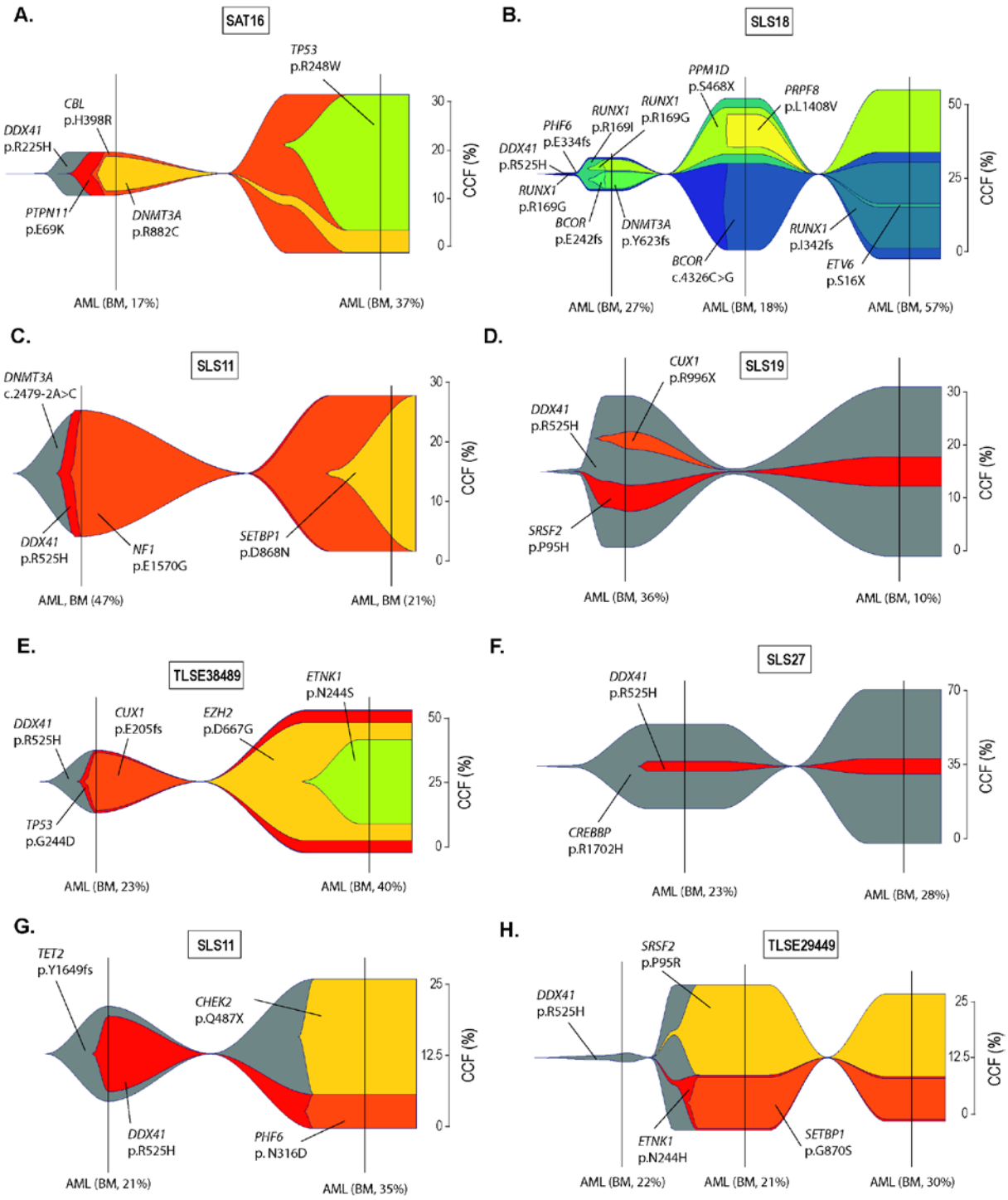
Supplemental figure 3: Outcome of patients with *DDX41*^{MutGL} compared to *DDX41*^{WT} AML. (A) CR/CRp rates after one induction course in *DDX41*^{MutGL} compared to *DDX41*^{WT} (Fav, Int/Adv) AML patients according to the trial. P-values from the bivariate regression for response are reported. Error bars represent the 95% confidence intervals calculated with the exact method. (B) OS censored at HSCT in CR1 in patients with *DDX41*^{MutGL} and *DDX41*^{WT} according to the ELN-2017 classification. (C) OS and (Dà DFS censored at HSCT in CR1 in patients with *DDX41*^{MutGL} (yellow) versus *DDX41*^{WT} ELN-2017 favorable (blue) and *DDX41*^{WT} ELN-2017 intermediate/adverse (grey) according to the trial.



Supplemental figure 4: Scaled Schoenfeld residuals plot versus time for the Cox proportional hazards model



Supplemental figure 5: Fishplots visualizing patterns of clonal evolution in relapse patients as inferred by CALDER algorithm.



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