DNMT3A mutation is associated with increased age and adverse outcome in adult T-cell acute lymphoblastic leukemia

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Received: May 24, 2018.
Accepted: January 10, 2019.
Pre-published: January 17, 2019.
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SUPPLEMENTARY MATERIAL

*DNMT3A* mutation is associated with increased age and adverse outcome in adult T-acute lymphoblastic leukemia

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Supplementary Methods:

The GRAALL-2003 and GRAALL-2005 studies: The GRAALL-2003 study was a multicenter Phase II trial, which enrolled 76 adults with T-ALL between November 2003 and November 2005. The multicenter randomized GRAALL-2005 study was the following Phase III trial, with the addition of a randomized evaluation of an intensified sequence of hyper-fractionated cyclophosphamide during induction and late intensification. 261 adults with T-ALL were enrolled in this study between May 2006 and September 2011. Informed consent was obtained from all patients at trial entry. Both studies were conducted in accordance with the Declaration of Helsinki and approved by local and multicenter research ethical committees. The complete study protocols are detailed in the Supplementary File ‘GRAALL-2003_2005 supplementary protocol’. Both trials were registered at http://www.clinicaltrials.gov as #NCT00222027 and #NCT00327678, respectively. With a data cut-off date on June 2015, the median follow-up was 5.7 years (6.0 and 5.4 years for GRAALL-2003 and GRAALL-2005 patients, respectively).
### Supplementary Table S1: Characteristics of the study patients and the remainder of the T-ALL cohort in the GRAALL-2003 and GRAALL-2005 studies.

|                          | Non-included cohort | Study cohort | p-value |
|--------------------------|---------------------|--------------|---------|
| Total, no.               | 139                 | 198          |         |
| GRAALL 2003/05 trial    | 40/99               | 36/162       | 0.025   |
| Sex ratio (Male/ Female) | 98/41               | 141/57       | 0.903   |
| Median age, y [Q1-Q3]    | 34.1 [25.5-47.2]    | 30.5 [23.4-40.4] | 0.022   |
| Median WBC, 10^9/L [Q1-Q3] | 16.7 [7.4-59.9]       | 32.6 [11.9-103.9] | 0.0001  |
| CNS involvement, no. (%) | 11 (7.9%)           | 24 (12.1%)   | 0.143   |
| CR, no. (%)              | 132 (95.0%)         | 183 (92.4%)  | 0.243   |
| CS, no. (%)              | 97 (69.8%)          | 108 (54.5%)  | 0.003   |
| CHS, no. (%)             | 76/134 (56.7%)      | 110/195 (56.4%) | 1.000   |
| SCT, no. (%)             | 39 (28.1%)          | 72 (36.4%)   | 0.126   |
| EFS at 5 years, % [95% CI] | 54.3% [45.6-62.3]   | 58.0% [50.6-64.5] | 0.606   |
| OS at 5 years, % [95% CI] | 61.5% [52.8-69.1]   | 66.0% [58.8-72.2] | 0.474   |

No: number; Q: Quartile; WBC: white blood cell count; CNS: central nervous system; CR: complete remission; CS: cortico-sensitive; CHS: chemo-sensitive; SCT: stem-cell transplantation; DFS: Disease-Free Survival; EFS: Event-Free Survival; OS: Overall Survival.
Supplementary Table S2: Primers used for direct sequencing of *DNMT3A*.

| Exon | Forward Primer                     | Reverse Primer                     |
|------|------------------------------------|------------------------------------|
| 7    | GAATGCTGTGGAAGAAAACCAG             | ATTCTTGTCCCCAGCATCG                |
| 8    | GCCTCGTGCACCACCTGTAATG             | CACTGAGAATTGCCGTCTCC               |
| 10   | GAGCCTGACCCATCTGCTTT              | CTTCTGGTG GCCAAGCC                |
| 14   | GCTTTCTGGAGTGTCGTACCA             | CAAGGTGCTACCTGGAATGG              |
| 15   | CAGACCCGCTCTTCCATTCC              | CGAAGAACATCTGGAGCCGG              |
| 19   | CTATGCAAGACAGCCCCAGCT             | ATCGCGAGATGTCCCTCTTG              |
| 23   | CGTGTCTGGCCAGCCTCAC              | CTTTGTCGTACCTCAGTTTG              |
# Supplementary Table S3: Details of *DNMT3A* mutations detected in this study.

| Case | Age (y) | Exon | Type        | Mutation       | Amino Acid | SIFT score | VAF (%) |
|------|---------|------|-------------|----------------|------------|------------|---------|
| 1    | 56.2    | 7    | Nonsense    | c.745C>T       | p.Q249*    | NA         | 40      |
| 1    | 56.2    | 14   | Missense    | c.1627G>T      | p.G543C    | 0.000      | 46      |
| 2    | 44.9    | 8    | Nonsense    | c.918G>A       | p.W306*    | NA         | 44      |
| 3    | 40.9    | 9    | Missense    | c.1117C>G      | p.L373V    | 0.013      | 18      |
| 4    | 42.8    | 10   | Missense    | c.1154C>T      | p.P385L    | 0.025      | 46      |
| 5    | 50.0    | 14   | Missense    | c.1628G>T      | p.G543V    | 0.000      | 59      |
| 6    | 40.3    | 14   | Missense    | c.1643T>C      | p.M548T    | 0.023      | 45      |
| 6    | 40.3    | 15   | Frameshift  | c.1688_1689delTG | p.V563GfsX14 | 0.000      | 43      |
| 7    | 50.9    | 14   | Missense    | c.1645T>C      | p.C549R    | 0.017      | 44      |
| 7    | 50.9    | 20   | Frameshift  | c.2383delT     | p.W795GfsX7 | 0.000      | 41      |
| 8    | 40.5    | 15   | Missense    | c.1687G>A      | p.V563M    | 0.197      | 50      |
| 9    | 42.9    | 16   | Missense    | c.1903C>T      | p.R635W    | 0.000      | 81      |
| 10   | 50.4    | 18   | Frameshift  | c.2153delC     | p.P718LfsX61 | 0.006      | 94      |
| 11   | 41.5    | 19   | Missense    | c.2185C>T      | p.R729W    | 0.111      | 84      |
| 12   | 59.0    | 22   | Missense    | c.2596A>T      | p.R866W    | 0.000      | 78      |
| 13   | 26.7    | 23   | Missense    | c.2644C>T      | p.R882C    | 0.000      | 38      |
| 14   | 40.8    | 23   | Missense    | c.2645G>A      | p.R882H    | 0.002      | 47      |
| 15   | 53.5    | 23   | Missense    | c.2644C>T      | p.R882C    | 0.000      | 46      |
| 16   | 58.1    | 23   | Missense    | c.2644C>T      | p.R882C    | 0.000      | 58      |
| 17   | 53.9    | 23   | Missense    | c.2645G>A      | p.R882H    | 0.002      | 30      |
| 18   | 36.4    | 23   | Missense    | c.2645G>A      | p.R882H    | 0.002      | 89      |

Age in years is shown. VAF: Variant Allele Frequency. All nucleotide changes were verified to have not been reported as single nucleotide polymorphisms (SNPs) in reference SNP databases, namely dbSNP (https://www.ncbi.nlm.nih.gov/SNP/) and Ensembl (http://www.ensembl.org/info/genome/variation/index.html). SIFT scores were calculated at the Provean website (http://provean.jcvi.org/index.php). All amino acid changes were predicted to be damaging, apart from the V563M and R729W substitutions, both of which have SIFT scores at the lowest range of predicted tolerability.
Supplementary Table S4: Bivariate analysis of CIR, EFS and OS including age and DNMT3A genotype as covariates.

|     | Age          | DNMT3A genotype |     |
|-----|--------------|------------------|-----|
|     | HR           | 95% CI           | p   | HR           | 95% CI           | p   |
| CIR | 0.99         | 0.96 - 1.02      | 0.432| 2.80         | 1.12 - 6.97      | 0.027|
| EFS | 1.01         | 0.99 – 1.04      | 0.211| 2.62         | 1.35 – 5.06      | 0.004|
| OS  | 1.03         | 1.00 - 1.05      | **0.034**| 2.05         | 1.02 - 4.12      | **0.043**|

CIR: Cumulative Incidence of Relapse. EFS: Event-free Survival. OS: Overall Survival. HR: Hazard Ratio. CI: Confidence Interval. Statistically significant results are shown in bold.
Supplementary Table S5: Bivariate analysis for prognostic impact of *DNMT3A* genotype and oncogenetic risk classifier on cumulative incidence of relapse (CIR), EFS, and OS.

|              | CIR          | EFS          | OS           |
|--------------|--------------|--------------|--------------|
|              | HR  | 95% CI | p  | HR  | 95% CI | p  | HR  | 95% CI | p  |
| *DNMT3A* mut.| 2.11| 0.95 - 4.69 | 0.066 | 2.86| 1.60 - 5.10 | <0.001 | 2.90| 1.55 - 5.42 | 0.001 |
| Risk Classifier* | 2.93| 1.60 - 5.10 | <0.001 | 2.67| 1.71 - 4.16 | <0.001 | 3.02| 1.84 - 4.94 | <0.001 |

*Oncogenetic Risk Classifier based on genotype for *NOTCH1, FBXW7, PTEN, NRAS* and *KRAS*, reported in Trinquand et al J Clin Oncol. 2013 Dec 1;31(34):4333-42.
WBC: White blood cell count.
*continuous variable
Supplementary Figure S1: Analysis of DNMT3A mutations in remission samples.

Direct sequencing confirmed the presence of the mutations c.2645G>A (Case 14) and c.2644C>T (Case 15) in DNMT3A Exon 23 in diagnostic samples. These mutations were not detected in remission samples in either case. The relevant nucleotides are indicated by red arrows. Patient samples are numbered according to the listing in Supplementary Table S3.
2 diagnostic T-ALL samples were separated into leukemic and non-leukemic fractions by immunophenotypic sorting. 1 case had *DNMT3A* mutations in the non-leukemic fractions (see Figure 2). One sample (Case 11 in Supplementary Table S3) had a probable homozygous c.2185C>T substitution in *DNMT3A* Exon 19 in the leukemic fraction only, as shown here. The relevant nucleotides are indicated by red arrows.
Supplementary Figure S3: Consort Diagram

GRAALL-2003 GRAALL-2005  T-ALL

Enrolled patients: n=337
G-2003: T-ALL n=76
G-2005: T-ALL n=261

See Supplementary Table 1 for details of non-included patients

Analysed (material available) : n = 198

DNMT3A mutated
n = 18/198 (9.1%)
G-2003: 1/36 (2.8%)
G-2005: 17/162 (10.5%)
5-year OS 38.8%
5-year EFS, 27.8%

DNMT3A Wild-type
n = 180/198 (90.9%)
G-2003:35/36 (97.2%)
G-2005: 145/162 (89.5%)
5-year OS, 68.7%
5-year EFS, 61.0%