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

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SUPPLEMENTARY MATERIAL

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Contents:

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	GCCTCGTGACCACTGTGTAATG	CACTGAGAATTTGCCGTCTCC
10	GAGCCTGACCCATCTGCCTT	CTTCTGGTGGCTCCAGGCC
14	GCTTTCTGGAGTGTGCGTACCA	CAAGGTGTGCTACCTGGAATGG
15	CAGACCCGGTCTTTCCATTCC	CGAAGAACATCTGGAGCCGG
19	CTATGCAGACAGCCCCAGCT	ATCGCGAGATGTCCCTCTTG
23	CTGGTCTGGCCAGCACTCAC	CTTTGTGTCGCTACCTCAGTTTG

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**

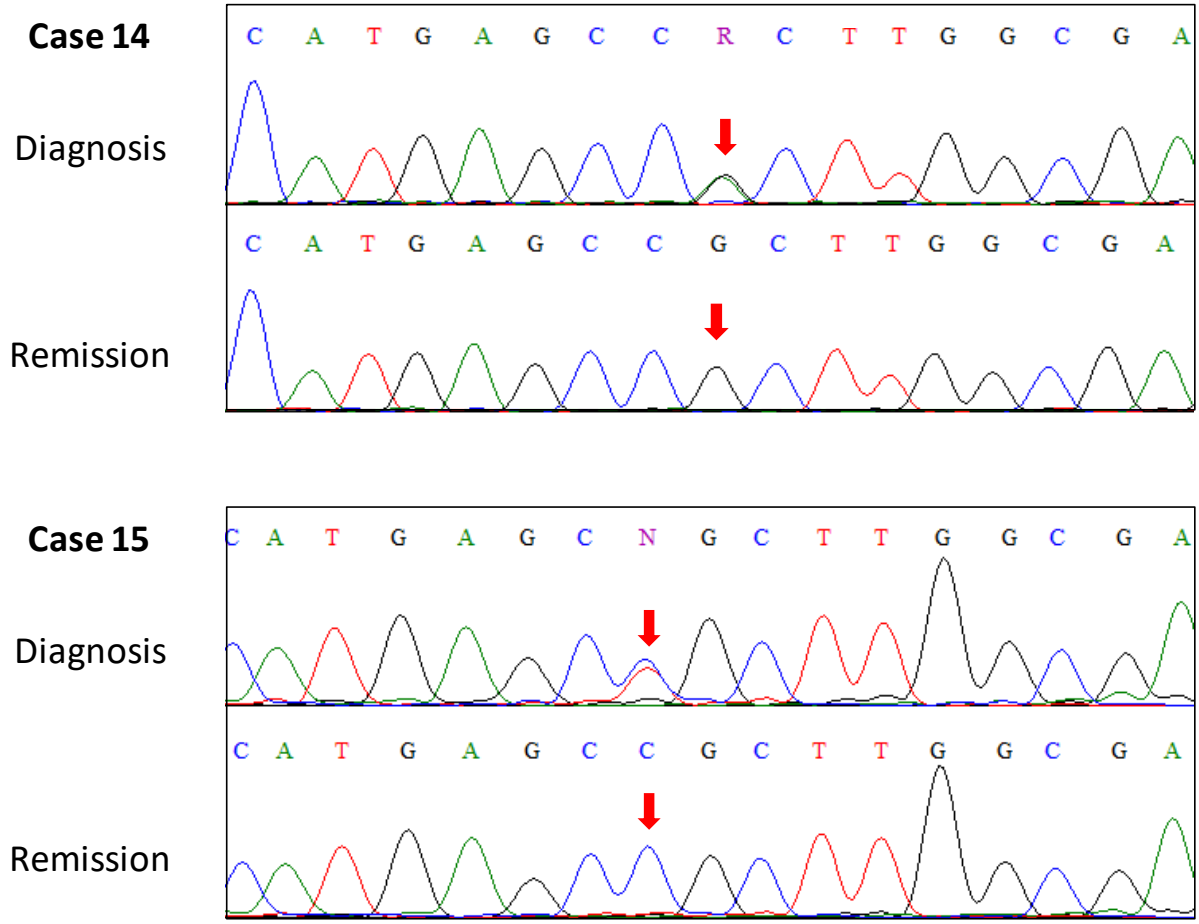
CIR: Cumulative Incidence of Relapse. EFS: Event-free Survival. OS: Overall Survival. HR: Hazard Ratio. CI: Confidence Interval. Statistically significant results are shown in bold.

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	1.68 – 5.10	< 0.001	2.67	1.71 – 4.16	<0.001	3.02	1.84 – 4.94	<0.001

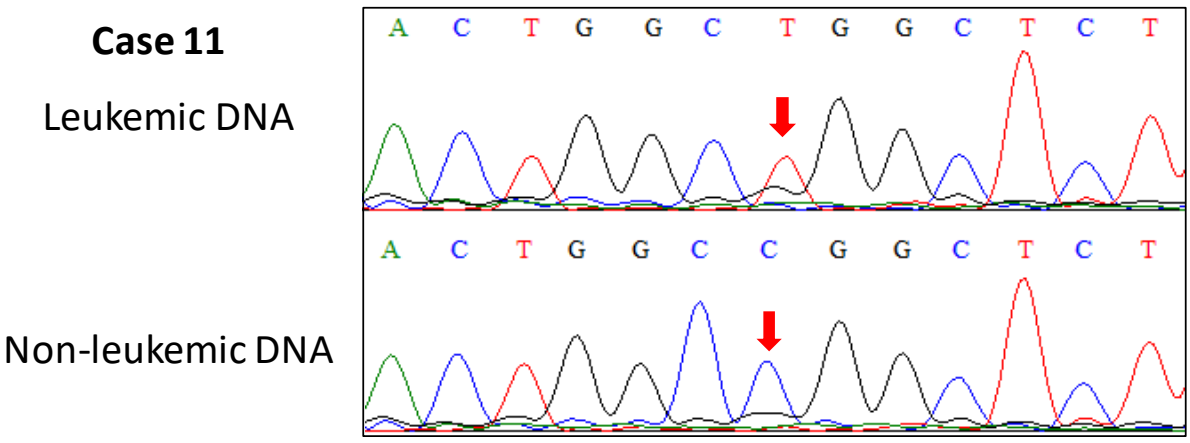
[#] Oncogenetic Risk Classifier based on genotype for *NOTCH1*, *FBXW7*, *PTEN*, *NRAS* and *KRAS,* reported in Trinquand et al J Clin Oncol. 2013 Dec 1;31(34):4333-42.
WBC: White blood cell count.
* continuous variable

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.

Supplementary Figure S2: Analysis of *DNMT3A* mutations in non-leukemic DNA.



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