Frequency, onset and clinical impact of somatic *DNMT3A* mutations in therapy-related and secondary acute myeloid leukemia

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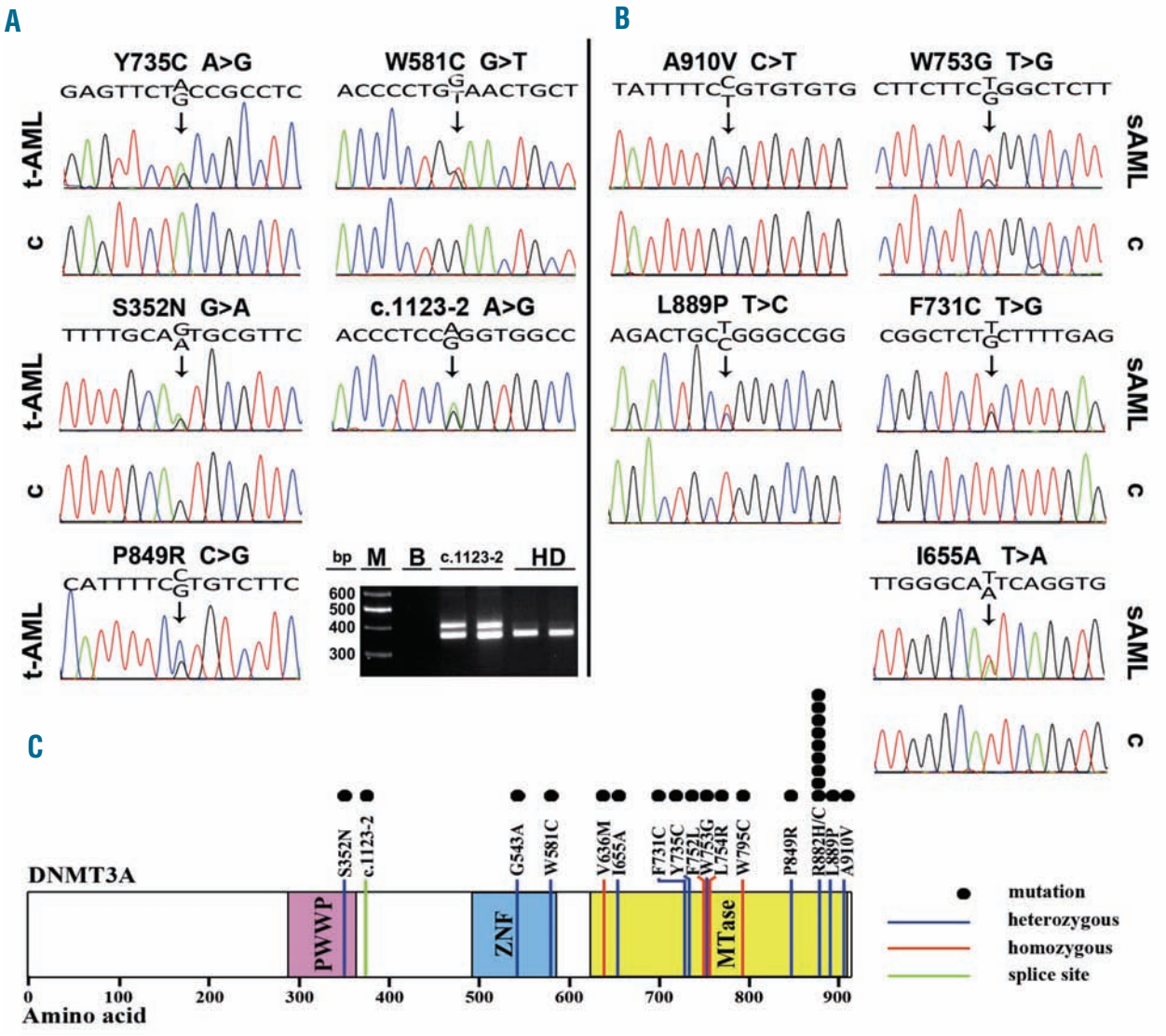

Online Supplementary Figure S1. (A) Electropherograms of newly identified heterozygous *DNMT3A* mutations in t-AML samples and corresponding constitutional material demonstrating the somatic nature of the mutation. The acceptor splice site mutation c.1123-2 resulted in the use of an alternative upstream 3' splice site thereby inserting a 40-bp intronic sequence in between exon 9 and exon 10 in the patient's mRNA (lower left panel; two lanes per sample represent technical replicates). As a consequence, a premature stop codon is introduced that is predicted to lead to a truncated protein with 432 amino acids lacking the zinc finger and methyltransferase domains. (B) Electropherograms of novel heterozygous *DNMT3A* mutation in sAML samples with corresponding constitutional material. L889P and F731C were detected in the same patient. (C) Schematic illustration of all *DNMT3A* mutations found and their relation to the functional domains of the DNMT3A protein. c: constitutional material; bp: base pair; M: marker; B: blank; HD: healthy blood donor as control. PWWP domain: characterized by highly conserved proline-tryptophan-tryptophan-proline motif; ZNF: zinc finger domain; MTase: methyltransferase domain.

Online Supplementary Table S1. DNMT3A DNA and cDNA sequencing primer (Transcript ID: ENST00000264709).

Primer name	Primer Sequence 5´- 3´	Annealing temperature (C°)	Product size (base pairs)
Exon 2 forward	CAGAAGGAGGAGCAACACCC	60	486
Exon 2 reverse	ACCCTGTCGTGAGCACTGAG	60	
Exon 3 forward	GCATATTACACAGCCCTGGAAG	60	360
Exon 3 reverse	GTCTTAAATGTCTCCAGGTCCC	60	
Exon 4 forward	TTATATGAACACAGTTTCTGGACA	60	502
Exon 4 reverse	GACCCAGACCATCCTTCCTG	60	
Exon 5 forward	CAGCTAAACGGCCAGAGGAC	60	283
Exon 5 reverse	GGATGTGTAAAGAAGGAGGAGG	60	
Exon 6 forward	CTGTGGGAAGGAGAGGAAGTG	60	553
Exon 6 reverse	GGCTCCCAGTAAGTTCTAAGGG	60	
Exon 7 forward	CCTTGACCTTCTGTCACTGTTC	60	449
Exon 7 reverse	GGTCAGGTGGAGAGAGCGAG	60	
Exon 8 forward	TCTGTCTTGCCTCATTCAGATG	60	455
Exon 8 reverse	TATCCCTACAGCTTCTTCCACC	60	
Exon 9 forward	CCTCCAGGGCTGAGACTGAC	60	346
Exon 9 reverse	GAGGTGGAGAGAAAGGAGTCG	60	
Exon 10 forward	GCGACTCCTTTCTCTCCACC	60	497
Exon 10 reverse	AGAGAGCAGGTCATTCAAGTCC	60	
Exon 11-12 forward	GCTGGAGTTTCCTGTCAGCC	60	420
Exon 11-12 reverse	AACCTTCCTAAGTGCCTCTGC	60	
Exon 13 forward	GCACCCTGGACTCTTTTCTG	60	281
Exon 13 reverse	GGACACAGTCAGCCAGAAGG	60	
Exon 14-15 forward	CAGGGCTTAGGCTCTGTGAG	60	694
Exon 14-15 reverse	AGGCTCCTAGACCCACACAC	60	
Exon 16 forward	AGGGTGTGTGGGTCTAGGAG	58	286
Exon 16 reverse	TGTGAAGCTAACCATCATTTCG	58	
Exon 17 forward	GACTTGGGCCTACAGCTGAC	58	377
Exon 17 reverse	GGCAAAGGGTGAAGAGAAAG	58	
Exon 18-19 forward	TCTCTTTCTTCCTGTCTGCCTC	60	561
Exon 18-19 reverse	GGATGAAGCAGCAGTCCAAG	60	
Exon 20 forward	TAGAGCAGCACTGTGCAATATG	60	545
Exon 20 reverse	CTATGGGTCATCCCACCTGC	60	
Exon 21 forward	TGTGAACTAGTGGCTGCTGG	60	279
Exon 21 reverse	CACTAGCTGGAGAAGCAGGC	60	
Exon 22 forward	TAGACGCATGACCAGTGTTGG	58	285
Exon 22 reverse	TGGAAAACAAGTCAGGTGGG	58	
Exon 23 forward	TCCTGCTGTGTGGTTAGACG	60	320
Exon 23 reverse	TCTCTCCATCCTCATGTTCTTG	60	
cDNA P forward	CTCGTTTTGCAGTGCGTTC	60	364
cDNA P reverse	CTCCTTGACCTTGGGCTTCT	60	

Online Supplementary Table S2. Comparison of baseline characteristics between patients with and without DNMT3A mutation.

	DNMT3A wild-type	DNMT3A mutated	*P* value
Sex			
female	41 (54.7%)	12 (52.2%)	1.000
male	34 (45.3%)	11 (47.8%)	
Age years (range)	66 (27-87)	67 (51-80)	0.313
WBC $\times 10^9$/L (range)	21.9 (1.0-270.0)	4.85 (0.9-245.0)	0.059
Platelets $\times 10^9$/L (range)	44 (8-304)	66 (20-197)	0.222
Hemoglobin g/dL (range)	9.6 (5.5-12.7)	9.2 (4.0-13.5)	0.362
IDH1/2 status			
mutated	2 (2.7%)	5 (21.7%)	0.007
unmutated	73 (97.3%)	18 (78.3%)	
Treatment*			
standard	32 (51.6%)	11 (55%)	0.999
allo-HSCT	12 (19.4%)	4 (20%)	1.000
palliative	30 (48.6%)	9 (45%)	0.999

** Full data on treatment were available in 82 patients. Patients with standard therapy received at least one cycle of induction chemotherapy with an anthracycline and cytarabine, whereas patients with palliative treatment either received low-dose cytarabine, hydroxyurea, aza-cytidine or best supportive care. Patients who underwent allogeneic hematopoietic stem cell transplantation for consolidation were also counted in the standard treatment group. WBC: white blood cell count; IDH: isocitrate dehydrogenase; allo-HSCT: allogeneic hematopoietic stem cell transplantation.*