ASXL1 mutations in younger adult patients with acute myeloid leukemia: a study by the German-Austrian Acute Myeloid Leukemia Study Group

Peter Paschka,[1] Richard F. Schlenk,[1] Verena I. Gaidzik,[1] Julia K. Herzig,[1] Teresa Aulitzky,[1] Lars Bullinger,[1] Daniela Späth,[1] Veronika Teleanu,[1] Andrea Kündgen,[2] Claus-Henning Köhne,[3] Peter Brossart,[4] Gerhard Held,[5] Heinz-A. Horst,[6] Mark Ringhoffer,[7] Katharina Götze,[8] David Nachbaur,[9] Thomas Kindler,[10] Michael Heuser,[11] Felicitas Thol,[11] Arnold Ganser,[11] Hartmut Döhner,[1] and Konstanze Döhner[1]

[1]Klinik für Innere Medizin III, Universitätsklinikum Ulm, Germany; [2]Klinik für Hämatologie, Onkologie und Klinische Immunologie, Universitätsklinikum Düsseldorf, Germany; [3]Klinik für Onkologie und Hämatologie, Klinikum Oldenburg, gGmbH, Germany; [4]Medizinische Klinik und Poliklinik III, Universitätsklinikum Bonn, Germany; [5]Medizinische Klinik und Poliklinik, Innere Medizin I, Universitätsklinikum des Saarlandes, Homburg, Germany; [6]II. Medizinische Klinik und Poliklinik, Universitätsklinikum Schleswig-Holstein, Kiel, Germany; [7]Medizinische Klinik III, Städtisches Klinikum Karlsruhe gGmbH, Germany; [8]III. Medizinische Klinik, Klinikum rechts der Isar der Technischen Universität München, Germany; [9]Universitätsklinik für Innere Medizin V, Medizinische Universität Innsbruck, Austria; [10]III. Medizinische Klinik und Poliklinik, Universitätsmedizin Mainz, Germany; and [11]Klinik für Hämatologie, Hämostaseologie, Onkologie und Stammzelltransplantation, Medizinische Hochschule Hannover, Germany

 doi:10.3324/haematol.2014.114157
Manuscript received on July 20, 2014. Manuscript accepted on December 31, 2014.
Correspondence: konstanze.doehner@uniklinik-ulm.de

Supplemental Appendix

The following AML Study Group (AMLSG) institutions and investigators participated in this study:

Günter Schlimok M.D., Klinikum Augsburg, Augsburg, Germany; Dietmar Reichert M.D., Ubbo-Emmius Klinik, Aurich, Germany; Peter Reichert M.D., Helios Klinikum, Bad Saarow, Germany; Jörg Westermann M.D., Charité Campus Virchow-Klinikum, Berlin, Germany; Dirk Strumberg M.D., Klinikum-Ruhr Universität, Bochum-Herne, Germany; Wolff Schmiegel M.D., Roland Schroers M.D., Knappschaftskrankenhaus, Bochum-Langendreer, Germany, Peter Brossart, M.D., Marie von Lilienfeld-Toal M.D., Universitätsklinikum Bonn, Bonn Germany; Jens Kersten M.D., Städtisches Klinikum, Braunschweig, Germany; Bernd Hertenstein M.D., Henrike Thomssen M.D., Klinikum Bremen-Mitte, Bremen, Germany; Helga Bernhard M.D., Klinikum Darmstadt, Darmstadt, Germany; Rainer Haas M.D., Andrea Kuendgen M.D., Universitätsklinikum Düsseldorf, Düsseldorf, Germany; Peter Reimer M.D., Mohammed Wattad M.D., Kliniken Essen Süd, Ev. Krankenhaus Essen-Werden gGmbH, Essen, Germany; Carsten Schwaenen M.D., Klinikum Esslingen, Esslingen, Germany; Nadezda Basara M.D., Malteser Krankenhaus St-Franziskus-Hospital, Flensburg, Germany; Elke Jäger M.D., Krankenhaus Nordwest GmbH, Frankfurt, Germany; Hans Martin M.D., Universitätsklinikum Frankfurt, Frankfurt, Germany; Hans Günter Derigs M.D., Klinikum Frankfurt-Höchst GmbH, Frankfurt, Germany; Michael Lübbert M.D., Universitätsklinikum Freiburg, Freiburg, Germany; Alexander Burchardt M.D., Matthias Rummel M.D., Universitätsklinikum Gießen, Gießen, Germany; Volker Runde M.D., Wilhelm-Anton-Hospital, Goch, Germany; Gerald Wulf M.D., Lorenz Trümper M.D., Universitätsklinikum Göttingen, Göttingen, Germany; Walter Fiedler M.D., Universitätsklinikum Hamburg-Eppendorf, Hamburg, Germany; Hans Salwender M.D., Asklepios Klinik Altona, Hamburg, Germany; Elisabeth Lange M.D., Evangelisches Krankenhaus Hamm, Hamm, Germany; Andrea Sendler M.D., Klinikum Hanau, Hanau, Germany; Arnold Ganser M.D., Jürgen Krauter M.D., Brigitte Schlegelberger M.D., Medizinische Hochschule Hannover, Hannover, Germany; Hartmut Kirchner M.D. KRH Klinikum Siloah, Hannover, Germany; Uwe Martens M.D., SLK-Kliniken GmbH Heilbronn, Heilbronn, Germany; Michael Pfreundschuh M.D., Gerhard Held M.D., Universitätsklinikum des Saarlandes, Homburg, Germany; David Nachbaur M.D., Günter Gastl M.D., Universitätsklinikum Innsbruck, Innsbruck, Austria; Mark Ringhoffer M.D., Martin Bentz M.D., Städtisches Klinikum Karlsruhe gGmbH, Karlsruhe, Germany; Heinz A. Horst M.D., Michael

Kneba M.D., Universitätsklinikum Schleswig-Holstein–Campus Kiel, Kiel, Germany; Stephan Kremers M.D., Caritas-Krankenhaus Lebach, Lebach, Germany; Frank Hartmann M.D., Klinikum Lemgo-Lippe, Lemgo, Germany; Andreas Petzer M.D., Krankenhaus der Barmherzigen Schwestern Linz, Linz, Austria; Michael Girschikofsky M.D., Krankenhaus der Elisabethinen Linz, Linz, Austria; Gerhard Heil M.D., Klinikum Lüdenscheid, Lüdenscheid, Germany; Thomas Fischer M.D., Universitätsklinikum Magdeburg, Magdeburg, Germany; Thomas Kindler M.D., Wolfgang Herr M.D., Matthias Theobald M.D., Universitätsklinikum Mainz, Mainz, Germany; Martin Grießhammer M.D., Johannes Wesling Klinikum, Minden Germany; Katharina Götze M.D., Christian Peschel M.D., Klinikum rechts der Isar der Technischen Universität München, München, Germany; Christoph Nerl M.D., Klinikum Schwabing, München, Germany; Holger Hebart M.D., Stauferklinikum Schwäbisch-Gmünd, Mutlangen, Germany; Ali-Nuri Hünerlitürkoglu M.D., Lukaskrankenhaus GmbH Neuss, Neuss; Germany; Claus-Henning Köhne M.D., Klinikum Oldenburg, Oldenburg, Germany; Frank Griesinger M.D., Pius-Hospital Oldenburg, Oldenburg, Germany; Thomas Südhoff M.D., Klinikum Passau, Passau, Germany; Otto Alexander Josef Kloke M.D., Elisabeth-Krankenhaus GmbH, Recklinghausen, Germany; Axel Matzdorff M.D., Caritas-Klinik St. Theresia, Saarbrücken, Germany; Richard Greil M.D., Gudrun Russ M.D., Universitätsklinikum der Paracelsus Medizinischen Universität Salzburg, Salzburg, Austria; Hans-Günther Mergenthaler M.D., Klinikum Stuttgart, Stuttgart, Germany; Else Heidemann M.D., Diakonie-Klinikum Stuttgart, Stuttgart, Germany; Thomas Kubin M.D., Matthias Burkert M.D., Klinikum Traunstein, Traunstein, Germany; Heinz Kirchen M.D., Krankenhaus der Barmherzigen Brüder, Trier, Germany; Rolf Mahlberg M.D., Mutterhaus der Borromäerinnen, Trier, Germany; Helmut Salih M.D., Lothar Kanz M.D., Universitätsklinikum Tübingen, Tübingen, Germany; Hartmut Döhner M.D., Konstanze Döhner M.D., Richard F. Schlenk M.D, Universitätsklinikum Ulm, Ulm, Germany; Wolfgang Brugger M.D., Schwarzwald-Baar Klinikum Villingen-Schwenningen GmbH, Villingen-Schwenningen, Germany; Elisabeth Koller M.D., Hanuschkrankenhaus, Wien, Austria; Aruna Raghavachar M.D., Helios-Klinikum Wuppertal, Wuppertal, Germany.

Supplemental material: Methods

ASXL1 mutation analysis

Pretreatment BM and/or PB specimens were enriched for mononuclear cells using Ficoll density gradient centrifugation. Genomic DNA (gDNA) was extracted from cryopreserved mononuclear cells using the AllPrep DNA/RNA Kit (Qiagen™, Hilden) or the DNAzol® reagent (Invitrogen™, Karlsruhe) according to the manufacturer's instructions. *ASXL1* mutation screening in patients enrolled on the AMLSG 07-04 trial covered the entire exon 12. Ten differently sized, overlapping PCR fragments were amplified in two multiplex PCR reactions (5 fragments in each multiplex PCR reaction) using reagents from the Type-it Microsatellite PCR Kit (Qiagen™, Hilden). The forward primer of each primer pair was labelled by a fluorescent dye (FAM or AT550 or AT565; Eurofins MWG Operon, Ebersberg, Germany) at the 5´-end. In addition, the reverse primer in each primer pair was modified by the addition of a "gtttctt" pigtail at the 5´-end with the aim to reduce the intensity of stutter peaks in the subsequent GeneScan analysis. The primer sequences and the labelling method are indicated in supplemental Table S1. One multiplex PCR reaction included primer pairs 1, 2, 3, 4, and 8; the other multiplex included primer pairs 5, 6, 7, 9 and 10 (Table S1). PCR reactions were checked for the successful amplification and appropriate size of the amplicons by gel-electrophoresis. Subsequently, amplicons from the two PCR reactions were pooled together and prepared for a GeneScan-based fragment analysis on Applied Biosystems® 3130xL or Applied Biosystems® 3500DxL Genetic Analyzer. Patients without a size altering mutation showed elektropherogramms containing 10 peaks (Figure S1); each peak corresponds to one PCR fragment that covers a distinct DNA region within exon 12. Patients presenting with more than 10 peaks were considered as mutated (Figure S2 as example), and the particular DNA region was re-amplified in a second PCR reaction using a non-labelled primer pair, and further analyzed by direct DNA sequencing. The mutational analysis in patients enrolled on the AML HD98A (n=733) and APL HD95 (n=70) protocol was performed in the same way, with the exception that the DNA regions in exon 12 encoding amino acids 1030-1232, 1402-1434, and 1500-1541 were not covered based on the fact that previous sequencing studies[1,2] did not identify mutations in these *ASXL1* regions. Indeed, in patients enrolled on the AMLSG 07-04 (n=893) trial no mutation was detected within regions omitted in the analysis of patients enrolled on AML HD98A and APL HD95 trials. In addition, results from other AML studies have shown that frameshift mutations in the omitted regions were not detected[3] or only extremely rare (<1%)[4]. To determine the sensitivity of the GeneScan screening serial plasmid dilutions of the most frequent *ASXL1* mutation (c.1934dupG) were used as templates for PCR reactions; the sensitivity to detect this mutation was estimated as at least 10%

(supplemental Figure S3). Base exchanges resulting in stop codons (non-sense mutations) are not detectable with our GeneScan-based screening approach. However, previous studies applying direct sequencing found, that non-sense mutations appear to be infrequent molecular alterations in younger AML patients; Metzeler et al.[3] did not find any non-sense mutation in 189 younger (<60 years) AML patients with a normal karyotype, and Chou et al.[2] detected only one (0.3%) non-sense mutation in 312 younger (<60 years) patients with unselected AML. Thus, we obviously do not dismiss a substantial proportion of *ASXL1* mutations in younger AML population with the screening approach developed for this study.

Supplemental Tables

Supplemental Table S1: Primer sequences for polymerase chain reaction (PCR) based amplification of fragments covering the entire *ASXL1* exon 12.

Primer pair	Primer sequence, 5‘→3‘	Dye
Primer pair 1		
Forward primer	tcacacagtcccaccagaaa	5‘-FAM
Reverse primer	*gtttctt*tacactttccaggggtgctc	
Primer pair 2		
Forward primer	acagatgggctaggagatgc	5‘-AT565
Reverse primer	*gtttctt*atcattcgatgggatgggta	
Primer pair 3		
Forward primer	tggaagaaatggattccaaag	5‘-FAM
Reverse primer	*gtttctt*ccttggcctgtaacattgct	
Primer pair 4		
Forward primer	ttaaggcaaatgccgagaac	5‘-FAM
Reverse primer	*gtttctt*acactggagcgagatgcttt	
Primer pair 5		
Forward primer	agcagtggtgatggtggtg	5‘-FAM
Reverse primer	*gtttctt*tcccactagagacggaatgg	
Primer pair 6		
Forward primer	cttgaaaaccaaggctctcg	5‘-AT550
Reverse primer	*gtttctt*tgggtttctcatccttgtcc	
Primer pair 7		
Forward primer	cctcgagaacacctcgtttc	5‘-AT550
Reverse primer	*gtttctt*agtccatgacaaagggcatc	
Primer pair 8		
Forward primer	gacagcagtgaggctgacac	5‘-AT550
Reverse primer	*gtttctt*agcaactgcatcacaagtgg	
Primer pair 9		
Forward primer	tcccagattccctactgctg	5‘-AT565
Reverse primer	*gtttctt*tctttcctggagcagcattt	
Primer pair 10		
Forward primer	cagtgcatcactttccttgc	5‘-FAM
Reverse primer	*gtttctt*gcaagagtgctcctgcctaa	

NOTE: A nucleotide sequence comprising “gtttctt” was added to the 5´-end of each reverse primer for reduction of the stutter peaks in the GeneScan analysis.

Supplemental Table S2: Clinical characteristics and outcomes of the entire study cohort.

Characteristic/Endpoint	All Patients, n=1696
Median age, yrs (range)	48.3 (18-61)
Male sex, n (%)	868 (51.2)
AML history, n (%)	
De novo	1529 (90.4)
Secondary after preceding MDS/MPN	74 (4.4)
Therapy-related	88 (5.2)
Median WBC count, $x10^9$/L (range)	13.5 (0.2-532.7)
Missing data, n	27
Median platelet count, $x10^9$/L (range)	53 (2-933)
Missing data, n	28
Median hemoglobin, g/dl (range)	9.0 (2.5-17.6)
Missing data, n	18
Median LDH in serum, U/l	422 (79-15098)
Median % blood blasts, (range)	37 (0-100)
Missing data, n	137
Median % bone marrow blasts, (range)	75 (2-100)
Missing data, n	139
Normal karyotype, n (%)	751 (47.7)
Missing data, n	123
Complete remission, %	73.1
Event-free survival	
Median, years	0.81
Event-free at 5 years, %	28.2
95%-CI	26.1-30.5
Relapse-free survival	
Median, years	2.1
Alive at 5 years, %	42.6
95%-CI	39.8-45.5
Overall survival	
Median, years	1.78
Alive at 5 years, %	40.2
95%-CI	37.6-42.9

Supplemental Table S3: *ASXL1* mutations identified in 103 of 1696 AML patients.

ASXL1* mutation	**Protein change[§]**	**Number of patients**
c.1934dupG	p.G646WfsX12	62
c.1900-1922del	p.E635RfsX15	17
c.1934delG	p.G645VfsX58	6
c.1770-1773delinsCTAACTTTGCG	p.Y591X	1
c.1772dupA	p.Y591X	1
c.1850_1851insC	p.K618X	1
c.1913-1914insGGGACAC	p.T638RfsX67	1
c.1926delA	p.G645VfsX58	1
c.1927-1928insA	p.G643EfsX15	1
c.1933_1934delGG	p.G645WfsX12	1
c.1935_1939delTGGCC	p.P647WfsX9	1
c.1960dupG	p.A654GfsX4	1
c.2081_2087delCACAACT	p.T693NfsX7	1
c.2085_2086insG	p.L696AfsX22	1
c.2129_2130insCCGG	p.T711RfsX8	1
c.2222delA	p.D741VfsX3	1
c.2383_2386delTCCT	p.S795GfsX22	1
c.2423delC	p.P808LfsX10	1
c.2583_2384ins50bp (ACAGTTCCACACCTGAATCCTCACC GACTGATTGCCTGCAGAACAGAGCA)	p.F862KfsX5	1
c.2921dupA	p.Y974X	1
c.3022_3031delCACCTCACGG	p.H1008RfsX13	1

NOTE. The last *ASXL1* exon was studied for mutations. Previous publications on *ASXL1* mutations have referred to this exon as exon 12, because *ASXL1* exon 3 only consists of 3 bases and was not considered as separate exon. However, according to the reference sequence version NM_015338.5 of the National Center for Biotechnology Information (NCBI) database the last *ASXL1* exon is numbered as exon 13. To keep conformity

with previous publications and to avoid misunderstandings, we refer in this manuscript to the last *ASXL1* exon as exon 12.

Abbreviations: AML, acute myeloid leukaemia; *ASXL1*, *Additional Sex Comb-Like 1*.

*The mutations are designated according the recommendations of the Human Genome Variation Society (http://www.hgvs.org/mutnomen/); the NCBI sequence version NM_015338.5 is used as reference and the nucleotide numbering uses the first base of the translation start codon as nucleotide „+1".

§The protein changes are theoretically deduced and designated according to the recommendations of the Human Genome Variation Society (http://www.hgvs.org/mutnomen)

Supplemental Table S4: Multivariable analysis on complete remission (n=1583).

Variable	Odds ratio	95%-Confidence interval	*P*
ASXL1$^{\text{mutated}}$	0.77	0.46 - 1.29	.32
RUNX1$^{\text{mutated}}$	0.98	0.63 - 1.53	.92
Interaction between *ASXL1*$^{\text{mutation}}$ and *RUNX1*$^{\text{mutation}}$	0.72	0.24 - 2.23	.57
NPM1$^{\text{mutated}}$	2.57	1.81 - 3.66	<.0001
CEBPA$^{\text{double mutated}}$	6.85	2.66 - 17.68	.0001
IDH2$^{\text{R172 mutated}}$	0.49	0.25 - 0.93	.03
FLT3-ITD$^{\text{mutated}}$	0.61	0.45 - 0.84	.002
IDH1$^{\text{mutated}}$	0.53	0.33 - 0.84	.007
IDH2$^{\text{R140 mutated}}$	1.08	0.65 - 1.78	.77
DNMT3A$^{\text{mutated}}$	1.46	1.03 - 2.06	.03
FLT3-TKD$^{\text{mutated}}$	1.38	0.83 - 2.31	.21
Favorable risk cytogenetics	3.17	2.00 - 5.02	<.0001
Adverse risk cytogenetics	0.49	0.36 - 0.69	<.0001
s-AML	0.36	0.21 - 0.61	.0001
t-AML	0.95	0.57 - 1.59	.85
Age*	0.84	0.75 - 0.95	.004
Log10(WBC)	0.66	0.52 - 0.83	.0004
Sex (male *vs* female)	0.84	0.66 - 1.07	.17
Blasts in BM	1.00	1.00 - 0.99	.65
Blasts in PB	1.00	0.99 - 1.00	.65
Hemoglobin	1.01	0.96 - 1.08	.61

NOTE. Odds ratio greater than (less than) 1 indicates an decreased (increased) risk for the category "present" for a dichotomous variable and for a higher value of a continuous variable.
s-AML indicates secondary acute myeloid leukaemia
t-AML indicates therapy-related acute myeloid leukemia.
*Odds ratio for difference of 10 y.

Supplemental Table S5: Multivariable analysis on event-free survival (n=1683).

Variable	Hazard ratio	95%-Confidence interval	*P*
ASXL1$^{\text{mutated}}$	0.88	0.67 - 1.15	.35
RUNX1$^{\text{mutated}}$	1.10	0.88 - 1.37	.40
Interaction between *ASXL1*$^{\text{mutation}}$ and *RUNX1*$^{\text{mutation}}$	1.31	0.76 - 2.27	.33
NPM1$^{\text{mutated}}$	0.44	0.37 - 0.52	<.0001
CEBPA$^{\text{double mutated}}$	0.42	0.30 - 0.57	<.0001
FLT3-ITD$^{\text{mutated}}$	1.49	1.29 - 1.74	<.0001
IDH2$^{\text{R172 mutated}}$	1.17	0.83 - 1.64	.36
IDH2$^{\text{R140 mutated}}$	1.01	0.80 - 1.28	.91
IDH1$^{\text{mutated}}$	1.16	0.92 - 1.46	.21
DNMT3A$^{\text{mutated}}$	1.01	0.86 - 1.19	.86
FLT3-TKD$^{\text{mutated}}$	0.84	0.66 - 1.07	.17
Favorable risk cytogenetics	0.33	0.27 - 0.41	<.0001
Adverse risk cytogenetics	1.60	1.36 - 1.90	<.0001
s-AML	1.29	0.99 - 1.67	.06
t-AML	1.07	0.83 - 1.39	.60
Age*	1.14	1.07 - 1.20	<.0001
Log10(WBC)	1.29	1.15 - 1.44	<.0001
Sex (male *vs* female)	1.23	1.09 - 1.38	.0007
Blasts in BM	1.00	1.00 - 1.00	.79
Blasts in PB	1.00	1.00 - 1.00	.07
Hemoglobin	0.97	0.76 - 2.27	.07

NOTE. Hazard ratio greater than (less than) 1 indicates an increased (decreased) risk for the category "present" for a dichotomous variable and for a higher value of a continuous variable.
s-AML indicates secondary acute myeloid leukaemia
t-AML indicates therapy-related acute myeloid leukemia.
*Hazard ratio for difference of 10 y.

Supplemental Table S6: Multivariable analysis on relapse-free survival (n=1228).

Variable	Hazard ratio	95%-Confidence interval	*P*
ASXL1$^{\text{mutated}}$	0.82	0.55 - 1.22	.34
RUNX1$^{\text{mutated}}$	0.89	0.65 - 1.21	.46
Interaction between *ASXL1*$^{\text{mutation}}$ and *RUNX1*$^{\text{mutation}}$	1.97	0.84 - 4.66	.12
NPM1$^{\text{mutated}}$	0.51	0.41 - 0.63	<.0001
CEBPA$^{\text{double mutated}}$	0.57	0.39 - 0.82	.003
FLT3-ITD$^{\text{mutated}}$	1.52	1.26 - 1.83	<.0001
IDH2$^{\text{R172 mutated}}$	0.57	0.34 - 0.98	.04
IDH1$^{\text{mutated}}$	1.21	0.90 - 1.64	.21
IDH2$^{\text{R140 mutated}}$	1.1	0.81 - 1.48	.55
DNMT3A$^{\text{mutated}}$	1.07	0.87 - 1.31	.52
FLT3-TKD$^{\text{mutated}}$	0.93	0.69 - 1.24	.61
Favorable risk cytogenetics	0.39	0.30 - 0.51	<.0001
Adverse risk cytogenetics	1.38	1.09 - 1.74	.007
s-AML	1.15	0.74 - 1.79	.54
t-AML	1.26	0.90 - 1.77	.17
Age*	1.23	1.14 - 1.33	<.0001
Log10(WBC)	1.35	1.16 - 1.57	.0001
Sex (male *vs* female)	1.14	0.98 - 1.33	.09
Blasts in BM	1.00	1.00 - 1.00	.96
Blasts in PB	1.00	1.00 - 1.00	.24
Hemoglobin	0.97	0.94 - 1.01	.21

NOTE. Hazard ratio greater than (less than) 1 indicates an increased (decreased) risk for the category "present" for a dichotomous variable and for a higher value of a continuous variable.
s-AML indicates secondary acute myeloid leukaemia
t-AML indicates therapy-related acute myeloid leukemia.
*Hazard ratio for difference of 10 y.

Legends for supplemental Figures

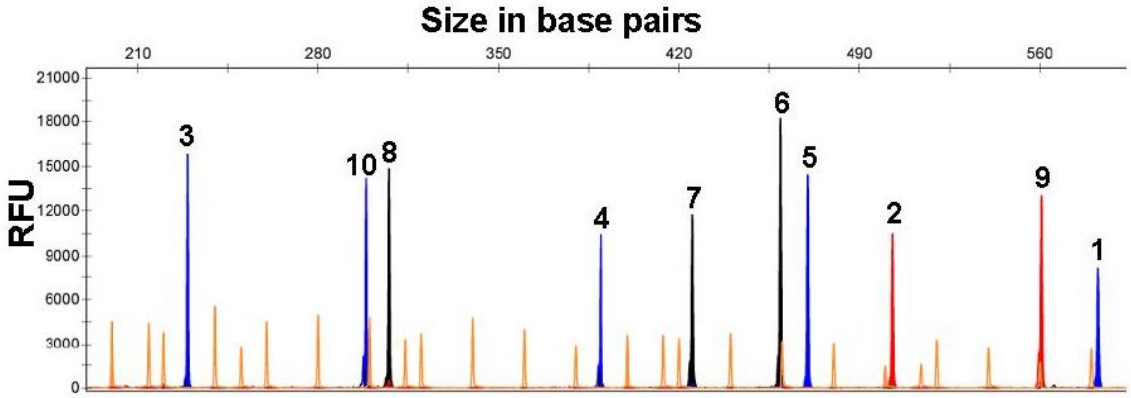
Figure S1: Exemplary GeneScan analysis of a patient with *ASXL1* wildtype in exon 12. The orange peaks represent the size standards. The number at the top of a particular peak indicates the primer pair from Table S1 used to generate this fragment.
Abbreviation: RFU, relative fluorescence units.

Figure S2: Exemplary GeneScan analysis of a patient with an *ASXL1* exon 12 mutation. The orange peaks represent the size standards. The number at the top of a particular peak indicates the primer pair from Table S1 used to generate this fragment. The doubled peak under number "1" indicates a duplication of one base pair; after sequencing the mutation was determined as c.1934dupG.
Abbreviation: RFU, relative fluorescence units.

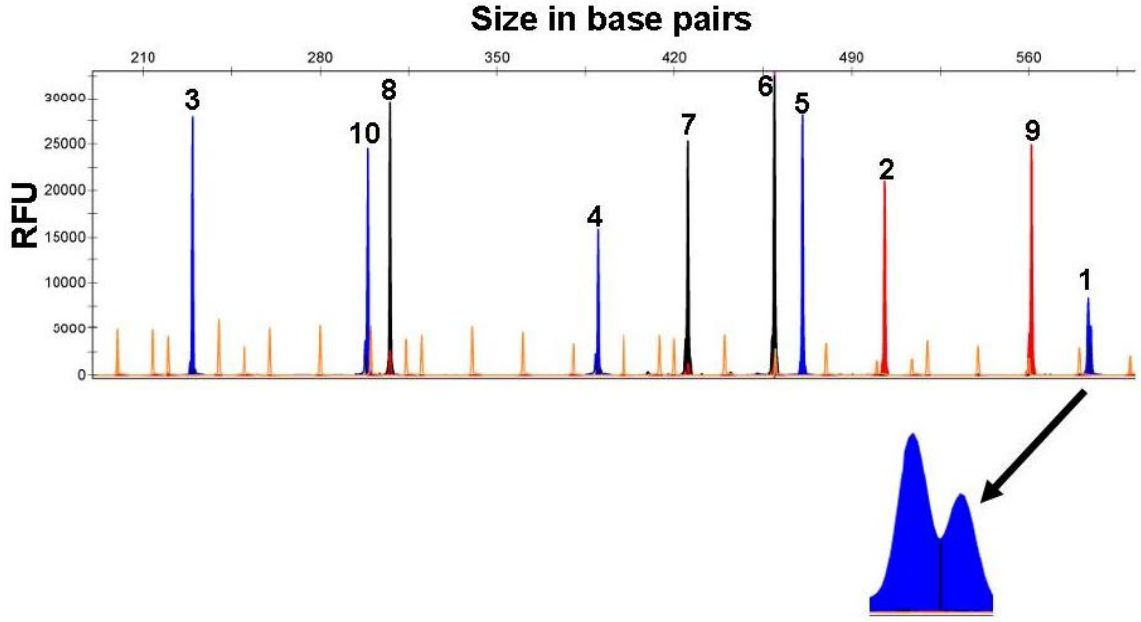
Figure S3: Sensitivity testing of the GeneScan screening. Serial plasmid dilutions of the most frequent *ASXL1* mutation (c.1934dupG) with wildtype were used as templates for PCR reactions. The amplicons were subsequently analyzed by the GeneScan analysis. The sensitivity to detect the mutation was estimated as at least 10%.

Supplemental Figures

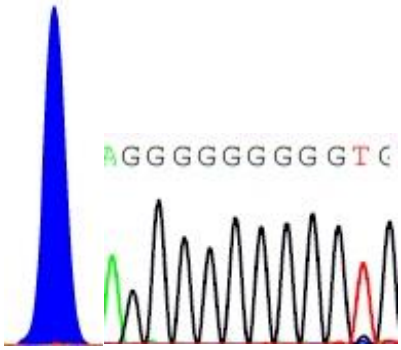
Supplemental Figure S1



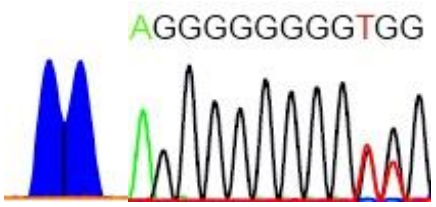
Supplemental Figure S2



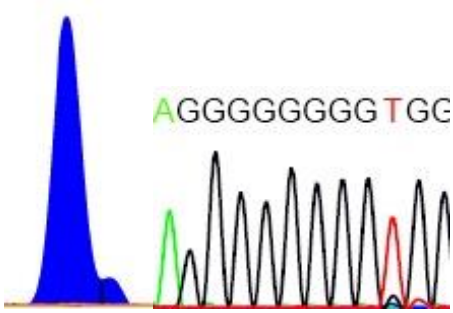
Supplemental Figure S3:



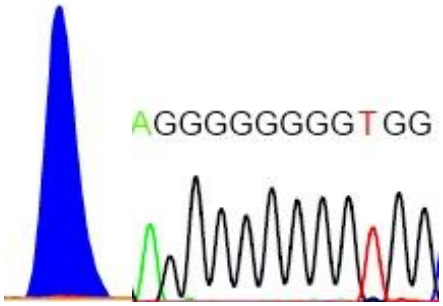
100% mutated allele



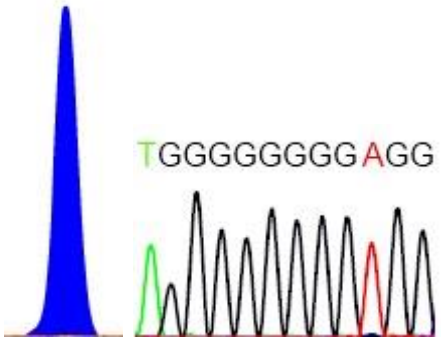
50% mutated allele



10% mutated allele



5% mutated allele



0% mutated allele

REFERENCES

1. Gelsi-Boyer V, Trouplin V, Adelaide J, et al. Mutations of polycomb-associated gene *ASXL1* in myelodysplastic syndromes and chronic myelomonocytic leukaemia. Br J Haematol. 2009;145(6):788-800.
2. Chou WC, Huang HH, Hou HA, et al. Distinct clinical and biological features of de novo acute myeloid leukemia with *additional sex comb-like 1* (*ASXL1*) mutations. Blood. 2010;116(20):4086-94.
3. Metzeler KH, Becker H, Maharry K, et al. *ASXL1* mutations identify a high-risk subgroup of older patients with primary cytogenetically normal AML within the ELN Favorable genetic category. Blood. 2011;118(26):6920-9.
4. Schnittger S, Eder C, Jeromin S, et al. *ASXL1* exon 12 mutations are frequent in AML with intermediate risk karyotype and are independently associated with an adverse outcome. Leukemia. 2013;27(1):82-91.