The spectrum of somatic mutations in high-risk acute myeloid leukaemia with -7/del(7q)

Megan E. McNerney,[1,2,3] Christopher D. Brown,[4] April L. Peterson,[1] Mekhala Banerjee,[5] Richard A. Larson,[3,5] John Anastasi,[2,3] Michelle M. Le Beau[1,3,5] and Kevin P. White[1,3,6]

[1]Institute for Genomics and Systems Biology, University of Chicago, [2]Department of Pathology, University of Chicago, [3]Comprehensive Cancer Center, University of Chicago, Chicago, IL, [4]Department of Genetics, University of Pennsylvania, Philadelphia, PA, [5]Section of Hematology/Oncology, Department of Medicine, University of Chicago, and [6]Section of Genetic Medicine, Department of Human Genetics, University of Chicago, Chicago, IL, USA

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Correspondence: Kevin P. White, Institute for Genomics and Systems Biology, The University of Chicago, 900 East 57th Street, KCBD 10100A, Chicago, IL 60637, USA.
E-mail: kpwhite@uchicago.edu

Summary

-7/del(7q) occurs in half of myeloid malignancies with adverse-risk cytogenetic features and is associated with poor survival. We identified the spectrum of mutations that co-occur with -7/del(7q) in 40 patients with *de novo* or therapy-related myeloid neoplasms. -7/del(7q) leukaemias have a distinct mutational profile characterized by low frequencies of alterations in genes encoding transcription factors, cohesin and DNA-methylation-related proteins. In contrast, RAS pathway activating mutations occurred in 50% of cases, a significantly higher frequency than other acute myeloid leukaemias and higher than previously reported. Our data provide guidance for which pathways may be most relevant in the treatment of adverse-risk myeloid leukaemia.

Keywords: myeloid leukaemia, monosomy 7, *CUX1*.

Cytogenetic abnormalities remain the strongest independent predictor for response to therapy and survival in myeloid malignancies. Adverse-risk cytogenetic abnormalities occur in 20–30% of *de novo* acute myeloid malignancies and 70% of therapy-related myeloid neoplasms (t-MN) (Leith *et al*, 1997; Smith *et al*, 2003; Grimwade *et al*, 2010). The median overall survival for patients with high-risk abnormalities is <1 year, a rate that has only minimally improved over the last three decades (Smith *et al*, 2003; Grimwade *et al*, 2010). The most common high-risk cytogenetic abnormality is -7/del(7q), identified in half of all t-MN patients and half of adverse-risk *de novo* acute myeloid leukaemia (AML) (Leith *et al*, 1997; Smith *et al*, 2003; Grimwade *et al*, 2010). While recent studies have focused on the genomics of low- and intermediate-risk AML, the genetic basis for adverse-risk AML/t-MN remains poorly understood. We previously mapped the commonly deleted segment of chromosome band 7q22 using RNA-sequencing and single nucleotide polymorphism (SNP)-array analysis (McNerney *et al*, 2013). We identified the gene encoding the CUX1 transcription-factor (*CUX1*) to be a highly conserved, haploinsufficient myeloid tumour suppressor located within 7q22 (McNerney *et al*, 2013). Herein, we identify the genome-wide spectrum of somatic mutations that co-occur with -7/del(7q) and *CUX1* loss. We found that the mutation profile of -7/del(7q) leukaemias is significantly different from other AMLs and reveals therapeutic opportunities for improving the outcome for patients with high-risk disease.

Materials and methods

Methods are provided in Data S1.

Results/Discussion

We identified the somatic mutations in 13 leukaemia samples with -7/del(7q) (University of Chicago, [UC] cohort). Three patients had *de novo* AML and ten had t-MN (Table S1). We included t-MN and *de novo* AML samples as they are indistinguishable morphologically and clinically (Schoch *et al*,

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2004), suggesting common biological features. It remains unknown, however, if t-MN and *de novo* AML with -7/del (7q) also have similar somatic mutations. Four samples had complex karyotypes, and three of these also had del(5q) (Table S1). Two cases had a recurrent genetic variation as defined by the 2008 World Health Organization category 'AML with recurrent genetic abnormalities' (Swerdlow *et al*, 2008), which was inv(3). Complex karyotype, del(5q), and inv (3) frequently co-occur with loss of 7q (Swerdlow *et al*, 2008). Paired tumour and normal exome-sequencing was performed on six cases; seven others underwent RNA-sequencing of the leukaemia sample with exome-sequencing of normal tissue. Thus, all samples received paired normal exome sequencing for somatic mutation detection. The median coverage of coding exons for tumour exomes was 130×, 72× for normal exomes, and 30× for RNA-sequenced tumours (Table S1). The median percentage of coding bases with sufficient depth for SNP identification (≥8× coverage) was 92·1% for tumour exomes, 83·6% for normal exomes, and 37·6% for RNA-sequenced tumours. Copy number analysis was available for eight leukaemia samples (McNerney *et al*, 2013).

We identified 40 mutations in the six exome-sequenced cases (Table I). Twenty-one mutations were Sanger sequenced with a 100% validation rate (Table S2). Thirty-nine mutations were identified in the RNA-sequenced cases of which 30 were verified, with a validation rate of 93·8% (Table S2). One RNA-sequenced sample had fusion events identified by RNA-sequencing (McNerney *et al*, 2013) (Table S1). The average number of single nucleotide mutations and indels per sample was six (0·16 mutations/Mb), which is lower than previous reports (Link *et al*, 2011; The Cancer Genome Atlas Research Network [TCGA] 2013). This is possibly due to conservative mutation calling parameters and lower coverage in the current study, particularly for the RNA-sequenced samples. The median number of mutations for the RNA-sequenced samples was 3, compared to 5·5 in the exomes. There was no difference in the mutation load for t-MN patients as compared to *de novo* AML; however, there are only three *de novo* AMLs in this cohort. The fraction of mutations that were transversions was 32·5% and was similar when restricting the analysis to the t-MN samples (36·1%), consistent with prior reports (Link *et al*, 2011; TCGA, 2013).

Driver mutations in AML genomes predominate in eight functional categories: tumour suppressors, signalling molecules, myeloid transcription factors, DNA-methylation regulators, chromatin modifiers, cohesin, spliceosome components and *NPM1* (Table S3) (TCGA, 2013). Of these, the most frequently altered in the UC cohort was the RAS pathway, with activating mutations in 8/13 (61·5%) samples (Fig 1A). The mutations were comprised of those associated with juvenile myelomonocytic leukaemia (JMML), including activating mutations of *NRAS* and *PTPN11*, and inactivating mutations of *CBL* (Table I). The next most frequently altered pathway involved chromatin modifiers (4/13 cases, 31%). There was a paucity of mutations in the other major pathways.

RNA-sequencing to detect somatic mutations is limited to identification of expressed mutations. Mutations in genes that are not expressed, expressed at low levels, or mutations that cause nonsense-mediated decay will be missed. Therefore, to extend our findings to a larger, independent cohort and to exclude the possibility that RNA-sequencing biased the discovery of mutations in specific pathways, mutations in -7/del(7q) AML samples from TCGA were assessed (TCGA, 2013). Of the 200 TCGA samples with exome or whole genome sequencing, 21 had -7/del(7q) by cytogenetic analysis. Six additional samples with >30 Mb deletions involving 7q identified by SNP array were also included, for a total of 27 cases with -7/del(7q) in the TCGA cohort. -7/del(7q) deletions spanned *CUX1* in 22/27 cases, the remaining five cases had deletions that spanned *EZH2* on 7q36.

The patterns of mutations seen in the TCGA -7/del(7q) samples reflected the results of the UC cohort (Fig 1B). RAS pathway activating mutations were prevalent, occurring in 44% of cases (Table S4). These included mutations of *NRAS, KRAS, RIT1,* and deletions or mutations of *NF1*. In contrast, RAS pathway mutations occurred in 19% of the other 173 TCGA samples (chi-squared $P = 0{\cdot}0033$). We note that RAS pathway mutations were restricted to those cases with deletions of *CUX1*, occurring in 12/22 (55%, $P = 0{\cdot}00014$). RAS pathway mutational status did not influence median overall survival within the -7/del(7q) TCGA subset (10·0 ± 22·8 months without RAS pathway mutations, $n = 15$; 9·4 ± 15·5 months for patients with RAS pathway mutations, $n = 12$).

The TCGA cohort replicated the finding that genes encoding chromatin modifiers were mutated at similar rates in -7/del(7q) cases (41%) as compared to others (30%, $P = 0{\cdot}24$), whereas alterations in other major leukemogenic pathways were underrepresented. There were fewer mutations in the genes encoding the signalling molecules, FLT3 or KIT, ($P = 0{\cdot}045$), the cohesin complex ($P = 0{\cdot}031$), and NPM1 ($P = 0{\cdot}0034$). Thirty percent of -7/del(7q) AML had alterations in the DNA methylation pathway, as compared to 46% of others, but this did not reach statistical significance ($P = 0{\cdot}12$).

Myeloid transcription factor alterations (Table S3) were decreased in -7/del(7q) leukaemias. Whereas 45% of AML samples without -7/del(7q) had disruption of at least one myeloid transcription factor gene, the frequency was 26% (7/27) in the TCGA -7/del(7q) cases ($P = 0{\cdot}061$). The frequency of myeloid transcription factor mutations was markedly lower within those TCGA samples with deletions of *CUX1*, occurring in only 18% (4/22) of cases ($P = 0{\cdot}014$), indicating that *CUX1* deletions are mutually exclusive with mutations of other myeloid transcription factor genes.

The high rate of *TP53* mutations or deletions (20% UC and 44% TCGA) in -7/del(7q) samples compared to others (5%, $P = 0{\cdot}0001$, TCGA cohort), is driven by the strong association

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Table I. University of Chicago cohort mutations from exome and RNA-sequencing.

Patient	Gene	Amino acid change	Deleteriousness (GERP score)	Cancer Gene Census gene	TCGA AML gene mutation frequency (%)	cBioPortal gene mutation frequency in other tumours
A24	*CCDC33*	V341M	2·67		0	7·1% bladder, 6·9% small cell lung, others
A24	*CSMD2*	c.8047C>G, synonymous	−0·0615		0	34·7% melanoma, 24·1% lung small cell, others
A24	*IMPG1*	c.2091G>A, synonymous	−8·35		0	12·4% melanoma, 6·2% lung squamous, others
A24	*NRAS*	G12D	5·23	Yes	8·0	30·8% melanoma, 18·0% multiple myeloma, others
A24	*ROCK2*	S823*	Nonsense		0	7·1% bladder, 5·6% endometrial, others
A24	*SMCHD1*	I183M	−2·61		0	6·0% endometrial, 5·1% cervical, others
A24	*SPEF2*	E1521V	4·38		0	17·2% melanoma, 13·8% lung small cell, others
A24	*TET2*	Q1553*	Nonsense	Yes	8·5	6·9% colorectal, 6·9% lung small cell, others
A24	*VNN2*	A253T	4·47		0	5·7% melanoma, 4·0% endometrial, others
A24	*ZRSR2*	G268D	5·09	Yes	0	2·4% endometrial, 2·3% bladder, others
A36	*COX7C*	R57G	3·7		0	1·8% pancreatic, 1·1% lung adenocarcinoma, others
A36	*FAM116B*	Q479R	4·72		0	5·6% colorectal, 1·8% pancreatic, others
A36	*HEATR5B*	A1534V	5·43		0	7·7% cervical, 7·1% bladder, others
A36	*KCTD17*	H94R	3·58		0	2·2% melanoma, 1·4% colorectal, others
A36	*TLN1*	R854H	5·56		0	11·1% colorectal, 6·6% melanoma, others
A74	*NRAS*	G12S	5·23	Yes	8·0	30·8% melanoma, 18·0% multiple myeloma, others
T03	*ANKRD32*	G875R	5·19		0	4·2% colorectal, 2·8% endometrial, others
T03	*DNAH1*	M2871T	4·61		0	16·7% colorectal, 12·4% melanoma, others
T03	*ELAC2*	M750T	4·65		0	4·2% colorectal, 3·6% melanoma, others
T03	*ETV6*	K403N	3·53	Yes	1·0	5·6% colorectal, 3·6% bladder, others
T03	*EWSR1*	c.1291C>T, synonymous	5·59	Yes	0·5	4·1% melanoma, 3·6% endometrial, others
T03	*EZH2*	G159R	5·73	Yes	1·5	4·8% endometrial, 4·1% head neck, others
T03	*FLT3*	D835Y	5·53	Yes	27·0	10·0% melanoma, 4·8% lung adenocarcinoma, others
T03	*FRY*	R1110*	Nonsense		0	11·1% colorectal, 9·1% melanoma, others
T03	*HDAC5*	V311M	3·98		0	4·2% colorectal, 3·6% endometrial, others
T03	*LILRA6*	L115M	−1·17		0	6·9% small cell lung, 3·6% bladder, others
T03	*MATR3*	R307G	2·49		0	3·3% melanoma, 2·8% endometrial, others
T03	*N4BP2L2*	Q441R	4·22		0	4·4% endometrial, 3·6% bladder, others
T03	*NUP153*	S902Y	5·71		0	7·1% bladder, 5·2% endometrial, others
T03	*PDE1B*	I371T	4·81		0	6·6% melanoma, 4·8% small cell lung, others
T03	*PROS1*	M192V	−5·88		0	10·3% small cell lung, 7·0% lung adenocarcinoma, others
T03	*PTPN11*	F71L	5·28	Yes	4·5	4·2% colorectal, 3·4% small cell lung, others
T03	*RIOK1*	M10T	5·82		0	7·0% pancreatic, 5·8% melanoma, others
T03	*TNPO2*	F873V	4·42		0	4·5% gastric, 3·6% endometrial, others
T03	*ZNF192*	L365V	4·5		0	3·4% lung small cell, 3·4% lung squamous, others

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Table I. (*Continued*)

Patient	Gene	Amino acid change	Deleteriousness (GERP score)	Cancer Gene Census gene	TCGA AML gene mutation frequency (%)	cBioPortal gene mutation frequency in other tumours
T03	*ZNF318*	Q219*	Nonsense		0	9·7% colorectal, 6·6% melanoma, others
T12	*CDK2AP1*	H23R	5·16		0	1·4% colorectal, 0·8% melanoma, others
T12	*FBXO18*	A495T	4·23		0	5·6% colorectal, 3·3% melanoma, others
T16	*NUP210*	L1504I	−8·3		0	11·6% melanoma, 5·6% colorectal, others
T16	*PPM1D*	S446*	Stop		0	4·4% endometrial, 4·2% colorectal, others
T16	*RUNX1*	R210K	4·62	Yes	9·0	3·4% breast, 3·2% endometrial, others
T18	*CBL*	Y368_E369insAD	Indel	Yes	1·0	5·5% melanoma, 4·4% endometrial, others
T18	*INPP1*	G178V	4·89		0	3·6% bladder, 2·6% cervical, others
T18	*SCN5A*	R367C	4·14		0	24·7% melanoma, 10·3% cervical, others
T20	*GSTM5*	N85S	3·43		0	2·2% melanoma, 1·7% lung squamous, others
T20	*HERC2*	G1886R	4·44		0·5	20·7% small cell lung, 19·4% colorectal, others
T20	*MPEG1*	F444V	5·38		0	4·2% colorectal, 3·4% lung small cell, others
T20	*NAP1L4*	K26N	−0·991		0	6·9% small cell lung, 4·4% endometrial, others
T20	*NRAS*	G12D	5·23	Yes	8·0	30·8% melanoma, 18·0% multiple myeloma, others
T45	*ADAMTS5*	N807S	5·48		0	9·2% lung adenocarcinoma, 7·7% gastric, others
T45	*FGF18*	R34H	4·24		0	2·2% melanoma, 1·6% lung adenocarcinoma, others
T45	*HIST1H2AL*	L24I	4·45		0	2·4% small cell lung, 2·0% bladder, others
T45	*NRAS*	G13C	5·23	Yes	8·0	30·8% melanoma, 18·0% multiple myeloma, others
T45	*PAPPA2*	C1167F	5·29		0·5	28·1% melanoma, 20·7% small cell lung, others
T46	*BRCA2*	T2310P	4·82	Yes	0	11·6% melanoma, 10·8% ovarian, others
T46	*CHM*	c.1361G>A, synonymous	−1·01		0	4·2% colorectal, 4% endometrial, others
T46	*GLB1L*	I514T	4·74		0	5·6% colorectal, 3·2% endometrial, others
T46	*LRP5*	c.1876G>A	Splice junction		0·5	10·3% cervical, 9·1% melanoma, others
T46	*MMP3*	K349 fs	Indel		0	3·5% pancreatic, 3·2% melanoma, others
T46	*NSD1*	Q1213*	Stop	Yes	0	10·8% head neck, 10·7% bladder, others
T47	*C10orf76*	Q267K	5·71		0·5	2·8% colorectal, 2·4% small cell lung, others
T47	*PLXNA2*	V475L	3·2		0	11·1% colorectal, 7·7% endometrial, others
T47	*TP53*	C275Y	4·57	Yes	7·0	94·6% ovarian, 89·7% lung small cell, others
T47	*TXLNA*	K427R	5·32		0	3·4% lung small cell, 2·2% melanoma, others
T50	*CCDC150*	T787I	1·12		0·5	4·4% endometrial, 3·2% melanoma, others
T50	*DLEC1*	c.2256C>T, synonymous	−9·17		0	10·0% melanoma, 5·6% endometrial, others
T50	*DNAH5*	c.3206C>G, synonymous	−9·74		0·5	52·7% melanoma, 25·0% colorectal, others
T50	*EWSR1*	Y170H	5·14	Yes	0·5	4·1% melanoma, 3·6% endometrial, others
T50	*GOLGA3*	Q122P	5·37		0	7·9% lung squamous, 5·9% gastric, others
T50	*HECTD1*	L330Q	5·72		0	7·7% endometrial, 7·1% bladder, others
T50	*NLGN4X*	R204H	3·55		0	8·3% lung adenocarcinoma, 7·4% melanoma, others
T50	*PTPN11*	A72T	5·28	Yes	4·5	4·2% colorectal, 3·4% small cell lung, others
T50	*SGOL1*	E212A	3·12		0	7·1% bladder, 3·5% prostate, others
T50	*SLC25A20*	S167N	5·32		0	1·6% endometrial, 1·1% lung squamous, others
T50	*SPTA1*	c.892G>A, synonymous	2·59		0	30·6% lung adenocarcinoma, 23·3% melanoma, others
T50	*TRPV4*	N678S	5·24		0	4·2% colorectal, 4·0% endometrial, others
T52	*CPSF2*	V208M	5·27		0	4·4% endometrial, 4·1% head neck, others

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Table I. (*Continued*)

Patient	Gene	Amino acid change	Deleteriousness (GERP score)	Cancer Gene Census gene	TCGA AML gene mutation frequency (%)	cBioPortal gene mutation frequency in other tumours
T52	*TMCO1*	I154N	5·82		0	1·8% pancreatic, 1·6% endometrial, others
T52	*TP53*	Y220C	4·93	Yes	7·0	94·6% ovarian, 89·7% lung small cell, others

GERP, genomic evolutionary rate profiling score (Cooper *et al*, 2005).
Cancer Gene Census data was downloaded March 2014 (Futreal *et al*, 2004).
cBioPortal data (Cerami *et al*, 2012) represents the two tumour types with the highest frequency of mutations in that gene (accessed March 2014).
*Indicates a stop codon.

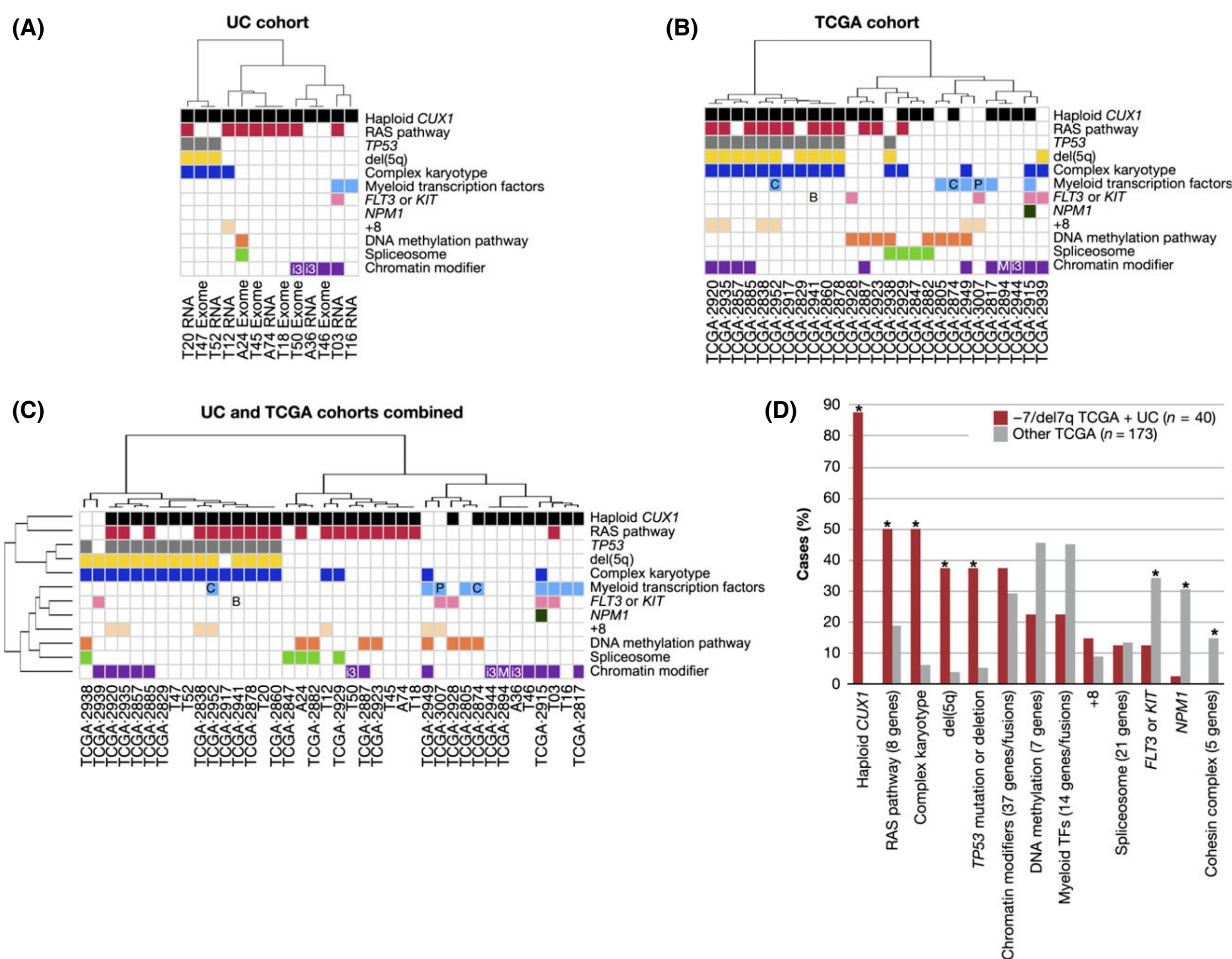

Fig 1. The pattern of somatic mutations in -7/del(7q) leukaemias is distinct from other acute myeloid leukaemia (AML) types. Categorization of genes within pathways is as defined (TCGA 2013) (Table S3). Mutations in genes not in these pathways are not shown. Samples are hierarchically clustered by Pearson correlation coefficients based on the presence or absence of mutations in these pathways using Ward's method. Mutated pathways are shown for the UC cohort (A), the TCGA cohort (B), and the combined UC and TCGA cohorts (C). (D) The frequency of the alteration in the combined UC (n = 13) and TCGA (n = 27) cohorts of -7/del(7q) leukaemias (red bars, n = 40) is shown in comparison to TCGA AML samples without -7/del(7q) (grey bars, n = 173). The number of genes per category is indicated in parentheses. * indicates chi-squared test $P < 0{\cdot}05$ comparing -7/del(7q) TCGA samples versus other TCGA samples. All recurrent genetic abnormalities according to the 2008 World Health Organization classification 'AML with recurrent genetic abnormalities' are indicated (Swerdlow *et al*, 2008), with an abbreviation within the relevant pathway. B: *BCR-ABL1* fusion; C: *CEBPA* mutation; i3: inv(3)(q21q26.2) or t(3)(3;3)(q21;q26.2); M: *MLLT3-KMT2A* fusion; and P: *PML-RARA* fusion. Abbreviations: TF, transcription factor. Within the UC cohort, t-MN samples are named by TXX and *de novo* AML samples are named by AXX.

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between del(5q) and *TP53* mutations (Figs. 1C and 1D). With one exception, all of the 15 *TP53* mutations or deletions in the combined cohorts occurred in samples that also had del(5q) (Cochran-Mantel-Haenszel test $P = 3{\cdot}5e\text{-}07$).

This is the first description of the genome-wide mutation burden in high-risk myeloid leukaemia with -7/del(7q). The analysis of additional patients in larger studies will be necessary to confirm the current findings. We did not observe differences in the mutational spectrum in t-MN or *de novo* AML. Across all -7/del(7q) cases, we observed a higher frequency of RAS pathway mutations (50% of UC and TCGA combined) than previously reported (14%) (Side *et al*, 2004), suggesting that haploinsufficiency of a gene(s) on chromosome 7 cooperates with RAS in AML pathogenesis. The finding of a low number of transcription factor alterations, particularly in those samples with a deletion of *CUX1*, is consistent with a transcription factor role for the gene(s) on chromosome 7, such as *CUX1* (McNerney *et al*, 2013). Of note, *CUX1* is mutated in 7–10% of endometrial carcinoma, gastric adenocarcinoma and melanoma (Cerami *et al*, 2012). Our analysis of TCGA data revealed that RAS pathway mutations are over twice as frequent in *CUX1*-mutated solid tumours within these three diseases ($P < 0{\cdot}01$). Indeed, a striking 80% of endometrial and melanoma cancers with mutated *CUX1* also have activating RAS pathway mutations, suggesting that cooperation between *CUX1* and RAS may be a tumourigenic mechanism that extends beyond haematological malignancies. As drugs targeting the RAS pathway advance, therapeutic inhibition of RAS, in addition to targeting pathways triggered by *CUX1* haploinsufficiency, may cooperate to improve the outcome for patients with high-risk myeloid neoplasms.

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Authorship contributions

M.E.M. designed research, performed experiments, analysed and interpreted data and wrote the manuscript; C.D.B. assisted in sequencing data analysis and edited the manuscript; A.L.P. generated exome libraries and performed Sanger sequencing; M.B. collected biospecimens and generated lymphoblastoid cell lines; R.A.L. collected biospecimens and edited the manuscript; J.A. performed morphological analysis, collected biospecimens and edited the manuscript; M.M.L. designed research, performed cytogenetic analysis of leukaemia samples, collected biospecimens and edited the manuscript; and K.P.W. designed research, interpreted data and edited the manuscript.

Conflict of interest

The authors do not have any competing financial interests in relation to the work described.

Supporting Information

Additional Supporting Information may be found in the online version of this article:

Table S1. University of Chicago cohort patient characteristics, sequencing statistics, and AML pathway mutations.

Table S2. University of Chicago mutations.

Table S3. Genes in pathways for Figure 1.

Table S4. Mutations in AML pathways in -7/del(7q) samples from The Cancer Genome Atlas.

Data S1. Materials and Methods.

References

Cerami, E., Gao, J., Dogrusoz, U., Gross, B.E., Sumer, S.O., Aksoy, B.A., Jacobsen, A., Byrne, C.J., Heuer, M.L., Larsson, E., Antipin, Y., Reva, B., Goldberg, A.P., Sander, C. & Schultz, N. (2012) The cBio cancer genomics portal: an open platform for exploring multidimensional cancer genomics data. *Cancer Discovery*, **2**, 401–404.

Cooper, G.M., Stone, E.A., Asimenos, G., Green, E.D., Batzoglou, S. & Sidow, A. (2005) Distribution and intensity of constraint in mammalian genomic sequence. *Genome Research*, **15**, 901–913.

Futreal, P.A., Coin, L., Marshall, M., Down, T., Hubbard, T., Wooster, R., Rahman, N. & Stratton, M.R. (2004) A census of human cancer genes. *Nature Reviews Cancer*, **4**, 177–183.

Grimwade, D., Hills, R.K., Moorman, A.V., Walker, H., Chatters, S., Goldstone, A.H., Wheatley, K., Harrison, C.J. & Burnett, A.K. (2010) Refinement of cytogenetic classification in acute myeloid leukemia: determination of prognostic significance of rare recurring chromosomal abnormalities among 5876 younger adult patients treated in the United Kingdom Medical Research Council trials. *Blood*, **116**, 354–365.

Leith, C.P., Kopecky, K.J., Godwin, J., McConnell, T., Slovak, M.L., Chen, I.M., Head, D.R., Appelbaum, F.R. & Willman, C.L. (1997) Acute myeloid leukemia in the elderly: assessment of multidrug resistance (MDR1) and cytogenetics distinguishes biologic subgroups with remarkably distinct responses to standard chemotherapy. A Southwest Oncology Group study. *Blood*, **89**, 3323–3329.

Link, D.C., Schuettpelz, L.G., Shen, D., Wang, J., Walter, M.J., Kulkarni, S., Payton, J.E., Ivanovich, J., Goodfellow, P.J., Le Beau, M., Koboldt, D.C., Dooling, D.J., Fulton, R.S., Bender, R.H., Fulton, L.L., Delehaunty, K.D., Fronick, C.C., Appelbaum, E.L., Schmidt, H., Abbott, R., O'Laughlin, M., Chen, K., McLellan, M.D., Varghese, N., Nagarajan, R., Heath, S., Graubert, T.A., Ding, L., Ley, T.J., Zambetti, G.P., Wilson, R.K. & Mardis, E.R. (2011) Identification of a novel TP53 cancer susceptibility mutation through whole-genome sequencing of a patient with therapy-related AML. *Journal of the American Medical Association*, **305**, 1568–1576.

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McNerney, M.E., Brown, C.D., Wang, X., Bartom, E.T., Karmakar, S., Bandlamudi, C., Yu, S., Ko, J., Sandall, B.P., Stricker, T., Anastasi, J., Grossman, R.L., Cunningham, J.M., Le Beau, M.M. & White, K.P. (2013) CUX1 is a haploinsufficient tumor suppressor gene on chromosome 7 frequently inactivated in acute myeloid leukemia. *Blood*, **121**, 975–983.

Schoch, C., Kern, W., Schnittger, S., Hiddemann, W. & Haferlach, T. (2004) Karyotype is an independent prognostic parameter in therapy-related acute myeloid leukemia (t-AML): an analysis of 93 patients with t-AML in comparison to 1091 patients with de novo AML. *Leukemia*, **18**, 120–125.

Side, L.E., Curtiss, N.P., Teel, K., Kratz, C., Wang, P.W., Larson, R.A., Le Beau, M.M. & Shannon, K.M. (2004) RAS, FLT3, and TP53 mutations in therapy-related myeloid malignancies with abnormalities of chromosomes 5 and 7. *Genes Chromosomes Cancer*, **39**, 217–223.

Smith, S.M., Le Beau, M.M., Huo, D., Karrison, T., Sobecks, R.M., Anastasi, J., Vardiman, J.W., Rowley, J.D. & Larson, R.A. (2003) Clinical-cytogenetic associations in 306 patients with therapy-related myelodysplasia and myeloid leukemia: the University of Chicago series. *Blood*, **102**, 43–52.

Swerdlow, S.H., Campo, E., Harris, N.L., Jaffe, E.S., Pileri, S.A., Stein, H., Thiele, J. & Vardiman, J.W. (2008) WHO Classification of Tumours of Haematopoieitc and Lymphoid Tissues. International Agency for Research on Cancer, Lyon, France.

TCGA (2013) Genomic and epigenomic landscapes of adult de novo acute myeloid leukemia. The Cancer Genome Atlas Research Network. *New England Journal of Medicine*, **368**, 2059–2074.

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