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The spectrum of somatic mutations in high-risk acute myeloid leukemia 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, Chicago, IL

[2]Department of Pathology, University of Chicago, Chicago, IL

[3]University of Chicago Comprehensive Cancer Center

[4]Department of Genetics, University of Pennsylvania, Philadelphia, PA

[5]Department of Medicine, Section of Hematology/Oncology, University of Chicago, Chicago, IL

[6]Department of Human Genetics, Section of Genetic Medicine, University of Chicago, Chicago, IL

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 forty patients with *de novo* or therapy-related myeloid neoplasms. -7/del(7q) leukemias 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 occur in 50% of cases, a significantly higher frequency than other AMLs and higher than previously reported. Our data provide guidance for which pathways may be most relevant in the treatment of adverse-risk myeloid leukemia.

Keywords

Acute myeloid leukemia; therapy-related myeloid neoplasm; monosomy 7; mutations; *CUX1*; RAS pathway

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 (AML) and 70% of therapy-related myeloid neoplasms (t-MN) (Leith *et al,* 1997; Smith *et al,* 2003; Grimwade *et al,* 2010). The

Corresponding author: Kevin P. White, Institute for Genomics and Systems Biology, The University of Chicago, 900 East 57th Street, KCBD 10100A, Chicago, IL 60637, kpwhite@uchicago.edu, phone: 773-834-3913, fax: 773-834-2877.

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median overall survival for patients with high-risk abnormalities is less than one 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* 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 SNP-array analysis (McNerney *et al,* 2013). We identified the gene encoding the *CUX1* transcription-factor to be a highly conserved, haploinsufficient myeloid tumor 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) leukemias 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 Supplemental Materials.

Results/Discussion

We identified the somatic mutations in thirteen leukemia 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,* 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) (Tables S1). Two samples had a recurrent genetic variation as defined by the 2008 WHO 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 tumor and normal exome-sequencing was performed on six cases; seven others underwent RNA-sequencing of the leukemia 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 tumor exomes was 130X, 72X for normal exomes, and 30X for RNA-sequenced tumors (Table S1). The median percentage of coding bases with sufficient depth for SNP identification (≥ 8X coverage) was 92.1% for tumor exomes, 83.6% for normal exomes, and 37.6% for RNA-sequenced tumors. Copy number analysis was available for eight leukemia samples (McNerney *et al.* 2013).

We identified 40 mutations in the 6 exome-sequenced cases (Table 1). 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, and the validation rate was 93.8% (Table 1 and 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; 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: tumor suppressors, signaling 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 (Figure 1A). The mutations were comprised of those associated with juvenile myelomonocytic leukemia (JMML), including activating mutations of *NRAS* and *PTPN11*, and inactivating mutations of *CBL* (Table 1). 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 pathways, mutations in -7/del(7q) AML samples from The Cancer Genome Atlas (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 5 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 (Figure 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=0.0033). We note that RAS pathway mutations were restricted to those cases with deletions of *CUX1*, occurring in 12/22 (55%, p=0.00014). 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=0.24), whereas alterations in other major leukemogenic pathways were underrepresented. There were fewer mutations in the genes encoding the signaling molecules, *FLT3* or *KIT*, (p=0.045), the cohesin complex (p=0.031), and *NPM1* (p=0.0034). 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=0.12).

Myeloid transcription factor alterations (Table S3) were decreased in −7/del(7q) leukemias. 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=0.061). 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=0.014), 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=0.0001, TCGA cohort), is driven by the strong association between del(5q) and *TP53* mutations. With one exception, all of the fifteen *TP53* mutations or deletions in the combined cohorts occurred in samples that also had del(5q) (Cochran-Mantel-Haenszel test p=3.5e-07).

This is the first description of the genome-wide mutation burden in high-risk myeloid leukemia 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 tumors within these three diseases (p<0.01). 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 tumorigenic mechanism that extends beyond hematologic 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.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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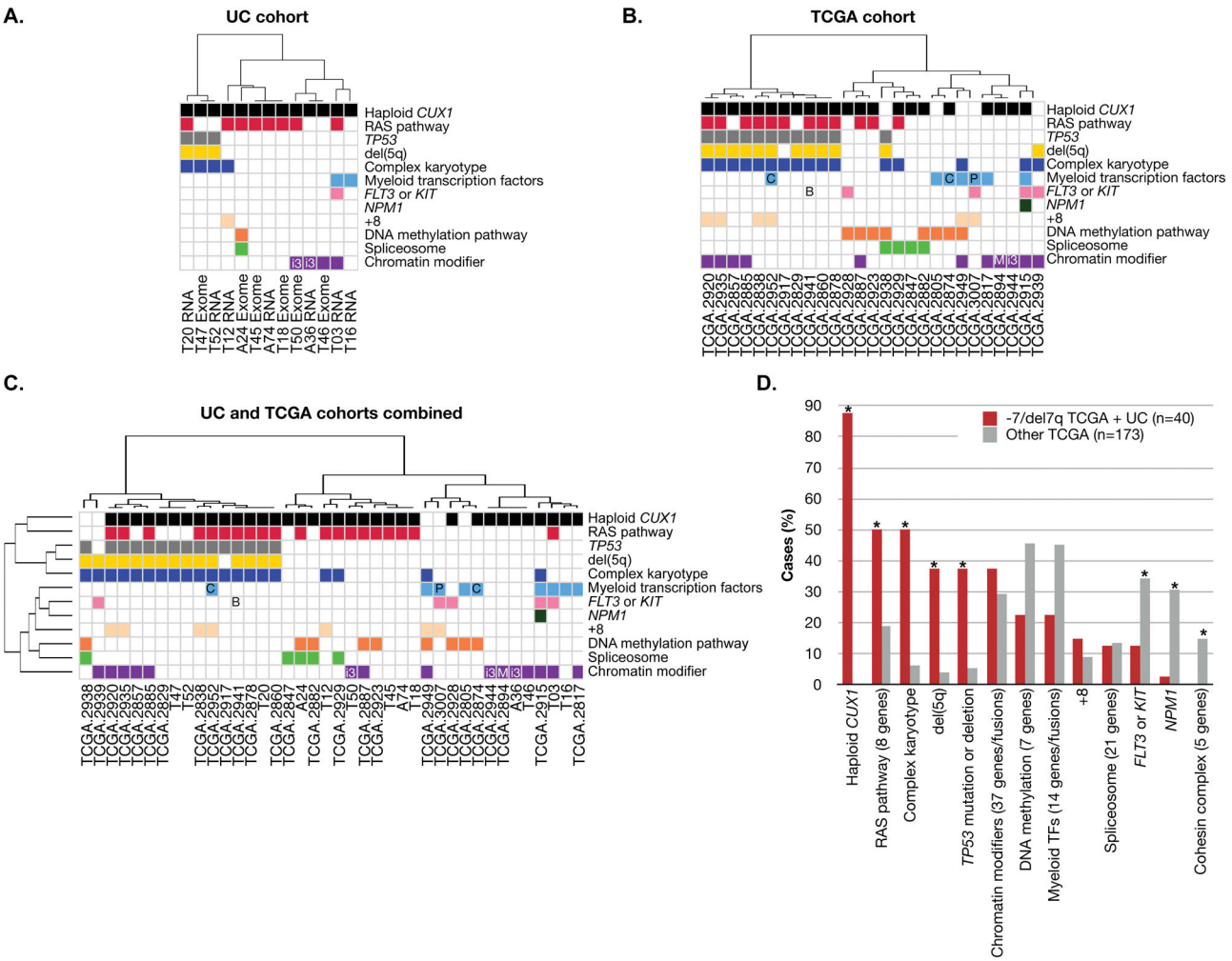

Figure 1.

The pattern of somatic mutations in -7/del(7q) leukemias is distinct from other AML types. Categorization of genes within pathways is as defined (TCGA 2013) (Table S4). 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) leukemias (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 $p < 0.05$ comparing -7/del(7q) TCGA samples versus other TCGA samples. All recurrent genetic abnormalities according to the 2008 WHO classification "AML with recurrent genetic abnormalities" are indicated (Swerdlow *et al.* 2008), with an abbreviation within the relevant pathway. B: BCR-ABL fusion; C: CEBPA mutation; i3: inv(3)(q21q26.2) or t(3)(3;3)(q21;q26.2); M: MLLT3-MLL fusion; and P: PML-RAR fusion. Abbreviations:

TF, transcription factor. Within the UC cohort, t-MN samples are named by T*XX* and *de novo* AML samples are named by A*XX*.

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Table 1

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

Sample	Gene	Amino acid change	Deleteriousness (GERP score)	Cancer Gene Census gene	TCGA AML gene mutation frequency	cBioPortal gene mutation frequency in other tumors
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 adeno., 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 adeno., 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

Sample	Gene	Amino acid change	Deleteriousness (GERP score)	Cancer Gene Census gene	TCGA AML gene mutation frequency	cBioPortal gene mutation frequency in other tumors
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 adeno., 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
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 adeno., 7.7% gastric, others
T45	FGF18	R34H	4.24		0%	2.2% melanoma, 1.6% lung adeno., 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	K349fs	indel		0%	3.5% pancreatic, 3.2% melanoma, others

Sample	Gene	Amino acid change	Deleteriousness (GERP score)	Cancer Gene Census gene	TCGA AML gene mutation frequency	cBioPortal gene mutation frequency in other tumors
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 adeno., 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 adeno, 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
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 Genome Research 15:901)

Cancer Gene Census data was downloaded March 2014 (Futreal, PA, *et al.* 2004 Nature Reviews Cancer 4:177).

cBioPortal data (Gao, J, *et al.* 2013 Science Signaling 6:pl1) represents the two tumor types with the highest frequency of mutations in that gene (accessed March 2014).