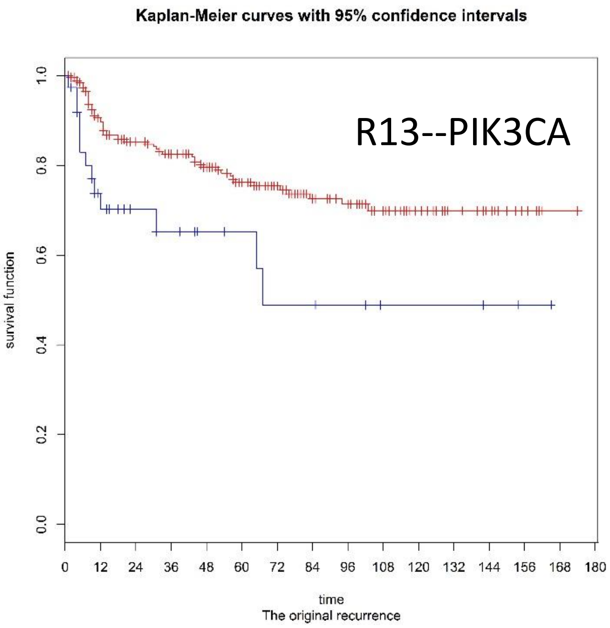
Somatic copy number alterations detected by ultra-deep targeted sequencing predict prognosis in oral cavity squamous cell carcinoma

Supplementary Material

The filtering process for SNVs reported by the Torrent Variant Caller.

All of the SNVs reported by the Torrent Variant Caller should satisfy each of the following conditions:

1. Allelic frequency ≧ 0.03.
2. Absence in the database of common variants, as defined by the dbSNP.
3. Absence in the SNP database of the International HapMap Project (general population database).
4. Absence in the SNP database of the 1000 Genomes Project (general population database).
5. Nonsynonymous SNV or SNV occurred in splicing sites.


Kaplan-Meier curves with 95% confidence intervals
R13--PIK3CA
survival function
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0.4
0.6
0.8
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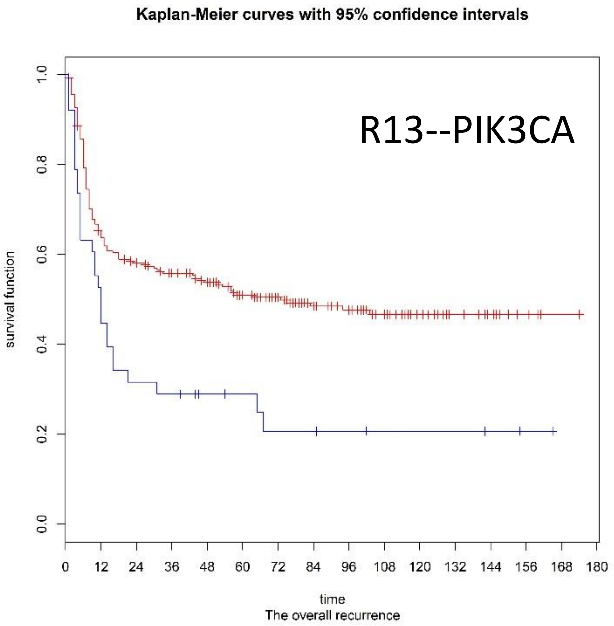
time
The original recurrence


Kaplan-Meier curves with 95% confidence intervals
R13--PIK3CA
survival function
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1.0
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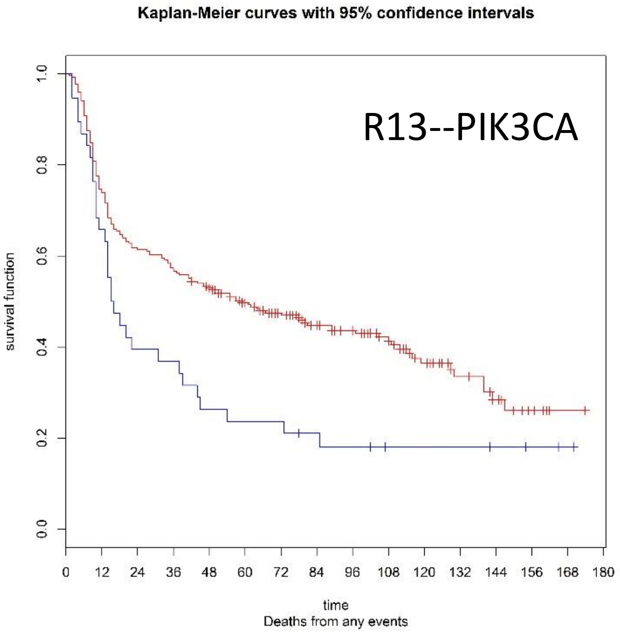
time
The overall recurrence


Kaplan-Meier curves with 95% confidence intervals
R13--PIK3CA
survival function
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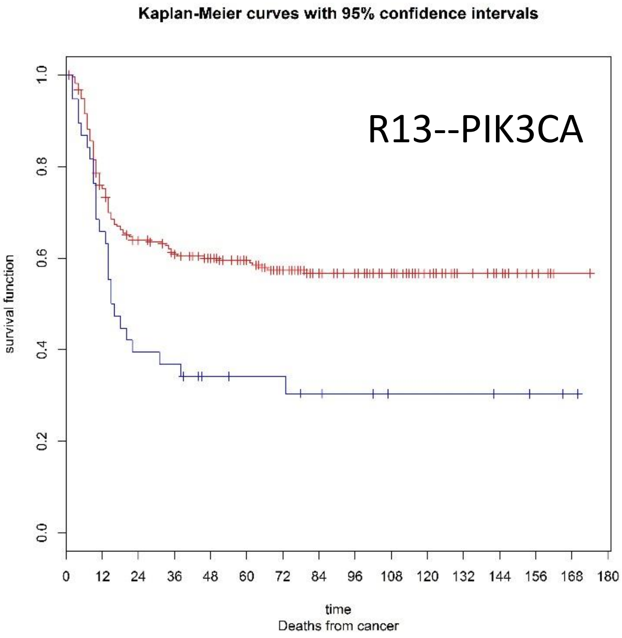
time
Deaths from any events


Kaplan-Meier curves with 95% confidence intervals
R13--PIK3CA
survival function
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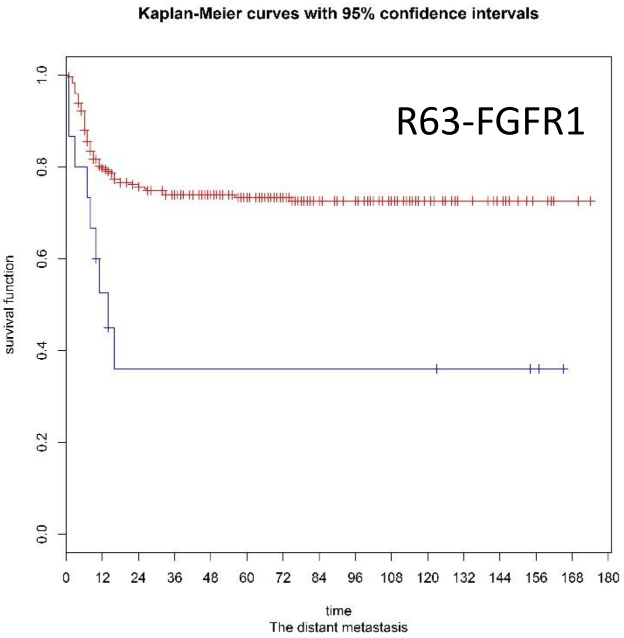
time
Deaths from cancer


Kaplan-Meier curves with 95% confidence intervals
R63-FGFR1
survival function
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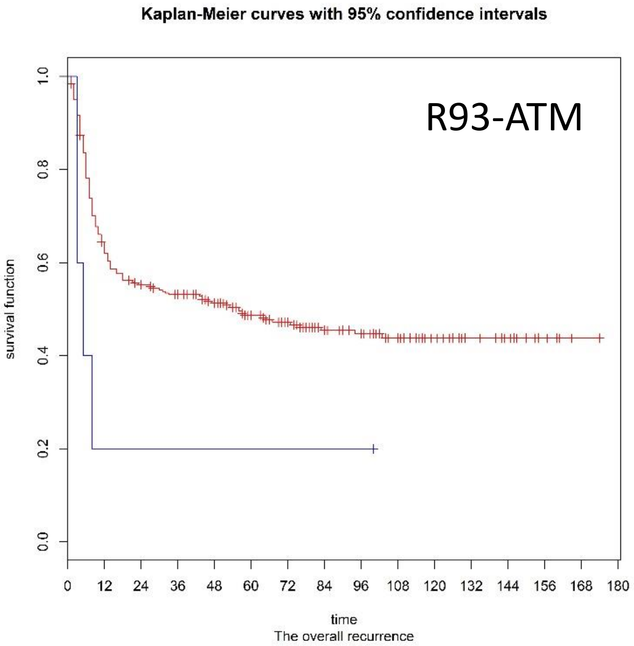
time
The distant metastasis


Kaplan-Meier curves with 95% confidence intervals
R93-ATM
survival function
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1.0
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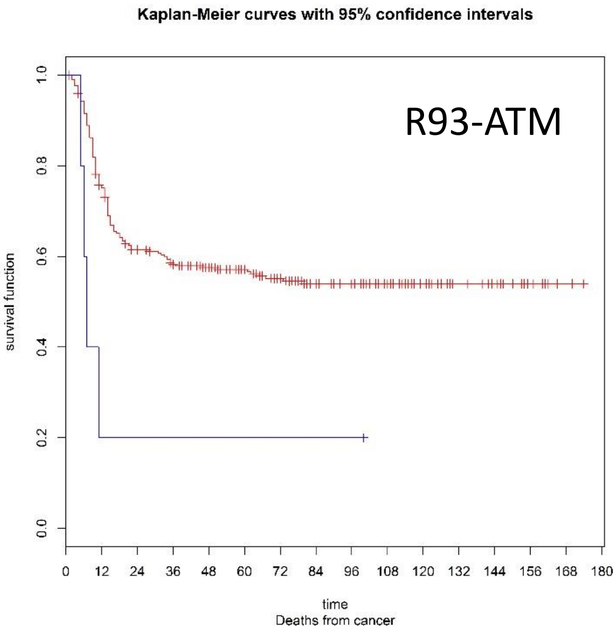
time
The overall recurrence


Kaplan-Meier curves with 95% confidence intervals
R93-ATM
survival function
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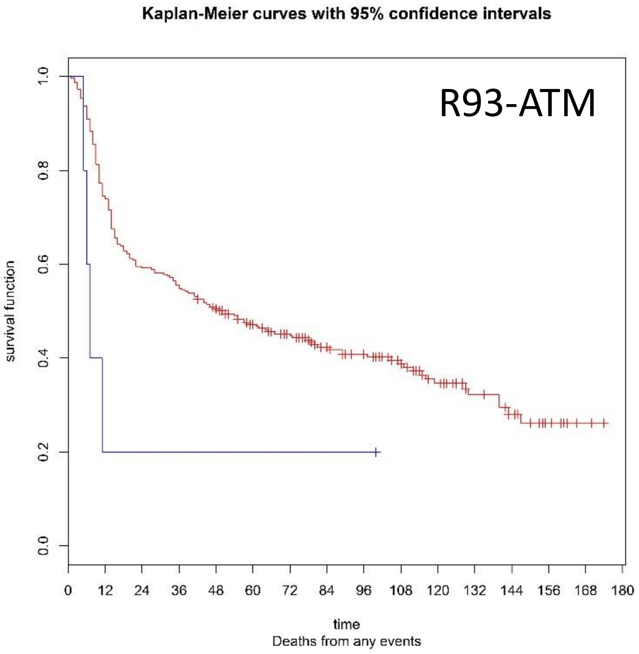
time
Deaths from cancer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R93-ATM
survival function
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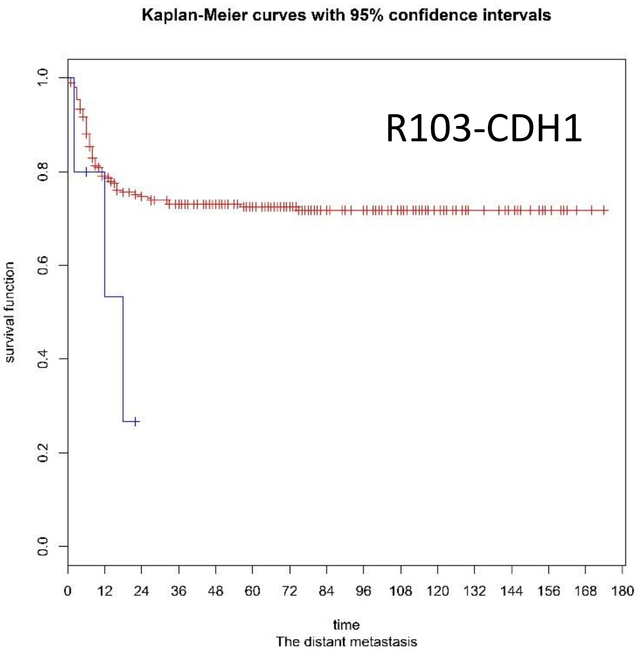
time
Deaths from any events


Kaplan-Meier curves with 95% confidence intervals
R103-CDH1
survival function
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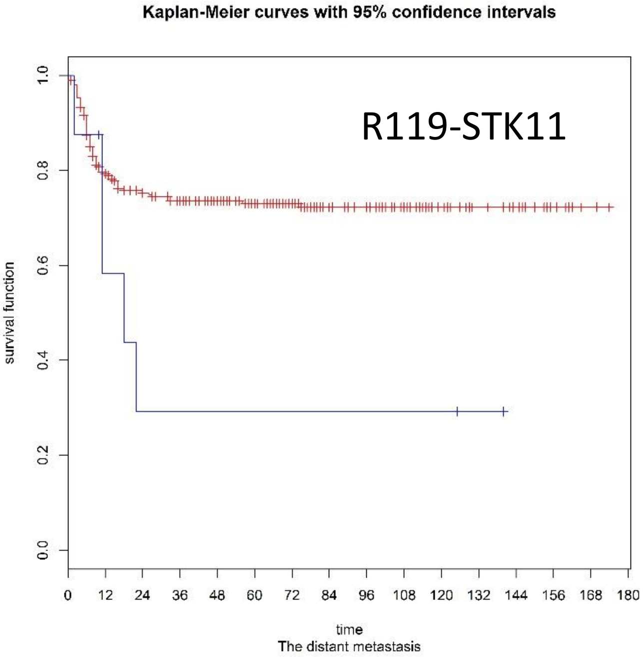
time
The distant metastasis


Kaplan-Meier curves with 95% confidence intervals
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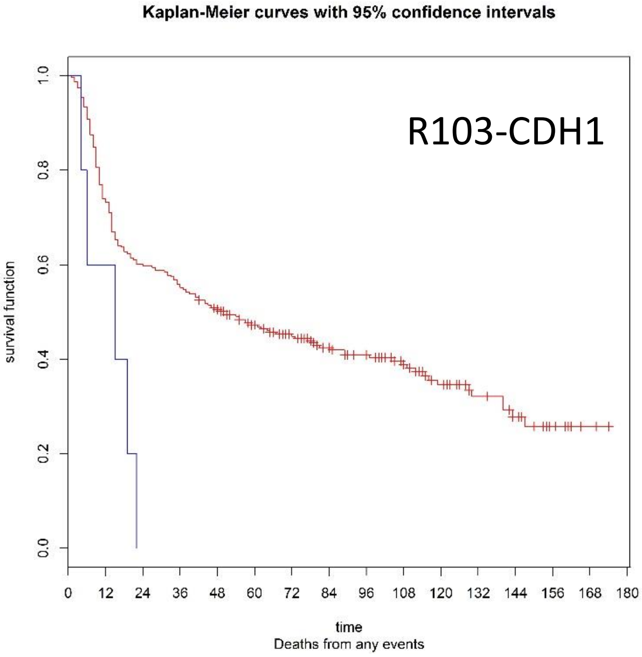
time
The distant metastasis


Kaplan-Meier curves with 95% confidence intervals
R103-CDH1
survival function
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0.8
1.0
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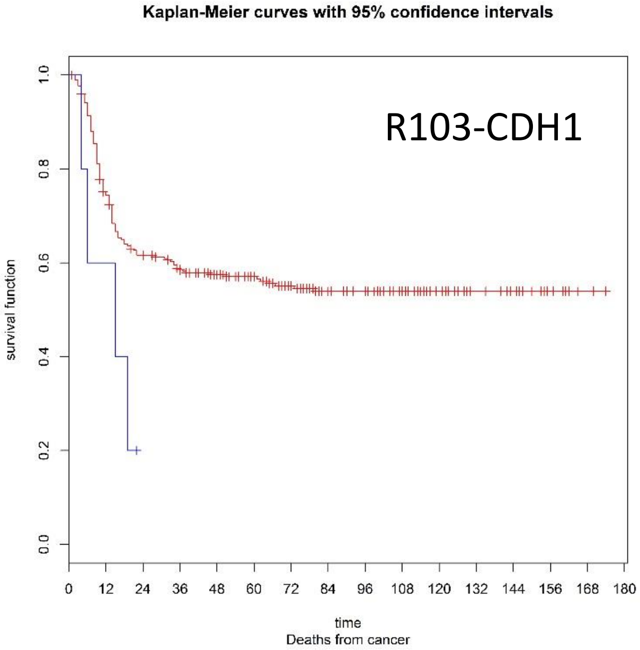
time
Deaths from any events


Kaplan-Meier curves with 95% confidence intervals
R103-CDH1
survival function
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0.4
0.6
0.8
1.0
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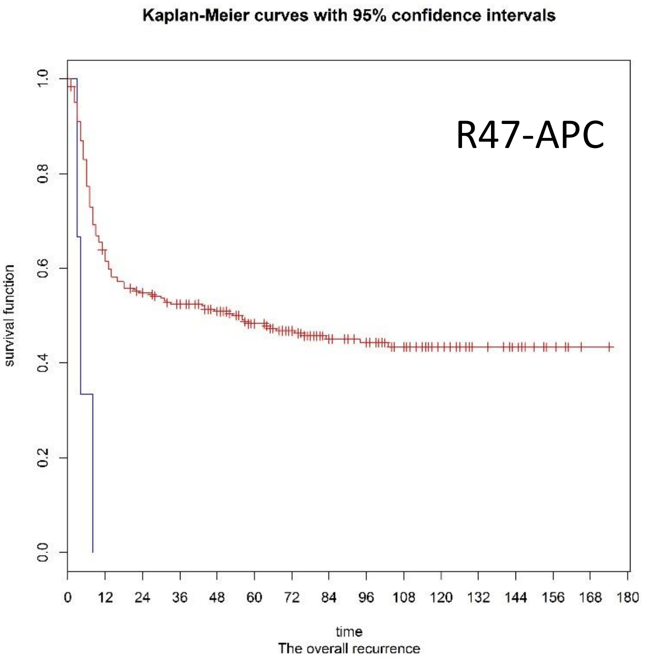
time
Deaths from cancer

Figure S1. Kaplan-Meier plots showing the associations between consistently replicated copy number amplifications and clinical outcomes.


Kaplan-Meier curves with 95% confidence intervals
R47-APC
survival function
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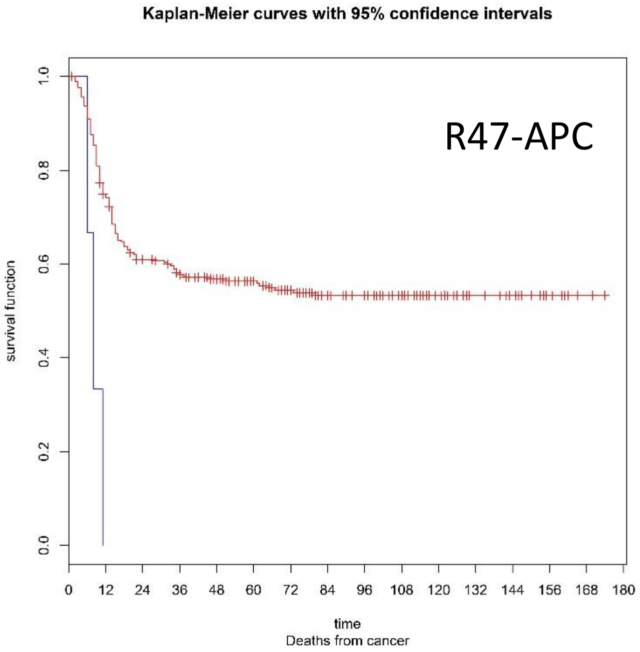
0.6
0.8
1.0
0 12 24 36 48 60 72 84 96 108 120 132 144 156 168 180
time
The overall recurrence


Kaplan-Meier curves with 95% confidence intervals
R47-APC
survival function
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0.4
0.6
0.8
1.0
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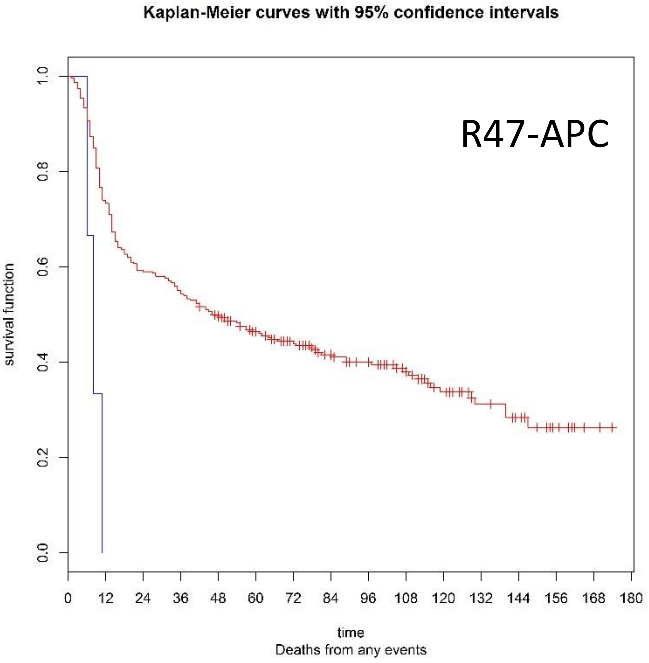
time
Deaths from cancer


Kaplan-Meier curves with 95% confidence intervals
R47-APC
survival function
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0.4
0.6
0.8
1.0
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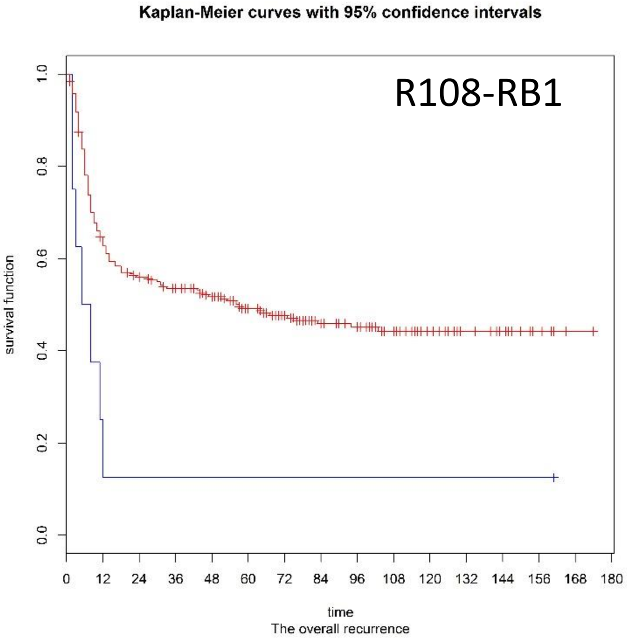
time
Deaths from any events


Kaplan-Meier curves with 95% confidence intervals
R108-RB1
survival function
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0.6
0.8
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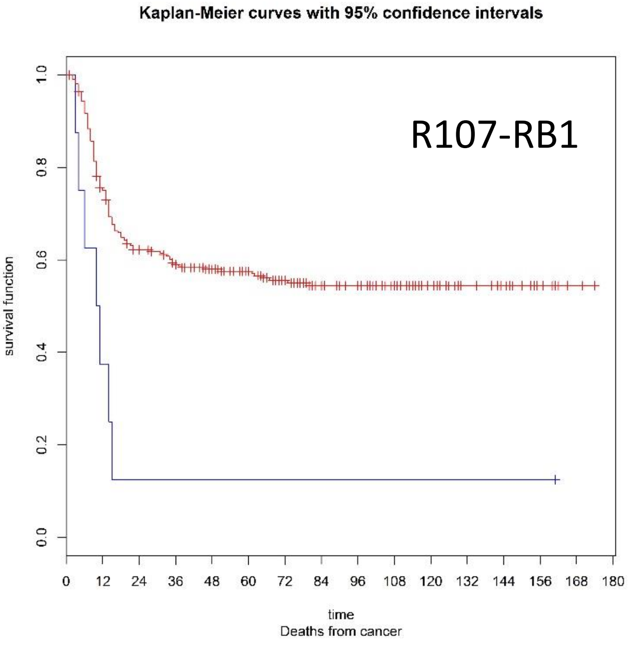
time
The overall recurrence


Kaplan-Meier curves with 95% confidence intervals
R107-RB1
survival function
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0.8
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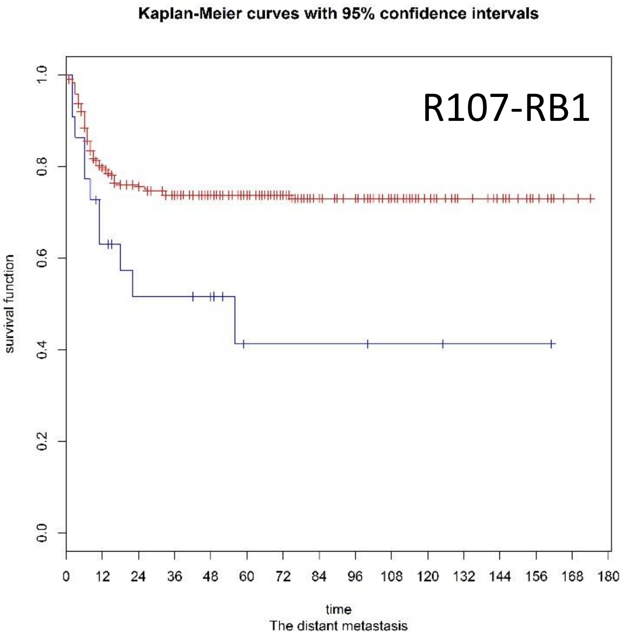
time
Deaths from cancer


Kaplan-Meier curves with 95% confidence intervals
R107-RB1
survival function
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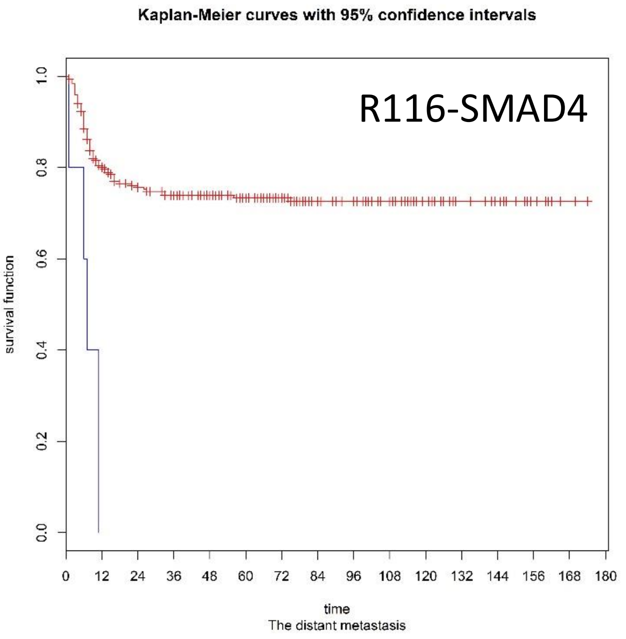
time
The distant metastasis


Kaplan-Meier curves with 95% confidence intervals
R116-SMAD4
survival function
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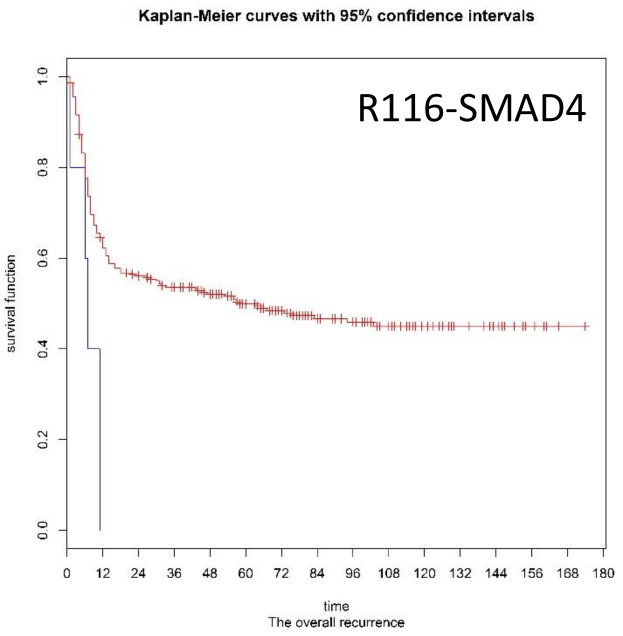
time
The distant metastasis


Kaplan-Meier curves with 95% confidence intervals
R116-SMAD4
survival function
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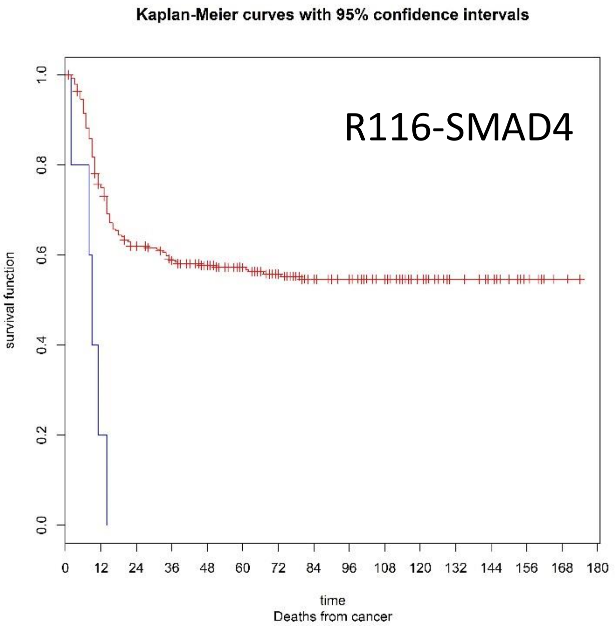
0 12 24 36 48 60 72 84 96 108 120 132 144 156 168 180
time
The overall recurrence


Kaplan-Meier curves with 95% confidence intervals
R116-SMAD4
survival function
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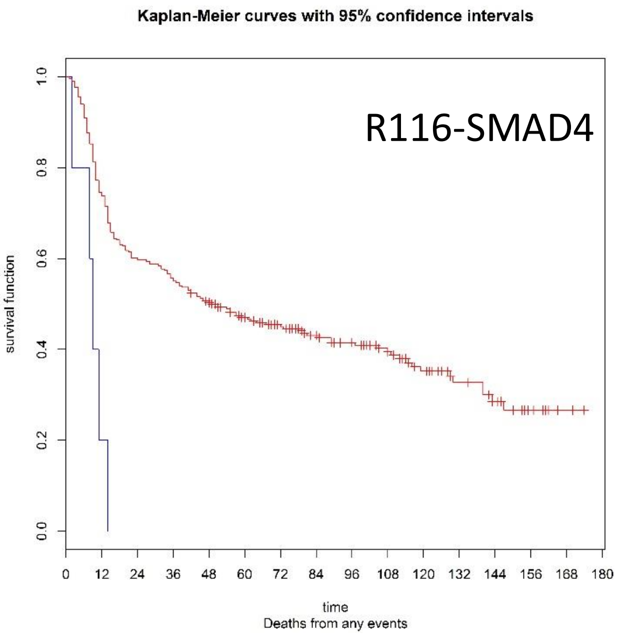
time
Deaths from cancer


Kaplan-Meier curves with 95% confidence intervals
R116-SMAD4
survival function
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0.6
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0.2
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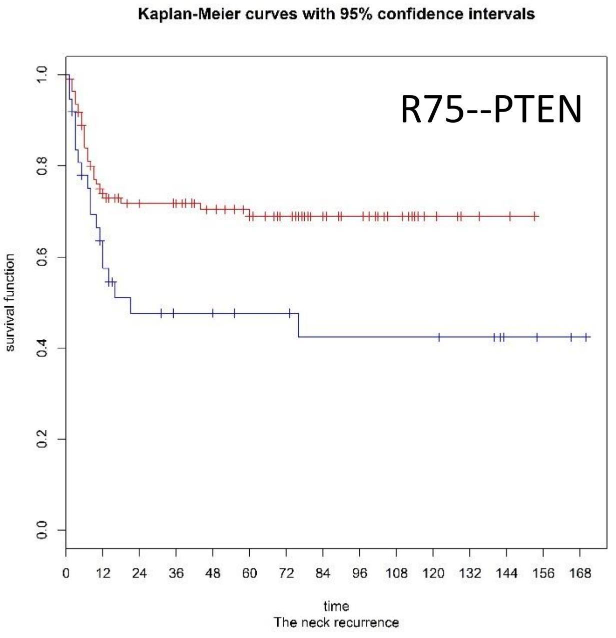
0 12 24 36 48 60 72 84 96 108 120 132 144 156 168 180
time
Deaths from any events


Kaplan-Meier curves with 95% confidence intervals
R75--PTEN
survival function
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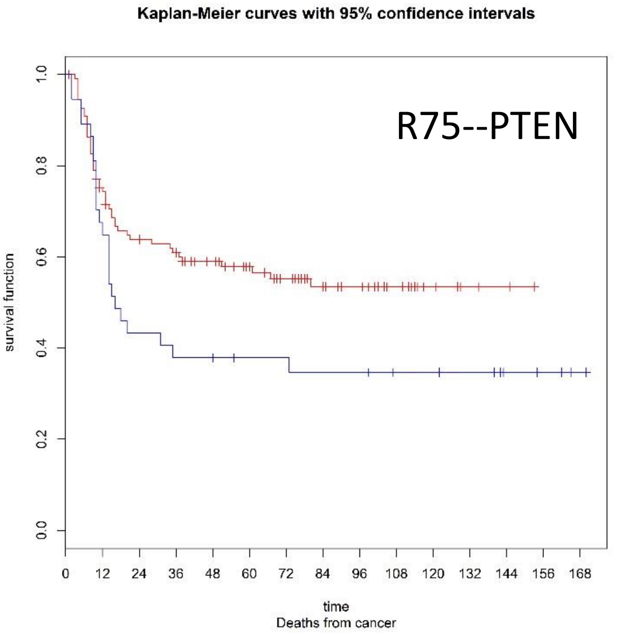
time
The neck recurrence


Kaplan-Meier curves with 95% confidence intervals
R75--PTEN
survival function
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0.6
0.4
0.2
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time
Deaths from cancer

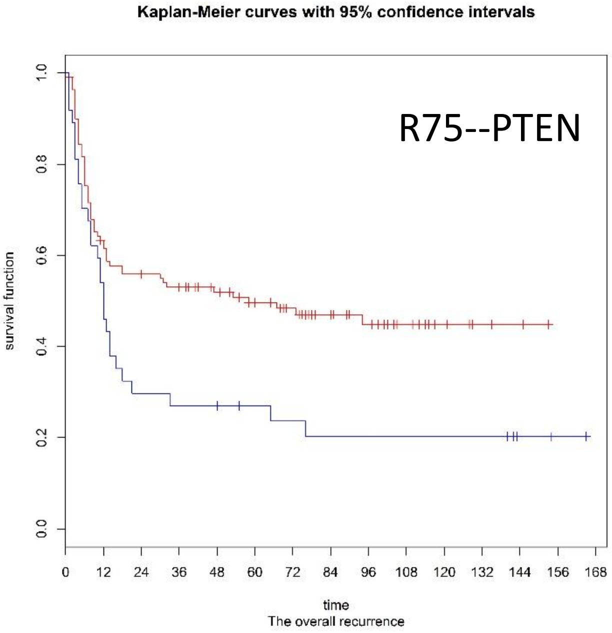

Figure S2. Kaplan-Meier plots showing the associations between consistently replicated copy number deletions and clinical outcomes.

Table S1. List of the 46 genes analyzed by ultra-deep targeted sequencing

ABL1
AKT1
ALK
APC
ATM
BRAF
CDH1
CDKN2A
CSF1R
CTNNB1
EGFR
ERBB2
ERBB4
FBXW7
FGFR1
FGFR2
FGFR3
FLT3
GNAS
HNF1A
HRAS
IDH1
JAK2
JAK3
KDR
KIT
KRAS
MET
MLH1
MPL
NOTCH1
NPM1
NRAS
PDGFRA
PIK3CA
PTEN
PTPN11
RB1
RET
SMAD4
SMARCB1
SMO
SRC
STK11
TP53
VHL

Table S2. Log-rank tests of different clinical outcomes in relation to focal copy number amplifications

Clinical outcome	**CNA-harboring gene**	**Hazard ratio**	**FDR adjusted P values**
Second primary tumors	ERBB4	5.711	0.05359
Second primary tumors	KDR	4.399	0.128281
Second primary tumors	RB1	3.524	0.11415
Second primary tumors	TP53	11.909	0.000389
Second primary tumors	ERBB2	9.707	0.006449
Local recurrence	PIK3CA	2.332	0.020028
Local recurrence	KIT	3.378	0.116609
Local recurrence	MET	2.147	0.018173
Local recurrence	JAK2	8.834	0.009555
Local recurrence	FGFR2	2.131	0.044413
Local recurrence	ATM	2.302	0.046334
Local recurrence	SMAD4	2.439	0.111215
Local recurrence	GNAS	2.424	0.04367
Local recurrence	SMARCB1	1.712	0.126397
Neck recurrence	ALK	1.720	0.045986
Neck recurrence	PIK3CA	1.793	0.118995

Neck recurrence	FBXW7	0.370	0.059376
Neck recurrence	APC	2.200	0.133036
Neck recurrence	JAK2	6.304	0.00691
Neck recurrence	ATM	24.163	0.006927
Neck recurrence	MPL	0.436	0.076583
Neck recurrence	RB1	8.131	0.01132
Neck recurrence	RET	23.474	0.007416
Distant metastases	MLH1	2.005	0.015232
Distant metastases	PDGFRA	3.285	0.015232
Distant metastases	KDR	2.696	0.086268
Distant metastases	APC	2.986	0.006234
Distant metastases	FGFR1	3.043	0.005267
Distant metastases	FGFR2	0.196	0.043997
Distant metastases	HRAS	1.881	0.095354
Distant metastases	FLT3	0.253	0.136246
Distant metastases	CDH1	3.182	0.125833
Distant metastases	ERBB2	3.535	0.086268
Distant metastases	STK11	66.444	0.000397
Distant metastases	GNAS	1.904	0.137368
Distant metastases	SMO	8.034	0.011414
Distant metastases	TP53	7.232	0.000422

Distant metastases	FGFR3	5.273	0.001057
Distant metastases	ERBB2	2.816	0.112271
Distant metastases	HNF1A	2.694	0.021986
Distant metastases	RB1	9.043	0.00683
Distant metastases	RET	13.325	0.031506
Disease-free survival	PIK3CA	1.987	0.002668
Disease-free survival	FBXW7	0.597	0.108512
Disease-free survival	APC	2.644	0.001777
Disease-free survival	MET	1.775	0.005425
Disease-free survival	JAK2	5.516	0.00285
Disease-free survival	ATM	14.102	0.027695
Disease-free survival	HRAS	1.814	0.024415
Disease-free survival	ERBB2	2.980	0.088398
Disease-free survival	SMO	4.069	0.128539
Disease-free survival	PDGFRA	31.941	0.00285
Disease-free survival	FGFR3	3.077	0.021433
Disease-free survival	STK11	31.098	0.003045
Disease-free survival	RB1	4.674	0.087732
Disease-free survival	RET	14.376	0.026579
Disease-specific survival	PIK3CA	1.945	0.006481
Disease-specific survival	MLH1	1.538	0.086341

Disease-specific survival	FBXW7	0.530	0.061604
Disease-specific survival	APC	2.655	0.002528
Disease-specific survival	MET	2.885	0.003454
Disease-specific survival	FGFR1	2.184	0.035701
Disease-specific survival	ABL1	1.546	0.067327
Disease-specific survival	JAK2	7.222	0.000383
Disease-specific survival	ATM	12.804	0.034817
Disease-specific survival	HRAS	1.738	0.057376
Disease-specific survival	CDH1	2.781	0.101434
Disease-specific survival	SMAD4	0.258	0.126938
Disease-specific survival	SMO	4.711	0.07245
Disease-specific survival	PDGFRA	31.674	0.00289
Disease-specific survival	TP53	4.274	0.09712
Disease-specific survival	FGFR3	3.009	0.042483
Disease-specific survival	STK11	31.244	0.002974
Disease-specific survival	ERBB2	2.658	0.050597
Disease-specific survival	RB1	5.431	0.047742
Disease-specific survival	RET	9.033	0.07245
Overall survival	PIK3CA	1.697	0.020329
Overall survival	FBXW7	1.724	0.050968
Overall survival	KDR	1.873	0.073175

Overall survival	APC	2.091	0.022218
Overall survival	MET	1.481	0.064844
Overall survival	JAK2	6.659	0.000668
Overall survival	ATM	11.899	0.0401
Overall survival	CDH1	3.323	0.023086
Overall survival	ERBB2	2.622	0.129804
Overall survival	SMAD4	0.322	0.064816
Overall survival	SMO	4.391	0.091564
Overall survival	PDGFRA	27.164	0.004311
Overall survival	TP53	3.982	0.122979
Overall survival	FGFR3	3.043	0.020864
Overall survival	STK11	27.273	0.004311
Overall survival	RB1	5.139	0.056585
Overall survival	RET	8.475	0.085023

Table S3. Log-rank tests of different clinical outcomes in relation to focal copy number deletions

Clinical outcome	CNA-harboring gene	Hazard ratio	FDR adjusted P values
Second primary tumors	ERBB4	9.363	0.115586
Second primary tumors	SMAD4	7.590	0.193956
Local recurrence	KIT	0.571	0.177049
Local recurrence	EGFR	3.220	0.163241
Local recurrence	ATM	0.615	0.17247
Local recurrence	HNF1A	9.266	0.099714
Local recurrence	RB1	4.998	0.007106
Local recurrence	SMO	2.438	0.092747
Local recurrence	PTEN	0.546	0.118786
Local recurrence	ERBB2	9.561	0.093865
Local recurrence	RET	2.303	0.123509
Neck recurrence	ERBB4	2.013	0.114452
Neck recurrence	APC	9.742	0.000499
Neck recurrence	SMAD4	16.956	5.06E-05
Neck recurrence	KIT	0.580	0.107829
Neck recurrence	TP53	3.277	0.05301
Neck recurrence	RB1	2.451	0.040416

Neck recurrence	BRAF	0.602	0.12575
Neck recurrence	PTEN	0.403	0.110695
Neck recurrence	PDGFRA	3.863	0.054354
Distant metastases	PTEN	66.158	0.000425
Distant metastases	RB1	2.270	0.039665
Distant metastases	TP53	2.025	0.0600823
Distant metastases	SMO	4.606	0.110999
Distant metastases	SMAD4	11.594	0.000123
Disease-free survival	ERBB4	1.900	0.049063
Disease-free survival	PDGFRA	3.646	0.036772
Disease-free survival	APC	5.702	0.011237
Disease-free survival	MET	4.128	0.144504
Disease-free survival	PTEN	31.155	0.003559
Disease-free survival	RB1	3.021	0.014776
Disease-free survival	SMAD4	5.556	6.89E-05
Disease-specific survival	ERBB4	1.872	0.086112
Disease-specific survival	KIT	0.651	0.092464
Disease-specific survival	APC	6.071	0.008054
Disease-specific survival	MET	5.472	0.050529
Disease-specific survival	ABL1	1.942	0.13819
Disease-specific survival	PTEN	31.532	0.003566

Disease-specific survival	RB1	3.600	0.003651
Disease-specific survival	SMAD4	5.426	0.00093
Overall survival	KIT	0.674	0.053378
Overall survival	APC	5.794	0.009984
Overall survival	MET	5.214	0.05303
Overall survival	PTEN	27.218	0.005174
Overall survival	RB1	2.558	0.049577
Overall survival	SMAD4	5.144	0.001345

Table S4 Pathogenic significance of genes with validated CNA in different human malignancies according to the published literature

Gene	Associated malignancy	
ABL1	Acute lymphoblastic leukemia	[1]
FGFR1	Oral squamous cell carcinoma	[2]
FLT3	Cutaneous myelomonocytic leukemia	[3]
KDR	Glioblastoma, primitive neuroectodermal tumors	[4-6]
KIT	Glioblastoma, primitive neuroectodermal tumors	[4-6]
PIK3CA	Oral squamous cell carcinoma	[7]
RB1	Oral squamous cell carcinoma, breast cancer, chronic lymphocytic leukemia, retinoblastoma	[8-11]
RET	Thyroid carcinoma	[12,13]
SMO	Melanoma	cBIO, 2014 (http://www.cbioportal.org/ctd2-dashboard-portal/index.do)
APC	Colorectal cancer, gastric adenocarcinomas	[14,15]
EGFR	Small cell lung cancer, non-small cell lung cancer	[16,17]
MET	Small cell lung cancer, non-small cell lung cancer	[18,19]
PTEN	Oral squamous cell carcinoma,	[20,21]

	prostate adenocarcinomas	
SMAD4	Oral squamous cell carcinoma	[22]
TP53	Oral squamous cell carcinoma	[9,23,24]

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