

Identification of new candidate therapeutic target genes in head and neck squamous cell carcinomas

Supplementary Materials

Supplementary Table S1: Selection of targeted therapies matching 42 selected druggable genes

Signalling pathway	Gene symbols	Names of genes	Chromosome location	Genbank accession number	Selected therapies
Growth factors, tyrosine kinase receptors, and ligands	<i>EGFR</i>	epidermal growth factor receptor	7p12	NM_005228.3	cetuximab, gefitinib, erlotinib, lapatinib, afatinib, neratinib, panitumumab
	<i>ERBB2</i>	erb-b2 receptor tyrosine kinase 2	17q12	NM_004448.3	trastuzumab, lapatinib, afatinib, neratinib
	<i>ERBB3</i>	erb-b2 receptor tyrosine kinase 3	12q13	NM_001982.3	pertuzumab, MM121
	<i>ERBB4</i>	erb-b2 receptor tyrosine kinase 4	2q33.3-q34	NM_005235.2	neratinib
	<i>FGFR1</i>	fibroblast growth factor receptor 1	8p11.23-p11.22	NM_015850.3	vandetanib, PD173074
	<i>FGFR2</i>	fibroblast growth factor receptor 2	10q26	NM_000141.4	vandetanib, PD173075
	<i>FGFR3</i>	fibroblast growth factor receptor 3	4p16.3	NM_000142.4	vandetanib, PD173076, dovitinib
	<i>IGF1R</i>	insulin-like growth factor 1 receptor	15q26.3	NM_000875.4	linsitinib, dalotuzumab
	<i>IGF2</i>	insulin-like growth factor 2	11p15.5	NM_000612.5	
	<i>MET</i>	MET proto-oncogene, receptor tyrosine kinase	7q31	NM_000245.2	crizotinib, foretinib
	<i>HGF</i>	hepatocyte growth factor (hepatopoietin A, scatter factor)	7q21.1	NM_000601.4	
	<i>ALK</i>	anaplastic lymphoma receptor tyrosine kinase	2p23	NM_004304.4	crizotinib
	<i>ROS1</i>	ROS proto-oncogene 1, receptor tyrosine kinase	6q22	NM_002944.2	
	<i>PDGFR4</i>	platelet-derived growth factor receptor, alpha polypeptide	4q12	NM_006206.4	imatinib, sunitinib, sorafenib, pazopanib, vatalanib, tandutinib, nilotinib, motesanib, vandetanib
	<i>PDGFRB</i>	platelet-derived growth factor receptor, beta polypeptide	5q33.1	NM_002609.3	
	<i>VEGFA</i>	vascular endothelial growth factor A	6p12	NM_003376.5	bevacizumab
	<i>VEGFB</i>	vascular endothelial growth factor B	11q13	NM_003377.4	afibercept
	<i>PGF</i>	placental growth factor	14q24.3	NM_002632.5	afibercept
	<i>VEGFR1</i>	fms-related tyrosine kinase 1	13q12	NM_002019.4	sunitinib, sorafenib, pazopanib, vatalanib, apatinib, motesanib, cediranib, vandetanib
	<i>VEGFR2</i>	kinase insert domain receptor	4q11-q12	NM_002253.2	sunitinib, sorafenib, pazopanib, vatalanib, apatinib, brivanib, motesanib, cediranib, vandetanib, foretinib
	<i>VEGFR3</i>	fms-related tyrosine kinase 4	5q35.3	NM_002020.4	sunitinib, sorafenib, pazopanib, vatalanib, apatinib, motesanib, cediranib, vandetanib
	<i>TEK</i>	TEK tyrosine kinase, endothelial	9p21	NM_000459.4	bevacizumab
	<i>KIT</i>	v-kit Hardy-Zuckerman 4 feline sarcoma viral oncogene homolog	4q12	NM_021099.3	imatinib, sunitinib, sorafenib, pazopanib, vatalanib, tandutinib, nilotinib, apatinib, motesanib
	<i>KITLG</i>	KIT ligand	12q22	NM_000899.4	imatinib, nilotinib, bafetinib
	<i>DDR2</i>	discoidin domain receptor tyrosine kinase 2	1q23.3	NM_006182.2	
	<i>ABL1</i>	ABL proto-oncogene 1, non-receptor tyrosine kinase	9q34.1	NM_005157.5	imatinib, nilotinib, dasatinib

	<i>CSF1R</i>	colony stimulating factor 1 receptor	5q32	NM_005211.3	sunitinib, vatalanib
	<i>SRC</i>	SRC proto-oncogene, non-receptor tyrosine kinase	20q12-q13	NM_005417.4	dasatinib, bosutinib, apatinib
	<i>RET</i>	ret proto-oncogene	10q11.2	NM_020630.4	sunitinib, motesanib
JAK-STAT pathway	<i>JAK2</i>	janus kinase 2	9p24	NM_004972.3	lestaurtinib
	<i>STAT3</i>	signal transducer and activator of transcription 3 (acute-phase response factor)	17q21.31	NM_003150.3	atiprimod
Cell Differentiation	<i>NOTCH1</i>	notch 1	9q34.3	NM_017617.3	MK0752, PF-03084014, RO4929097, R4733
	<i>NOTCH2</i>	notch 2	1p13-p11	NM_024408.3	MK0752, PF-03084014, RO4929097, R4733, tarextumab
	<i>NOTCH4</i>	notch4	6p21.3	NM_004557.3	MK0752, PF-03084014, RO4929097, R4733
	<i>DLL4</i>	delta-like 4 (Drosophila)	15q14	NM_019074.3	REGN421
	<i>SMO</i>	smoothened, frizzled class receptor	7q32.3	NM_005631.4	vismodegib, patidegib, erismodegib, taladegib
PI3K-AKT-mTOR	<i>AKT1</i>	v-akt murine thymoma viral oncogene homolog 1	14q32.32	NM_005163.2	AZD5363, MK2206, perifosine, ipatasertib (GDC-0068)
	<i>PIK3CA</i>	phosphatidylinositol-4,5-bisphosphate 3-kinase, catalytic subunit alpha	3q26.3	NM_006218.2	buparlisib (BKM120), copanlisib, taselisib (GDC-0032)
Cell Cycle	<i>CDK4</i>	cyclin-dependent kinase 4	12q14.3-q15	NM_000075.3	palbociclib, RGB-286638, R547, P1446-05, BAY1000394, ZK304709
	<i>CDK6</i>	cyclin-dependent kinase 6	7q21-q22	NM_001259.6	palbociclib, R547
	<i>MDM2</i>	MDM2 proto-oncogene, E3 ubiquitin protein ligase	12q14.3-q15	NM_002392.5	idasanutlin, RO5503781
Immune response	<i>PDL1/CD274</i>	CD274 molecule	9p24	NM_014143.3	avelumab, tremelimumab, atezolizumab, BMS-936559
Controls	<i>CCND1</i>	cyclin D1	11q13	NM_053056.2	_
	<i>MKI67</i>	marker of proliferation Ki-67	10q26.2	NM_002417.4	_

Supplementary Table S2: Primers used for quantitative RT-PCR for the 42 selected genes and *CCND1*, *MKI67*, *TBP* controls

Gene	Upper sequence (5' à 3')	Lower sequence (5' à 3')	Amplicon size (pb)
<i>EGFR</i>	5' GGA GAA CTG CCA GAA ACT GAC C 3'	5' GCC TGC AGC ACA CTG GTT G 3'	106
<i>ERBB2</i>	5' AGC CGC GAG CAC CCA AGT 3'	5' TTG GTG GGC AGG TAG GTG AGT T 3'	147
<i>ERBB3</i>	5' GTCTGTGTGACCCACTGCAACT 3'	5' GGGTGGCAGGAGAAGCATT 3'	80
<i>ERBB4</i>	5' GGC TGC TGA GTT TTC AAG GAT G 3'	5' GCT TCA TAC GAT CAT CAC CCT GA 3'	74
<i>FGFR1</i>	5' GAA TTG GAG GCT ACA AGG TCC GTT 3'	5' GGT TGA TGC TGC CGT ACT CAT TCT 3'	118
<i>FGFR2</i>	5' GGC CGT GAA GAT GTT GAA AGA TG 3'	5' CCT GTG TGC AGG CTC CAA GAA 3'	128
<i>FGFR3</i>	5' GGCTGAAGAACGGCAGGGAGT 3'	5' CTGCTGATGCCGCAGCTTGA 3'	68
<i>IGF1R</i>	5' CCAAGGCCTGAAAACCTCCATCT 3'	5' ACACATTCTCGCTGATCCTCAACT3'	116
<i>IGF2</i>	5' CGA CCG TGC TTC CGG ACA AC 3'	5' AGG CGC TGG GTG GAC TGC TT 3'	85
<i>MET</i>	5' ATG GGT CAA TTC AGC GAA GTC C 3'	5' GAT CGA GAA ACC ACA ACC TGC AT 3'	119
<i>HGF</i>	5' CCC TGG TGT TTC ACA AGC AAT C 3'	5' GGT CAT GCA TTC AAC TTC TGA ACA 3'	81
<i>KIT</i>	5' TCC TCG CCT CCA AGA ATT GTA T 3'	5' CTT GAT GTC TCT GGC TAG ACC AAA 3'	111
<i>KITLG</i>	5' ACG TCA CTA AAT TGG TGG CAA ATC 3'	5' GCT TAT CCA ACA ATG ACT TGG CA 3'	98
<i>RET</i>	5' GCC ACC GAC CAG CAG ACC T 3'	5' GCC TCC TCG GCC ACA TAT GA 3'	80
<i>CSF1R</i>	5' GATGGGTGGCAGGAAGGTGA 3'	5' GGGCCCTGGGATGACTTTCT 3'	67
<i>PDGFRA</i>	5' CAT TTA CAT CTA TGT GCC AGA CCC A 3'	5' ATG GCA GAA TCA TCA TCC TCC AC 3'	93
<i>PDGFRB</i>	5' CCC CAG TGC CGA GTT AGA AGA C 3'	5' GCA CGT AGC CGC TCT CAA CC 3'	116
<i>ALK</i>	5' CGG AGG ATA TAT AGG CGG CAA T 3'	5' ATG CCC AGT GGA CTG ATG AAG GA 3'	90
<i>SRC</i>	5' ACC ACC TTT GTG GCC CTC TAT G 3'	5' GCC ACC AGT CTC CCT CTG TGT T 3'	109
<i>DDR2</i>	5' TCC AGT CAC CGA CCA CTC CAT 3'	5' GTA AGA CAC CAA GCC ATC TAG CCA 3'	82
<i>ROS1</i>	5' GATGGCAACGTTTTATGGTCCTT 3'	5' TCTAGCTGCCAGATCCCTGTGA 3'	126
<i>VEGFA</i>	5' CTT GCC TTG CTG CTC TAC CTC C 3'	5' CAT CCA TGA ACT TCA CCA CTT CGT 3'	103
<i>VEGFB</i>	5' GGT GCC CAG CTG CGT GAC T 3'	5' CCG GAT CAT GAG GAT CTG CAT C 3'	112
<i>VEGFR1</i>	5' ATC ATT CCG AAG CAA GGT GTG AC 3'	5' TCC TTC TAT TAT TGC CAT GCG CT 3'	122
<i>VEGFR2</i>	5' TGG GAA CCG GAA CCT CAC TAT C 3'	5' GTC TTT TCC TGG GCA CCT TCT ATT 3'	132
<i>VEGFR3</i>	5' GTC ACG CTG CGC TCG CAA A 3'	5' GTG CAG CAG TGG CGT GGA CA 5'	99
<i>PGF</i>	5' GCTCGTCAGAGGTGGAAGTGGT 3'	5' CTCGCTGGGGTACTCGGACA 3'	103
<i>STAT3</i>	5' GCT TCC TGC AAG AGT CGA ATG TT 3'	5' GGC TTC TCA AGA TAC CTG CTC TGA 3'	85
<i>MDM2</i>	5' GCG TGC CAA GCT TCT CTG TGA 3'	5' CTG AGT CCG ATG ATT CCT GCT GA 3'	96
<i>ABL1</i>	5' GCCAGTACGGGGAGGTGTACGA 3'	5' CTCCATGGTGTCTCCTTCAAGGT 3'	96
<i>NOTCH1</i>	5' TGC GTG CAG CGC GTC AAT GAC T 3'	5' CAG CGG CGC CCG GTG TGA 3'	62
<i>NOTCH2</i>	5' CAC CTG CCC ACA AGG CTA CA 3'	5' CAC AAG GAT TGC TAT TGG CCA T 3'	80
<i>NOTCH4</i>	5' GTGCATCTGCCTGCCTGGATT 3'	5' CCCACCATGGCACAGGGA 3'	85
<i>JAK2</i>	5' GACTTTTGTCTGTTCGAGCGAGAA 3'	5' CTTTGTCCCACTGAGGTTGTACTCTT 3'	94
<i>SMO</i>	5' GCT ACT TCC TCA TCC GAG GAG TCA 3'	5' GGC GCA GCA TGG TCT CGT T 3'	108
<i>TEK</i>	5' GTC AGC TTG CTC CTT CTG GAA CT 3'	5' GAG GCA ATG CAG GTG AGA GAT GT 3'	107
<i>AKT1</i>	5' CCC AGG TCA CGT CGG AGA CT 3'	5' ACT CCA TGC TGT CAT CTT GGT CA 3'	99
<i>PIK3CA</i>	5' CCTGATCTTCCTCGTGCTGCTC 3'	5' ATGCCAATGGACAGTGTTCCTCTT 3'	92
<i>CDK4</i>	5' CCA ATG TTG TCC GGC TGA TG 3'	5' CTA CAT GCT CAA ACA CCA GGG TTA C 3'	84
<i>CDK6</i>	5' CCC AAC GTG GTC AGG TTG TTT 3'	5' GTG GTC AAG TCT TGA TCG ACA TGT T 3'	101
<i>PDL1/CD274</i>	5' GCTGAATTGGTCATCCCAGAACTAC 3'	5' AAACGGAAGATGAATGTCAGTGCTAC 3'	95
<i>DLL4</i>	5' AAT GGA GGC AGC TGT AAG GAC C 3'	5' AAG GTG CTG TGT TCA CAA TGC AG 3'	90
<i>MKI67</i>	5' ATT GAA CCT GCG GAA GAG CTG A 3'	5' GGA GCG CAG GGA TAT TCC CTT A 3'	105

CCND1	5' GGATGCTGGAGGTCTGCGA 3'	5' AGAGGCCACGAACATGCAAG 3'	128
TBP	5' TGCACAGGAGCCAAGAGTGAA 3'	5' CACATCACAGTCCCCACCA 3'	112

Supplementary Table S3: Association between clinical and biological characteristics of the 46 HNSCC patients with high expression levels of *PIK3CA*, compared to the patients with low expression levels of *PIK3CA*

	Whole cohort	<i>PIK3CA</i> high expression	<i>p</i> ^a
	Number of patients (%)	Number of patients (%)	
Total	96	46 (47.9)	
<i>Age</i>			
< 56	46 (53.1)	24 (52.2)	0.42
> 56	50 (46.9)	22 (44)	
<i>Sexe</i>			0.0089
male	77 (80.2)	42 (54.5)	
female	19 (19.8)	4 (21.1)	
<i>HPV status</i>			
positive	12 (12.5)	3 (25.0)	0.089
negative	84 (87.5)	43 (51.1)	
<i>Alcool</i> ^b			
Yes	50 (70.4)	28 (56.0)	0.013
No	21 (29.6)	5 (23.8)	
<i>Tobacco</i> ^c			
Yes	58 (72.5)	31 (53.4)	0.036
No	22 (27.5)	6 (27.3)	
<i>AJCC Stage</i>			
stage I	10 (10.4)	2 (20)	0.014
stage II	15 (15.6)	3 (20)	
stage III	12 (12.5)	7 (58.3)	
stage IV	59 (61.5)	34 (57.6)	
<i>Tumor Location</i>			
Oral cavity	43 (44.8)	13 (30.2)	0.0011
Oropharynx	20 (20.8)	9 (45)	
Larynx	17 (17.7)	10 (58.8)	
Hypopharynx	16 (16.7)	14 (87.5)	
<i>HRAS, NRAS, PIK3CA</i> mutations	12 (12.5)	5 (41.6)	0.64
<i>HRAS, NRAS, PIK3CA</i> wild type	84 (87.5)	41 (48.8)	

^aChi2 test.

^bTobacco use was considered at 10 packyears or more. Information available for 71 patients.

^cAlcohol use was considered at 10 gr/day or more (ie. alcohol unit). Information available for 80 patients.