Supplemental Information

Co-targeting mTORC and EGFR signaling as a therapeutic strategy in HNSCC

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Supplemental Tables

Table S1. List of cell lines used, sources, and culture conditions.

HPV Status	Cell line	Tumor description	Sources, yr	Culture condition
HPV-negative	UM-SCC1	(1)	Dr. Thomas E. Carey, University of Michigan, 2001	DMEM with 4.5 g/dL glucose, 10% FBS, 1% hydrocortisone, penicillin (100 units/mL), streptomycin (100 mg/mL)
	TU-138	(2)	Dr. Jennifer Grandis, University of Pittsburgh, 2006	
HPV-positive	UD-SCC2	(3)	Dr. Thomas Carey, with permission of Dr. Henning Bier, Technical University Munich, Munich, Germany, 2010	DMEM with 4.5 g/dL glucose, 10% FBS, penicillin (100 units/mL), streptomycin (100 mg/mL),
	UM-SCC47		Dr. Thomas E. Carey, University of Michigan, 2010	
	UPCI-SCC90		Dr. Robert Ferris, University of Pittsburgh, 2010	

Table S2. Primary antibodies used in study.

Antibody	Abbreviation used in Text	Assay, Dilution	Source	Company	Catalog #
AKT	AKT	WB – 1:1000	Mouse	Cell Signaling Technology (CST), Danvers, MA	2920
alpha-tubulin	α-Tub	WB – 1:2000	Mouse	Calbiochem	CP06
Cleaved Caspase 3	Cl-Caspase3	IHC – 1:1000	Rabbit	CST	9661
EGFR	EGFR	IHC - 1:200	Rabbit	CST	4267
EGFR	EGFR	WB – 1:1000	Mouse	CST	2239
GAPDH	GAPDH	WB- 1:10000	Mouse	CST	97166
GAPDH	GAPDH	WB – 1:1000	Rabbit	CST	5174
Ki-67	Ki-67	IHC – 1:200	Rabbit	CST	9027
mTOR	mTOR	WB – 1:1000	Rabbit	CST	2983
mTOR	mTOR	WB – 1:1000	Rabbit	CST	2983
phospho-4E-BP1(T37/46)	p-4E-BP1	IHC - 1:400 WB – 1:1000	Rabbit	CST	2855
phospho-Akt (Ser473)	pAkt	IHC - 1:100	Rabbit	CST	3787
phospho-AKT (Ser473)	p-AKT_S473	WB – 1:1000	Rabbit	CST	4060
phospho-AKT (Thr308)	p-AKT_T308	WB – 1:1000	Rabbit	CST	13038
phospho-AKT(Thr308)	pAkt_T308	WB – 1:1000	Rabbit	CST	4056
phospho-EGFR (Tyr1173)	pEGFR	IHC - 1:500	Rabbit	CST	4407
phospho-mTOR (Ser2448)	p-mTOR	WB – 1:1000	Rabbit	CST	5536
phospho-p44/42 MAPK (Erk1/2) (Thr202/Tyr204)	pMAPK1	IHC - 1:400 WB – 1:1000	Rabbit	CST	4370
p44/42 MAPK (Erk1/2)	MAPK1	WB – 1:2000	Mouse	CST	4696
phospho-p70 S6 Kinase (Thr389)	p-p70S6K_T389	WB – 1:1000	Rabbit	CST	9205
phospho-S6 Ribosomal Protein (S235/326)	p-S6 RP	WB – 1:1000	Rabbit	CST	4858
phosphor-EGFR(Tyr1068)	p-EGFR_Y1068	WB – 1:1000	Rabbit	CST	3777
PI3 Kinase p110α	PI3KCA	WB – 1:1000	Rabbit	CST	4249
PTEN	PTEN	IHC - 1:100	Rabbit	CST	9559

Table S3. siRNA oligos used in study.

siRNA target	Description	Vendor	Catalog #
mTOR	ON-TARGETplus human MTOR (2475) SMARTpool	GE Dharmacon	L-003008-00-0005
PIK3CA	ON-TARGETplus human PIK3CA (5290) SMARTpool	GE Dharmacon	L-003018-00-0005
EGFR	ON-TARGETplus Human EGFR (1956) SMARTpool	GE Dharmacon	L-003114-00-0005
Non-targeting	ON-TARGETplus human Non-targeting pool	GE Dharmacon	D-001810-10-05

Supplemental Figure and Legends

Figure S1

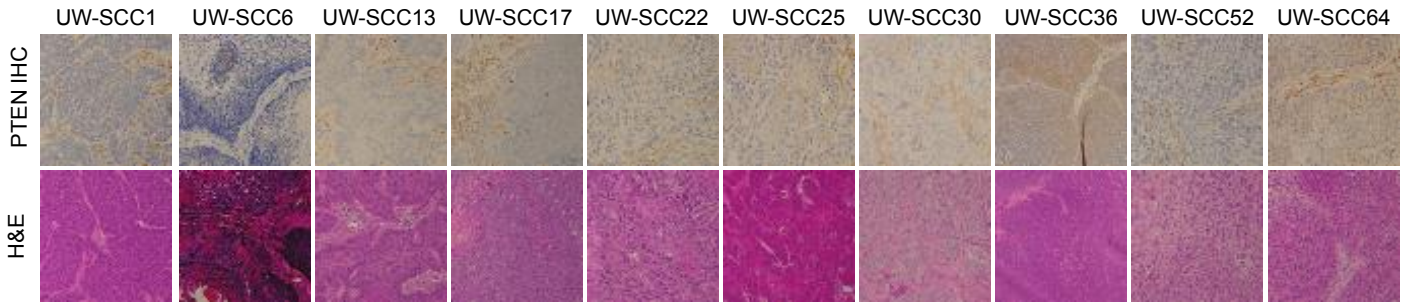


Figure S1. PTEN IHC staining of PDX FFPE tissue from untreated tumors. Basal PTEN staining of the tumor tissue was scored on a 1-low, 2-medium, 3-high by a board-certified pathologist specializing in head and neck cancer (RH). PTEN expression was internally controlled by comparing the tumor cells to adjacent stroma, sections where no staining was observed in stroma could not be included in the evaluation. This scoring was used to evaluate tumors for potential PTEN loss or reduced expression, with results summarized in Figure 1A. Images are representative 20X fields of multiple fields analyzed scoring purposes. Paired H&E sections are shown to confirm tissue quality and morphology.

Figure S2

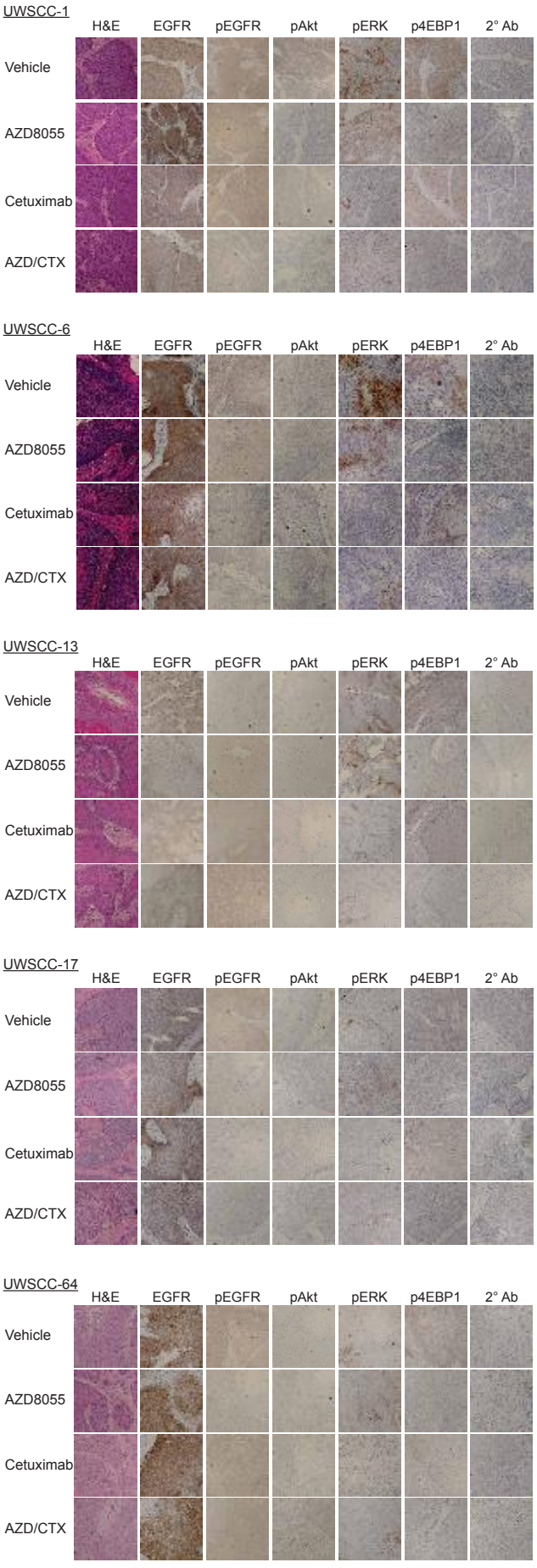
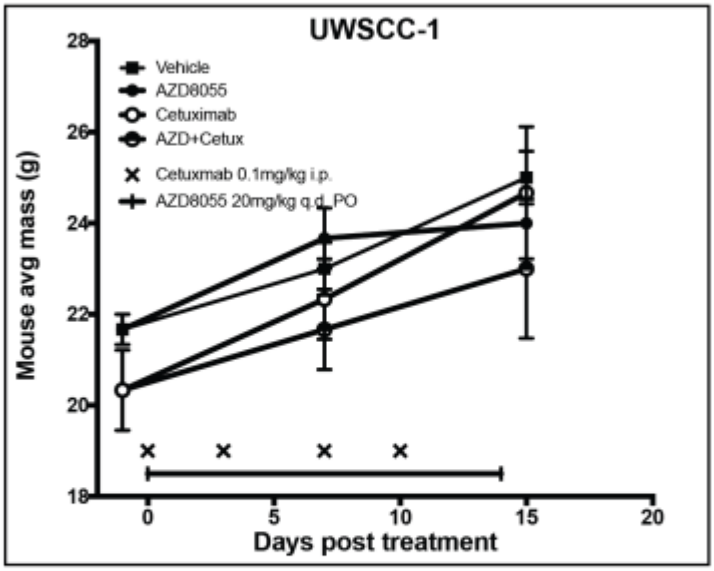
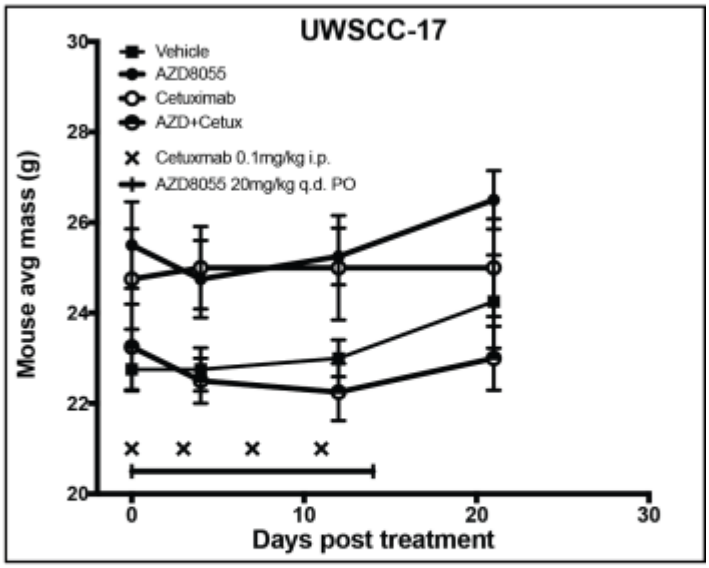
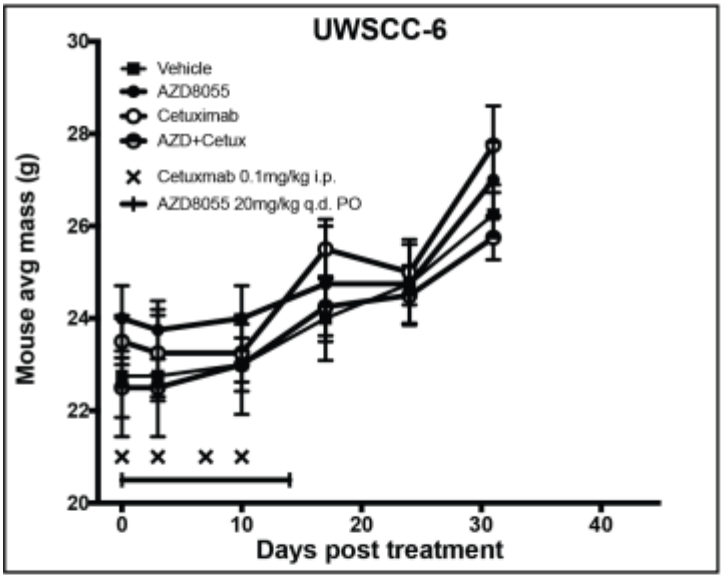

UWSCC-1
H&E
EGFR
pEGFR
pAkt
pERK
p4EBP1
2° Ab
Vehicle
AZD8055
Cetuximab
AZD/CTX
UWSCC-6
H&E
EGFR
pEGFR
pAkt
pERK
p4EBP1
2° Ab
Vehicle
AZD8055
Cetuximab
AZD/CTX
UWSCC-13
H&E
EGFR
pEGFR
pAkt
pERK
p4EBP1
2° Ab
Vehicle
AZD8055
Cetuximab
AZD/CTX
UWSCC-17
H&E
EGFR
pEGFR
pAkt
pERK
p4EBP1
2° Ab
Vehicle
AZD8055
Cetuximab
AZD/CTX
UWSCC-64
H&E
EGFR
pEGFR
pAkt
pERK
p4EBP1
2° Ab
Vehicle
AZD8055
Cetuximab
AZD/CTX

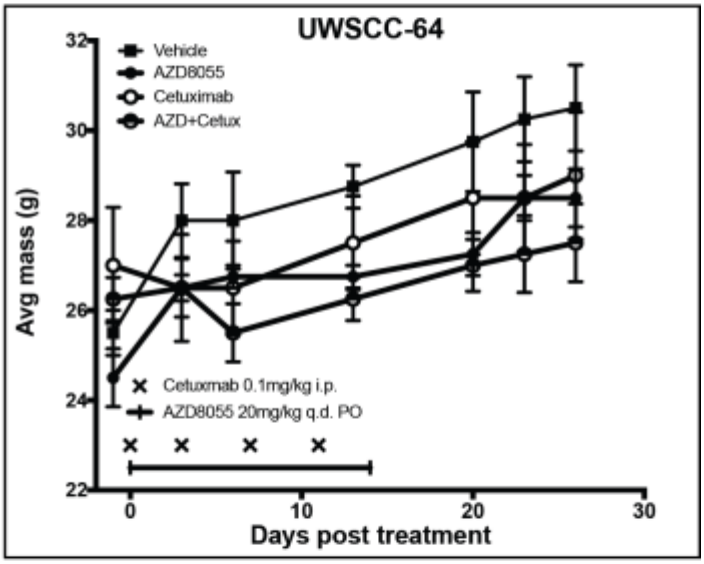
Figure S2. IHC staining of FFPE tissues harvested 2 hrs post initial treatment of indicated therapeutics. Images shown are representative 20X field of overall staining. pEGFR and pAkt staining was impacted as expected for given drug/drug combination, but effect was less robust than was observed for pMAPK1and p4EBP1. A secondary antibody only control was included for each tumor to ensure specificity of the immunostaining.

Figure S3


UWSCC-1
Vehicle
AZD8055
Cetuximab
AZD+Cetux
Cetuxmab 0.1mg/kg i.p.
AZD8055 20mg/kg q.d. PO
Mouse avg mass (g)
Days post treatment


UWSCC-17
Vehicle
AZD8055
Cetuximab
AZD+Cetux
Cetuxmab 0.1mg/kg i.p.
AZD8055 20mg/kg q.d. PO
Mouse avg mass (g)
Days post treatment


UWSCC-6
Vehicle
AZD8055
Cetuximab
AZD+Cetux
Cetuxmab 0.1mg/kg i.p.
AZD8055 20mg/kg q.d. PO
Mouse avg mass (g)
Days post treatment


UWSCC-64
Vehicle
AZD8055
Cetuximab
AZD+Cetux
Cetuxmab 0.1mg/kg i.p.
AZD8055 20mg/kg q.d. PO
Avg mass (g)
Days post treatment

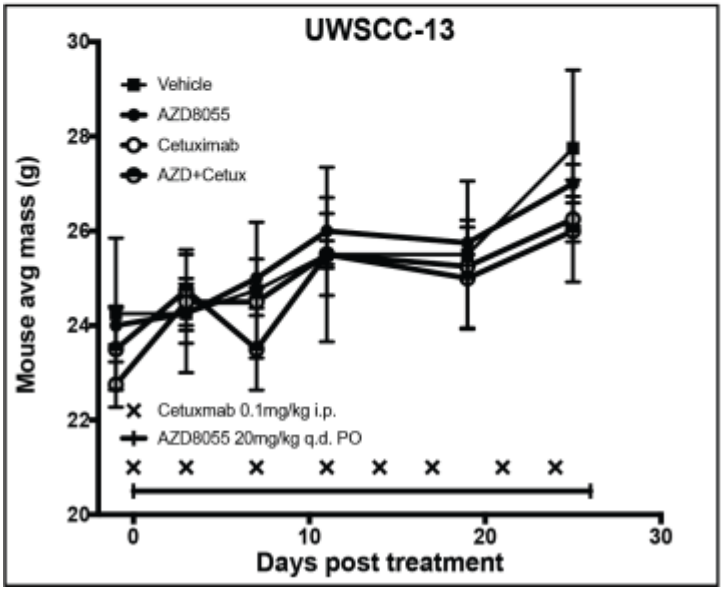

UWSCC-13
Vehicle
AZD8055
Cetuximab
AZD+Cetux
Cetuxmab 0.1mg/kg i.p.
AZD8055 20mg/kg q.d. PO
Mouse avg mass (g)
Days post treatment

Figure S3. Mice mass during and following treatment. Mice were weighed at least weekly during and following treatment period. Symbols indicate treatment group mean mass in grams with SEM error bars.

Figure S4

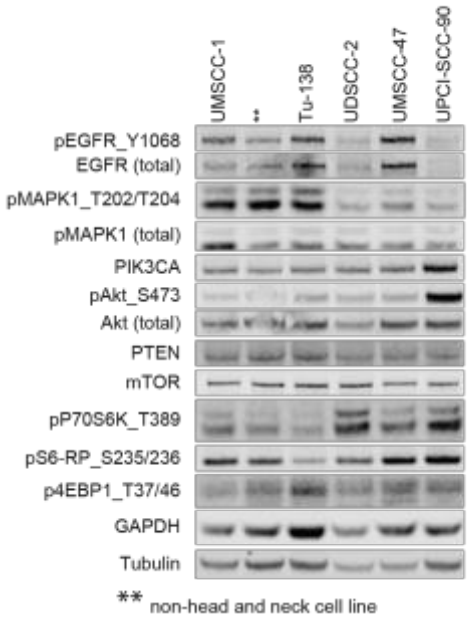


Figure S4. Unedited blot from Figure 1D.

Supplemental Methods

Cell lines, strains, and drugs

Head and neck cancer cell lines derived from HPV-negative patients: UM-SCC1 and TU-138 and from HPV-positive patients: UD-SCC2, UM-SCC47, and UPCI-SCC90, were obtained from indicated sources and cultured according to standard conditions (Supplementary Table S1). The identity of all cell lines was confirmed via short-tandem repeat testing within 6 months of use (see short-tandem repeat reports, below). For drug inhibition assays, low passage (p3-p10) UWSCC-64 cells were cultured in keratinocyte serum-free media (Cat #17005042, Invitrogen) supplemented with 5%FBS at 37°C in a humidified atmosphere of 5% CO_2. AZD8055 and NVP-BEZ-235 were purchased from LC-Labs and resuspended in DMSO at 10mM stock concentration. Cetuximab (IMC-225, Erbitux) was purchased from the University of Wisconsin Hospital Pharmacy.

Hotspot mutational analysis

Total genomic DNA was isolated from PDX FFPE tissue and HNSCC cell lines and sequenced using the Illumina TruSeq Cancer Amplicon panel run on a MiSeq2000 as described previously(4). Briefly, H&E slides were used to identify regions of SCC tissue and exclude normal and necrotic areas, then marco-dissected from consecutive unstained slides.The tissue was deparaffinized and DNA isolated with the Qiagen GeneRead FFPE DNA isolation kit with RNase digestion to remove RNA. Genomic DNA from immortalized HNSCC lines was isolated with Qiagen DNeasy Blood and Tissue kit with RNase digestion. DNA was submitted to the University of Wisconsin-Madison Biotechnology Center. DNA concentration was verified using the Qubit® dsDNA HS Assay Kit (Life Technologies, Grand Island, NY). Sequencing libraries were prepared using the Illumina TruSeq Amplicon Cancer Panel according to the manufacturer's instructions. The panel comprises 212 amplicons from 48 genes that are simultaneously amplified in a single-tube reaction. Per manufacturer's instructions, probes containing oligonucleotide pairs specific to the TSACP panel regions were hybridized to each genomic DNA sample. Amplicons were generated by extension and ligation of the bound oligonucleotides, followed by PCR amplification. Products were amplified using primers that included individual per-sample indexes, as well as common adapters and stem sequences for sequencing cluster generation. After PCR cleanup, library quality as assessed using a Bioanalyzer High Sensitivity chip (Agilent Technologies, Santa Clara,CA). Libraries were normalized, and equal volumes of each sample were combined, diluted, and heat denatured prior to MiSeq sequencing, using a MiSeq

2x150 bp sequencing run and MiSeq 300 bp v2 kit. Images were analyzed using the standard Illumina Pipeline, version 1.8.2.

DNA sequencing reads were adapter and quality(Q20) trimmed and aligned to the reference genome, GRCh37.. Variants were called using MuTect(5) version 1.4 using the standard (STD) method followed by annotation with SnpEff(6). We used SnpSift(7) to filter variants by effect and RTG-tools version 3.6 to determine the distribution of variants for each effect class. Variants were further filtered by setting a minimum allele frequency at 5% and annotated by comparing to published studies searchable on cBioPortal for Cancer Genomics http://www.cbioportal.org/ (8,9). Further details available in the supplemental methods.

Growth inhibition assay

Cells were plated in 96-well plates at a density of 1,000-7,500 cells/well dependent on cell type growth rate. 24 hrs post-plating cells were treated at indicated doses and allowed to incubate for 72-96 hrs. Cells per well were counted using a SpectraMax i3 MiniMax 300 Imaging Cytometer plate reader using the optical imaging module and transmitted light setting. The Softmax-Pro v6.4 software was trained to count each cell type and used to determine the number of cells/well at the endpoint. Treated wells were normalized to DMSO only controls and IC50 values were calculated with GraphPad Prism v7 using the inhibitor concentration vs response (three parameter) function. Each condition was assayed with triplicate wells. IC50 curves represent average of three biological replicates.

Clonogenic survival assay

250 to 750 cells/well (dependent on cell type, adjusted to higher density for high drug concentrations) were plated in 12-well plates. Cells were treated at indicated doses 24 hrs post plating and allowed to incubate in media containing the inhibitors for 7 to 21 days. When DMSO control wells had colonies of 50 or more cells all plates for that cell type were washed 1X with PBS and then fixed and stained with methanol/crystal violet. Plates were rinsed in diH2O and allowed to dry. Plates were imaged with a low power microscope and colonies of 50 or more cells were counted. Surviving fraction was determined and plotted using GraphPad Prism v7. Bar plots represent mean values of at least three independent experiments of quadruplicate wells. Error bars are S.E.M.

BrdU assay

Cells were plated in 96-well plates at a density of 1,000-7,500 cells/well dependent on cell type growth rate. 24 hrs post-plating cells were treated at indicated doses and allowed to incubate for 72-96 hrs. BrdU incorporation was measured using a BrdU ELISA approach according to manufacturer instructions (Roche ref:11 647 229 001). Two hours

of exposure to BrdU was performed immediately prior to fixing and staining the cells. Absorbance was read with a SpectraMax i3 MiniMax, with values normalized to DMSO controls. Bar plots represent mean of quadruplicate wells.

Apoptosis assay

HNSCC cells were plated in 96-well plates at a density of 10,000 cells/well. 24 hrs post-plating cells were treated at indicated doses and allowed to incubate for 48 hrs. The image cytometer function was used to count cells/well prior to assay**.** Apoptosis was measured by caspase activity with the Caspase-Glo 3/7 assay (Promega G8091); reagent was added at a 1:1 ratio with culture media, incubated for 30 mins and luciferase activity was read with a SpectraMax i3 MiniMax. Luciferiase intensity was normalized to number of cells/well and expressed relative to DMSO controls. Staurosporine treatment of each cell line was included to ensure the function of the reaction (data not shown). Bars represent mean of triplicate wells with SD error.

Immunoblotting

Following treatment, cells were lysed with RIPA buffer supplemented with protease/phosphatase inhibitor cocktail (CST #5872) and were sonicated. Equal amounts of protein were analyzed by SDS-PAGE (~50mg/well) and transferred to polyvinylidene difluoride (PVDF) membranes (Immobilon-FL), were probed by specific primary antibodies by overnight incubation at 4ºC, and were detected via incubation with anti-mouse or anti-rabbit as appropriate, NIR-conjugated secondary antibodies (LiCOR) imaged on a LiCOR Odyssey FC. Antibodies and sources are listed in Supplementary Table S2.

siRNA knockdown experiments

SiRNA oligos were obtained from sources indicated in Table S3. SiRNAs were resuspended in 1x Dharmacon siRNA buffer at a stock concentration of 10 μM, aliquoted and stored at -20ºC. Cells were transfected in suspension at indicated concentrations using Lipofectamine RNAiMax reagent (Life Technologies). RNAiMax was added at a ratio of 1 ul per 15pmol of siRNA. Cells received a total of 50nM siRNA; 50nM of non-targeting(NT) siRNA for controls, 25nM NT and 25 nM of mTOR, PIK3CA, or EGFR for single treatments or 25 nM EGFR and 25 nM of either mTOR or PIK3CA for combination treatments. For western blots, cells were incubated with siRNA for 72 hrs prior to harvest and processing as described above. For growth inhibition assay, cells were diluted at plated at densities indicated above, with counting and analysis as described above.

Mice

Six to eight week old female NOD-SCID gamma (NSG, NOD.Cg-*Prkdc*^{scid} *Il2rg*^{tm1Wjl}/SzJ) mice (Jackson Laboratories) were used for PDX revival and tissue amplification; six to eight week old female Hsd:athymic Nude-*Foxn1*^{nu} (Harlan Laboratories) were used for therapy studies(10). Mice were kept in the Association for Assessment and Accreditation of Laboratory Animal Care-approved Wisconsin Institute for Medical Research (WIMR) Animal Care Facility. Animals were housed in specific pathogen free rooms, and their clinical health was evaluated weekly. Studies involving the mice were carried out in accordance with an animal protocol approved by the University of Wisconsin.

PDX cetuximab response studies

Head and neck cancer patient derived xenografts were generated in our group as described previously(10). Of the more than 20 tumors that were successfully engrafted and could be passaged through multiple generations, 10 were selected to evaluate response to cetuximab. Cryo-perserved PDX tissue was revived by implantation into NSG mice and allowed to grow for ~30 days. When tumors had reached sufficient size, PDX tissue was harvested by euthanizing the mice and collecting all available tumor tissue in a petri dish. The tissue was mixed 1:1 mixture of media (Dulbecco's Modified Eagle Medium with 10% fetal bovine serum, 1% penicillin/streptomycin, and 2.5 μg/mL amphotericin B) and matrigel (catalog #354230, BD Biosciences, Inc) minced into less than 1 mm^3 pieces and injected subcutaneously into flanks of nude mice. Tumor volume was assessed twice weekly with Vernier calipers and calculated according to the equation $V = (\pi/6) \times (\text{large diameter}) \times (\text{small diameter})^2$. Tumors were allowed to grow for 20-40 days; when average volume reached 250mm^3, mice were randomized into control or cetuximab treatment groups such that average tumor size was uniform across all groups. Mice received cetuximab, dosed twice a week at 10mg/kg by intraperitoneal injection(i.p.) for two weeks, vehicle mice received saline i.p. injections. Measurement continued twice weekly for the treatment period and successive 4-6 weeks. At two weeks post end of treatment, size was measured of both vehicle and treated mice and used to calculate treated/control (T/C) ratios. GraphPad Prism v7 was used to generate waterfall style plots and highlight response by HPV status and mutation profile.

PDX combination therapy studies

UW-SCC-1, 6, 13, 17, 64 PDXs were previously established in the lab (10,11).Tumor tissue was reanimated and amplified in NSG mice and distributed to nude mice for treatment studies, with tumor measurement as described above. Tumors were allowed to grown for 20-40 days; when average volume reached 250mm^3, mice were randomized into control (vehicle), AZD8055, cetuximab, or combination treated groups (n=4-6 mice/10-16 tumors per group) such that average tumor size was uniform across all groups. AZD8055 was formulated in 30% Capsitol and delivered once a day at

20mg/kg by oral gavage(p.o.) as described previously (12). Cetuximab was dosed twice a week at 10mg/kg by intraperitoneal injection(i.p.). Vehicle and single treatment mice received 30% Capsitol p.o. or saline i.p. as appropriate. Mice were sacrificed at 40 days post start of treatment or when tumors reached 2000mm^3. Weights were recorded weekly to monitor mouse health and adverse drug effects. At times indicated post-treatment, additional tumor-bearing mice were sacrificed and tumors were harvested. Tissue from each tumor was divided in two, with half going for formalin fixed paraffin embedded (FFPE) preservation while other tumor chunks were fast frozen in liquid nitrogen. Growth curves were statistically evaluated with the non-parametric Friedman's test; evaluation of these and other xenograft growth studies has demonstrated that these data are not normally distributed with a slight right-shift prescribing the use of a non-parametric test.

Histology and immunohistochemistry

FFPE preserved PDX tissue was microtomed for 5µm sections and stained for H&E. Additional sections were IHC stained for various targets as described previously (13). Briefly, slides were deparaffinized, rehydrated and underwent heat-induced epitope retrieval followed by blocking steps for endogenous peroxidase activity and nonspecific antibody interactions. Overnight incubations at 4°C were performed with primary antibody dilutions prepared in 1% goat serum for the protein targets of interest (Supplemental Table S1). The next day anti-rabbit horseradish peroxidase-conjugated secondary antibodies (#8114; Cell Signaling Technology, Danvers, MA) were applied to the TMA for 30 mins followed by a 2 min development with 3,3'-diaminobenzidine (DAB) (#SK-4100; Vector Laboratories, Burlingame, CA). Finally, slides were counterstained with hematoxlyin, dehydrated, dipped in fresh xylenes and coverslipped with Cytoseal XYL (Thermo Fisher Scientific, Waltham, MA) and a number 1.5 coverslip. A no primary antibody control slide was stained for each tumor to ensure specificity of the reaction.

References

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11. Stein AP, Saha S, Liu CZ, Hartig GK, Lambert PF, Kimple RJ. Influence of handling conditions on the establishment and propagation of head and neck cancer patient derived xenografts. PLoS One **2014**;9:e100995
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Short Tandem Repeat Analysis

School of Medicine and Public Health
UNIVERSITY OF WISCONSIN-MADISON

Department of Pathology and Laboratory Medicine
TRIP Laboratory (Molecular)
http://www.pathology.wisc.edu/research/trip

1111 Highland Ave, WIMR 2062
Madison, WI 53705-8550
(608) 265-9168

Sample Report:
SCC1
Sample Name on Tube: 1
486.4 ng/µL, (A260/280=2.09)
Sample Type: DNA
Cell Count: N/A

Requestor:
Kwang Nickel
Randy Kimple Lab

Sample Date: N/A
Receive Date: 03/21/17
Assay Date: 03/21/17
File Name: STR 170322 wmr
Report Date: 03/24/17

STR Locus	STR Genotype Repeat #	STR Genotype
FGA	16–18,18.2,19,19.2,20,20.2,21,21.2,22, 22.2, 23, 23.2, 24, 24.2, 25, 25.2, 26–30, 31.2, 43.2, 44.2,45.2, 46.2	22,22
TPOX	6-13	8,11
D8S1179	7-18	13,16**
vWA	10-22	15,18
Amelogenin	X,Y	X,X
Penta_D	2.2, 3.2, 5, 7-17	9,9
CSF1PO	6-15	10,12**
D16S539	5, 8-15	12,13
D7S820	6-14	9,12
D13S317	7-15	8,11
D5S818	7-16	10,13**
Penta_E	5-24	13,13
D18S51	8-10, 10.2, 11-13, 13.2, 14-27	18,18
D21S11	24,24.2,25,25.2,26-28,28.2,29,29.2, 30, 30.2,31, 31.2,32,32.2,33,33.2, 34,34.2,35,35.2,36-38	27,27
TH01	4-9,9.3,10-11,13.3	6,6
D3S1358	12-20	18,18

Results: Based on the SCC1 DNA submitted by Kwang Nickel – Randy Kimple Lab dated and received on 03/21/17, this sample (Label on Tube: 1) exactly matches the STR profile of the human cell line UM-SCC-1 (Zhao, et al, Clin Cancer Res; 17(23) December 1, 2011). It is comprised of 23 allelic polymorphisms across the 15 STR loci analyzed.

Interpretation: No STR polymorphisms other than those corresponding to the human UM-SCC-1 cell line were detected however, allelic imbalance (denoted by ** in table above) was observed at the D8S1179, CSF1PO, D5S818 loci and could be the result of chromosomal gains and/or losses in this cell line. The concentration of DNA required to achieve an acceptable STR genotype (signal/ noise) was equivalent to that required for the standard procedure (~1 ng/amplification reaction) from human genomic DNA. This result suggests that the SCC1 sample submitted corresponds to the UM-SCC-1 cell line and was not contaminated with any other human cells.

Sensitivity: Sensitivity limits for detection of STR polymorphisms unique to either this or other human cell lines is ~2-5%.

X *RMB* **Digitally Signed on 03/24/17**

Rebecca M. Baus
TRIP Laboratory, Molecular

X *WMR* **Digitally Signed on 03/24/17**

William M. Rehrauer, PhD, Director / Co-Director
UWHC Molecular Diagnostics Laboratory / UWSMPH TRIP Laboratory

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Short Tandem Repeat Analysis

School of Medicine and Public Health
UNIVERSITY OF WISCONSIN-MADISON

Department of Pathology and Laboratory Medicine
TRIP Laboratory (Molecular)
http://www.pathology.wisc.edu/research/trip

1111 Highland Ave, WIMR 2062
Madison, WI 53705-8550
(608) 265-9168

Sample Report:
SCC2
Sample Name on Tube: 2
257.7 ng/µL, (A260/280=2.02)
Sample Type: DNA
Cell Count: N/A

Requestor:
Kwang Nickel
Randy Kimple Lab

Sample Date: N/A
Receive Date: 03/21/17
Assay Date: 03/21/17
File Name: STR 170322 wmr
Report Date: 03/24/17

STR Locus	STR Genotype Repeat #	STR Genotype
FGA	16–18,18.2,19,19.2,20,20.2,21,21.2,22, 22.2, 23, 23.2, 24, 24.2, 25, 25.2, 26–30, 31.2, 43.2, 44.2,45.2, 46.2	20,27
TPOX	6-13	8,10
D8S1179	7-18	13,15
vWA	10-22	18,18
Amelogenin	X,Y	X,Y
Penta_D	2.2, 3.2, 5, 7-17	9,13
CSF1PO	6-15	11,12**
D16S539	5, 8-15	11,13**
D7S820	6-14	9,9
D13S317	7-15	8,8
D5S818	7-16	10,11
Penta_E	5-24	10,10
D18S51	8-10, 10.2, 11-13, 13.2, 14-27	12,17
D21S11	24,24.2,25,25.2,26-28,28.2,29,29.2, 30, 30.2,31, 31.2,32,32.2,33,33.2, 34,34.2,35,35.2,36-38	30,31.2
TH01	4-9,9.3,10-11,13.3	8,9
D3S1358	12-20	14,16

Results: **Based on the SCC2 DNA submitted by Kwang Nickel – Randy Kimple Lab dated and received on 03/21/17, this sample (Label on Tube: 2) exactly matches the STR profile of the human cell line UD-SCC-2 (CVCL_E325) (Cellosaurus) with the exception of the D7S820 loci (9,9) where Cellosaurus observed 8,9 and the vWA loci (18,18) where Cellosaurus observed 15,18. Standards for cell line authentication have been promoted with suggested algorithms to determine degrees of "relatedness" and have been recommended as a simple and effective way to interpret results from STR profiling of human cell lines. In general, ≥80% match is common between related samples, whereas ≤ 50% match is unrelated (Int. J. Cancer: 132, 2510-2519. 2013). Using this criteria, across the 15 microsatellite STR loci (tested by both Cellosaurus and TRIP Lab), determined the SCC2 sample displays a 28/30 allelic match (93% match), thus we would call this sample a match to UD-SCC-2 (CVCL_E325). It is comprised of 26 allelic polymorphisms across the 15 STR loci analyzed.**

Interpretation: **No STR polymorphisms other than those corresponding to the human SCC2 cell line were detected however, allelic imbalance (denoted by ** in table above) was observed at the D16S539 and CSF1PO loci and could be the result of chromosomal gains and/or losses in this cell line. The concentration of DNA required to achieve an acceptable STR genotype (signal/ noise) was equivalent to that required for the standard procedure (~1 ng/amplification reaction) from human genomic DNA. This result suggests that the SCC2 sample submitted corresponds to the SCC2 cell line, matches UD-SCC-2 (CVCL_E325), and was not contaminated with any other human cells.**

Sensitivity: **Sensitivity limits for detection of STR polymorphisms unique to either this or other human cell lines is ~2-5%.**

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1111 Highland Ave, WIMR 2062
Madison, WI 53705-8550
(608) 265-9168

Sample Report:
SCC47
Sample Name on Tube: 47
191.4 ng/µL, (A260/280=2.11)
Sample Type: DNA
Cell Count: N/A

Requestor:
Kwang Nickel
Randy Kimple Lab

Sample Date: N/A
Receive Date: 03/21/17
Assay Date: 03/21/17
File Name: STR 170322 wmr
Report Date: 03/24/17

STR Locus	STR Genotype Repeat #	STR Genotype
FGA	16–18,18.2,19,19.2,20,20.2,21,21.2,22, 22.2, 23, 23.2, 24, 24.2, 25, 25.2, 26–30, 31.2, 43.2, 44.2,45.2, 46.2	23,25
TPOX	6-13	10,11
D8S1179	7-18	15,15
vWA	10-22	18,18
Amelogenin	X,Y	X,Y
Penta_D	2.2, 3.2, 5, 7-17	9,10**
CSF1PO	6-15	11,13**
D16S539	5, 8-15	8,13
D7S820	6-14	11,11
D13S317	7-15	8,11
D5S818	7-16	11,12**
Penta_E	5-24	12,13
D18S51	8-10, 10.2, 11-13, 13.2, 14-27	18,18
D21S11	24,24.2,25,25.2,26-28,28.2,29,29.2, 30, 30.2,31, 31.2,32,32.2,33,33.2, 34,34.2,35,35.2,36-38	29,30
TH01	4-9,9.3,10-11,13.3	7,9.3
D3S1358	12-20	15,15

Results: Based on the SCC47 DNA submitted by Kwang Nickel – Randy Kimple Lab dated and received on 03/21/17, this sample (Label on Tube: 47) exactly matches the STR profile of the human cell line UM-SCC-47 (Zhao, et al, Clin Cancer Res; 17(23) December 1, 2011). It is comprised of 25 allelic polymorphisms across the 15 STR loci analyzed.

Interpretation: No STR polymorphisms other than those corresponding to the human UM-SCC-47 cell line were detected however, allelic imbalance (denoted by ** in table above) was observed at the D5S818, CSF1PO, and Penta_D loci and could be the result of chromosomal gains and/or losses in this cell line. The concentration of DNA required to achieve an acceptable STR genotype (signal/ noise) was equivalent to that required for the standard procedure (~1 ng/amplification reaction) from human genomic DNA. This result suggests that the UM-SCC-47 sample submitted corresponds to the SCC47 cell line and was not contaminated with any other human cells.

Sensitivity: Sensitivity limits for detection of STR polymorphisms unique to either this or other human cell lines is ~2-5%.

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1111 Highland Ave, WIMR 2062
Madison, WI 53705-8550
(608) 265-9168

Sample Report:
SCC90
Sample Name on Tube: 90
106.0 ng/µL, (A260/280=2.05)
Sample Type: DNA
Cell Count: N/A

Requestor:
Kwang Nickel
Randy Kimple Lab

Sample Date: N/A
Receive Date: 03/21/17
Assay Date: 03/21/17
File Name: STR 170322 wmr
Report Date: 03/24/17

STR Locus	STR Genotype Repeat #	STR Genotype
FGA	16–18,18.2,19,19.2,20,20.2,21,21.2,22, 22.2, 23, 23.2, 24, 24.2, 25, 25.2, 26–30, 31.2, 43.2, 44.2,45.2, 46.2	20,20
TPOX	6-13	8,8
D8S1179	7-18	10,11,12**
vWA	10-22	17,17
Amelogenin	X,Y	X,X
Penta_D	2.2, 3.2, 5, 7-17	11,11
CSF1PO	6-15	11,12**
D16S539	5, 8-15	12,13**
D7S820	6-14	9,10**
D13S317	7-15	11,11
D5S818	7-16	11,12
Penta_E	5-24	11,12
D18S51	8-10, 10.2, 11-13, 13.2, 14-27	14,18**
D21S11	24,24.2,25,25.2,26-28,28.2,29,29.2, 30, 30.2,31, 31.2,32,32.2,33,33.2, 34,34.2,35,35.2,36-38	29,31**
TH01	4-9,9.3,10-11,13.3	7,7
D3S1358	12-20	14,14

Results: Based on the SCC90 DNA submitted by Kwang Nickel – Randy Kimple Lab dated and received on 03/21/17, this sample (Label on Tube: 90) matches the STR profile of the human cell line UPCI:SCC090 (ATCC® CRL-3239™, 8 loci tested) with the exception of the Amelogenin loci (X,X) where ATCC observed (X,Y). It should be noted that this loss of the Y chromosome is inconsistent with both ATCC and the DSMZ database (https://www.dsmz.de) and that the Amelogenin loci is not included in our calculations of allelic match for "relatedness." This line is comprised of 24 allelic polymorphisms across the 15 STR loci analyzed.

Interpretation: While the major STR polymorphisms reported correspond to the human SCC90 cell line, we observed allelic variants at a low level (~10% of the major alleles) (alleles 10 and 11) relative to the major allele (allele 12) at the D8S1179 loci. Additionally, triploid genotypes at the D8S1179 loci and allelic imbalance (denoted by ** in the table above) was observed at the D8S1179, CSF1PO, D16S539, D7S820, D18S51, D21S11 loci. These observations could be the result of chromosomal gains, losses and/or amplifications in this cell line. The concentration of DNA required to achieve an acceptable STR genotype (signal/ noise) was equivalent to that required for the standard procedure (~1 ng/amplification reaction) from human genomic DNA. This result suggests that the SCC90 sample submitted corresponds to the UPCI:SCC090 cell line and was not contaminated with any other human cells.

Sensitivity: Sensitivity limits for detection of STR polymorphisms unique to either this or other human cell lines is ~2-5%.

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1111 Highland Ave, WIMR 2062
Madison, WI 53705-8550
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Sample Report:
SCC1483
Sample Name on Tube: 1483
183.2 ng/µL, (A260/280=2.16)
Sample Type: DNA
Cell Count: N/A

Requestor:
Kwang Nickel
Randy Kimple Lab

Sample Date: N/A
Receive Date: 03/21/17
Assay Date: 03/21/17
File Name: STR 170322 wmr
Report Date: 03/24/17

STR Locus	STR Genotype Repeat #	STR Genotype
FGA	16–18,18.2,19,19.2,20,20.2,21,21.2,22, 22.2, 23, 23.2, 24, 24.2, 25, 25.2, 26–30, 31.2, 43.2, 44.2,45.2, 46.2	18.2,18.2
TPOX	6-13	10,11
D8S1179	7-18	13,13
vWA	10-22	15,16**
Amelogenin	X,Y	X,X
Penta_D	2.2, 3.2, 5, 7-17	14,14
CSF1PO	6-15	12,13**
D16S539	5, 8-15	8,9
D7S820	6-14	10,10
D13S317	7-15	12,12
D5S818	7-16	12,12
Penta_E	5-24	12,12
D18S51	8-10, 10.2, 11-13, 13.2, 14-27	14,14
D21S11	24,24.2,25,25.2,26-28,28.2,29,29.2, 30, 30.2,31, 31.2,32,32.2,33,33.2, 34,34.2,35,35.2,36-38	28,29
TH01	4-9,9.3,10-11,13.3	8,8
D3S1358	12-20	14,14

Results: **Based on the SCC1483 DNA submitted by Kwang Nickel – Randy Kimple Lab dated and received on 03/21/17, this sample (Label on Tube: 1483) exactly matches the STR profile of the human cell line Tu-138 (Zhao, et al, Clin Cancer Res; 17(23) December 1, 2011). It is comprised of 20 allelic polymorphisms across the 15 STR loci analyzed. It does NOT match 1483 (Zhao, et al, Clin Cancer Res; 17(23) December 1, 2011).**

Interpretation: **No STR polymorphisms other than those corresponding to the human SCC1483 cell line were detected however, allelic imbalance (denoted by ** in table above) was observed at the CSF1PO and vWA loci and could be the result of chromosomal gains and/or losses in this cell line. The concentration of DNA required to achieve an acceptable STR genotype (signal/ noise) was equivalent to that required for the standard procedure (~1 ng/amplification reaction) from human genomic DNA. This result suggests that the SCC1483 sample submitted corresponds to the SCC1483 cell line, matches Tu-138, does NOT match 1483 (Zhao, et al), and was not contaminated with any other human cells.**

Sensitivity: **Sensitivity limits for detection of STR polymorphisms unique to either this or other human cell lines is ~2-5%.**

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