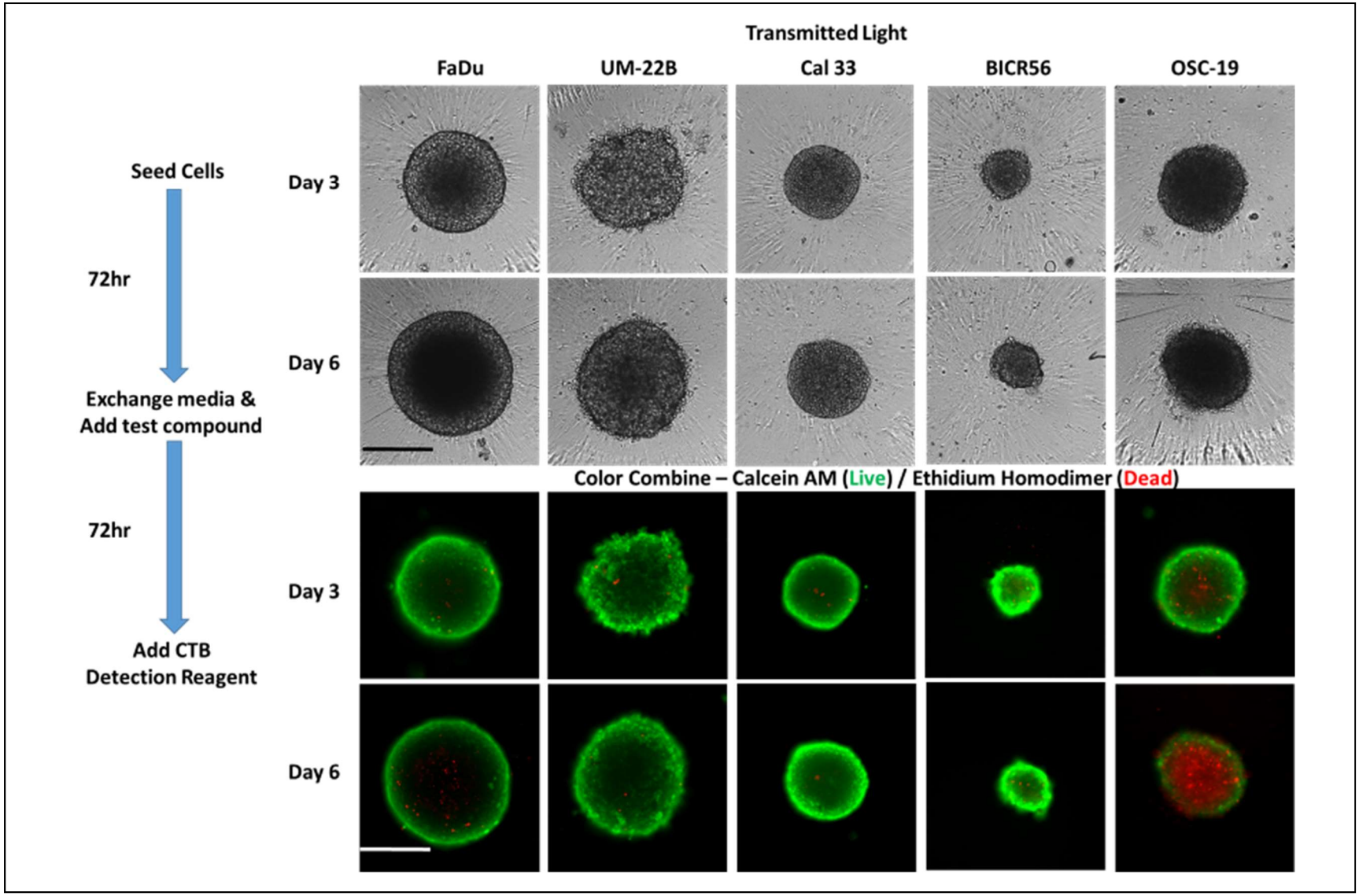
Supplemental materials for:

Maximizing the Value of Cancer Drug Screening in Multicellular Tumor Spheroid Cultures: A Case Study in Five Squamous Cell Carcinoma Cells Lines. Kochanek et al.

Supplemental Figures 1 and 2, and Supplemental Tables 1-5, and Supplemental References 51-65.

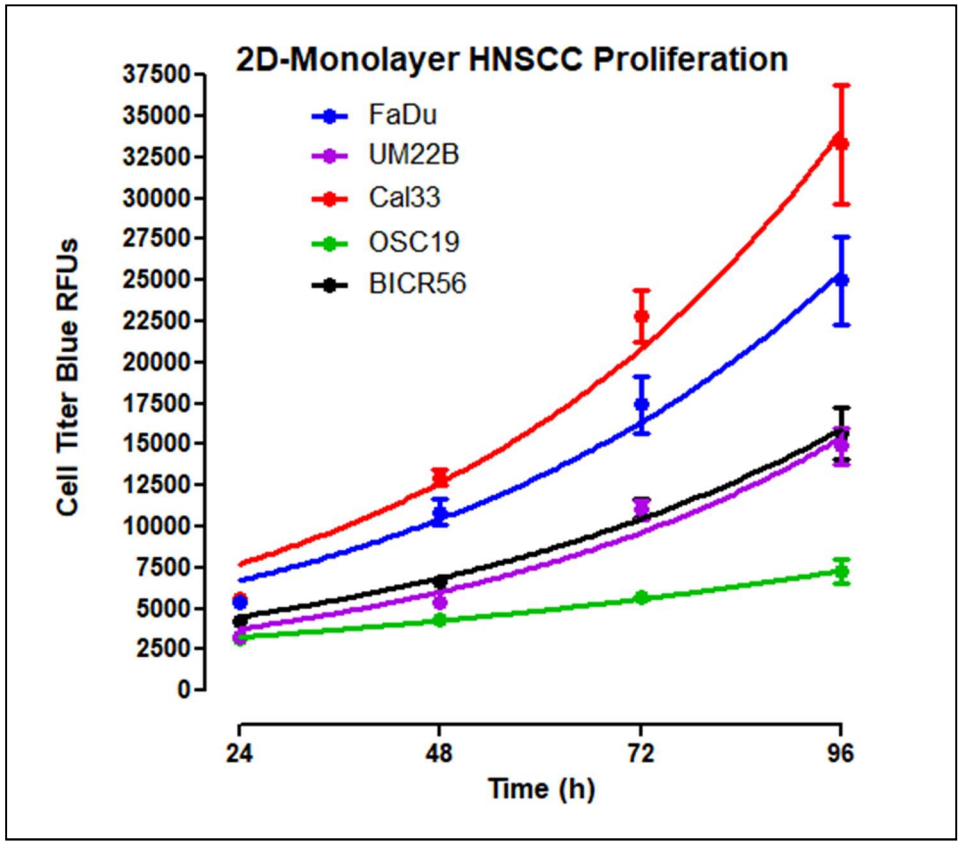
Corresponding Author: Paul A. Johnston Ph.D., Associate Professor, Department of Pharmaceutical Sciences, School of Pharmacy, Room 4101 Pittsburgh Technology Center, 700 Technology Drive, Pittsburgh PA 15219. Email: paj18@pitt.edu

Supplemental Figure 1. Head and Neck Squamous Cell Carcinoma Multicellular Tumor Spheroid Growth Inhibition Drug Screening Assays; Transmitted Light and Live/Dead Color Composite Images of 3- & 6-day HNSCC MCTSs.



The five HNSCC cell lines were seeded in 384-well ULA-plates and cultured for 3 days to allow for MCTS formation, before the test compounds and plate controls were added to assay plates which were returned to the incubator for an additional 72h. MCTSs were then stained with the live cell CAM (green) and dead cell EHD (red) reagents and 4X images were acquired on the IXM in the TL, FITC and Texas Red channels. Greyscale TL images are presented along with color composite fluorescent images of live cell CAM and dead cell EHD staining depicted as green and red, respectively. Representative images from multiple independent experiments are presented. All scale bars represent 300 µm.

Supplemental Figure 2. 2D Monolayer Head and Neck Squamous Cell Carcinoma Cell Titer Blue® Growth Curves.



The five HNSCC cell lines were seeded into 384-well assay plates at 500 cells per well and cultured for the indicated time periods before the addition of the CTB detection reagent and subsequent measurement of RFU signals. CTB RFUs were measured in 32 wells (*n*=32) and are presented as the mean ± SD for the following HNSCC cell lines; FaDu (blue circle, ●), UM-22B (magenta circle, ●), Cal33 (red circle, ●), OSC-19 (green circle, ●), and BIRC56 (black circle, ●). The data were then analyzed GraphPad Prism 6 software using an exponential growth equation Y=Y0*exp(k*X) which describes the growth with a constant doubling time. Y0 is the Y value (RFU's) when X (time in h) is zero. K is the rate constant, expressed in reciprocal of the X axis time units (h). Tau is the time constant, expressed in the same units as the X axis and computed as the reciprocal of K. The doubling-time in h is computed as ln(2)/K. The 2D HNSCC monolayers exhibited exponential growth rates (r^2 >0.93) and their doubling times provided a rank order of growth; Cal33 (33.6h) = UM-22B (35.5h) = FaDu (37.4h) < BIRC56 (39.5h) << OSC19 (61.8h). Representative data from one of three independent experiments are presented.

Supplemental Table 1. Anticancer Test Drug Set: Mechanism of Action and Approved Cancer Therapy.

Compound	Max Conc (μM)	MOA	Approved Cancer Therapy for:
5-FU*	500	Antimetabolite	HNSCC, breast, colorectal, stomach & pancreatic
Methotrexate*	20	Antimetabolite & antifolate	HNSCC, breast, leukemia, lung, lymphoma, & osteosarcoma
Bleomycin*	20	Single- & double-stranded DNA breaks	HNSCC, Hodgkin's lymphoma, non-Hodgkin's lymphoma, testicular, ovarian & cervical cancers
Docetaxel*	100	Tubulin stabilizer	HNSCC, breast, prostate, stomach, & non-small cell lung cancers
Cisplatin*	500	Intra- & inter-strand DNA cross-links	HNSCC, testicular, ovarian, cervical, breast, bladder, lung, mesothelioma, brain tumors & neuroblastoma
Gefitinib	100	EGF-R tyrosine kinase inhibitor	Non-small cell lung cancer
Erlotinib	500	EGF-R tyrosine kinase inhibitor	Non-small cell lung & pancreatic cancer
Dasatinib	20	Src family kinase inhibitor	Acute & chronic myeloid leukemia
Sunitinib	100	Multi-receptor tyrosine kinase inhibitor	Advanced renal cell carcinoma & imatinib-resistant gastrointestinal stromal tumors
Ruxolitinib	200	Janus kinase inhibitor	Myelofibrosis & polycythemia vera
Doxorubicin	200	Topoisomerase II inhibitor	Several different cancer types
Etoposide	100	Topoisomerase II inhibitor	Small cell lung, & testicular cancer
Topotecan	1	Topoisomerase I inhibitor	Small cell lung, cervical & ovarian cancer
Dactolisib	50	Phosphatidylinositol 3-kinase inhibitor	Clinical trials for recurrent triple negative breast cancer, or high grade serous ovarian cancer
Buparlisib	20	Phosphatidylinositol 3-kinase inhibitor	Clinical trials for locally advanced or metastatic HER2 negative breast cancer, or advanced pancreatic neuroendocrine tumors
Romidepsin	0.5	Histone deacetylase inhibitor	Cutaneous or peripheral T-cell lymphoma
Bortezomib	0.5	26S proteasome inhibitor	Clinical trials for multiple myeloma or acute lymphoblastic leukemia
Everolimus	100	mTOR inhibitor	Clinical trials as combination therapy for different cancers
Ganetespib	1	Heat shock protein 90 inhibitor	Clinical trials for breast cancer

Max Conc = maximum concentration.
MOA = Mechanism of Action.
HNSCC = head and neck squamous cell carcinoma.

* = drugs approved for HNSCC therapy.
EGFR = epidermal growth factor receptor
Drug mechanisms of action and clinical trial information were extracted from the National Cancer Institute (NCI) drug dictionary (https://www.cancer.gov/publications/dictionaries/cancer-drug) and clinical trial databases (https://www.cancer.gov/about-cancer/treatment/clinical-trials/search).

Supplemental Table 2. 2D Monolayer and Multicellular Tumor Spheroid Head and Neck Squamous Cell Carcinoma Culture Cell Titer Blue® Growth Inhibition Assay Performance Statistics.

HNSCC		2D monolayer cultures		MCTS cultures	
Cell Line	**Plate #**	**S:B ratio**	**Z'-factor coefficient**	**S:B ratio**	**Z'-factor coefficient**
FaDu	1	13.8	0.93	9.97	0.71
FaDu	2	11.2	0.84	8.4	0.63
UM-22B	1	18.3	0.95	8.09	0.86
UM-22B	2	15.9	0.92	7.9	0.78
Cal33	1	16.3	0.88	6.19	0.81
Cal33	2	13.6	0.9	5.19	0.83
BICR56	1	17.5	0.91	7.65	0.81
BICR56	2	14.6	0.89	7.04	0.8
OSC-19	1	12.8	0.89	10.7	0.86
OSC-19	2	12.2	0.86	10.5	0.79

To define 2D-monolyer and MCTS HNSCC culture growth inhibition assay signal windows and normalize the data for GI_{50} determinations, we used 0.2% DMSO control wells to represent uninhibited growth (0% GI, Max controls, n=32), and 200μM Doxorubicin + 0.2% DMSO control wells to represent fully inhibited growth (100% GI, Min controls, n=32) respectively, and to calculate signal-to-background (S:B) ratios and Z'-factor coefficient assay performance statistics

Supplemental Table 3. 2D Monolayer and Multicellular Tumor Spheroid Head and Neck Squamous Cell Carcinoma Culture Growth Inhibition Drug Impact Scores

	2D Monolayer HNSCC CTB GI_{50} Drug Impact Scores						MCTS HNSCC CTB GI_{50} Drug Impact Scores					
Compound	**FaDu**	**UM-22B**	**Cal33**	**BIRC56**	**OSC19**	**Sum**	**FaDu**	**UM-22B**	**Cal33**	**BIRC56**	**OSC19**	**SUM**
5-FU*	1.0	1.0	1.0	1.0	1.0	5.0	0.5	1.0	0.0	0.5	0.0	2.0
Methotrexate*	1.0	1.0	1.0	1.0	1.0	5.0	0.0	0.0	0.0	0.0	0.0	0.0
Bleomycin*	1.0	1.0	1.0	1.0	1.0	5.0	0.0	1.0	0.5	0.5	0.5	2.5
Docetaxel*	1.0	1.0	1.0	1.0	1.0	5.0	1.0	1.0	0.0	1.0	1.0	4.0
Cisplatin*	1.0	1.0	1.0	1.0	1.0	5.0	1.0	1.0	1.0	1.0	1.0	5.0
Gefitinib	1.0	1.0	1.0	1.0	1.0	5.0	0.0	0.0	0.0	0.0	0.0	0.0
Erlotinib	0.5	1.0	0.5	0.5	0.5	3.0	0.0	0.0	0.0	0.0	0.0	0.0
Dasatinib	1.0	1.0	1.0	1.0	0.5	4.5	0.0	0.5	0.0	0.0	0.0	0.5
Sunitinib	1.0	1.0	1.0	1.0	1.0	5.0	1.0	1.0	1.0	1.0	1.0	5.0
Ruxolitinib	1.0	1.0	1.0	1.0	1.0	5.0	0.0	0.0	0.0	1.0	0.5	1.5
Doxorubicin	1.0	1.0	1.0	1.0	1.0	5.0	1.0	1.0	1.0	1.0	1.0	5.0
Etoposide	1.0	1.0	1.0	1.0	0.5	4.5	0.0	0.0	0.0	0.0	0.0	0.0
Topotecan	0.5	0.5	0.5	0.5	0.5	2.5	0.0	0.0	0.0	0.0	0.0	0.0
Dactolisib	1.0	0.5	1.0	0.5	0.5	3.5	0.0	0.0	0.0	0.0	0.0	0.0
Buparlisib	0.5	0.5	1.0	0.0	0.5	2.5	0.0	0.0	0.0	0.0	0.0	0.0
Romidepsin	0.5	0.5	1.0	0.5	0.0	2.5	0.0	0.0	0.0	0.0	0.0	0.0
Bortezomib	1.0	1.0	1.0	1.0	1.0	5.0	0.0	0.0	0.0	0.0	0.0	0.0
Everolimus	1.0	1.0	1.0	1.0	1.0	5.0	1.0	1.0	1.0	1.0	1.0	5.0
Ganetespib	0.5	0.5	0.5	0.5	0.5	2.5	0.0	0.5	0.0	0.0	0.0	0.5

CTB GI drug impact scores for the 19 test drugs in both 2D-monolayer and MCTS HNSCC culture formats were based on GI_{50} determination concentration response curves (Table 1). If a drug produced a calculable GI_{50} it scored 1. If a drug didn't produce a calculable GI_{50} but exhibited a concentration dependent GI response and achieved ≥25% GI it scored 0.5. If a drug didn't produce a calculable GI_{50} or exhibit a concentration dependent GI response with ≥25% GI it received a score of 0. Maximum drug impact score per HNSCC culture = 1, and across all cultures = 5. Minimum drug impact score per HNSCC culture = 0, and across all cultures = 0. * = Drugs approved by the Federal Drug Administration for HNSCC therapy.

Supplemental Table 4A. Cancer Drug Effects on Head and Neck Squamous Cell Carcinoma Multicellular Tumor Spheroid Calcein AM and Ethidium Homodimer Mean Integrated Fluorescent Intensity Values.

	Calcein AM Live MIFI % of DMSO Control					EHD Dead MIFI % of DMSO Control				
Compound	**FaDu**	**UM2-22B**	**Cal33**	**BIRC56**	**OSC-19**	**FaDu**	**UM2-22B**	**Cal33**	**BIRC56**	**OSC-19**
5-FU*	21.2	78.4	82.6	74.7	99.4	47.1	152	398	107	362
Methotrexate*	26.5	43.2	97.4	58.6	79.0	117	96.3	144	63.6	103
Bleomycin*	34.2	34.0	87.0	89.8	51.0	54.5	130	99.6	93.3	124
Docetaxel*	48.1	64.1	95.9	72.4	74.6	321	567	360	297	328
Cisplatin*	50.3	77.5	94.4	56.9	91.4	406	639	474	268	491
Gefitinib	27.0	39.5	68.3	47.7	42.9	202	415	185	253	230
Erlotinib	47.0	58.5	83.9	126	94.0	75.8	368	255	89.6	113
Dasatinib	59.4	38.1	96.4	79.9	86.2	215	77.7	166	151	200
Sunitinib	11.4	21.9	29.7	33.7	61.0	197	278	129	158	170
Ruxolitinib	46.7	52.1	106	64.8	84.2	275	368	563	180	162
Doxorubicin	-	-	-	-	-	-	-	-	-	-
Etoposide	19.9	26.8	118	70.6	79.9	123	128	306	82.3	155
Topotecan	24.0	67.4	130	115	75.9	140	225	220	88.6	106
Dactolisib	53.8	46.7	82.0	86.5	86.1	71.7	46.7	153	164	100
Buparlisib	67.7	61.2	112	103	96.6	251	175	395	197	165
Romidepsin	57.7	82.3	100	77.1	112	275	315	308	219	249
Bortezomib	101	85.1	153	86.7	104	457	489	686	130	232
Everolimus	34.6	129	189	50.2	39.2	347	1042	675	402	274
Ganetespib	58.6	66.5	155	91.6	111	193	95.6	585	136	268

MIFI = mean integrated fluorescent intensity.
EHD = ethidium homodimer dead stain
CAM = Calcein AM live stain
CAM & EHD dead cell MIFI values were normalized and expressed as % of DMSO controls.
5-FU = 5-fluorouracil
* = Drugs approved by the Federal Drug Administration for HNSCC therapy.
There are no MIFI values for Doxorubicin because of autofluorescence and potential cross talk in the FITC and Texas Red channels. [22, 31]

Supplemental Table 4B. Head and Neck Squamous Cell Carcinoma Multicellular Tumor Spheroid Culture Live and Dead Staining Cancer Drug Impact Scores.

	Calcein AM Live Stain Drug Impact Score						Ethidium Homodimer Dead Stain Drug Impact Score					
Compound	**FaDu**	**UM2-22B**	**Cal33**	**BIRC56**	**OSC-19**	**Total**	**FaDu**	**UM2-22B**	**Cal33**	**BIRC56**	**OSC-19**	**Total**
5-FU*	1.0	0.5	0.0	0.5	0.0	2.0	0.0	1.0	1.0	0.0	1.0	3.0
Methotrexate*	1.0	1.0	0.0	0.5	0.5	3.0	0.0	0.0	0.5	0.0	0.0	0.5
Bleomycin*	1.0	1.0	0.0	0.0	0.5	2.5	0.0	0.5	0.0	0.0	0.0	0.5
Docetaxel*	1.0	0.5	0.0	0.5	0.5	2.5	1.0	1.0	1.0	1.0	1.0	5.0
Cisplatin*	0.5	0.5	0.0	0.5	0.0	1.5	1.0	1.0	1.0	1.0	1.0	5.0
Gefitinib	1.0	1.0	0.5	1.0	1.0	4.5	1.0	1.0	1.0	1.0	1.0	5.0
Erlotinib	1.0	0.5	0.0	0.0	0.0	1.5	0.0	1.0	1.0	0.0	0.0	2.0
Dasatinib	0.5	1.0	0.0	0.5	0.0	2.0	1.0	0.0	1.0	1.0	1.0	4.0
Sunitinib	1.0	1.0	1.0	1.0	0.5	4.5	1.0	1.0	0.5	1.0	1.0	4.5
Ruxolitinib	1.0	0.5	0.0	0.5	0.0	2.0	1.0	1.0	1.0	1.0	1.0	5.0
Doxorubicin	-	-	-	-	-	NA	-	-	-	-	-	NA
Etoposide	1.0	1.0	0.0	0.5	0.5	3.0	0.5	0.5	1.0	0.0	1.0	3.0
Topotecan	1.0	0.5	0.0	0.0	0.5	2.0	0.5	1.0	1.0	0.0	0.0	2.5
Dactolisib	0.5	1.0	0.0	0.0	0.0	1.5	0.0	0.0	1.0	1.0	0.0	2.0
Buparlisib	0.5	0.5	0.0	0.0	0.0	1.0	1.0	1.0	1.0	1.0	1.0	5.0
Romidepsin	0.5	0.0	0.0	0.5	0.0	1.0	1.0	1.0	1.0	1.0	1.0	5.0
Bortezomib	0.0	0.0	0.0	0.0	0.0	0.0	1.0	1.0	1.0	0.5	1.0	4.5
Everolimus	1.0	0.0	0.0	0.5	1.0	2.5	1.0	1.0	1.0	1.0	1.0	5.0
Ganetespib	0.5	0.5	0.0	0.0	0.0	1.0	1.0	0.0	1.0	0.5	1.0	3.5

CAM live and EHD dead drug impact scores based on the % of MIFI data presented in Supplemental Table 4A. In CAM stained MCTS cultures, if a drug reduced the CAM MIFI by ≥50% relative to DMSO controls it scored 1, if it reduced the CAM MIFI by ≥20% but <50% it scored 0.5, and if it reduced the CAM MIFI by <20% it scored 0. In EHD stained MCTS cultures, if a drug increased the EHD MIFI by ≥50% relative to DMSO controls it scored 1, if it increased the EHD MIFI by ≥20% but <50% it scored 0.5, and if it increased the EHD MIFI by <20% it scored 0. Maximum CAM/EHD drug impact score per HNSCC MCTS culture = 1, and across all cultures = 5. Minimum drug CAM/EHD impact score per HNSCC MCTS culture = 0, and across all cultures = 0. * = Drugs approved by the Federal Drug Administration for HNSCC therapy. No scores for Doxorubicin due to autofluorescence and potential cross talk in the FITC and Texas Red channels. [22, 31]

Supplemental Table 5A. Cancer Drug Effects on Head and Neck Squamous Cell Carcinoma Multicellular Tumor Spheroid Morphology

	FaDu			UM-22B			Cal33			BIRC56			OSC-19		
Compound	**P/S**	**D/C**	**ΔD**	**P/S**	**D/C**	**ΔD**	**P/S**	**D/C**	**ΔD**	**P/S**	**D/C**	**ΔD**	**P/S**	**D/C**	**ΔD**
5-FU*	Rounded	Loose	Dec	Irregular	Loose	Inc	Rounded	Tight	Unc	Rounded	Tight	Unc	Irregular	Dispersed	Dec
Methotrexate*	Rounded	Loose	Dec	Rounded	Tight	Dec	Rounded	Tight	Unc	Rounded	Tight	Dec	Rounded	Loose	Unc
Bleomycin*	Rounded	Tight	Unc	Irregular	Tight	Dec	Rounded	Tight	Dec	Rounded	Tight	Unc	Rounded	Loose	Dec
Docetaxel*	Irregular	Dispersed	Inc	Irregular	Dispersed	Inc	Irregular	Dispersed	Dec	Irregular	Loose	Inc	Irregular	Dispersed	Inc
Cisplatin*	Rounded	Loose	Inc	Irregular	Dispersed	Inc	Irregular	Tight	Inc	Rounded	Tight	Dec	Irregular	Loose	Inc
Gefitinib	Rounded	Loose	Dec	Irregular	Dispersed	Inc	Irregular	Loose	Inc	Rounded	Tight	Unc	Irregular	Loose	Dec
Erlotinib	Rounded	Tight	Dec	Rounded	Loose	Dec	Rounded	Tight	Dec	Rounded	Tight	Unc	Irregular	Loose	Dec
Dasatinib	Irregular	Loose	Dec	Rounded	Tight	Dec	Rounded	Tight	Inc	Rounded	Loose	Unc	Irregular	Loose	Inc
Sunitinib	Rounded	Loose	Dec	Irregular	Dispersed	Dec	Rounded	loose	Unc	Rounded	Tight	Dec	Irregular	Loose	Inc
Ruxolitinib	Rounded	Loose	Dec	Irregular	Loose	Dec	Rounded	Tight	Dec	Irregular	Loose	Inc	Irregular	Loose	Inc
Doxorubicin	Rounded	Loose	Inc	Irregular	Dispersed	Inc	Rounded	Loose	Inc	Irregular	Loose	Unc	Irregular	Loose	Inc
Etoposide	Rounded	Loose	Dec	Rounded	Loose	Dec	Rounded	Tight	Dec	Rounded	Tight	Unc	Rounded	Loose	Dec
Topotecan	Rounded	Loose	Dec	Rounded	Tight	Dec	Rounded	Tight	Inc	Rounded	Tight	Unc	Rounded	Loose	Unc
Dactolisib	Rounded	Tight	Dec	Rounded	Tight	Dec	Rounded	Tight	Dec	Rounded	Loose	Dec	Rounded	Loose	Dec
Buparlisib	Irregular	Loose	Dec	Irregular	Tight	Dec	Rounded	Tight	Dec	Irregular	Loose	Dec	Irregular	Loose	Inc
Romidepsin	Irregular	Loose	Dec	Irregular	Loose	Dec	Rounded	Loose	Dec	Irregular	Loose	Inc	Irregular	Loose	Inc
Bortezomib	Irregular	Loose	Inc	Irregular	Dispersed	Inc	Rounded	Tight	Inc	Rounded	Tight	Dec	Irregular	Dispersed	Inc
Everolimus	Irregular	Loose	Inc	Irregular	Dispersed	Inc	Irregular	Loose	Inc	Irregular	Tight	Unc	Irregular	Dispersed	Inc
Ganetespib	Rounded	Loose	Dec	Irregular	Tight	Dec	Rounded	Loose	Dec	Irregular	Dispersed	Inc	Irregular	Loose	Inc

5-FU = 5-fluorouracil
* = Drugs approved by the Federal Drug Administration for HNSCC therapy.
P/S = perimeter or shape of MCTS
D/C = density or compactness of MCTS
ΔD = change in diameter
Dec = decrease in diameter
Inc = increase in diameter
Unc = diameter unchanged

Supplemental Table 5B. Head and Neck Squamous Cell Carcinoma Multicellular Tumor Spheroid Culture Morphology Cancer Drug Impact Scores.

	HNSCC MCTS Morphology Drug Impact Scores					
Compound	**FaDu**	**UM2-22B**	**Cal33**	**BIRC56**	**OSC-19**	**Total**
5-FU*	1.0	0.5	0.0	0.0	1.0	2.5
Methotrexate*	1.0	1.0	0.0	0.5	0.0	2.5
Bleomycin*	0.0	1.0	0.5	0.0	0.5	2.0
Docetaxel*	1.0	1.0	1.0	1.0	1.0	5.0
Cisplatin*	1.0	1.0	1.0	0.5	1.0	4.5
Gefitinib	1.0	1.0	1.0	0.0	1.0	4.0
Erlotinib	0.5	1.0	0.5	0.0	1.0	3.0
Dasatinib	1.0	1.0	0.5	0.5	1.0	4.0
Sunitinib	1.0	1.0	0.5	0.5	1.0	4.0
Ruxolitinib	1.0	0.5	0.5	1.0	1.0	4.0
Doxorubicin	1.0	1.0	1.0	1.0	1.0	5.0
Etoposide	1.0	1.0	0.5	0.0	0.5	3.0
Topotecan	1.0	1.0	0.5	0.0	0.0	2.5
Dactolisib	0.5	1.0	0.5	1.0	0.5	3.5
Buparlisib	1.0	1.0	0.5	1.0	1.0	4.5
Romidepsin	1.0	0.5	1.0	1.0	1.0	4.5
Bortezomib	1.0	1.0	0.5	0.5	1.0	4.0
Everolimus	1.0	1.0	1.0	0.5	1.0	4.5
Ganetespib	1.0	1.0	1.0	1.0	1.0	5.0

The morphology drug impact scores are based on Supplemental Table 5A which summarizes the changes in MCTS morphology parameters relative to DMSO controls in MCTS cultures exposed to the top concentrations of the 19 drugs. If a drug altered ≥2 of the 3 MCTS morphology parameters it scored 1, if it only altered 1 of 3 morphology parameters it scored 0.5, and if it did not change any of the morphology parameters it scored 0. Maximum morphology drug impact score per HNSCC MCTS culture = 1, and across all cultures = 5. Minimum morphology drug impact score per HNSCC MCTS culture = 0, and across all cultures = 0. * = Drugs approved by the Federal Drug Administration for HNSCC therapy.

Supplemental References 51-65

51. Schmidt, M., Scholz, CJ, Polednik, C, Roller, J. Spheroid-based 3-dimensional culture models: Gene expression and functionality in head and neck cancer. *Oncology Reports* **2016,** *35*, 2431-2440.
52. Grantab, R., Sivananthan, S, Tannock, IF. The penetration of anticancer drugs through tumor tissue as a function of cellular adhesion and packing density of tumor cells. *Cancer Res.* **2006,** *66*, 1033-1039.
53. Grantab, R., Tannock, IF. Penetration of anticancer drugs through tumour tissue as a function of cellular packing density and interstitial fluid pressure and its modification by bortezomib. *BMC Cancer* **2012,** *12*.
54. Tredan, O.; Galmarini, C. M.; Patel, K.; et al. Drug resistance and the solid tumor microenvironment. *J Natl Cancer Inst* **2007,** *99*, 1441-54.
55. Kyle, A., Huxham, LA, Chiam, AS, et al. Direct assessment of drug penetration into tissue using a novel application of three-dimensional cell culture. *Cancer Res.* **2004,** *64*, 6304-6309.
56. Kyle, A., Huxham, LA, Yeoman, DM, Minchinton, AI. Limited tissue penetration of taxanes: a mechanism for resistance in solid tumors. *Clin. Cancer Res.* **2007,** *13*, 2804-2810.
57. Kuh, H., Jang, SH, Wientjes, MG, et al. Determinants of paclitaxel penetration and accumulation in human solid tumor. *J Pharmacol Exp Ther* **1999,** *290*, 871-880.
58. Au, J., Jang, SH, Zheng, J, et al. Determinants of drug delivery and transport to solid tumors. *J Control Release.* **2001,** *74*, 31-46.
59. Zheng, J., Chen, CT, Au, JL, Wientjes, MG. Time- and concentration-dependent penetration of doxorubicin in prostate tumors. *AAPS PharmSci.* **2001,** *3*, E15.
60. Cribbes, S., Kessel, S, McMenemy, S, et al. A Novel Multiparametric Drug-Scoring Method for High-Throughput Screening of 3D Multicellular Tumor Spheroids Using the Celigo Image Cytometer. *SLAS Discov.* **2017,** *22*, 547-557.
61. Kang, H., Kiess, A, Chung, CH. Emerging biomarkers in head and neck cancer in the era of genomics. *Nat Rev Clin Oncol.* **2015,** *12*, 11-26.
62. Network, T. C. G. A. Comprehensive genomic characterization of head and neck squamous cell carcinomas. *Nature* **2015,** *517*, 576–582.
63. Sun, W., Califano, JA. Sequencing the head and neck cancer genome: implications for therapy. *Ann N Y Acad Sci.* **2014,** *1333*, 33-42.
64. Holohan, C., Van Schaeybroeck, S, Longley, DB, Johnston, PG. Cancer drug resistance: an evolving paradigm. *Nat Rev Cancer.* **2013,** *13*, 714-26.

65. Lovly, C., Shaw, AT. Molecular pathways: resistance to kinase inhibitors and implications for therapeutic strategies. *Clin Cancer Res.* **2014,** *20*, 2249-56.