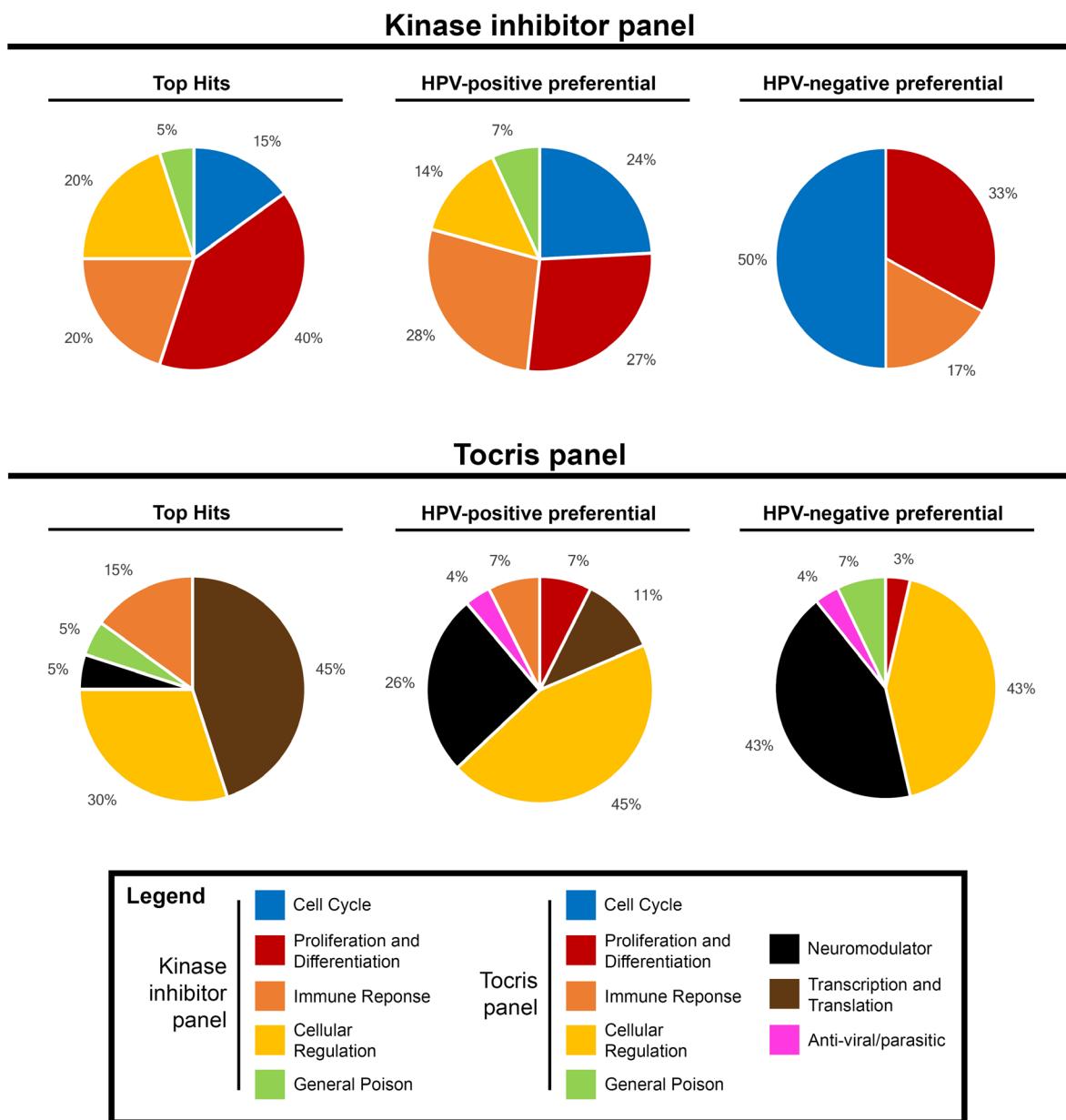
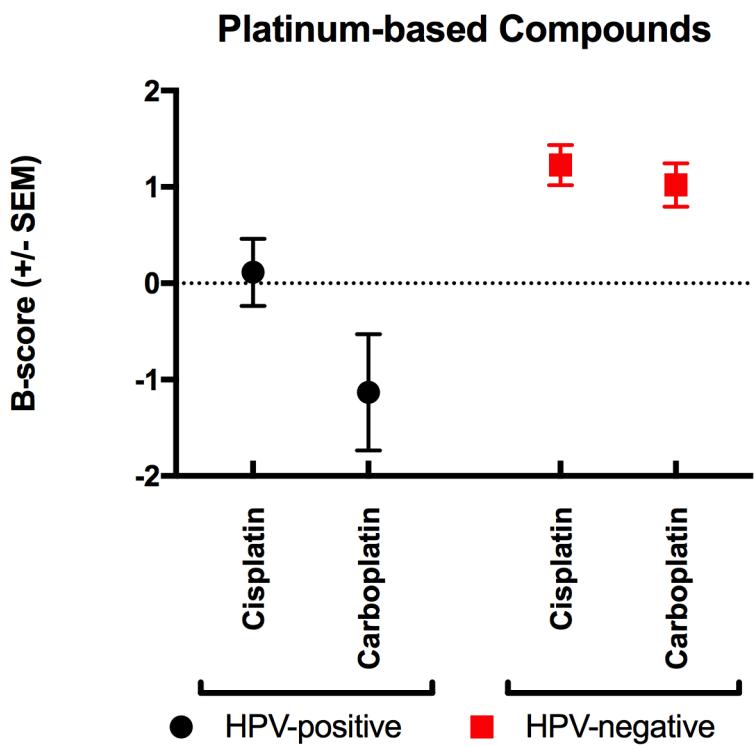


High-throughput testing in head and neck squamous cell carcinoma identifies agents with preferential activity in human papillomavirus-positive or negative cell lines

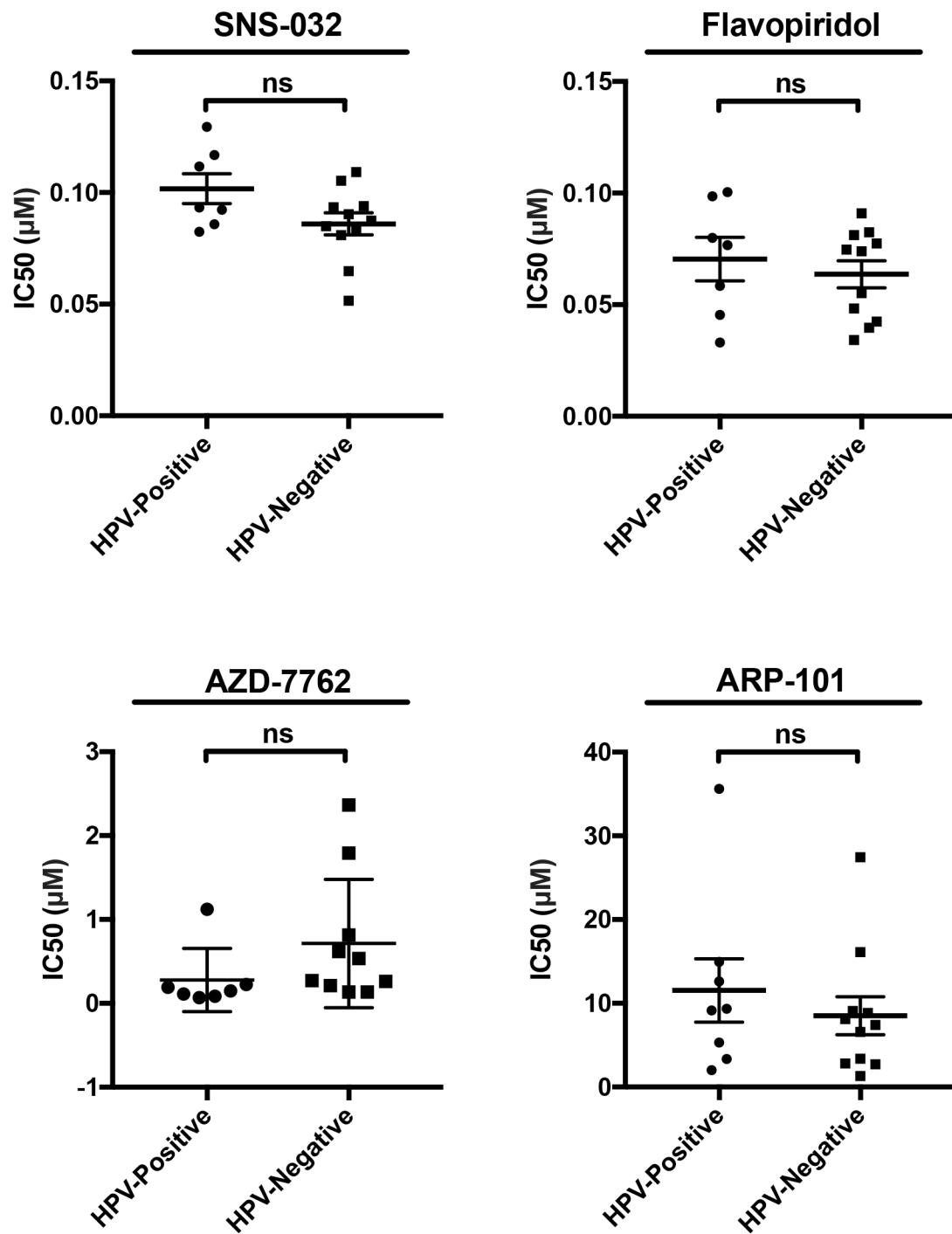
SUPPLEMENTARY MATERIALS



Supplementary Figure 1: Compounds identified as top hits in potency, preferential activity against HPV-positive and preferential activity against HPV-negative cell lines using the Kinase inhibitor and Tocris panels grouped by their mechanism of action.



Supplementary Figure 2: Cisplatin and carboplatin, chemotherapeutics in routine use head and neck cancer, were included in the compound panels, but did not meet the set criteria for significant activity (B-score < -2) at a dose of $4\mu\text{M}$.



Supplementary Figure 3: Validation studies on SNS-032, Flavopiridol, AZD-7762 and ARP-101 did not reveal significantly selective potency against HNSCC cell lines stratified by HPV status ($p=0.07$, 0.54 , 0.19 and 0.48 respectively). (ns = not significant).

Supplementary Table 1: Top 20 compounds with highest potency from the kinase inhibitor and tocris panels against HNSCC cell lines

Compound	All Cell lines		Primary mechanism of action	Category
	Mean	SEM		
Top 20 overall Hits from Kinase Inhibitor Panel				
Staurosporine	-4.66	0.46	Non-selective protein kinase inhibitor	General Poison
TCS 2312 dihydrochloride	-3.73	0.54	Potent Chk1 inhibitor	Cell Cycle
GDC-0941 bismesylate	-3.45	0.41	Inhibitor of class I PI3 kinase	Proliferation & Differentiation
IMD 0354	-3.36	0.33	Inhibitor of IKK β	Immune Response
ER 27319 maleate	-3.11	0.31	Selective Syk kinase inhibitor	Immune Response
IC261	-3.08	0.35	Selective casein kinase 1 δ and 1 ϵ inhibitor	Cellular Regulation
Alsterpaullone	-3.04	0.51	Inhibitor of multiple CDKs	Cell Cycle
BI 78D3	-2.81	0.72	Competitive JNK inhibitor	Immune Response
Dasatanib	-2.72	0.56	Inhibitor of Abl, Src and c-Kit	Proliferation & Differentiation
NVP-BEZ235	-2.70	0.24	Inhibitor of PI3K and mTOR	Proliferation & Differentiation
PIK-75	-2.64	0.52	Inhibitor of p110 α	Proliferation & Differentiation
A-443654	-2.63	0.39	Inhibitor of Akt	Proliferation & Differentiation
TAE-684	-2.48	0.51	Inhibitor of ALK	Proliferation & Differentiation
5-Iidotubercidin	-2.45	0.35	Potent adenosine kinase inhibitor	Cellular Regulation
FAK Inhibitor 14	-2.43	0.45	Selective FAK inhibitor	Proliferation & Differentiation
Ryuvidine	-2.40	0.58	Inhibitor of SETD8 and CDK4	Cellular Regulation
Lestaurtinib	-2.31	0.23	Inhibitor of JAK2, FLT3 and TrkA	Immune Response
NVP-AEW541, AEW541	-2.11	0.35	Inhibitor of IGF-1R/InsR	Cellular Regulation
SB-505124 hydrochloride hydrate	-2.06	0.67	Inhibitor of ALK5	Proliferation & Differentiation
NSC 663284	-2.05	0.43	Inhibitor of Cdc25 phosphatase	Cell Cycle
Top 20 Overall Hits from Tocris panel				
Pyrrolidinedithiocarbamate ammonium	-19.71	1.92	Inhibits NF- κ B, prevents increase in NOS mRNA	Immune Response
Daunorubicin hydrochloride	-16.78	1.53	Inhibits topoisomerase II	Transcription and translation
Actinomycin D	-16.64	1.64	Inhibits RNA polymerase	Transcription and translation
SCS	-15.54	2.18	Selective GABAA receptor antagonist	Neuromodulators
NSC 146109 hydrochloride	-15.41	1.23	activates p53-dependent transcription	Transcription and translation
Doxorubicin hydrochloride	-14.70	1.88	Inhibits DNA topoisomerase II	Transcription and translation
Homoharringtonine	-14.62	1.26	Inhibitor of protein synthesis	Transcription and translation
A23187, free acid	-14.34	1.49	Calcium ionophore	General Poison
BNTX maleate	-14.31	1.20	Standard δ 1 selective antagonist	Cellular regulation & physiology
MG 132	-13.67	1.32	Inhibitor of Proteasome, calpain and NF- κ B activation	Immune Response
Brefeldin A	-13.61	1.21	Disrupts protein translocation to Golgi	Transcription and translation
Bay 11-7085	-13.32	1.38	Inhibitor of TNF- α -induced I κ B α phosphorylation	Immune Response
Diphenyleneiodonium chloride	-13.21	1.15	GPR3 agonist; also inhibits NOS and NADPH oxidases	Cellular regulation & physiology
NSC 632839 hydrochloride	-13.11	1.11	Inhibitor of ubiquitin isopeptidase activity	Cellular regulation & physiology
SN 38	-12.50	1.17	Inhibitor of DNA topoisomerase I	Transcription and translation
Cycloheximide	-12.14	1.16	Inhibitor of protein synthesis	Transcription and translation
JTC 801	-12.00	1.10	Selective NOP antagonist	Cellular regulation & physiology
SCH 79797 dihydrochloride	-11.72	1.05	Selective casein kinase 1 δ and 1 ϵ inhibitor	Cellular regulation & physiology
Camptothecin	-11.61	1.25	DNA topoisomerase inhibitor	Transcription and translation
Cantharidin	-11.56	1.17	Inhibitor of Protein phosphatase 1/2A	Cellular regulation & physiology

Supplementary Table 2: Complete list of kinase inhibitor panel and Tocris panel compound activity against individual HNSCC cell lines

See Supplementary File 1

Supplementary Table 5: Source, HPV status and growth media of cell lines in the high-throughput study (HTS) and dose-response validation studies (DRVS)

Cell line	HPV status	Source	Study	Media
HMS-001	Positive	Rocco	HTS & DRVS	DMEM/F12
UM-SCC47	Positive	Carey	HTS & DRVS	DMEM/F12
93-VU-147T	Positive	deWinter	HTS & DRVS	DMEM/F12
UPCI:SCC090	Positive	Gollin	HTS & DRVS	DMEM/F12
UPCI:SCC154	Positive	Gollin	HTS & DRVS	DMEM/F12
UD-SCC2	Positive	Rocco	HTS & DRVS	DMEM/F12
UWO37	Positive	In House	DRVS	DMEM/F12
UWO23	Positive	In House	DRVS	DMEM/F12
SCC9	Negative	ATCC	HTS	DMEM/F12
FaDu	Negative	ATCC	HTS	DMEM/F12
Detroit 562	Negative	ATCC	HTS & DRVS	DMEM/F12
SCC15	Negative	ATCC	HTS & DRVS	DMEM/F12
SCC4	Negative	ATCC	HTS	DMEM/F12
SCC25	Negative	ATCC	HTS & DRVS	DMEM/F12
Cal 27	Negative	ATCC	HTS & DRVS	DMEM/F12
HSC2	Negative	JHSF	HTS & DRVS	DMEM/F12
JHU011	Negative	Rocco	HTS & DRVS	DMEM/F12
Cal33	Negative	DSMZ	HTS & DRVS	DMEM
JHU029	Negative	Rocco	HTS & DRVS	DMEM/F12
PCI 6B	Negative	Ferris	HTS & DRVS	DMEM/F12
PCI 13	Negative	Ferris	HTS	DMEM/F12
PCI 22B	Negative	Ferris	HTS	DMEM/F12
PCI 6A	Negative	Ferris	HTS	DMEM/F12
RF 15A	Negative	Ferris	HTS & DRVS	DMEM/F12
RF 15B	Negative	Ferris	HTS	DMEM/F12
RF 22A	Negative	Ferris	HTS	DMEM/F12
RF 37B	Negative	Ferris	HTS & DRVS	DMEM/F12
JHU 006	Negative	Rocco	HTS	DMEM/F12

Legend: **HPV** – human papillomavirus; **HTS** – High-throughput study; **DRVS** – Dose-response studies; **Rocco** - Dr. James Rocco, Harvard Medical School; **Carey** - Dr. Tom Carey, University of Michigan; **deWinter** - Dr. Johann deWinter, Free University of Amsterdam; **Gollin** - Dr. Suzanne Gollin, University of Pittsburgh Cancer Institute; **JHSF** - Japan Health Sciences Foundation; **DSMZ** - Deutsche Sammlung von Mikroorganismen und Zellkulturen; **Ferris** - Dr. Robert Ferris, University of Pittsburgh Cancer Institute; **In House**; Developed in our laboratory at Western University.

