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Functional precision medicine identifies novel druggable targets and therapeutic options in head and neck cancer

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Abstract

Purpose—Head and neck squamous cell carcinoma (HNSCC) is the sixth most common cancer worldwide with high mortality and a lack of targeted therapies. To identify and prioritize druggable targets, we performed genome analysis together with genome-scale siRNA and oncology drug profiling using low passage tumor cells derived from a patient with a treatment-resistant HPV-negative HNSCC.

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Declarations

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Experimental design—A tumor cell culture was established and subjected to whole exome sequencing, RNA sequencing, comparative genome hybridization, and high-throughput phenotyping with siRNA library covering the druggable genome and an oncology drug library. Secondary screens of candidate target genes were performed on the primary tumor cells and two non-tumorigenic keratinocyte cell cultures for validation and to assess cancer-specificity. siRNA screens of the kinome on two isogenic pairs of p53-mutated HNSCC cell lines were used to determine generalizability. Clinical utility was addressed by performing drug screens on two additional HNSCC cell cultures derived from patients enrolled in a clinical trial.

Results—Many of the identified copy number aberrations and somatic mutations in the primary tumor were typical of HPV(-) HNSCC, but none pointed to obvious therapeutic choices. In contrast, siRNA profiling identified 391 candidate target genes, 35 of which were preferentially lethal to cancer cells, most of which were not genomically altered. Chemotherapies and targeted agents with strong tumor specific activities corroborated the siRNA profiling results and included drugs that targeted the mitotic spindle, the proteasome and G2/M kinases *WEE1* and *CHK1*. We also show the feasibility of *ex-vivo* drug profiling for patients enrolled in a clinical trial.

Conclusions—High-throughput phenotyping with siRNA and drug libraries using patient derived tumor cells prioritizes mutated driver genes and identifies novel drug targets not revealed by genomic profiling. Functional profiling is a promising adjunct to DNA sequencing for precision oncology.

Keywords

Next generation Sequencing; CGH; RNAi; Drug Screen; Gene Targets; Head and Neck Cancer

Introduction

Genomic profiling of tumors holds great promise to guide cancer therapy. However, a central challenge is the difficulty of matching effective drugs to genomic profiles, which are often complex and unique to each patient. Furthermore, many commonly mutated cancer genes, such as tumor suppressor genes, are difficult to target. Even for genes that might be targetable, it is not always clear which one should be prioritized or which drug would be effective. A second, no less significant challenge is the shortage of druggable targets and associated therapeutic agents. A recent estimate of the number of genes currently targeted by FDA approved drugs is 109 genes, yet there are ~22,000 genes in the human genome, of which ~10,000 are considered druggable (1). Even in cases where genome-guided targeted therapy works, development of resistance is common, highlighting the need for new targeted agents or combination therapies.

Head and neck squamous cell carcinoma (HNSCC) is the sixth most common cancer worldwide, with about 600,000 new cases each year and a mortality rate around 50% (2). Patients are often diagnosed with locally advanced disease and subjected to intensive treatment with a combination of surgery, radiation, and chemotherapy. Although these treatments can increase loco-regional control, they often induce high-grade toxicities (3). Resistance to chemotherapy and radiation contributes to tumor recurrence and options for those patients are limited.

Genomic analysis of HNSCC has revealed a complex mutational profile. In HPV-negative HNSCC, mutation in the tumor suppressor gene *TP53* is by far the most common event, detected in 74% of the TCGA HNSCC patient cohort (4), and is associated with poor clinical outcome (5). Oncogenic mutations or amplifications are present at lower frequencies such as *CCND1* (~27%), *ERBB2* (~5%), *HRAS* (4–12%), *MYC* (~13%), *FADD* (6%), *BIRC2* (6%), and *PIK3CA* (6–21%) (4). Despite detailed genomic characterization and clear evidence that *TP53* plays a central role in HNSCC malignancy, targeted therapies for HNSCC are lacking.

To identify new targets and therapeutic strategies, we established tumor cultures from a patient with an aggressive, cisplatin-resistant oral cancer, and performed comprehensive genomic analysis as well as genome-scale RNA interference and oncology focused drug profiling. We illustrate how integration of tumor genomics with siRNA and drug profiling efficiently prioritizes driver from passenger genomic aberrations and identifies novel targetable vulnerabilities, some matched to candidate chemotherapeutics or targeted agents. We identified not only strategies to capitalize on the tumor's *TP53* mutant status via synthetic lethality with G2/M checkpoint regulators such as *WEE1* and *CHK1*, but also vulnerabilities associated with mitotic spindle function, developmental and growth factor pathways, protein degradation machinery, and apoptosis.

Methods

Cell lines

This study was approved by the institutional review board (IRB) of the University of Washington (UW) and the IRB of the Fred Hutchinson Cancer Research Center (FHCRC). Tissue specimens were collected with written consent obtained from a previously untreated 59-year-old male with a floor-of-mouth squamous cell carcinoma (T2N2b, HPV-negative) during surgery treatment. All procedures were conducted in accordance with International Ethical Guidelines for Biomedical Research Involving Human Subjects (CIOMS). Tumor tissue was disinfected in 1% povidone-iodine solution for 5 minutes, minced with a scalpel, and digested with 2–4 mg collagenase and 0.5 mg DNase I (Sigma, St. Louis, MO) in PBS at 37°C for 120 minutes. Cells were obtained by filtration through a 70µm strainer and centrifugation at 1000 rpm for 3 minutes. The cells were plated onto collagen IV (Sigma) coated tissue culture flasks and cultured in Keratinocyte-SFM medium supplemented with pen-strep, fungizone, glutamine, and non-essential amino acids (Thermo Fisher Scientific, Grand Island, NY). Epithelial tumor cells were enriched by selective culturing and magnetic-activated cell sorting with EpCAM microbeads (Miltenyi Biotec, San Diego, CA). The established primary tissue culture, which was named FHCRC-SCC-1, was immunohistochemically stained with anti-cytokeratin antibody ab961 and anti-vimentin antibody EPR868(2) (Abcam, Cambridge, MA) to confirm epithelial enrichment. Tumorigenicity was confirmed by inoculating cultured cells subcutaneously into the tongue of NOD/SCID interleukin-2 gamma null mice. When tumors reached a palpable mass of >50 mm³ the tumor was harvested for histological analysis. Isolation and propagation of FHCRC-SCC-7A and FHCRC-SCC-10A HNSCC primary cultures was performed as above. These two cultures were established from two patients enrolled in our phase I clinical trial

testing AZD1775 alone and in combination with neoadjuvant chemotherapy in previously untreated, metastatic HNSCC (NCT02508246). The normal oral keratinocyte (NOK) cell line (6) was obtained from Dr. Duvvuri at University of Pittsburgh (Pittsburgh, PA). The cells were maintained in the same medium as FHCRC-SCC-1. Primary human foreskin keratinocytes (HFK-1) were obtained from Dr. Galloway at FHCRC (7). The cells were maintained in EpiLife® supplemented with human keratinocyte growth supplement (HKGS), glutamine, and non-essential amino acids (Thermo Fisher Scientific).

Two pairs of HNSCC cell lines were used: UM-SCC-14A and UM-SCC-14C, obtained from Dr. Carey at University of Michigan (Ann Arbor, MI), and PCI-15A and PCI-15B, obtained from Dr. Ferris at University of Pittsburgh (Pittsburgh, PA). Each pair was derived from the same patient's primary tumor and subsequent recurrence in the case of UM-SCC-14C or the metastatic lymph node in the case of PCI-15B (8). The HNSCC cell lines were maintained in DMEM supplemented with 10% FBS, glutamine, and non-essential amino acids, and penicillin/streptomycin (Thermo Fisher Scientific).

DNA and RNA extraction

DNA and RNA from the primary tumor and HNSCC cell lines were extracted using the AllPrep DNA/RNA Mini kit (QIAGEN, Valencia, CA). DNA was also extracted from the patient's tumor-free salivary gland formalin-fixed block using QIAamp® DNA FFPE Tissue kit (QIAGEN). Extracted DNA and RNA were assessed with ND-1000 Spectrophotometer (NanoDrop Technologies, Wilmington, DE) and Agilent 2200 TapeStation (Agilent Technologies, Santa Clara, CA). Quantity of double stranded DNA was determined by Quant-iT™ PicoGreen® dsDNA Assay Kit (Thermo Fisher Scientific). Quantity of RNA was also determined by Trinean DropSense96 spectrophotometer (Caliper Life Sciences, Hopkinton, MA) before sequencing.

Exome sequencing

Next generation exome sequencing was performed on FHCRC-SCC-1 at the UW Northwest Genomics Center using the Nimblegen SeqCap probes on an Illumina platform. Paired tumor-free DNA was used as reference to remove germline SNPs. Exome sequencing results were analyzed using VarScan (v2.3.6) (9) and MuTect (v1.1.4) (10) with default parameter settings. SNP and indel calls were generated in reference to human genome build hg19 (Supp. Table 1, Figure 1E track 2). Targeted sequencing of FHCRC-SCC-7A and FHCRC-SCC-10A was done using UW Oncoplex version 5 (<http://tests.labmed.washington.edu/UW-OncoPlex>).

Comparative genome hybridization

Comparative genome hybridization (CGH) was performed at the FHCRC Genomics Core using Agilent's SurePrint G3 Human High-Resolution Discovery Microarray 1×1M (Design ID 023642, Agilent Technologies). Male human genomic DNA G147A (Promega, Madison, WI) was used as normal genome reference for making copy number aberration calls. The array was scanned at 2 µm resolution using Agilent DNA Microarray Scanner. Hybridization signal was extracted from raw images and normalized using Feature Extraction software (Agilent Technologies, v9.5). Copy number aberrations were detected using the ADM-2

algorithm (Threshold = 6, Fuzzy Zero = on) in Agilent's Genomic Workbench Software (Agilent Technologies, v.7.0). Genomic regions with signal $\geq 3/2$ were deemed *amplified* and those with signal $\leq 1/2$ were deemed *deleted* (Supp. Table 2, Figure 1E track 4).

RNA sequencing

Next generation RNA sequencing was performed at the FHCRC Genomics Core. Briefly, RNA-seq libraries were prepared from total RNA using the TruSeq RNA Sample Prep Kit (Illumina, Inc., San Diego, CA, USA) and a Sciclone NGSx Workstation (PerkinElmer, Waltham, MA, USA). Library size distributions were validated using an Agilent 2200 TapeStation (Agilent Technologies). Additional library QC, blending of pooled indexed libraries, and cluster optimization were performed using Invitrogen Qubit® 2.0 Fluorometer (Life Technologies, Carlsbad, CA, USA). RNA-seq libraries were pooled (5-plex) and clustered onto a flow cell lane. Sequencing was performed using HiSeq 2500 (Illumina) in "rapid run" mode employing a paired-end, 100 base read length (PE100) sequencing strategy. Image analysis and base calling were performed using Real Time Analysis software (Illumina, v1.18), followed by 'demultiplexing' of indexed reads and generation of FASTQ files, using bcl2fastq Conversion Software (Illumina, v1.8.4).

Confirmation of tumor genetic profile

Copy number aberrations and mutations of selected cancer-related genes were confirmed using next generation sequencing-based technology by Resolution Bioscience Inc. (Bellevue, WA). Mutation in *TP53* was further confirmed by capillary electrophoresis-based sequencing method using Applied Biosystems (ABI) 3730xl DNA Analyzers (Thermo Fisher Scientific) with primer sets as previously described (8).

Integrative analysis of patient tumor and TCGA HNSCC profiles

Genome mutations: TCGA HNSCC mutation data (277 patients $\times$ 14,693 genes) was obtained from Synapse (syn1571579) as part of the Pan-Cancer 12 TCGA data (11) and used to calculate gene mutation frequency. *Transcriptome:* To facilitate incorporation of patient tumor FHCRC-SCC-1 RNA-seq data with that from TCGA for downstream analysis, the 'TCGA mRNA-seq Pipeline for UNC data' was implemented locally to generate 'Level 3, RNASeq Version 2' processed data from our samples. In brief, reads of poor quality were discarded prior to alignment to hg19 using MapSplice (v2.1.8) (12), followed by quantitation with RSEM (v1.1.13) (13). Genes with undetected transcripts were excluded and the remaining signals were normalized using the weighted trimmed mean of M-values approach from the edgeR package (14). A small constant was added to all normalized counts and the mean value of the normal samples was calculated to obtain base 2 log fold-change. Genes with log fold-change >1 were deemed *highly expressed* and those with log fold change <-1 were deemed *lowly expressed*.

We used a CIRCOS plot with 4 concentric tracks to visualize our integrative findings (Figure 1E). Track 1 illustrates the mutation frequency of genes in the TCGA HNSCC patient cohort (11). Tracks 2–4 visualize the genomic profile of FHCRC-SCC-1. Track 2 presents the mutation results as detected by WES, including: non/missense, readthrough, splice site, flanking, UTR, RNA and intergenic mutations, as well as in-frame and frame-shift indels.

Track 3 shows the base 2 log normalized RNA-seq transcript counts. Track 4 visualizes the normalized base 2 log ratio of tumor to normal CNV signal as detected by CGH. The amplified and deleted regions are highlighted with black border. On the periphery of the CIRCOS plot we highlight genes of interest. We first compiled a list “cancer genes” by aggregating three sets of genes that may be actionable for precision oncology (i.e. Foundation One®, MSK-IMPACT™, and UW OncoPlex™). We used this list to filter the highly expressed genes (shown in red), the highly expressed and amplified genes (shown in bold red), and the lowly expressed genes, which were also harboring deletions (shown green). Mutated genes in FHCRC-SCC-1 that were also mutated in TCGA at 1% or higher frequency are shown in black. A subset of them that were also amplified on one hand, or lowly expressed and deleted on the other, are highlighted in bold.

To identify frequently over-expressed genes in the TCGA HNSCC patient cohort, we used the tumor (syn1571420) and normal (syn1571422) RNA-seq transcript profiles and after filtering non-detected transcripts calculated the average transcript levels for each gene. Genes with average tumor expression at least one standard deviation above the average normal transcript level (FDR < 0.05) were deemed *over-expressed*. Similarly, we identify frequently amplified genes in TCGA using tumor (syn1571428) and normal (syn1571430) genome-wide SNP6 array (CNV) profiles. Genes with average tumor CNV at least one standard deviation above the average normal were deemed *amplified*.

RNA interference druggable genome screen

High-throughput robotic siRNA-mediated gene knockdown on FHCRC-SCC-1 cells was performed at the UW Quellos Screening Core as previously described (8). Briefly, optimal transfection conditions were established by comparing cell viability after knockdown of KIF11 with Hs_KIF11_6 siRNA (QIAGEN Inc, Cat #SI02653693), which causes cell arrest in mitosis, to non-targeting control siRNA (NTC) and reagent-only mock controls. siRNA-mediated druggable genome profiling was performed using the MISSION® siRNA Human Druggable Genome library (Sigma, N = 6,659, Supp. Figure 2) and an additional panel of DNA damage and repair genes (N = 318). In total, 6,977 genes were screened (Supp. Table 7, Supp. Figure 3). Each gene was targeted by a pool of three siRNAs at a final concentration of 25nM in a one-gene-per-well, 384-well plate format with 0.1% Lipofectamine® RNAiMAX transfection reagent (Thermo Fisher Scientific). All siRNA-mediated gene knockdown tests were performed in triplicate with Hs_KIF11_6 and NTC siRNA as positive and negative controls, respectively. Cell viability was quantified 96 hours after gene knockdown with CellTiter-Glo® Luminescent Cell Viability Assay (Promega).

Raw luminescence signal was mock-normalized per plate to calculate the percent viabilities for each gene and a homoscedastic *t*-test *p*-value was computed. Replicate signals were summarized into a mean value, Z-transformed and used to rank genes. Genes that were both in the bottom 10% and had a *p*-value < 0.05 (FDR < 0.23) were deemed *strong hits*. Genes were also analyzed on a per plate basis. A robust Z-score (MAD=1.4826) was computed using the plate signal as reference (15). Genes with at least one replicate with a robust Z-score value < -2 and *p*-value < 0.05 were deemed *weak hits*. A total of 391 candidate target genes (CTGs) were prioritized. We performed functional annotation and pathway

enrichment analysis of the identified genes using the g:ProfileR package version 0.5.3 with FDR as the multiple testing correction method (16), which integrates data from the Gene Ontology (17), Corum (18), KEGG (19), and REACTOME (20) databases, among others.

RNA interference kinome screening of HNSCC cell lines

High-throughput RNA interference kinome screens were performed using MISSION® siRNA Human Gene Family Set Kinase Panel (713 kinases) on four HNSCC cell lines as previously described (8). Each gene was targeted by a pool of three siRNAs at a final concentration of 25nM in a one-gene-per-well, 384-well plate format with 0.1% Lipofectamine® RNAiMAX transfection reagent (Thermo Fisher Scientific). Gene knockdown tests were performed in triplicate with Hs_KIF11_6 and NTC siRNA as positive and negative controls. Cell viability was quantified 72 hours after gene knockdown with CellTiter-Glo® Luminescent Cell Viability Assay. Strong and weak hits were identified in the HNSCC cell lines as described above for the druggable genome screen. A total of 71, 71, 66, and 77 CTGs were prioritized respectively in UM-SCC-14A, PCI-15A, PCI-15B, and UM-SCC-14C cells (Supp. Table 4).

Predicted compounds for target prioritization

To further prioritize targets for intermediate scale validation, we used predictive modeling to associate CTGs without targeted compounds with alternative compounds. To extract drug response biomarkers, we trained a Gene-wise Prior Bayesian Group Factor Analysis model (21) with 200 hidden components, using copy number, expression, mutation, and drug response data for a total of 138 compounds and 16,291 genes from the Genomics of Drugs Sensitivity in Cancer data portal (22). The top-ranked (0.1%) predicted biomarkers were selected for each compound, intersected with the 391 CTGs and further filtered yielding a set of 49 targets (Supp. Table 5).

Confirmatory retesting of 178 prioritized gene candidates

siRNAs were independently designed and manufactured by GE Dharmacon (Lafayette, CO, Supp. Table 6). Each prioritized gene target was targeted with a pool of 4 siRNAs. Gene knockdown was manually performed in triplicate as described above in a one-gene-per-well, 96-well plate format and cell viability was quantified 72 hours after siRNA-mediated gene knockdown. A homoscedastic *t*-test *p*-value was computed for each gene against NTC siRNA. Targeted genes with less viability than NTC siRNA ($p < 0.05$) were deemed confirmed (Supp. Table 10).

Drug screens and confirmatory retesting

High-throughput robotic drug screening on FHCRC-SCC-1 and NOK cells was performed at the UW Quellos Screening Core. All drugs were tested in duplicate using a series of 10-point dilutions ranging from 0.5nM to 10μM in a 384-well plate format. Cell viability was quantified 72 hours after drug treatment using CellTiter-Glo® Luminescent Cell Viability Assay. Prioritized small molecules were manually retested in triplicate in a 96-well plate format following the same procedure. Raw luminescence signals were normalized to vehicle control for each drug and analyzed with XLfit (IDBS, Alameda, CA). A four-parameter dose

response curve was fit for each drug. The area under the curve (AUC), IC₅₀, EC₅₀, Y-minimum, Y-maximum, and R-squared were calculated for each compound using default parameters. Drug screening of FHCRC-SCC-7A and FHCRC-SCC-10A cells was performed using a drug library assembled by SEngine Precision Medicine which includes FDA approved targeted agents and chemotherapies as well as agents in clinical development for the majority of solid tumors (see Supp. Table 9). Cells were tested in 2D under optimal growth condition for HNSCC and data evaluated as described in Pauli et al (23).

Results

Patient background and primary tumor culture for ex-vivo functional genomic profiling

The overall strategy is shown in Figure 1A. To determine if comprehensive functional profiling could inform therapeutic strategies in a clinical setting, we chose an aggressive, treatment-resistant tumor as a case study. The patient presented with a previously untreated, HPV-negative, floor-of-mouth T2N2b squamous cell carcinoma. This patient received surgery followed by concurrent radiation and cisplatin treatment. The tumor recurred, however, and the patient died within a year of diagnosis.

A primary culture was established from a biopsy taken at the initial surgery and designated as “FHCRC-SCC-1”. Following a purification step, the culture was composed mainly of epithelial cells with no significant stromal cell contamination (Figure 1B). Upon intralingual injection into immunodeficient NOD SCID gamma (NSG) mice, these cells formed tumors with typical HNSCC pathological characteristics including abundant abnormal mitotic figures (Figure 1C). Immunostaining showed high levels of nuclear p53 and sequencing identified a *TP53* mutation P278S (Figure 1B–C). The original tumor was intrinsically cisplatin resistant and gain of function p53 mutations are associated with chemoresistance (24,25), so we examined the effect of cisplatin on p53 activity. Cisplatin treatment of FHCRC-SCC-1 cells did not increase the already high levels of p53 nor did it lead to induction of the p53 target gene p21, while it did so in p53 wild type HNSCC cells (Figure 1D).

Molecular profiling of FHCRC-SCC-1

We characterized the mutation spectra, copy number aberrations, and gene expression patterns of FHCRC-SCC-1 with whole exome sequencing (WES), comparative genome hybridization (CGH), and RNA-sequencing, respectively. The CIRCOS plot in Figure 1E serves as a bird’s eye view of the mutational landscape of this tumor with respect to these three parameters. In total, 581 somatic point mutations and 102 indels were identified in 568 genes (Supp. Table 1). Of these, 210 genes harbor somatic point mutations (n = 192) and/or indels (n = 21) that could potentially alter the gene function (Supp. Table 1). CGH analysis detected copy number changes, several of which are also commonly observed in HNSCC, such as amplification in 3p12.1 - p11.2, 11p13 - p12, 11q12.3 - q13.4, and 20q11.21 - q12, and deletion in 9p21.3 and 18q11.2 - q23. A total of 1,299 and 831 genes were located respectively in amplified and deleted regions (Supp. Table 2). Compared to the normalized gene expression levels in 44 HNSCC normal controls (see Materials and Methods), 773

genes in FHCRC-SCC-1 were highly expressed and 12,063 genes were lowly expressed, as identified by RNA-seq (Supp. Table 3).

Identification of potential cancer-associated genetic events in FHCRC-SCC-1

Multi-gene panel tests are increasingly used to identify aberrations in common cancer genes that may be actionable for precision oncology (i.e. Foundation One®, MSK-IMPACT™, and UW OncoPlex™). The outside ring of the CIRCOS plot in Figure 1E highlights 104 genomic alterations or expression changes from FHCRC-SCC-1 for genes tested by these panels. These include tumor suppressor genes (*TP53* and *CDKN2A/B*); DNA repair genes (*RAD21*, *51* and *54L*; *FANCD2* and *FANCF*); mitotic regulation/complex (*AURKA*, *AURKB*, *CHEK2*, and *DNAH5*); *FAT2*; and receptor tyrosine kinases and oncogenic drivers (*EPHA3*, *EIF3E* and *DDR2*), among others.

The innermost ring of the CIRCOS plot shows mutational frequencies from the TCGA HNSCC dataset for comparison (11). Among the altered genes in FHCRC-SCC-1 genome, eighteen were also altered at frequency of 3% or higher in HNSCC, providing preliminary evidence that these genes could be generally involved in HNSCC pathogenesis (Table 1). FHCRC-SCC-1 harbors a *TP53* mutation, consistent with the high prevalence of *TP53* mutation in HPV-negative HNSCC (4). Amplification of 11q13 in FHCRC-SCC-1 is also frequently observed in solid tumors including one quarter of HNSCC cases. Several genes in this amplicon, such as the *FGF3*, *FGF4*, *FGF19*, and *CCND1*, are associated with tumorigenesis in several cancers including HNSCC (26,27). Other alterations in FHCRC-SCC-1 that are frequently observed in HNSCC include deletion of the tumor suppressors *CDKN2A* and *CDKN2B*, amplification of oncogenes *PIK3CA* and *MYC*, and mutation in the receptor tyrosine kinase (RTK) ephrin receptor *EPHA3*. Another mutated RTK was *DDR2* encoding the discoidin domain receptor 2, which is involved in a wide range of carcinogenic processes such as epithelial-to-mesenchymal transition. Mutations in this gene were recently shown in advanced and recurrent HNSCC tumors and conferred sensitivity to the kinase inhibitor dasatinib (28,29). However, the *DDR2* mutation in FHCRC-SCC-1 was in the 3'UTR making it less likely to be functional.

The frequent alteration of these eighteen genes in HNSCC is suggestive of a functional role in FHCRC-SCC-1 pathogenesis, but beyond well-studied genes such as *TP53*, *CDKN2A* or *MYC*, this evidence is not definitive and insufficient to guide treatment. In summary, the genomic landscape of FHCRC-SCC-1 is typical for HPV-negative HNSCC in that it has a high mutation load, has substantial copy number variation, and carries aberrations in known cancer genes. This patient's genomic profile is also typical in that despite carrying many somatic mutations, these do not point to obvious therapeutic choices. Given deletion of *CDKN2A* and amplification of *CCND1*, one could hypothesize an overactive CDK4/6 pathway and sensitivity to CDK4 inhibitors such as palbociclib. Alternatively, mutations in FGF genes might confer sensitivity to FGF/FGFR/RTK inhibitors and mutations in other RTKs such as *EPHA3* and *DDR2* might predict sensitivity to dasatinib. In the absence of functional information, there is no straightforward way to prioritize these targets or potential therapeutic options.

Functional testing to prioritize driver events and identify new targets

We used two complementary approaches to identify novel targets and potentially effective therapeutic strategies: functional genomic profiling using genome-scale arrayed siRNA in a one gene per well approach, and drug profiling using an oncology focused drug library.

We performed siRNA functional profiling of FHCRC-SCC-1 cells using siRNAs targeting 6,977 genes, which includes a 6,659-gene druggable genome library and a 318-gene DNA damage and repair library (Supp. Figure 1–3). Compared to the reagent controls, the knockdown of 1,023 genes affected cell viability with 391 causing growth inhibition and 632 growth stimulation (Figure 2A; Supp. Table 4). Here, we focused only on genes whose knock-down caused growth inhibition, which we will refer to as “*candidate target genes*” (CTGs). Functional annotation and pathway enrichment analysis of the 391 CTGs revealed enrichment of genes involved in cell cycle regulation, DNA damage response, microtubule spindle complex, proteolysis, cell signaling, morphogenesis, immune system, and locomotion (Figure 2B).

We next sought to identify actionable targets among somatically mutated genes by testing whether siRNA-mediated knockdown impacted viability of FHCRC-SCC-1 derived cell cultures. Because functional testing and genomic characterization were both performed on the same patient derived cells, we could address functionality in the same cells in which these mutations arose. Of the 210 genes with potential function-altering mutations, 60 were included in the siRNA screen. siRNA-mediated knockdown of nine of these genes significantly reduced cell viability (Figure 2C, left panel), while siRNAs to the remaining 51 were neutral or even growth enhancing. Interestingly, four of the nine genes that were both mutated and CTGs, *TP53*, *DNAH5*, *EPHA3*, and *FAT2*, are also mutated in the TCGA HNSCC dataset at a frequency of 3% or higher (Table 1). siRNA to *EPHA2*, related to *EPHA3*, also reduced viability of FHCRC-SCC-1 cells and *EPHA2* is mutated in HNSCC at ~4% frequency and *FAT1*, related to *FAT2*, is mutated at ~23% frequency (4). This combination of mutational and functional evidence suggests that, in addition to *TP53*, mutations in *DNAH5*, *EPHA3/EPHA2*, and *FAT2/FAT1* could have a driver role in FHCRC-SCC-1 and possibly other head and neck cancers with these mutations.

Similarly, intersection of the genes included in the siRNA library with genes amplified or highly expressed in FHCRC-SCC-1 revealed that knockdown of only ~5.3% (20/379) of copy number amplified genes and ~7.6% (18/238) of highly expressed genes led to a reduction in viability (Figure 2C, middle and right panels). As in the case of mutated genes, the majority of amplified or highly expressed genes tested were phenotypically neutral in this assay.

In summary, only a small fraction of mutated, amplified, or highly expressed genes showed evidence of essentiality, suggesting most events are phenotypically neutral. In contrast, functional genomic testing with siRNA profiling identified novel vulnerabilities that would not be revealed by descriptive genotyping approaches.

Cancer-specificity of gene targets

From the set of 391 CTGs derived from our siRNA profiling, we prioritized 174 genes for further testing based on the following criteria: 1) the top 1% of profiled genes according to negative viability Z-score ($n = 69$); 2) genes with available or predicted (see Materials and Methods) small molecule inhibitors ($n=49$); 3) genes that harbor non-silent point mutations and that were also mutated or deleted at rate of 1% or higher in TCGA HNSCC cases ($n = 14$); 4) genes that are frequently over-expressed ($n = 7$) or amplified ($n = 53$) in HNSCC; 5) genes that were also identified as CTGs from siRNA kinome screens of HNSCC cell lines (described below) ($n = 19$); and 6) genes based on biological rationale ($n=14$) (Supp. Table 5). We retested these prioritized targets using an independent set of siRNAs with at least three of four siRNAs targeting a different region of the gene as compared to the siRNAs used for the primary screen. siRNA-mediated knockdown of 121 out of 174 of the retested genes (70%) significantly reduced viability of FHCRC-SCC-1 cells ($p<0.05$, Figure 3A), and the remaining 53 trended in the same direction indicating a high level of reproducibility of results from the primary screen. To determine if candidate targets showed preferential activity against tumor cells, the same set of 174 siRNAs was tested against two non-tumorigenic keratinocyte cell lines, NOK and HFK1 (6,7). siRNAs to 35 of the tested genes resulted in greater lethality in tumor cells compared to both normal cells and an additional 23 to one or the other normal cells ($p<0.05$, Figure 3A, Supp. Table 10). This set of “tumor-specific” targets included proteasome genes, G2/M regulators (*AURKA*, *CHEK1*, *WEE1*), mitotic spindle genes, *TP53*, the Notch ligand *JAG1*, the Wnt pathway gene *WISP2*, the antiapoptotic gene *MCL1* and others (Figure 3A–B). These confirmed tumor-specific gene targets were not necessarily the most lethal hits from the primary siRNA screen, demonstrating the value of using multiple parameters besides the top Z-score to prioritize targets. In summary, we tested 6,977 genes and identified 391 candidate target, from which we selected 174 genes for confirmation. Retesting confirmed 121 genes as potential targets against FHCRC-SCC-1 and 35 genes were identified as tumor-specific gene targets (Figure 3C). *TP53* was both mutated (P278S) and identified as a CTG in FHCRC-SCC-1 (Figure 3A), consistent with a gain of function and pro-survival activity for this mutation as previously shown in lung cancer (24).

Kinome siRNA profiling of *TP53* mutant HNSCC cell lines

To further prioritize and nominate highly druggable gene targets, we performed kinome-focused siRNA profiling on four *TP53*-mutant HNSCC cell lines (8). One pair of cells was derived from a primary tumor and a subsequent recurrence (UM-SCC-14A/C) and another pair from a primary tumor and metastatic lymph node (PCI-15A/B). Comparison of the top target kinases from the four cell lines revealed 19 kinases that were also essential for FHCRC-SCC-1, including *AURKA*, *WEE1*, *CHK1*, *EPHA3*, and *EPHA2* (Figure 4). The shared kinases were involved in the regulation of microtubule-based processes and cytoskeleton organization, mitotic centrosome separation, and G2/M transition of mitotic cell cycle, confirming that pathway dependencies noted in the patient tumor apply to other HNSCC cell lines. Two kinases implicated in response to radiation, *TLK2* and *CHK1* (30,31), were essential in FHCRC-SCC-1, the recurrent UM-SCC-14C and metastatic lymph node derived PCI-15B cells. Of the 19 shared candidate target kinases, 15 were re-tested, of which 9 successfully validated as targets in FHCRC-SCC-1 (Figure 3A).

Drug response profiling

To complement the siRNA profiling results and to enhance the potential for clinical translation, we performed drug profiling of FHCRC-SCC-1 cells using 332 small molecules, including FDA approved drugs, drugs in clinical development, and experimental agents targeting cancer pathways (Supp. Table 8). The majority of the drug library was assembled by The National Cancer Institute's Cancer Target Discovery and Development Network (CTD², manuscript in preparation). Based on potency, preferential toxicity to FHCRC-SCC-1 relative to NOK cells, and potential for clinical application, we prioritized 41 candidate tumor-specific drugs, including 25 chemotherapies and 15 targeted agents (Figure 5). Drugs that showed strong tumor specific activities included chemotherapies that target the mitotic spindle (paclitaxel, docetaxel, Ixabepilone, vincristine, vinblastine) and targeted agents that inhibit G2/M kinases *WEE1* and *CHK1* (AZD1775 and AZD7762). FHCRC-SCC-1 tumor cells were 15 times more sensitive to the *WEE1* inhibitor AZD1775 (IC₅₀ = 0.13 μ M vs. 1.9 μ M, Figure 5B) relative to NOK cells. Additional drugs that showed preferential activity toward FHCRC-SCC-1 included the proteasome inhibitor carfilzomib, the AKT inhibitor (–)-Deguelin, topoisomerase inhibitors etoposide and topotecan, the NF- κ B activation inhibitor IV, a JNK inhibitor IX, a PDE4 inhibitor ML-030, and a TRPC4/5 inhibitor ML204.

We also tested dasatinib, a pan kinase inhibitor that inhibits EPHA receptor kinase activity as *EPHA2* and *EPHA3* were both identified as candidate targets of FHCRC-SCC-1 with some tumor specificity (Figure 3A). FHCRC-SCC-1 tumor cells were 7 times more sensitive to dasatinib compared to NOK cells (IC₅₀ = 0.06 μ M vs. 0.44 μ M, Figure 5B).

Interestingly, despite amplification of *FGF3*, *FGF4*, *FGF19*, and *FGFR1*, FHCRC-SCC-1 cells were not responsive to FGFR1 inhibitors AZD4547, dovitinib, erdafitinib, lucitanib, PD173074, Ki8751, thalidomide, FGF RTK Inhibitor 341608 (CAS #192705-79-6), and the FGF/PDGF/VEGF RTK inhibitor 341610 (CAS #144335-37-5) (Supp. Table 8).

Similarly, despite FHCRC-SCC-1 harboring a *CDKN2A* deletion which includes p16INK4A, cells' response to *CDK4* inhibitor palbociclib, did not show preferential activity toward FHCRC-SCC-1 relative to NOK cells (Supp. Table 8). Additional *CDK4* inhibitors, such as abemaciclib and ribociclib did not significantly affect the viability of FHCRC-SCC-1 cells at any of the doses tested (up to 10 μ M) after 3 or 6 days of drug treatment. Consonant with the lack of effect of these targeted agents, siRNAs to either *FGFR1* or *CDK4* did not reduce viability of FHCRC-SCC-1 cells (Supp. Table 4). This lack of response to palbociclib is also observed in metastatic breast cancers harboring *CDKN2A* genetic alterations (32). The drug library contained five mTOR inhibitors, rapamycin, PI-103, PP121, GSK1059615, and BEZ235. FHCRC-SCC1 tumor cells were resistant to all these compounds relative to NOK cells despite increased *PIK3CA* copy number (Supp. Table 8).

In summary, a subset of chemotherapies and targeted agents that were active against FHCRC-SCC-1 cells impinge on the same cellular processes and genes that were identified from the siRNA functional profiling providing two independent lines of evidence that FHCRC-SCC-1 cells are vulnerable to disruption of G2/M regulation, the mitotic spindle, and proteasome function (Supp. Figure 4). In contrast, and in agreement with the siRNA

results, an additional set of targeted agents that were predicted to be active based on FHCRC-SCC-1 genomics did not show preferential activity.

High throughput drug profiling and genomic analysis of tumor cells derived from patients on a clinical trial

To determine if *ex-vivo* functional testing can identify patient-specific vulnerabilities in a clinically-relevant setting, we generated two additional primary HNSCC cell cultures, FHCRC-SCC-7A and FHCRC-SCC-10A, from patients enrolled in a phase I clinical trial. This trial tests the WEE1 inhibitor AZD1775 alone followed by a combination with cisplatin/docetaxel given to previously untreated advanced HNSCC patients (NCT02508246). During the four week trial period, tumor of the patient corresponding to cell culture FHCRC-SCC-7A had a partial response (Figure 6A, left panel). In contrast, tumor of the patient corresponding to cell culture FHCRC-SCC-10A progressed rapidly through treatment (Figure 6A, right panel). Cell cultures from these two patients were subjected to the SEngine Precision Medicine CLIA approved PARIS test, which determines sensitivity to 120 oncology focused drugs (23). In parallel, tumor biopsies corresponding to these primary cultures were interrogated with the CLIA approved UWOncoPlex test which provides next-generation sequencing data for 262-cancer relevant genes (tests.labmed.washington.edu/UW-OncoPlex). The overall drug sensitivities for these two patients are shown in Figure 6B and Supp. Table 9. Responses are compared to a database of additionally tested cells to evaluate relative sensitivities across patients. Cells FHCRC-SCC-7A derived from the patient with a positive clinical response, showed relative sensitivity to AZD1775 (normalized AUC=0.42, IC₅₀= 9.1×10^{-7} M, Z-score=-0.44), while cells FHCRC-SCC-10A, from the patient who did not respond to treatment, showed relative resistance (normalized AUC=0.51, IC₅₀= 1.2×10^{-6} M, Z-score=-0.07), (Figure 6C). We have found that the Z-score based on AUC is the most relevant metric to determine the relative sensitivity to a given drug (23). FHCRC-SCC-7A cells were also sensitive to the *PIK3CA* inhibitor apitolisib. Of note, this patient's tumor carried a mutation in *PIK3CA* (E545K) and a recent phase I study of apitolisib reported a partial response in two patients whose tumors carried the same *PIK3CA* mutation (33). Finally, as seen in our FHCRC-SCC1 primary culture, despite FHCRC-SCC-7A having a mutation in *CDKN2A* (R58X), this was not associated with sensitivity to any of the CDK2/4/6 inhibitors tested. Therefore, broadening these studies to additional cases will help define biomarkers of response to these targeted agents.

In summary, we show the feasibility of combining genomics with *ex-vivo* drug testing using cultures established from patients enrolled in a clinical trial. Depending on the size and quality of the biopsy, the establishment of primary cultures, drug screening, and analysis can occur in under four weeks, which is a similar time frame for genomic analysis. For the two patients tested, the *ex-vivo* responses to the trial drug AZD1775 were consistent with patient responses.

Discussion

Here we demonstrate the feasibility and potential for clinical translation of high-throughput functional profiling of patient-derived tumor cells using both siRNA and oncology focused drug libraries together with in depth genomic profiling. To illustrate this approach on the subtype of head and neck cancers with the greatest unmet need, we studied a patient with HPV-negative p53 mutant oral cavity HNSCC who died of disease in less than one year despite the most aggressive treatments with surgery and cisplatin chemoradiation.

Comprehensive genomic analysis of this patient's tumor revealed a heterogeneous mutational profile that is typical for HPV-negative HNSCC, with mutations or deletions in tumor suppressor genes such as *TP53* and *CDKN2A*, amplification of oncogenes such as *MYC* and *CCND1* and over 200 additional somatic variants and many copy number aberrations overall pointing to defects in cell cycle regulation, cell adhesion/polarity, and growth factor signaling (Supp. Figure 4). Despite this comprehensive genomic characterization, identifying a drug or drug target that might be effective for this patient remained elusive.

Functional analysis of mutated or amplified genes found in cancers has historically relied on the use of unrelated cell lines or animal models. Because we used the same tumor derived cells for both genomic and functional profiling, we were able to study the functional consequences of somatically altered genes in the appropriate genetic, epigenetic and cellular context. This comprehensive and patient-focused approach led to several general conclusions. First, there was a high rate of confirmation of the candidate targets identified from the druggable genome siRNA screen (>70%). Second, it enabled the nomination of several mutated driver genes, such as *EPHA3*, a highly druggable tyrosine kinase receptor for pre-clinical exploration. Third, the majority of genes that were mutated or amplified in this patient had minimal impact on viability when targeted by siRNA, suggesting most of these alterations are bystander events. Fourth, siRNA profiling that was unbiased, yet focused on the druggable genome, identified a much greater number of potential targets than did genomic profiling. Many of these novel targets lack clinically available inhibitors highlighting opportunities for drug development. Finally, drug profiling in combination with siRNA testing was useful to confirm gene targets and pathway dependencies and also to prioritize therapeutic strategies as described below.

Results from two orthogonal functional profiling schemes converged on the same cellular processes and gene targets as vulnerabilities for FHCRC-SCC-1 (Supp. Figure 4). Nearly a third (20/69) of the top 1% of genes, based on viability Z-score, were components of the ubiquitin proteasome system highlighting both the sensitivity of FHCRC-SCC-1 cells to disruption of protein recycling machinery (34) and the ability of our functional screening platform to identify not just isolated genes but entire pathways. Similarly, nearly one third (11/35) of essential tumor-specific genes were involved in G2/M regulation or mitotic spindle function including kinases and spindle components. siRNAs to *WEE1* and *CHK1* as well as specific inhibitors to WEE1 and CHK1 and five chemotherapies that disrupt the mitotic spindle also showed preferential sensitivity toward FHCRC-SCC-1 over non-tumorigenic keratinocytes. *In-vivo* tumor growth of FHCRC-SCC-1 showed frequent

abnormal mitotic figures (Figure 1C), including lagging chromosomes, tripolar mitotic figures and anaphase bridges consistent with aberrant regulation of mitosis.

TP53 mutations result in loss of the G1 checkpoint leading to an increased reliance on the G2 checkpoint to maintain genome stability. Thus, WEE1 and CHK1 inhibitors have been developed with the hopes that they would be selectively lethal against p53 mutant cancer cells, particularly in combination with genotoxic therapy (35–37). We and others have shown that inhibiting WEE1 in p53 mutant cells leads to mitotic cell death and treatment with AZD1775 either alone or in combination with cisplatin blocked growth of p53 mutant HNSCC xenografts in mice (8,37,38). Based on these results, we recently opened a phase I clinical trial with AZD1775 in combination with neoadjuvant chemotherapy in previously untreated, metastatic HNSCC (NCT02508246). The early results of this trial are encouraging and support further investigation of WEE1 as a target in the metastatic setting of HNSCC (39). The relative sensitivity to AZD1775 of tumor cells cultured from two of these patients was concordant with the patient response or non-response *in-vivo*. Both tumors had *TP53* mutations, while the non-responder had an *HRAS* mutation, potentially linked to the lack of response to targeted agents (40). Antitumor activity of AZD1775 as a single agent has been shown in a recent phase I trial with confirmed partial responses seen in two patients carrying *BRCA* mutations, one of which was a head and neck cancer patient (41). These findings emphasize the need of functional testing of individual tumor samples to define biomarkers of sensitivity.

Our functional analysis also implicates mutant p53 as a driver of this patient's aggressive tumor and non-responsiveness to cisplatin. Gain of function activity has been documented for mutations in amino acids 278, 280, and 282 (24,42,43), suggesting a gain of function hot spot at this DNA binding region of p53. Gain of function mutations in p53 can protect cells from deregulated *MYC* and chemotherapy induced apoptosis (44). FHCRC-SCC-1 cells had amplification of *MYC* and were very sensitive to depletion of the antiapoptotic gene *MCL1*, indicating these cells are primed to undergo apoptosis, which is blocked by MCL-1. This finding is consistent with the identification of MCL-1 as a MYC synthetic lethal target (45,46). In addition, gain of function mutations in p53 lead to abrogation of the G2/M checkpoint after DNA damage, centrosome amplification, and aberrant mitosis (44,47), which could explain the strong dependence of FHCRC-SCC-1 cells on disruption of spindle function and G2/M regulation. In summary, targeting both the G2/M vulnerability of p53 mutant cells, as well as inhibiting antiapoptotic proteins such as MCL-1, may provide a valuable drug combination approach for head and neck cancer with dual alterations of *MYC* and *TP53*.

Integrating genomic, siRNA, and drug profiling implicate EPH receptor signaling as another potentially targetable pathway. *EPHA3* was mutated in FHCRC-SCC-1 and both *EPHA2* and *EPHA3* are mutated in ~3% of HNSCC cases. Both *EPHA2* and *EPHA3* were identified as vulnerabilities by siRNA profiling in both FHCRC-SCC-1 and HNSCC cell lines. Dasatinib, which inhibits EPH receptor kinase activity (48), also showed preferential activity to FHCRC-SCC-1 consistent with reliance of FHCRC-SCC-1 cells on EPH kinase activity. *EPHA3* is overexpressed in a number of cancer types and both *EPHA2* and *EPHA3* are candidate anticancer targets (49–51). However, context may be important as *EPHA2* is

reported to have tumor suppressor activity in murine squamous cell carcinoma (52). Functional profiling also helped clarify that *DDR2* mutation was not likely a driver mutation as knockdown did not decrease viability.

In conclusion, we demonstrate the utility of genome-scale functional testing as an adjunct to genomic analysis in the execution of precision oncology for treatment-refractory HNSCC. Functional profiling prioritizes candidate actionable targets but also reveals novel targets that are not genomically altered. Functional profiling using high-throughput drug screening with a clinically relevant collection of drugs within the context of a clinical trial offers the opportunity to address mechanisms of resistance and biomarkers of response in real time. Finally, the dependency of p53 mutant HNSCC cells on *WEE1* and *CHK1* reinforces the therapeutic potential of targeting G2/M checkpoint regulators as a therapeutic option for these aggressive cancers.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Statement of translational relevance

For the majority of cancer patients, genomic analysis of their tumor is insufficient to point to obvious therapeutic options. To advance precision oncology there is a need to identify additional targetable vulnerabilities and effective therapies matched to patient-specific features. Here we show for the first time the feasibility of high-throughput functional testing with both siRNA and drug libraries on patient derived tumor cultures. Combining this large scale functional data with genomic analysis of the same tumor facilitates the nomination of driver from passenger genomic aberrations and identifies many candidate targets and potentially effective drugs that could not be predicted *a priori*. We also show the feasibility of this approach to inform an ongoing clinical trial. Although here we focused on aggressive treatment resistant head and neck squamous cell carcinoma (HNSCC), this functional precision oncology approach is applicable to other cancer types for which live biopsies can be obtained.

Figure 1. Integrative genomics for personalized medicine

A) Schematic overview of an integrative high-throughput strategy combining genomic profiling with genome-scale siRNA and drug profiling of a patient with advanced HNSCC; B) Enrichment of cytokeratin positive tumor cells (red) from vimentin positive stromal cells (green); C) Tumorigenicity of FHCRC-SCC-1: mouse xenograft confirms tumor morphology (left panel), prominent nuclear p53 staining (middle panel), and mitotic figures (right panels); D) Cisplatin treatment did not induce p21 in FHCRC-SCC-1 cells but did induce p21 in p53 wild-type UMSCC-74A cells; E) Integration of patient tumor genomics and TCGA HNSCC mutation profiles. *Track 1: Gene mutation frequency in TCGA HNSCC*

patient cohorts. *Track 2:* Selective mutations in FHCRC-SCC-1 as detected by WES, including nonsense and readthrough (red), missense (blue), splice site (green), flanking, UTR, RNA and intergenic (grey) mutations, in-frame (orange) and frame-shift (green) indels. *Track 3:* FHCRC-SCC-1 log-scale transcript level as detected by RNA-seq, with highly expressed genes highlighted with black border. *Track 4:* FHCRC-SCC-1 genome copy number aberrations as detected by CGH: log-ratio of tumor to normal CNV signal is shown, where green and red tracks indicate partial deletion and amplification regions, respectively, and the line in between showing no change. A total of 87 deleted (ratio $-1/2$) and 56 amplified (ratio $3/2$) regions are highlighted with black borders. *On the periphery:* highlighted genes of interest were filtered by potentially actionable gene lists aggregated from Foundation One[®], MSK-IMPACT[™], and UW OncoPlex[™]. Those include: highly expressed genes (red), highly expressed genes in amplified regions (bold red), lowly expressed genes in deleted regions (green), mutated genes that were also mutated in TCGA HNSCC (>1%) (black), and mutated genes that were also lowly expressed or in an amplified or deleted region (bold black).

Figure 2. Genome scale siRNA profiling to identify candidate target genes in FHCRC-SCC-1
A) 3D volcano plot of FHCRC-SCC-1 druggable genome siRNA-mediated genome knockdown screen. Red and green dots show respectively candidate target genes and suppressor genes; B) Top enriched pathway categories of candidate target genes; C) Top: Viability Z-score plots highlighting essential genes in red that were also mutated (left), amplified (center), or highly expressed (right) in FHCRC-SCC-1 cells. Bottom: Venn-diagrams summarizing the gene sets overlaps.

Figure 3. Retesting and tumor-specificity of prioritized targets

A) Bar-graph showing % viability of FHCRC-SCC-1 cells, normal oral keratinocytes (NOK) and human foreskin keratinocytes (HFK1) after siRNA targeting 174 candidate genes ordered by most essential to least in FHCRC-SCC-1 cells. Red bars indicate genes validated as tumor cell targets relative to NTC control (blue bars, $p < 0.05$) and in comparison with NOK and HFK1 (grey bars, $p < 0.05$). Kif11 is shown in green for reference; B) Functional categorization of the tumor-specific gene targets; C) Summary of target prioritization.

Figure 4. Private and shared vulnerabilities between FHCRC-SCC-1 and p53-mutated HNSCC cell lines

The UM-SCC-14A/C and PCI-15A/B HNSCC cell lines were screened using the MISSION[®] siRNA Human Gene Family Set Kinase Panel (713 kinases) and candidate target genes (CTGs) were identified using the same analytical protocol as in FHCRC-SCC-1 (see Materials and Methods). Edges link genes (circular nodes) to cell lines/primary culture (diamond and hexagon nodes), in which a given gene was identified as CTG, respectively. Genes are color-coded by degree of generalization, ranging from shared by 4 cell lines/primary culture to CTGs unique to each.

Figure 5. Drug profiling of FHCRC-SCC-1 and NOK cells

A) The heatmap shows clustering of the 320 drugs tested based on their AUC values for the two cell lines. The scatterplot shows a further comparison of drug response between FHCRC-SCC-1 (X-axis) and NOK cells (Y-axis). Drugs showing tumor-specific inhibitory effect, as defined by at least 2-fold AUC difference, are labeled and highlighted in blue. The size of the dot is proportional to the absolute differential of AUC values between the two cell cultures; B) Twenty selected tumor-specific agents were retested. Shown are dose response curves (compound concentration [log(M)] vs. percent viability) of FHCRC-SCC-1 cells (blue) and NOK cells (red).

Figure 6. High throughput *ex-vivo* drug profiling of patients enrolled in a clinical trial
A) Selected genomic features and images from two patients enrolled in clinical trial NCT02508246. Images were taken before and after neoadjuvant therapy (4 weeks apart) with AZD1775 alone on the first week, and in combination with cisplatin/docetaxel for 3 additional weeks. Left panel: PET scans of patient who corresponds to culture FHCRC-SCC-7A showed a RECIST response of -16% and histological response of -95%. Right panel: CT images of patient who corresponds to cultures FHCRC-SCC-10A showed a RECIST progression of 21% . Yellow arrows depict tumor in the lateral tongue for FHCRC-SCC-7A and buccal space and lymph node for FHCRC-SCC-10A. Compared to the partial

loss of PET signal in the former, there is significant progression of disease in the latter. B) Primary tumor cultures from these patients were subjected to SEngine PARIS drug test. Shown are normalized AUC and Z-score for 120 agents tested. C) Drug response curves to AZD1775 in FHCRC-SCC-7A and 10-A (red curves) compared to the average response of other tumor lines (black curves).

Table 1
Genes altered in FHCRC-SCC-1 that may be associated with tumor progression

Gene	FHCRC-SCC-1	MUT	CNV	TCGA
TSG	TP53	Mut	P278S	72%
	CDKN2A	Del	-5	32%
	CDKN2B	Del	-5	28%
	SMAD4	Del	-2	4%
ONC	CCND1	Amp	2	24%
	PIK3CA	Amp	3	21%
	MYC	Amp	1	12%
RTK	FGFR1	Amp	1	7%
	EPHA3	Mut	C106S	3%
GF	FGF3	Amp	2	25%
	FGF4	Amp	2	25%
	FGF19	Amp	2	24%
MISC	DNAH5	Mut	E550K	14%
	PAPPA2	Mut	Q546H	9%
	DCHS2	Mut	G2070A	7%
	FAT2	FS/Del	T1305ter	7%
	CNTNAP2	Mut	H565Y	6%
	BIRC6	Mut	S4523A	5%