Cancer Therapy: Preclinical
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Functional Kinomics Identifies Candidate Therapeutic Targets in Head and Neck Cancer

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Abstract

**Purpose:** To identify novel therapeutic drug targets for p53-mutant head and neck squamous cell carcinoma (HNSCC).

**Experimental Design:** RNAi kinome viability screens were performed on HNSCC cells, including autologous pairs from primary tumor and recurrent/metastatic lesions, and in parallel on murine squamous cell carcinoma (MSCC) cells derived from tumors of inbred mice bearing germline mutations in Trp53, and p53 regulatory genes: Atm, Prkdc, and p19Arf. Cross-species analysis of cell lines stratified by p53 mutational status and metastatic phenotype was used to select 38 kinase targets. Both primary and secondary RNAi validation assays were performed on additional HNSCC cell lines to credential these kinase targets using multiple phenotypic endpoints. Kinase targets were also examined via chemical inhibition using a panel of kinase inhibitors. A preclinical study was conducted on the WEE1 kinase inhibitor, MK-1775.

**Results:** Our functional kinomics approach identified novel survival kinases in HNSCC involved in G2–M cell-cycle checkpoint, SFK, PI3K, and FAK pathways. RNAi-mediated knockdown and chemical inhibition of the WEE1 kinase with a specific inhibitor, MK-1775, had a significant effect on both viability and apoptosis. Sensitivity to the MK-1775 kinase inhibitor is in part determined by p53 mutational status, and due to unscheduled mitotic entry. MK-1775 displays single-agent activity and potentiates the efficacy of cisplatin in a p53-mutant HNSCC xenograft model.

**Conclusions:** WEE1 kinase is a potential therapeutic drug target for HNSCC. This study supports the application of a functional kinomics strategy to identify novel therapeutic targets for cancer. Clin Cancer Res; 20(16); 4274–88. ©2014 AACR.

Introduction

Patients with head and neck squamous cell carcinoma (HNSCC) are treated aggressively with surgery followed by radiation, often together with cisplatin (1). Although these treatments increase loco-regional control, they are frequently disfiguring and induce high-grade toxicities limiting their effectiveness (2). Furthermore, resistance to cisplatin and radiation contributes to tumor recurrence, and options for those who do not respond are limited to palliative care. Targeted therapies for HNSCC are currently limited to experimental agents targeting the EGF receptor (3).

Mutations in the tumor-suppressor gene p53 are very common in HNSCC, with an estimated frequency of >50% (4, 5). Mutations in p53 have been associated with metastasis, resistance to radiation, and poor patient survival (6–8). Despite the strong implication of p53 in the biology and clinical outcome of HNSCC, there are no available therapies that specifically target p53-mutant cancer cells.

Here, we hypothesized that HNSCC cancer cells, in particular those with p53 mutations, are dependent on particular kinases for survival and that targeting these kinases...
In this study, we address the unmet need to find novel therapies for p53-mutant head and neck squamous cell carcinoma (HNSCC). We used a functional kinomics approach and human–murine interspecies comparison of high-throughput siRNA viability screens to identify conserved survival pathways in SCC. The rationale for targeting kinases in cancer is significant, and as such we focused on the kinome to identify druggable and clinically relevant survival kinases in HNSCC. Our findings reveal vulnerabilities of p53-mutant HNSCC cells to inhibition of G2–M, SFK, PI3K, and FAK pathways. For proof-of-concept and mechanism, we performed preclinical validation studies on one of our top kinase targets, WEE1. Our preclinical data demonstrate the vulnerability of p53-mutant HNSCC cells to deregulation of the G2–M transition, and support initiation of clinical trials with MK-1775 or other G2–M checkpoint inhibitors for HNSCC, particularly in combination with cisplatin.

Translational Relevance

In this study, we address the unmet need to find novel therapies for p53-mutant head and neck squamous cell carcinoma (HNSCC). We used a functional kinomics approach and human–murine interspecies comparison of high-throughput siRNA viability screens to identify conserved survival pathways in SCC. The rationale for targeting kinases in cancer is significant, and as such we focused on the kinome to identify druggable and clinically relevant survival kinases in HNSCC. Our findings reveal vulnerabilities of p53-mutant HNSCC cells to inhibition of G2–M, SFK, PI3K, and FAK pathways. For proof-of-concept and mechanism, we performed preclinical validation studies on one of our top kinase targets, WEE1. Our preclinical data demonstrate the vulnerability of p53-mutant HNSCC cells to deregulation of the G2–M transition, and support initiation of clinical trials with MK-1775 or other G2–M checkpoint inhibitors for HNSCC, particularly in combination with cisplatin. Could have therapeutic potential. To identify these cancer-specific survival kinases, we used an unbiased and genome scale high-throughput siRNA gene–silencing strategy. We surveyed the entire human kinome to identify those kinases that are required for survival of HNSCC cells stratified by p53 mutational status and metastatic propensity. We included pairs of HNSCC cells derived from primary tumors and either recurrent or metastatic lesions. The cell lines derived from the recurrent or metastatic tumors have been shown by us and others to have more aggressive features than their primary tumor autologous pairs, as measured by migration, avoidance of anoikis, and metastatic potential in mouse orthotopic xenografts (Materials and Methods for details; ref. 9). Recurrent metastatic tumors are generally resistant to standard-of-care therapies and as such are most in need of novel targeted therapies.

The rationale for targeting kinases in human cancer is significant. These enzymes regulate multiple cellular processes that contribute to tumor development and progression, and many human tumors display aberrant activation of kinases caused by genetic alterations. For tumors that are dependent on kinase activity for survival, targeted drugs could be effective.

Understanding that human cancer cell lines exhibit genetic and phenotypic heterogeneity, which can hamper the identification of robust drug targets, we performed a parallel siRNA kinome screen using a set of low passage murine squamous cell carcinoma (MSCC) cells. These cancer cells were derived from tumors of inbred mice bearing germline mutations in Tp53 and p53 regulatory genes Atm, Prkdc, and p19ARF10 (10–13). This set of p53 pathway-deficient cancer cells share the same culture history and genetic background and were derived from tumors sharing the same etiology. Comparative analysis of siRNA screen results between mouse and human cells identified kinases relevant to SCC survival. We reasoned that these evolutionarily conserved kinases might represent more robust therapeutic targets. Through an efficient in vitro and in vivo prioritization and validation scheme, we identified the G2–M cell-cycle regulatory kinase WEE1 as one of several clinically promising targets, and show that inhibition of WEE1 with a highly specific small-molecule inhibitor impaired growth of p53-mutant HNSCC tumors in vivo.

Materials and Methods

Cell lines

The following human HNSCC cell lines were used: UM-SCC14A, UM-SCC14C, PCI-15A, PCI-15B, JHU-019, UM-SCC22A, UM-SCC22B, UM-SCC38, UM-SCC17A, SCC-61, and three HPV(+) cell lines: UM-SCC47, UPCI:SCC090, and UM-SCC104 (Supplementary Table S1). Three cell line pairs were derived from primary tumors and subsequent recurrences or metastatic cervical lymph nodes from the same patients: UM-SCC14A and UM-SCC14C; PCI-15A and PCI-15B; and UM-SCC22A and UM-SCC22B. The cell line JHU-019 was derived from a late-stage patient with oral SCC (Supplementary Table S1). For the paired lines, wound-healing assays revealed that the migration rate of cell lines derived from metastatic HNSCC (i.e., UM-SCC-14A and PCI-15B) was higher than those derived from the primary tumor (i.e., UM-SCC-14A and PCI-15A) and that JHU-019 had the fastest migration rate (9). In addition, JHU-019 and PCI-15B cell lines tested in mouse xenografts by orthotopic injection into the tongue produced squamous carcinoma at the sites of injection and cervical lymph node metastasis (9). Given a previous report raising concerns of JHU-019 contamination with prostate adenocarcinoma cells (14), we performed immunohistochemistry staining of 4 paraffin-embedded blocks from JHU-019 tumors orthotopically-injected in the tongue of NOD/SCID IL2 gamma null mice (NSC) with antibodies against 3 markers used clinically to identify both squamous cell and prostate carcinoma (EP1601Y for Cytokeratin 5 (CK5); BC4A4 for p63; and PSA for Prostate Specific Antigen). Staining and evaluation of the immunohistochemical stains cited above were determined by the CLIA-certified UW Medicine Pathology Laboratories. There was uniformly positive staining for p63 in all blocks, uniformly positive staining for CK5 in two blocks and variably positive staining in the other two blocks. There was no staining for PSA in any of the blocks (data not shown). To determine p53 mutational status, we designed primers to amplify exons 2–11 of the blocks (data not shown). To determine p53 mutational status or metastatic cervical lymph nodes from the same patients: UM-SCC14A and UM-SCC14C; PCI-15A and PCI-15B; and UM-SCC22A and UM-SCC22B. The cell line JHU-019 was derived from a late-stage patient with oral SCC (Supplementary Table S1). For the paired lines, wound-healing assays revealed that the migration rate of cell lines derived from metastatic HNSCC (i.e., UM-SCC-14A and PCI-15B) was higher than those derived from the primary tumor (i.e., UM-SCC-14A and PCI-15A) and that JHU-019 had the fastest migration rate (9). In addition, JHU-019 and PCI-15B cell lines tested in mouse xenografts by orthotopic injection into the tongue produced squamous carcinoma at the sites of injection and cervical lymph node metastasis (9). Given a previous report raising concerns of JHU-019 contamination with prostate adenocarcinoma cells (14), we performed immunohistochemistry staining of 4 paraffin-embedded blocks from JHU-019 tumors orthotopically-injected in the tongue of NOD/SCID IL2 gamma null mice (NSC) with antibodies against 3 markers used clinically to identify both squamous cell and prostate carcinoma (EP1601Y for Cytokeratin 5 (CK5); BC4A4 for p63; and PSA for Prostate Specific Antigen). Staining and evaluation of the immunohistochemical stains cited above were determined by the CLIA-certified UW Medicine Pathology Laboratories. There was uniformly positive staining for p63 in all blocks, uniformly positive staining for CK5 in two blocks and variably positive staining in the other two blocks. There was no staining for PSA in any of the blocks (data not shown). To determine p53 mutational status, we designed primers to amplify exons 2–11 using Primer3 software (Whitehead Institute, Cambridge, MA). Primer specificity was confirmed by gel electrophoresis. PCR-amplified fragments were purified and sequenced using an ABI 3730xl DNA Analyzer with ABI’s BigDye Terminator Cycle Sequencing method. Sequencing results are aligned to GenBank TP53 sequence NG_017013.1 using Sequencher 4.10.1 (Gene Codes, Ann Arbor, MI). Cell lines were characterized for metastatic potential as described (9).

Materials and Methods

Cell lines

The following human HNSCC cell lines were used: UM-SCC14A, UM-SCC14C, PCI-15A, PCI-15B, JHU-019, UM-SCC22A, UM-SCC22B, UM-SCC38, UM-SCC17A, SCC-61, and three HPV(+) cell lines: UM-SCC47, UPCI:SCC090, and UM-SCC104 (Supplementary Table S1). Three cell line pairs were derived from primary tumors and subsequent recurrences or metastatic cervical lymph nodes from the same patients: UM-SCC14A and UM-SCC14C; PCI-15A and PCI-15B; and UM-SCC22A and UM-SCC22B. The cell line JHU-019 was derived from a late-stage patient with oral SCC (Supplementary Table S1). For the paired lines, wound-healing assays revealed that the migration rate of cell lines derived from metastatic HNSCC (i.e., UM-SCC-14A and PCI-15B) was higher than those derived from the primary tumor (i.e., UM-SCC-14A and PCI-15A) and that JHU-019 had the fastest migration rate (9). In addition, JHU-019 and PCI-15B cell lines tested in mouse xenografts by orthotopic injection into the tongue produced squamous carcinoma at the sites of injection and cervical lymph node metastasis (9). Given a previous report raising concerns of JHU-019 contamination with prostate adenocarcinoma cells (14), we performed immunohistochemistry staining of 4 paraffin-embedded blocks from JHU-019 tumors orthotopically-injected in the tongue of NOD/SCID IL2 gamma null mice (NSC) with antibodies against 3 markers used clinically to identify both squamous cell and prostate carcinoma (EP1601Y for Cytokeratin 5 (CK5); BC4A4 for p63; and PSA for Prostate Specific Antigen). Staining and evaluation of the immunohistochemical stains cited above were determined by the CLIA-certified UW Medicine Pathology Laboratories. There was uniformly positive staining for p63 in all blocks, uniformly positive staining for CK5 in two blocks and variably positive staining in the other two blocks. There was no staining for PSA in any of the blocks (data not shown). To determine p53 mutational status, we designed primers to amplify exons 2–11 using Primer3 software (Whitehead Institute, Cambridge, MA). Primer specificity was confirmed by gel electrophoresis. PCR-amplified fragments were purified and sequenced using an ABI 3730xl DNA Analyzer with ABI’s BigDye Terminator Cycle Sequencing method. Sequencing results are aligned to GenBank TP53 sequence NG_017013.1 using Sequencher 4.10.1 (Gene Codes, Ann Arbor, MI). Cell lines were characterized for metastatic potential as described (9). To determine if a p53 mutation is disruptive, we used criteria established by Poeta et al. (7).

MSCC cells were derived from NIH/Ola strain mice with germline mutations in p53 pathway genes and...
included: MSCC-CK101 (HrasQ61L, Trp53+/+), MSCC-CK102 (HrasQ61L, Trp53/−), MSCC-CK103 (HrasQ61L, p19Arf/−), MSCC-CK104 (KrasQ138, Atm+/−), MSCC-CK1 (HrasQ61L, p53+/+), and MSCC-CK4 (HrasQ61L, p53+/−, C0+/+, p53+/−, C0/C0). All mice were subjected to the identical DMBA/TPA (7,12-dimethylbenz(a)anthracene/(Supplementary Table S2) (13). All mice were subjected to gene, but mutations in refs. 10–12]. MSCC-CK105 (HrasQ61L, Prkdcsmu/mu) cells were from SCID-mutant mice of a mixed C3H/Balb/c background (Supplementary Table S2) (13). All mice were subjected to the identical DMBA/TPA (7,12-dimethylbenz(a)anthracene/12-O-tetradecanoylphorbol-13-acetate) two-stage carcinogen protocol to induce SCC. Tumors induced by this protocol principally harbor an activating mutation in the Hras oncogene, but mutations in Kras have also been noted (15, 16). Carcinomas arising from both p19Arf, and p53-deficient mice are highly aggressive and metastatic (11, 12, 17, 18). Mouse SCC lines were derived using a standard outgrowth explant method. Briefly, carcinoma tissue was washed in sterile PBS, sliced into 2-mm pieces using a sterile razor blade, and placed into a 60-mm tissue culture plate with Dulbecco’s Modified Eagle Medium, 10% fetal calf serum and penicillin/streptomycin. Media were replaced every 72 hours until cell outgrowths reached 70% to 90% confluence, and were subsequently passaged and/or frozen at low passage number. Total RNA was isolated from the MSCC-CK1 line with TRIzol and cDNA generated using Superscript 3 reverse transcripase (Life Technologies). The p53 cDNA transcript spanning exons 2 to 11 was PCR amplified as previously described (19), and cloned into a TOPO TA vector (Life Technologies), competent cells transformed, and several colonies sequenced using an ABI 3730xl DNA Analyzer with the ABI’s BigDye competent cells transformed, and several colonies sequenced using an ABI 3730xl DNA Analyzer with the ABI’s BigDye terminator Cycle Sequencing method for mutations in the p53 gene (Supplementary Table S2).

High-throughput RNA interference kinome screens

Kinome-wide siRNA screens were performed with viability as the phenotypic endpoint on five HNSCC lines: JHU-019; PCI115A and 15B; UM-SCC14A and 14C; and five MSCC lines: MSCC-CK101, MSCC-CK102, MSCC-CK103, MSCC-CK104, and MSCC-CK105. Normal human foreskin fibroblasts (HFF) were screened to control for nonspecific cell toxicity (UW-Quellos facility proprietary data). Briefly, culturing of normal HFFs was performed as previously described (20). Kinome-wide RNA interference screens were performed on two cultures of HFFs (HFF1, HFF3) using the Ambion kinome library (Ambion-Life Technologies). An HFF exclusion plot was generated using this kinome screen information to determine whether RNAi-mediated knockdown of kinase targets compromised cell viability in both HFF cultures, with <70% viability (>30% cell death) as a threshold (Supplementary Fig. S2; partial data shown). siRNA libraries targeting 713 human (MISSION siRNA Human Gene Family Set; Sigma) and 572 murine kinases (Ambion) were constructed and used in pools of three independent siRNAs targeting each gene, in one gene per well approach. RNAi screens were performed in 384-well format using robotics instrumentation (21). Transfection feasibility of each cell line was established using a factorial optimization. Mock condition and a nontargeting universal siRNA control were used as negative controls, whereas an siRNA directed at KIF11 (kinesin-like protein), which arrests cells in mitosis, was used as a positive control. All reagent conditions were statistically evaluated using a simple Z-factor score to evaluate differentials and variability of replicates (i.e., potent cell killing with KIF11 at the lowest toxicity possible in the mock universal controls) to select an optimized transfection condition for each cell line (22). All kinases were tested in triplicate to establish experimental variability and statistical validity. Scrambled siRNA-negative controls were used to monitor dynamic range and off-target effects and the results were standardized to mock-transfected cells. Viability and apoptosis were quantified using an Envision Multilabel detector/plate reader (PerkinElmer) with either a CellTiter-Glo assay (Promega), or Apotox assay (Promega), the former measures metabolic ATP via a standard curve to mock/universal siRNA at all conditions. Raw luminescence values were mock normalized per plate and then Z-transformed per cell line and plotted for distribution and data mining (Miner 3D software; version 7.3). All high-throughput kinome screens and subsequent validation screens on MSCC and HNSCC cells were statistically evaluated using published methods (23).

Comparison of human and mouse kinome screens

The 713 human (Supplementary Table S3) and 572 murine kinase (Supplementary Table S4) sets were cross-referenced using mouse genome informatics (http://www.informatics.jax.org/) and National Center for Biotechnology Information (http://www.ncbi.nlm.nih.gov/) database nomenclature to generate a common list of 508 kinases referred to as the interspecies kinase (Supplementary Table S5). Using this common list, prioritization of screen results then followed on the basis of the viability scores from the five HNSCC cells and five MSCC cell lines. Mean viabilities (μi2) from five HNSCC cell lines [μi2 all (human) = μi (O19) + μi (14A) + μi (114C) + μi (15A) + μi (15B)] and from five MSCC cell lines were calculated [μi2 all (murine) = μi (wild-type) + μi (Trp53+/−) + μi (p19Arf−/−) + μi (Atm−/−) + μi (Prkdc mu/mu)] for each of 508 kinases (i = 1, 2, 3, 4, . . . , 508), where μi is the triplicate of pooled siRNAs (three distinct siRNAs) average normalized viability for each individual gene per cell line. Mean viabilities (μi2) for each gene were then Z-transformed using the equation, Z = μi2 − μ/σ, where μ is the mean viability and σ the SD for all siRNAs per well for all five HNSCC cell lines and five MSCC cell lines, respectively (Supplementary Table S8). Mean viabilities (μi2 all; μi3 p53 murine; μi4 metastatic) and Z-score transformations were calculated for both human and murine lines per genotype and phenotype: (i) all human and murine cell lines; (ii) p53-mutant/deficient human and murine cell lines; and (iii) metastatic human and murine cell lines (Supplementary Table S8). Cartesian plots (Z-scorehuman, Z-scorermurine) of all 508 kinases in common with murine and human kinomes were then generated for each of the three comparisons. Population mean viabilities (Meanμi, Meanμi3, Meanμi4 all) were calculated for each pairwise comparison using a one-sided Student’s t-test.

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Functional Kinomics Identifies Therapeutic Targets in HNSCC

Mean Met) and Z-score transformations (Z-scoreMean Met; Z-score{p53 mutant}All; Z-score{p53 mutant}All; Z-scoreMean Met) were calculated for specific genotypic and phenotypic comparisons and used in the color-coded overlay on the cartesian plots for selection per comparison (Fig. 1B; Supplementary Table S8). Kinase targets were selected on the basis of the Z-score threshold from each of the cartesian plots: Z-scoreMean All < -1.0; Z-score{p53 mutant}All < -2.0; Z-scoreMean Met < -1.5 (Supplementary Table S8), in which kinases were data mined from more than one comparison and duplicates were removed for a final selection of 38 kinase targets from each of the cartesian plots: kinase targets (38 kinases) removed for a final selection of 38 kinase targets from all from more than one comparison and duplicates were removed for a final selection of 38 kinase targets from all comparisons, kinase targets (38 kinases) = kinase targetsAll + kinase targets{p53 mutant}All + kinase targetsMean Met (Fig. 1B and C; Supplementary Table S8). Kinases were further prioritized on the basis of those whose expression or activity was increased in SCC and that play known functions in SCC pathogenesis.

Primary validation of kinase targets with siRNA

Twenty-eight kinases were selected for follow-up and validation based on the interspecies kinome comparison and HFF kinase exclusion. Two small-scale time-course primary validation RNAi screens were performed in parallel on four HNSCC cell lines (UM-SCC14A, UM-SCC14C, PCI-15A, and PCI-15B) in 384-well formats with an independent set of siRNAs (Qiagen; Supplementary Table S6), with three separate siRNAs plus pooled siRNAs per gene target in triplicate for an N = 12 for each gene target. All phenotypic endpoints of cell viability and caspase-3/7–dependent apoptosis were measured in parallel screens in a time-course format at 1.5, 3, and 4.5 days posttransfection using the CellTiter-Glo assay (Promega) and ApoptoGlo (CaspaseGlo-3/7 reagent) assay (Promega) per the manufacturer’s specifications and an Envision multilabel plate reader (PerkinElmer). Primary screen ‘hits’ were assessed using a single endpoint for cell viability at 4.5 days posttransfection termed, absolute viability, by both a negative control–independent analysis: triplicate siRNAs versus population mean of the screen, Z-score threshold and the unpaired t test, Z-score < -1.0, P < 0.1 scored as hit (Supplementary Table S13; Column U), as well as a negative control–dependent analysis: triplicate siRNAs versus universal negative control siRNAs, ANOVA with Dunnett posttest, mean difference >0 and P < 0.05 scored as hit (Column AA; Summary of “hits”; Supplementary Tables S13 and S14). Comparing posttransfection effects on absolute viability (day 4.5) for each kinase target with either the universal negative control siRNA or the population mean yielded similar results (Supplementary Tables S13 and S14). Differential viabilities (days 4.5–1.5) based on the mean of both the N = 12 data (i.e., all three distinct siRNAs per target plus pooled siRNAs in triplicate) and N = 3 data (i.e., pooled siRNAs alone) were calculated for all 28 kinase targets (Supplementary Tables S10–S12). AUC (area under the curve) estimates of caspase-dependent apoptosis (AUC{caspase}) using all three data points with both the N = 12 data and N = 3 pooled siRNAs were calculated for all kinase targets (Supplementary Tables S15–S17). Statistical significance of RNA interference–mediated knockdown of the 28 kinases was assessed via ANOVA with Dunnett posttest for multiple comparisons (P < 0.05 as significant) on differential viability (days 4.5–1.5), and AUC analysis of caspase-3/7–dependent apoptosis versus universal negative siRNA control (Supplementary Tables S10–S12 and S15–S17).

Secondary validation of kinase targets with siRNA

Ten kinase targets were further validated in a 96-well format in five additional HNSCC cell lines (UM-SCC22A, UM-SCC22B, UM-SCC38, UM-SCC47A, and JHU-019). In addition, the 10 HFF exclusion kinase targets were included in the low-throughput assay (i.e., 20 kinase targets). This assay consisted of three independent siRNAs per well (pooled siRNAs) assayed in triplicate (Qiagen; Supplementary Table S7) for cell viability and apoptosis measured at 1.5, 3, and 4.5 days posttransfection using the ApoptoGlo assay (Promega) as per the manufacturer’s specifications using a Synergy H4 Hybrid Multi-Mode microplate reader (BioTek). Statistical significance of RNA interference–mediated knockdown of the 20 kinases was assessed via ANOVA with Dunnett posttest for multiple comparisons on absolute viability (day 4.5), differential viability (days 4.5–1.5), and AUC analysis of caspase-dependent apoptosis versus the negative siRNA control (Supplementary Tables S18–S20).

Dose–response curves with kinase inhibitors

Kinase inhibition dose–response curves were performed with six kinase inhibitors [MK-1775 (a.k.a., AZD-1775), TAE684, Pl828, PIK93, PP2, and PF-562271] against kinase targets [WEE1, ALK, PI3K, PIK4CB, FYN, and FAK (ILK surrogate)], respectively. Kinase inhibitors: MK-1775 (S1525), PIK93 (S1489), and TAE684 (S1108) were obtained from Selleck Chemicals; Pl828 (2814), PP2 (1407) from Tocris Bioscience, and PF-562271 from SYNkinase. All HNSCC (UM-SCC17A, UM-SCC47A, PCI-15A, PCI-15B, UM-SCC14A, and UM-SCC14C) and MESC (CK1, p53+/− and CK4, p53−/−) cells were plated at approximately 5 to 10 × 10^5 cells per 100 μL per well, and incubated at 37°C for approximately 24 hours on 96-well assay plates (Corning Inc.). Serial dilutions of the kinase inhibitors and vehicle control (dimethyl sulfoxide, DMSO) were prepared in 1 mL assay blocks at 3× working concentration to generate dose–response curves ranging from 100 to 0.03 μmol/L. All serial dilutions were prepared using cell culture media. Approximately 72 hours after treatment, cells were assessed for metabolic activity via ATP using CellTiter-Glo (Promega), following the protocol outlined by the manufacturer using an FLx800, and/or a Synergy H4 Hybrid multimode reader (BioTek). All assays were performed in triplicate and normalized to wells with no treatment. Dose–response curves and IC50 values were generated using GraphPad Prism Version 5 [parameters, nonlinear regression fit; equation = log (inhibitor) vs. response − variable slope (four parameters); single constraint).
COSMIC public database of drug sensitivity data

Using the Catalogue of Somatic Mutations in Cancer (COSMIC) website, TP53 gene mutational status was extracted from the Sanger Cancer Cell Line Project, which contains information on 820 cancer cell lines. In addition, the genomics of the Drug Sensitivity Project (released July 2, 2012) contains 541 cancer cell lines that were treated with a WEE1/CHK1 inhibitor, 681640 (EMD Millpore), a pyrrrolcarbazole compound that acts as a potent, ATP-binding site inhibitor of WEE1 (IC50 = 11 nmol/L). Drug sensitivity was measured with nine different concentrations of 681640 and IC50 values presented as natural log (µmol/L; Supplementary Table S21). The sign test was applied to test the median difference in sensitivity by TP53 status. We performed a similar analysis on only the SCC cell lines. Forty-two squamous cell lines were identified from COSMIC annotation, eight were p53 wild-type and the remaining 34 had a p53 mutation.

Mitotic entry, cell-cycle analysis, and apoptotic assays

Mitotic entry was assessed as previously described (24). Briefly, HNSCC cells (PCI-15B, UMSCC-17A) were treated with 1 µmol/L MK-1775 for 8 and 24 hours, and all cells were harvested, washed, and incubated with rabbit monoclonal antibody to phospho-histone H3 (Serine10; Cell Signaling Technology; Cat. no. 3465) for 2 hours at room temperature, washed, and DNA stained with 20 µg/mL propidium iodide; RNaseA in PBS (Sigma-Aldrich; Cat. nos. P4170, R6513). Flow cytometric analysis was performed using a BD FACSCanto II, and profiles analyzed with BD Cell Quest software (Becton Dickinson). AUCs were calculated for all treatments and vehicle (DMSO) for all cell lines using two measurements over a 48-hour period with three concentrations (30 nmol/L, 100 nmol/L, and 1 µmol/L) of MK-1775.

NSG xenograft tumor model

PCI-15B cells were inoculated subcutaneously into the right flanks of 8–10-week-old NOD/SCID interleukin-2 gamma (NSG) null mice provided by the Olson laboratory at the Fred Hutchinson Cancer Research Center Laboratory Animal Care and Use Committee.

Immunoblotting

Tumor tissues were minced and homogenized on ice in M-PER Mammalian Protein Extraction Reagent supplemented with Halt Protease and Phosphatase Inhibitor Cocktail (Thermo Fisher Scientific). Extracted proteins were quantified by a bicinechonic acid protein assay (Thermo Fisher Scientific). Fifty micrograms of each protein specimen was revealed on a NuPAGE 4% to 12% Bis-Tris mini gel (Life Technologies) and transferred onto an Immobilon-P polyvinylidene difluoride membrane (Millipore). Anti-WEE1 (Cat. no. 4936), anti–phospho-WEE1 (Ser642; Cat. no. 4910), anti-CDC2 (Cat. no. 9112), and anti–phospho-CDC2 (Tyr15; Cat. no. 4539) antibodies were purchased from Cell Signaling Technology. The secondary antibodies used were ZyMax horseradish peroxidase (HRP)–conjugated goat-anti-rabbit immunoglobulin G (Life Technologies). HRP was detected with the SuperSignal West Pico Chemiluminescent Substrate Kit (Thermo Fisher Scientific). Densitometry on immunoblot analysis was performed with ImageJ software (Wayne Rasband, NIH, Bethesda, MD) and raw data normalized to β-actin loading control per lane.

Statistical analysis

All column and curve data points presented as mean ± SEM, unless otherwise noted. All statistical analyses were performed using unpaired two-tailed t tests unless otherwise indicated. All statistical analysis of RNAi interference primary and secondary screening data is described above and all calculations used for significance testing are presented in Supplementary Tables. Statistical tests were all performed using GraphPad Prism versions 5 and 6 (GraphPad Software Inc.) (Supplementary Tables S9–S20).

Results

RNA interference kinome screens of SCC

We performed kinome-wide siRNA viability screens on a set of five HNSCC cell lines (UM-SCC14A; UM-SCC14C; PCI-15A; PCI-15B; and JHU-019). Two pairs of these cells (UM-SCC14A, UM-SCC14C and PCI-15A, PCI-15B) were derived from primary and subsequent posttreatment recurrences or metastatic cervical lymph nodes from the same patients and all carried mutations in p53 (Supplementary Table S1). A total of 713 kinases were interrogated using an arrayed siRNA platform that quantified cell viability following knockdown with a pool of three siRNAs per gene per well. All assays were performed in triplicate. Cell viability was monitored 4 days after siRNA transfection using the ATP-based, CellTiter-Glo assay (see Materials and Methods for details). In parallel, we performed kinome-focused screens on a set of five low passage cancer cells derived from SCC (Supplementary Table S2). These cells were isolated from carcinoma-bearing inbred mice harboring germline...
mutations in the p53 pathway genes Atm, Prkdc, p19Arf, and Trp53 (10–13).

Next, we derived an interspecies kinase (508 kinases shared between both species) to identify kinase targets that had the highest shared impact on cell viability. Human and mouse cell lines were sorted into each of three comparisons: All cells (left), p53-mutant cells (middle), and metastatic cells (right). Candidate kinase targets with Z-scores greater than one SD from the mean cell viability per comparison are shown in red; Supplementary Table S8 for details. C, the Venn diagram of selection of 38 kinase targets from interspecies comparison; inclusion in diagram represents kinase targets that met a certain threshold in each comparison.
cells with mutant p53 and metastatic phenotype (Fig. 1C; Supplementary Table S8). Many of these putative HNSCC survival kinases are implicated in signaling pathways such as focal adhesion and integrin signaling (CAMK2B, FYN, ILK, EPHA3, EIF2AK4, and TRIB2), PI3K (phosphoinositide 3-kinase) signaling (PIK4CB, PIK3CB, PIP5K1B, TRIB2, FGFR3, and ALK), SRC signaling (FYN, TXK, and CAM2KB), and G2–M cell-cycle regulation (WEE1, NEK4, TTK, AURKA, and CHK1).

To prioritize targets for preclinical validation, we used primary cultures of HFFs to assess whether inhibition of these kinases caused toxicity to normal cells. Ten kinases caused >30% loss in cell viability in both HFF cultures (Supplementary Fig. S2) and were not included in the primary validation screen. The remaining 28 kinase targets were retested with independent siRNAs on the same two pairs of autologous HNSCC cell lines (UM-SCC14A and 14C; PCI-15A and 15B) using a format of three separate siRNAs per gene plus a pool of all three siRNAs, each in triplicate (i.e., N = 12/gene; Supplementary Fig. S1). Both cell viability and caspase-3/7–dependent apoptosis were measured in parallel at 1.5, 3, and 4.5 days posttransfection. Differential viability (days 4.5–1.5), absolute viability (day 4.5), and apoptosis was calculated for each kinase (Supplementary Tables S9–S17; Material and Methods for details). Differential viabilities were calculated to measure posttransfection effects over time (days 4.5–1.5) and statistically evaluated versus the universal negative control (Supplementary Fig. S2 and Supplementary Fig. S3). For example, RNAi-mediated knockdown of TK2 and TRIB2 was more effective in cells isolated from the primary versus the recurrent/metastatic lesions (Fig. 2A and Supplementary Fig. S3). Several kinases, such as WEE1 and NEK4, remained equally effective in both primary and recurrent/metastatic tumors.

**Small-molecule inhibition of kinases confirms role in HNSCC cell survival**

We further prioritized kinase targets using commercially available small-molecule inhibitors. This step not only provides independent chemical confirmation of siRNA results but also provides lead compounds to test in *in vivo* models. Dose–response curves using kinase inhibitors were performed for WEE1 (MK-1775), ALK (TAE684), PIK4CB (PIK93), FAK (PF-562271), PIK3CB (PIB28), and FYN (PP2; Fig. 3). In agreement with our siRNA knockdown experiments, p53-mutant HNSCC cell lines were sensitive to small-molecule inhibitors targeting WEE1, ALK, PIK4CB, and FAK. The WEE1 kinase inhibitor, MK-1775 had the broadest and most significant effect on cell survival in both primary and recurrent/metastasis-derived HNSCC cells, with an IC50 ranging from 220 nmol/L to 3.1 μmol/L (Fig. 3).

**Preclinical validation of WEE1 as a drug target for HNSCC in *vitro* and in *vivo***

Results from our cross-species comparative analysis of kinome screens, validation assays, and small-molecule inhibitor studies nominated WEE1, a G2–M regulator, as a promising target against p53-mutant HNSCC. To further explore the sensitivity of p53-deficient cells to WEE1 inhibition, we performed dose–response curves with MK-1775 in pairs of p53 wild-type and p53-mutant/deficient SCC cells. The IC50 for MK-1775 was 20-fold lower in p53−/−...
Figure 2. RNA interference validation screens on kinase targets. A, RNAi primary validation screen; left bar graphs, differential cell viability (days 4.5–1.5) of RNAi-mediated knockdown of 28 kinase targets in autologous pairs of HNSCC cell lines derived from primary tumor and recurrent/metastatic site (14A, 14C, 15A, and 15B); kinase target versus universal negative siRNA control (UNI in yellow), $P < 0.05$ (green); right bar graphs, caspase-dependent apoptosis integrated over 4.5-day time-course of RNAi-mediated knockdown of 28 kinase targets, kinase target versus UNI, $P < 0.05$ (blue); results ranked by differential viability for each kinase target per the HNSCC cell line. KIF11 positive control for differential viability (white). B, RNAi secondary validation assays in five additional HNSCC cell lines (22A, 22B, 38, 47, and 019); left bar graphs, differential cell viability (days 4.5–1.5) of RNAi-mediated knockdown of 20 kinase targets in HNSCC cell lines, kinase target versus the negative siRNA control (SINC in yellow), $P < 0.05$ (green); right bar graphs, caspase-dependent apoptosis integrated over 4.5-day time-course of RNAi-mediated knockdown of 20 kinase targets in HNSCC cell lines, kinase target versus SINC, $P < 0.05$ (blue), results ranked by differential viability for each kinase target per the HNSCC cell line. KIF11 positive control for differential viability (white). C, kinase targets significance in HNSCC. RNAi-mediated knockdown of kinase targets ranked by the percentage of HNSCC cell lines in which kinase target reached statistical significance versus negative siRNA control. Differential viability (top); caspase-3/7–dependent apoptosis (bottom).
MSCC cells compared with p53 wild-type cells (0.22 vs. 4.5 μmol/L; Fig. 4A). The IC50 for MK-1775 in p53-mutant PCI-15A and PCI-15B HNSCC cells (0.14–0.17 μmol/L) and p53 wild-type SCC-61 and UMSCC-17A cells (2.8–4.5 μmol/L) showed a similar differential sensitivity to MK-1775 as the mouse SCC cells. Interestingly, the HPV+, p53 wild-type UMSCC-47, UPCI:SCC090, and UMSCC104 cells showed IC50 values (0.29–0.84 μmol/L) closer to p53-mutant cell lines (Fig. 4B and C).

As WEE1 regulates mitotic entry, this suggests p53-deficient cells are sensitive to deregulation of the G2–M transition. CHK1, a kinase required for the DNA damage induced G2–M checkpoint, and AURKA, a kinase involved in spindle assembly during mitosis, were also identified as putative survival kinases (Fig. 2). Consistent with the WEE1 inhibitor results, p53-deficient MSCC cells were also more sensitive to the CHK1 inhibitor, AZD7762 than p53 wild-type cells (IC50 0.13 vs. 2.2 μmol/L; Fig. 4A).

Data from the Genomics of Drug Sensitivity project contain dose–response measurements on 820 genomically characterized cancer cell lines treated with 138 different compounds (26). One compound in this collection, labeled 681640, is a dual WEE1/CHK1 inhibitor (27). We used mutational profiling data from the Sanger Cancer Cell Line Project to classify cell lines based on p53 mutational status and tested for correlation with sensitivity to 681640 (Supplementary Table S21; ref. 28). Examination of the 42 cells that were derived from SCCs of the head and neck (19), esophagus (11), lung (five), cervix (three), vulva (three), and skin (one), showed that, despite a broad range of

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**Figure 3.** Chemical inhibition of SRC family kinase (FYN), phosphatidylinositol kinases (PIK3CB, PIK4CB), focal adhesion kinase (FAK), tyrosine kinase receptor (ALK), and G2–M mitotic kinase (WEE1) impair viability of autologous pairs of p53-mutant HNSCC cells. Dose–response curves performed with six kinase inhibitors (MK-1775, TAE684, PF-562271, PIK28, PIK33, and PP2) against kinase targets (WEE1, ALK, PIK3CB, PIK4CB, FYN, and FAK; 8-point, mean (N = 3), range 100 μmol/L to 30 nmol/L, R2 > 0.85 for all curves. Autologous HNSCC cell pairs (UMSCC-14A, UMSCC-14C, PCI-15A, and PCI-15B) derived from primary and recurrent/metastatic site from the same patient (14, 15).
sensitivities in both wild-type and mutant groups, on average p53-mutant SCC cells had increased sensitivity to 681640 compared with p53 wild-type cells (median IC₅₀, 5.34 vs. 29.23 μmol/L; P = 0.005; Fig. 4D). However, the correlation between p53 status and sensitivity to 681640 was not observed in the overall collection of 499 cell lines.

Figure 4. Drug sensitivity of MSCC and HNSCC cells to chemical inhibition of G₂-M mitotic kinases determined by loss-of-function mutational status of p53. A, differential sensitivity of MSCC p53⁺/+ and p53⁻/⁻ cells to the WEE1 inhibitor MK-1775 and CHK1/CHK2 inhibitor AZD7762. Of note, 12-point dose-response curves, mean ± SEM (N = 3), range, 100 μmol/L–0.3 nmol/L, R² > 0.95 for all curves. B, differential sensitivity of HNSCC cell lines to MK-1775. Eight and 11-point dose-response curves, mean ± SEM (N = 3–4), range 100 mmol/L to 1 nmol/L, R² > 0.86 for all curves. C, bar graph of MK-1775 IC₅₀ values for each of the nine cell lines. *, statistically significant differences in IC₅₀ values between p53 wild-type (WT) versus either p53 wild-type, HPV(+) or p53-mutant HNSCC cell lines, ANOVA with the Holm–Sidak posttest; **, P < 0.05. D, box plots of TP53 mutation status versus IC₅₀ values following treatment with the dual WEE1/CHK1 inhibitor 681640 based on data from the Genomics of Drug Sensitivity project. Left, comparison of TP53 wild-type (n = 8) and TP53-mutant (n = 34) SCC cells. Right, comparison of TP53 wild-type (n = 177) and TP53-mutant (n = 322) status across all cell lines excluding SCC lines, representing a diversity of tumor types.
which excluded the 42 SCC cell lines and represents a broader variety of tumor types. In fact, the trend between p53-mutant status and 681640 sensitivity was reversed (median IC_{50}, 12.83 vs. 7.75 μmol/L; \( P = 1.348 \times 10^{-3} \); Fig. 4D). This analysis emphasizes the importance of validating candidate synthetic lethal interactions or drug sensitivities in specific tumor contexts and it indicates that other factors besides p53 mutational status affect sensitivity to 681640.

We next used flow cytometric cell-cycle analysis to determine the basis for the enhanced sensitivity of p53-mutant SCC cells to WEE1 inhibition. Treatment of cells with MK-1775 led to unscheduled mitotic entry in p53 mutant but not wild-type cells as measured by phospho-histone H3 (serine 10) (Fig. 5A). This was accompanied by an increase in sub-G_{1} DNA content, a loss of 4N DNA content, and activation of the apoptotic marker, caspase-3/7 (Fig. 5B and C). This indicates that WEE1 inhibition by MK-1775 in p53-mutant SCC cells caused unscheduled mitotic entry leading to mitotic catastrophe and apoptotic cell death.

To determine whether WEE1 inhibition was effective against p53-mutant HNSCC in a preclinical tumor model, we performed a four-arm double-blind study on PCI-15B xenograft-bearing mice. On the basis of our previous research PCI-15B demonstrates high metastatic potential as determined by cell migration and anchorage-independent growth assays, as well as the ability to metastasize to lymph nodes in an orthotopic mouse model of HNSCC (9). In addition, the PCI-15B line is also relatively resistant to cisplatin and radiation (data not shown). When tumors reached a palpable mass of >50 mm³, mice were randomized into four treatment arms and treated with vehicle, MK-1775, cisplatin, or cisplatin plus MK-1775. Cisplatin is the standard chemotherapeutic agent for HNSCC and cisplatin plus MK-1775 was used to determine whether inhibition of WEE1 would synergize with DNA-damaging therapy, as p53-mutant tumor cells would be expected to depend on G_{2}-M arrest after DNA-damaging treatment to repair DNA. Twice weekly oral gavage of MK-1775 increased growth of HNSCC tumors by 66% over the 4-week protocol as compared with vehicle (Fig. 6A and B, \( P = 0.06 \)). Cisplatin alone led to partial tumor regression, but also caused significant weight loss (Supplementary Fig. S4). However, MK-1775 given 24 hours after cisplatin therapy further augmented tumor regression (60% reduction with cisplatin alone vs. 80% reduction with cisplatin plus MK-1775, \( P = 0.003 \)). Tumor lysates from MK-1775–treated mice probed with WEE1 and CDC2 antibodies showed reduced phosphorylation of WEE1 and its substrate CDC2, indicating that oral administration of MK-1775 effectively blocked WEE1 kinase activity in tumors (Fig. 6C and D).

**Discussion**

One of the most significant clinical challenges in the management of patients with HNSCC is recurrent disease. In addition to being resistant to radio- or chemotherapy, these tumors can present with distant metastases, leaving palliative care as the only option. Here, we applied a functional kinomic approach to identify new candidate therapeutic targets for aggressive p53-mutant tumors. To prioritize targets, we also screened isogenic murine SCC cells with germline mutations (Atm, DNA-PKcs, p19Arf, and Tp53) in the p53 pathway. The rationale for this cross-species analysis was to identify evolutionary conserved survival pathways/kinases, the inhibition of which was associated with loss of cellular viability in p53-mutant cells. Retesting of these prioritized targets with independent siRNAs using both viability and apoptosis endpoints identified those that were effective in most or all cells tested, as well as those that were cell line or condition specific.

Comparing siRNA kinase screening results from cells derived from primary and recurrent/metastatic lesions revealed a high degree of concordance, implying that tumor cells isolated at different times or locations from the same patient share common vulnerabilities. In addition, recurrent/metastatic cell lines tended to be less responsive to kinase knockdown relative to cells from...
the primary lesion, suggesting development of resistance to target knockdown induced cell death. Altogether, these findings indicate this functional kinomic platform can reliably identify profiles of essential survival kinases specific to individual patients.

To further validate candidate therapeutic targets, we tested several small-molecule kinase inhibitors as a confirmatory step to support the RNAi results as well as to identify those inhibitors that might be effective for testing in vivo. Overall, this strategy identified the WEE1 kinase for further validation in vivo as RNAi-mediated knockdown of WEE1 led to a significant reduction in cell viability and a concomitant increase in apoptosis in all nine HNSCC cell lines tested. Moreover, p53-deficient MSCC and HNSCC cells were highly sensitive to the specific WEE1 inhibitor MK-1775 relative to p53 wild-type cells, a finding that has been observed in other settings (29–31). Additionally, HPV+ p53 wild-type cell lines were more sensitive to MK-1775 than the p53 wild-type cell lines, consistent with the idea that functional loss of p53, either by the E6 viral component of HPV or by somatic mutation is associated with greater sensitivity to the WEE1 inhibitor, MK-1775 (Fig. 4C). Mechanistically, WEE1 inhibition in p53-mutant cells, but not wild-type cells, led to unscheduled mitotic entry, mitotic catastrophe, and apoptosis, consistent with previous reports (24, 32). The G2 checkpoint kinase CHK1 was also a top candidate from our screen and p53-deficient SCC cells showed an increased sensitivity to both a CHK1 and a dual WEE1/CHK1 inhibitor. Collectively, this suggests p53-deficient SCC cells may be particularly vulnerable to deregulation of the G2–M transition.

As preclinical validation, we demonstrated that oral administration of MK-1775 inhibited the growth of p53-mutant HNSCC xenografts and also cooperated with cisplatin to induce tumor regression. This xenograft protocol was designed and implemented after careful examination of previous preclinical studies using genotoxic agents and/or MK-1775, and it was determined that the greatest responses to the WEE1 inhibitor MK-1775 would likely be obtained in p53-mutant HNSCC when given after genotoxic treatment (i.e., cisplatin; refs. 29–31). Our comparably conservative MK-1775 dosing regimen of two times per week, 24 hours pre- and post-cisplatin treatment was performed in the context of limiting potential toxicities from cisplatin treatment. Given that this current regimen was well tolerated as evidenced in Supplementary Fig. S4 (body weight of MK-1775–treated mice), it is quite possible that higher doses of the MK-1775 inhibitor would also be well tolerated and high efficacy could be attained either as a single-agent or in combination with genotoxic treatment.

Currently, cisplatin chemotherapy for the treatment of HNSCC is given either in the neoadjuvant setting or...
concurrently with radiotherapy. Unfortunately, the associated toxicities of combining cisplatin with other chemo-
therapeutic agents or the three potentially toxic cisplatin doses of 100 mg/m² administered during radiation treat-
ment can limit the clinical applicability of these regimens. Thus, the degree by which MK-1775 enhances response to
cisplatin would not only increase the effectiveness of exist-
ing therapy, but would open the possibility of reducing
cisplatin dosing to minimize side-effects and broaden
patient candidacy to these regimens.

MK-1775 has been shown to sensitize other p53-mutant
tumors to DNA-damaging agents (30, 31, 33). Molecular
analysis of HNSCC tumor lysates showed reduced phos-
phorylation of the WEEl substrate CDC2, indicating
that MK-1775 inhibited its intended target. We previously found
amplification of 11q13.1 in metastatic HNSCC tumor cells
with corresponding overexpression of cyclin B, the activat-
ing subunit of CDC2 (34), which could exacerbate the
sensitivity of HNSCC cells to WEE1 inhibition.

In addition to WEE1 and CHK1, siRNAs to other mitotic
kinases, including AURKA and NEK4, reduced viability and
increased apoptosis in the majority of HNSCC cells, includ-
ing those derived from recurrent/metastatic lesions, suggest-
ing potential as therapeutic targets. AURKA and CHK1 are
being pursued as drug targets (35–37), whereas NEK4 a
member of the NIMA family of kinases modulates sensitiv-
ty to microtubule poisons and DNA damage (38–40).
siRNAs to several Src family kinases (SFK) or related
signaling proteins (FYN, TXK, and CAM2KB) also reduced
viability in one or more HNSCC cell lines and were prior-
itized as candidates in the cross species comparisons. FYN is
an SFK involved in many prooncogenic processes such as
cellular proliferation, integrin-mediated and PI3K signal-
ing, and TXK is a tyrosine kinase activated by the SRC family
kinase LYN (41–43). SFKs are activated by mitogenic signals
to induce HNSCC cell proliferation and LYN mediates cell
motility and tumor growth in head and neck cancer (43,44).
Furthermore, SRC/FAK signaling correlates strongly
with phenotypes associated with tumor progression such
as invasion and metastasis (44, 45) and FAK (focal adhesion
kinase) itself is amplified in HNSCC (48), providing further
support for targeting the SFK pathway in more aggressive
subtypes of HNSCC (49,50).

In summary, our cross-species functional kinomic
approach using autologous pairs of primary and recur-
rent/metastatic p53-mutant HNSCC lines, coupled with
isogenic mouse SCC cells with defined mutations along
the p53 pathway has identified several survival kinases as
candidate therapeutic targets for aggressive HNSCC. These
kinases regulate a range of cellular processes such as phos-
phatidylinositol, focal adhesion, and Src signaling path-
ways, and the G2–M cell-cycle transition, suggesting func-
tional targets for therapeutic intervention. Discovery and
development of multiple targets may prove to be a useful
strategy, as tumors frequently develop resistance to single
agents and targeting multiple vulnerabilities simultane-
ously may be a required to achieve long-term remission.

Our preclinical data on WEE1 illustrate not only the
vulnerabilities of p53-mutant HNSCC cells to deregulation
of the G2–M transition, but also support the initiation of
clinical trials with MK-1775 or other G2–M checkpoint
inhibitors for HNSCC, particularly in combination with
cisplatin. More generally, this study illustrates the utility of
integrating functional genomic approaches with more tra-
ditional descriptive genomic and molecular profiles to
identify therapeutic targets in cancer.

Disclosure of Potential Conflicts of Interest

W.G. Yarbrough reports receiving a commercial research grant from
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