Targeting the DNA Damage Response in OSCC with *TP53* Mutations

Journal of Dental Research
2018, Vol. 97(6) 635–644
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for Dental Research 2018
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DOI: 10.1177/0022034518759068
journals.sagepub.com/home/jdr

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Abstract

Oral squamous cell carcinoma (OSCC) is the most common type of oral cancer worldwide and in the United States. OSCC remains a major cause of morbidity and mortality in patients with head and neck cancers. Tobacco and alcohol consumption alone or with chewing betel nut are potential risk factors contributing to the high prevalence of OSCC. Multimodality therapies, including surgery, chemotherapy, biologic therapy, and radiotherapy, particularly intensity-modulated radiotherapy (IMRT), are the current treatments for OSCC patients. Despite recent advances in these treatment modalities, the overall survival remains poor over the past years. Recent data from whole-exome sequencing reveal that *TP53* is commonly mutated in human papillomavirus–negative OSCC patients. Furthermore, these data stressed the importance of the *TP53* gene in suppressing the development and progression of OSCC. Clinically, *TP53* mutations are largely associated with poor survival and tumor resistance to radiotherapy and chemotherapy in OSCC patients, which makes the *TP53* mutation status a potentially useful molecular marker prognostic and predictive of clinical response in these patients. Several forms of DNA damage have been shown to activate p53, including those generated by ionizing radiation and chemotherapy. The DNA damage stabilizes p53 in part via the DNA damage signaling pathway that involves sensor kinases, including ATM and ATR and effector kinases, such as Chk1/2 and Wee1, which leads to posttranscriptional regulation of a variety of genes involved in DNA repair, cell cycle control, apoptosis, and senescence. Here, we discuss the link of *TP53* mutations with treatment outcome and survival in OSCC patients. We also provide evidence that small-molecule inhibitors of critical proteins that regulate DNA damage repair and replication stress during the cell cycle progression, as well as other molecules that restore wild-type p53 activity to mutant p53, can be exploited as novel therapeutic approaches for the treatment of OSCC patients bearing p53 mutant tumors.

Keywords: oncology, biomarkers, cancer biology, tumor biology, treatment planning, oral carcinogenesis

Introduction

Oral squamous cell carcinoma (OSCC) is one of the most prevalent tumors of the head and neck region, with more than 300,000 new patients diagnosed annually worldwide (Haddad and Shin 2008; Ferlay et al. 2010). In the United States alone, more than 30,000 new cases, which account for approximately 3% of all malignancies, and over 13,000 deaths of OSCC are recorded each year (Haddad and Shin 2008; Siegel et al. 2016). The histological grade of the OSCC can differ from well-differentiated keratinizing to undifferentiated nonkeratinizing carcinoma with a high tendency to metastasize. The risk factors for OSCC include smoking, alcohol consumption, viral infections (Epstein-Barr virus, human papillomavirus [HPV], and herpes simplex virus), betel nut chewing, occupational exposure to carcinogens, immunodeficiency, irradiation, diet, and genetic predisposition (Argiris et al. 2008; Hashibe et al. 2009). Furthermore, owing to demographic factors, the incidence and mortality rates for OSCC are disproportionately distributed (Vigneswaran and Williams 2014). For instance, OSCC is more prevalent in male than in female individuals. In the Indian subcontinent and Taiwan, mouth cancers are more common due to the practice of chewing betel nut. In the United States, despite a decreasing incidence of OSCC in African

American individuals over the past 2 decades, the mortality rate for this disease is still higher in this race than in Caucasian patients (DeSantis et al. 2013). Management of OSCC often requires multimodality treatments, including surgery, radiation, and/or platinum-based chemotherapy. Currently, there are no molecular biomarkers to guide these management decisions. *TP53* is the most commonly mutated gene (65%–85%) in OSCC, and mutations in *TP53* have been shown to have predictive significance for response to platinum-based therapy. The p53 protein is composed of several domains, including a DNA-binding domain, a transactivation region, and an oligomerization site. Most *TP53* mutations in OSCC are missense mutations, frequently occurring within the central region of the

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A supplemental appendix to this article is available online.

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protein that serves as the p53 DNA-binding domain. Recently, extensive efforts have been set forth to understand the mechanistic basis for *TP53* mutations and their impacts on the development of tumor resistance to therapies and poor survival. This mechanism is discussed in details in the supplemental appendix. The prognostic significance of *TP53* mutations has been demonstrated in several studies (Poeta et al. 2007; Lindenberg-van der Plas et al. 2011). In an effort to accurately predict the clinical impact of *TP53* mutations, we created and validated a novel algorithm, termed evolutionary action score of *TP53* EAp53 (EAp53, <http://mammoth.bcm.tmc.edu/EAp53/>), in which each *TP53* missense mutation is scored based on the functional sensitivity of p53 to amino acid sequence variations and classified as either high or low risk (Neskey et al. 2015).

In this review, we discuss the association of EAp53 mutations with treatment outcome and survival in OSCC patients, and we also describe novel therapeutic approaches for the treatment of OSCC patients bearing p53 mutant tumors.

Current Treatment of OSCC

Given the significant diversity of the anatomical subsites and molecular pathogenesis of OSCC, treatment of this disease often incorporates multimodal approaches administered by a multidisciplinary team of surgeons and clinical oncologists. Early stage OSCC disease is treated relatively well with single-modality therapy, which includes surgery or in some cases radiotherapy. However, most patients (more than 66%) present with advanced disease (stage III and IV) and are treated with multidisciplinary therapy including surgery, chemotherapy, biologic therapy, and radiotherapy (particularly intensity-modulated radiotherapy [IMRT]). Addition of cetuximab, an anti-epidermal growth factor receptor (EGFR) monoclonal antibody to first-line chemotherapy, improves the survival of recurrent or metastatic OSCC patients (Vermorken et al. 2008). Despite recent advances in imaging, surgery, radiation, and systemic therapies, overall survival has not improved substantially over the past 20. Thus, OSCC remains a major clinical challenge, and currently there are no available biomarkers to guide therapeutic decisions. Treatment plans for OSCC are selected based on different clinical and histological parameters, including the tumor site, TNM stage, and general medical condition. However, due to discernable biological and molecular pathogenesis of OSCC, tumors at similar stages respond very differently to the same treatment. Furthermore, the HPV-positive head and neck squamous cell carcinoma (HNSCC) tumors are typically wild-type (wt) *wtTP53*, as the p53 protein is inactivated by the viral oncoprotein (E6) and HPV-infected tumors respond favorably to chemoradiotherapy and show better survival. The presence of the viral oncoproteins and p16 in these tumors can be used as a reliable biomarker to stratify patients and optimize treatment in OSCC. Therefore, identifying reliable biomarkers and novel molecular targets is vital to stratify patients for precision and personalized treatment plans. This strategy can be more effective and less toxic than standard chemotherapy and radiotherapy.

TP53 Mutation as a Prognostic Biomarker for Poor Outcomes in OSCC

Recent evidence supported by the results of genomic sequencing analyses of HNSCC and The Cancer Genome Atlas (TCGA) data on HNSCC suggests that genetic alterations in specific molecular pathways, including the p53 pathway, play critical roles in HNSCC tumorigenesis and progression (Agrawal et al. 2011; Pickering et al. 2013; Cancer Genome Atlas 2015). Most *TP53* mutations are predominantly localized in the DNA-binding domain (Fig. 1), which effectively block wtp53 from binding to the transcription response elements and transactivating downstream target genes. Some p53 mutant proteins have a dominant negative effect as they can bind and inhibit the function of protein translated from the remaining wtp53 allele (Brosh and Rotter 2009). Moreover, other mutant p53 can exhibit oncogenic properties, termed *gain-of-function*, which are independent of wtp53 functions (Brosh and Rotter 2009). These gain-of-function p53 mutants enhance cell transformation, increase tumor formation in mice, and confer cellular resistance to chemotherapy (Brosh and Rotter 2009; Muller and Vousden 2013; Xie et al. 2013). Therefore, the functional impact of mutations with high evolutionary action scores is significant. We demonstrated that in both in vitro and in vivo preclinical models of OSCC, expression of high-risk *TP53* mutations endowed tumor cells with more invasive and tumorigenic characteristics than expression of low-risk mutations (Neskey et al. 2015). Using 168 TCGA HNSCC samples as a training set and 96 samples from our center as a validation set, we demonstrated that patients with high-risk mutant p53 had worse survival outcomes and a higher rate of distant metastases than those with low-risk mutations while the survival of patients with the low-risk mutation was not significantly different from those with wtp53 tumors (Neskey et al. 2015). Such data were further validated in the recently updated TCGA cohort with 450 patients with HPV-negative HNSCC (Fig. 2A, B). When overall survival was compared between a combined low-risk group (consisting of 93 patients with low-risk mutant p53 mutations and 96 patients with wtp53) and a combined high-risk group (consisting of 124 patients with high-risk mutant p53 and 137 patients with nonsense/indel mutant p53), the combined high-risk group showed worse survival outcomes. A previous study revealed that truncated mutations were associated with poor outcomes in HNSCC (Lindenberg-van der Plas et al. 2011). Therefore, in our study, we included the nonsense/indel mutations into the high-risk group. In addition, we showed that the truncated mutations were highly resistant to cisplatin and associated with decreased survival in HNSCC, consistent with the previous study (unpublished observation).

TP53 Mutations as a Predictive Biomarker of Platinum Response in OSCC

Although *TP53* status can predict therapy response of OSCC, an effective and reliable system of evaluating *TP53* status for

Figure 1. Histogram of number of cases with TP53 mutations by p53 codon position and the top 8 most frequently mutated p53 codons located in the DNA-binding domain (DBD) in head and neck squamous cell carcinoma (HNSCC) The Cancer Genome Atlas (TCGA) ($n = 446$). Most of the HNSCC patients are oral squamous cell carcinoma (OSCC) types.

Figure 2. Overall survival of head and neck squamous cell carcinoma (HNSCC), including oral squamous cell carcinoma (OSCC) The Cancer Genome Atlas (TCGA) cohort by EAp53 status. (A) Kaplan-Meier curves comparing overall survival of HNSCC patients with wild-type p53 (WT), low-risk mutant p53 (LR), high-risk mutant p53 (HR), and nonsense or indel mutation of p53 (Nonsense/Indel). (B) Kaplan-Meier curves for WT plus LR and HR plus Nonsense/Indel groups. Difference in overall survival between the groups was analyzed by log-rank test, and hazard ratio (HR) with 95% confidence interval (CI) and P value are shown.

stratifying patients into different response categories is still lacking. To examine if the EAp53 algorithm can predict response to cisplatin in patients with OSCC, the *TP53* mutational status was determined by sequencing in a cohort of 68 patients with locally advanced OSCC treated with cisplatin-based induction chemotherapy followed by surgical resection (Osman, Neskey, et al. 2015). Given the similar outcomes, patients with tumors having low-risk mutations were combined with wtp53 (Osman, Neskey, et al. 2015). We identified that 13 of 14 patients with high-risk mutant p53 were significantly resistant to cisplatin-based chemotherapy compared to patients with low-risk mutant p53 or wtp53 tumors (Osman, Neskey, et al. 2015). We also identified that 22 patients with wtp53 and 8 patients with low-risk mutant p53 did not respond

to cisplatin-based therapy, respectively (Osman, Neskey, et al. 2015). Compared with the low-risk mutant p53 group, the high-risk mutant p53 group was 10-fold more likely to have residual disease following cisplatin-based treatment. Likewise, OSCC cells bearing various high-risk *TP53* mutations showed significant resistance to cisplatin-based treatment compared to their wild-type and low-risk counterparts in vitro. Interestingly, sensitivity to cisplatin in OSCC cells expressing low-risk mutant p53 was comparable to that in OSCC cells expressing wtp53 (Osman, Neskey, et al. 2015). Similar results were observed in vivo in an orthotopic mouse model of tongue cancer, in which high-risk mutant p53 tumor-bearing animals were associated with decreased response to cisplatin and shorter survival (Osman, Neskey, et al. 2015). Collectively, these results

Figure 3. Proposed signaling pathways for induction of DNA damage response and replication stress leading to cell death in oral squamous cell carcinoma tumor cells in response to DNA-damaging agents and cell cycle inhibitors.

indicated that the *TP53* mutational status can be a useful biomarker for predicting response to cisplatin-based chemotherapy in OSCC patients and should be considered for integration into clinical practice.

Novel Molecularly Targeted Therapy for Targeting OSCC with *TP53* Mutations

Given the fact that platinum-refractory OSCC frequently bears *TP53* mutations, we have great interest in the development of novel therapeutic approaches to overcome this resistance. Since p53 activation leads to cell cycle arrest and initiation of DNA repair in response to DNA damage, inhibitors of critical proteins that regulate DNA damage response (DDR) and cell cycle progression have potential to sensitize OSCC cells with mutant p53 to chemotherapy (Fig. 3). Recently, this therapeutic vulnerability has been exploited by targeting the cell cycle using small-molecule inhibitors of the Ataxia-telangiectasia mutated (ATM), the ATM-Rad3 related (ATR), the DNA-dependent protein kinase (DNA-PK), the checkpoint kinase-1/2 (Chk1/2), and the Wee1 kinase. Targeting OSCC cells

expressing mutant p53 with Chk1/2 and Wee1 inhibitors has become an especially intensive area of investigation. Combining these inhibitors with other molecularly targeted agents, chemotherapeutics, and/or radiotherapy can improve the efficacy of the checkpoint blockade in OSCC.

DNA Damage Response and Replication Stress as Therapeutic Targets in OSCC

Activation of p53 can occur in response to a number of cellular stresses, including DNA damage, hypoxia, and nucleotide deprivation. Several forms of DNA damage have been shown to activate p53, including those generated by ionizing radiation and chemotherapy. The DNA damage stabilizes p53 in part via the DNA damage signaling pathway that involves the sensor kinases, including ATM and ATR and effector kinases, such as Chk1/2 and Wee1, which leads to the transcriptional regulation of a variety of genes involved in cell cycle control and apoptosis (Coutts and La Thangue 2006). Several studies have demonstrated that *TP53* mutant cells can be sensitized to genotoxic agents in a synthetic lethal strategy through inhibition of ATR, ATM, Chk1/2, and Wee1.

High levels of DNA damage leading to induction of DNA single- or double-strand breaks or premature mitosis may interfere with DNA replication and hamper its progression (Allen et al. 2011). If nucleotides or components of the replication machinery are not properly supplied or assembled to complete DNA replication, the cells become “stressed.” This is manifested by stalling of replication forks and binding of replication protein A (RPA) to the single-strand DNA segments (Dobbelstein and Sorensen 2015). Stalled replication forks occur selectively in cancer cells owing to a loss of tumor suppressors, including mitotic and S-phase cell cycle inhibitors (Suram and Herbig 2014). This is in turn followed by the activation of the DNA sensors such as ATR, ATM, and DNA-PK, which recruit their immediate downstream target proteins, including Chk1, p53, and Wee1, to inhibit new origins of replication from firing, stabilize advancing replication forks, prevent mitosis, and arrest cell cycle. This activation step ensures that errors in replication can be primarily repaired through excision of double-stranded DNA breaks or Rad51-mediated homologous recombination (Pfister et al. 2015). If the mechanisms that deal with faithful replication become faulty, more DNA breaks occur and gradually accumulate, leading to genomic instability and extreme lethality (Suram and Herbig 2014; Pfister et al. 2015). The ATR-ATM-Chk1-Wee1 signaling pathway is a crucial component of the general surveillance mechanism of the replication phase and is activated at low

thresholds even during the unperturbed S-phase (Shechter et al. 2004; Syljuasen et al. 2005). Therefore, cells undergoing replication stress can be activated to undergo senescence or apoptosis through inhibition of this pathway at one or more levels (Fig. 3) (Suram and Herbig 2014; Pfister et al. 2015).

Treatment with the histone deacetylase (HDAC) inhibitor, vorinostat, or knockdown of HDAC8 showed preferential cytotoxicity for p53 mutant rather than p53 wt or null tumor cells, providing the rationale for a novel anticancer strategy using vorinostat-based regimens for treatment of mutant p53-specific tumors (Li et al. 2011; Yan et al. 2013). Interrelation and dependence between p53 and the DNA damage response proteins and HDAC targeted for therapy are described in details in the supplemental appendix.

ATM and ATR Inhibitors

ATM and ATR are members of the phosphatidylinositol 3-kinase-related kinase (PIKK) family of serine/threonine protein kinases (Shechter et al. 2004). These kinases are key mediators of the DDR, which induce cell cycle arrest and DNA repair machinery via their downstream targets. Resistance to genotoxic treatments can be associated with increased DDR. Many cancers have defects in some components of the DDR, making them highly dependent on the remaining DNA repair pathways for survival of cancer cells. As a result, inhibition of the DDR has become an attractive approach in cancer therapy. ATM and ATR act as primary regulators of the response to DNA double-strand breaks and replication stress, respectively, with overlapping but nonredundant activities (Shechter et al. 2004). Preclinical data have provided a strong rationale for clinical testing of ATR and ATM inhibitors either alone or in combination with chemotherapy, radiotherapy, and molecularly targeted therapies to treat tumors with deficiencies in certain DDR components. Mutations in ATR (4% to 10%) and ATM (1% to 16%) occur in HNSCC, providing the rationale for targeting DNA repair pathway in these tumors, including OSCC (Seiwert et al. 2015). Currently, the small-molecule inhibitors of ATR, AZD6738 and VX-970 (also known as VE-822 or berzosertib), are in phase I/II clinical trials in solid tumors, including HNSCC (NCT02264678; NCT02567422). Most ATM inhibitors are still under preclinical evaluation, and only AZD0156 has reached phase I clinical trial as monotherapy or in combination with poly (ADP-ribose) polymerase (PARP) inhibitor olaparib and other cytotoxic agents in patients with advanced solid tumors (NCT02588105) (Table).

Chk Inhibitors

Chk1 and Chk2 are structurally unrelated yet functionally overlapping serine/threonine kinases activated in response to diverse genotoxic insults. These 2 kinases regulate the fundamental cellular functions of DNA replication, cell cycle progression, chromatin remodeling, and apoptosis (Bartek and Lukas 2003). In the absence of functional p53, cell cycle arrest and DNA repair rely on the function of Chk1/2 for faithful

DNA repair and replication. Thus, we inhibited Chk1/2 in OSCC cells with mutant p53 with the small-molecule Chk1/2 inhibitor AZD7762 and showed that AZD7762 sensitized these cells to cisplatin through induction of mitotic catastrophe (Gadhikar et al. 2013), a form of cell death that is characterized by the appearance of multinucleated cells and polyploidy. Recently, other Chk inhibitors have been reported to possess synergistic effects on killing cancer cells when combined with inhibitors targeting important cell cycle proteins such as Wee1 kinase (Guertin et al. 2012). Interestingly, in some *in vitro* and *in vivo* cancer models, Chk1 inhibitors displayed single-agent efficacy in cells under replication stress (Chen et al. 2009; Ferrao et al. 2012; Gadhikar et al. 2018). Several early phase clinical trials using small-molecule inhibitors of Chk1/2 in combination with various chemotherapeutic agents have been conducted in OSCC (Table).

Wee1 Inhibitors

Wee1 is a serine/threonine kinase that responds to DNA damage and induces G2-M arrest by inactivating cyclin dependent kinase 1 (CDK1, also known as CDC2) through phosphorylation of the Tyr15 residue (Squire et al. 2005). While Wee1 is essential to prevent cells with DNA damage from entering mitosis, inhibition of this kinase activity can override G2 cell cycle arrest, leading to accumulation of cells with extensive DNA damage and subsequent cell death through mitotic catastrophe (Portugal et al. 2010; De Witt Hamer et al. 2011). Thus, inhibition of Wee1 kinase activity may sensitize p53 mutant cancer cells to DNA-damaging therapy. Several studies with AZD1775 (formerly known as MK-1775), a specific inhibitor of Wee1, and Wee1-siRNA-mediated depletion indicate that Wee1 inhibition abrogates the G2 checkpoint and selectively sensitizes tumor cells defective of p53 to various DNA-damaging agents, such as gemcitabine, cisplatin, and radiotherapy, to inhibit tumor growth *in vivo* (Wang et al. 2004; Hirai et al. 2009; Hirai et al. 2010; Bridges et al. 2011; Rajeshkumar et al. 2011). In light of the findings, AZD1775 has been tested in a phase I clinical trial as a chemosensitizer in patients with advanced solid tumors and showed good tolerability with minimal collateral side effects, and it has currently advanced to phase II clinical trials in solid tumors (Table) (Leijen et al. 2010; Leijen et al. 2016). We have recently demonstrated that AZD1775 can overcome cisplatin resistance in HPV-negative OSCC cells expressing high-risk mutant p53 through mitotic arrest followed by senescence rather than apoptosis both *in vitro* and *in vivo* (Osman, Monroe, et al. 2015). Intriguingly, AZD1775 displays single-agent activity and significantly potentiates the response of HPV-positive HNSCC cells to cisplatin both *in vitro* and *in vivo* (Tanaka et al. 2015). Unlike HPV-negative OSCC cells, AZD1775 induces apoptosis triggered by selective degradation (or cleavage) of the antiapoptotic proteins Mcl-1 and XIAP in HPV-positive HNSCC cells. Collectively, our data suggest that cancer cells undergoing replication stress can be targeted with Wee1 kinase inhibitor.

Table. Cell Cycle Inhibitors That Target DNA Repair and Replication Stress and Currently in Clinical Trials.

Compound Name	Main Target	Phase of Development	Clinical Trials: Details	Disease	Identifier
AZD7762	Chk1/2	Phase I	Combination with gemcitabine	Solid tumors and advanced solid malignancies	NCT00937664/ NCT00413686
MK-8776 (SCH 900776)	Chk1	Phase I	Combination with irinotecan	Solid tumors	NCT00473616
		Phase II	Combination with cytarabine	Adult relapsed acute myeloid leukemia	NCT01870596
		Phase I	Combination with cytarabine	Acute leukemia	NCT00907517
		Phase I	Combination with gemcitabine	Solid tumors and lymphoma	NCT00779584
		Phase I	Combination with hydroxyurea	Advanced malignant solid tumors	NCT01521299
LY2603618 (Rabusertib)	Chk1	Phase II	Combination with pemetrexed	Advanced or metastatic NSCLC	NCT00988858
		Phase I/II	Combination with gemcitabine	Pancreatic cancer	NCT00839332
		Phase I/II	Combination with cisplatin or pemetrexed	NSCLC	NCT01139775
		Phase I	Combination with pemetrexed	Cancer	NCT00415636
		Phase I	Combination with gemcitabine	Solid tumors	NCT01341457
		Phase I	Drug interaction study to assess the effect of LY2603618 on the metabolic pathway of desipramine	Solid tumors	NCT01358968
			Drugs: desipramine, pemetrexed, gemcitabine		
		Phase I	CI 4 study	Advanced and/or metastatic solid tumors	NCT01296568
			Combination with pemetrexed or gemcitabine		
LY2606368 (Prexasertib)	Chk1/2	Phase II	Alone	Ovarian, breast, and prostate cancer	NCT02203513
		Phase II	Alone	Extensive stage disease small cell lung cancer	NCT02735980
		Phase II	Alone	Advanced cancers with replicative stress or homologous repair deficiency	NCT02873975
		Phase I	Combination with olaparib	Advanced solid tumor	NCT03057145
		Phase I	Combination with cytarabine and fludarabine	Leukemia	NCT02649764
		Phase I	Alone	Recurrent or refractory solid tumors	NCT02808650
		Phase I	Combination with ralimetinib	Advanced or metastatic cancer	NCT02860780
		Phase I	Combination with chemotherapy (cisplatin or cetuximab) and radiation	Head and neck cancer	NCT02555644
		Phase I	Alone	Advanced cancer	NCT02778126
		Phase I	Alone	Advanced cancer	NCT02514603
		Phase I	Alone	Advanced cancer	NCT01115790
		Phase I	Combination with other anticancer drugs (cisplatin, cetuximab, pemetrexed, fluorouracil, or LY3023414)	Advanced cancer	NCT02124148
GDC-0425	Chk1	Phase I	Combination with gemcitabine	Refractory solid tumors or lymphoma	NCT01359696
SAR-020106	Chk1	Preclinical	Alone		
AZD1775 (MK-1775) ^a	Wee1	Phase II	Combination with cisplatin	Recurrent or metastatic head and neck cancer	NCT02196168
		Phase I	Combination with cisplatin and radiotherapy	Head and neck cancer	NCT03028766
		Phase I	Combination with cisplatin	Recurrent or metastatic head and neck cancer	NCT02196168
		Phase I	Combination with cisplatin and radiotherapy	Intermediate/high-risk HNSCC	NCT02585973
		Phase I	Combination with cisplatin and docetaxel	Stage III to IVB HNSCC	NCT02508246

(continued)

Table. (continued)

Compound Name	Main Target	Phase of Development	Clinical Trials: Details	Disease	Identifier
AZD0156	ATM	Phase I	Combination with olaparib/other anticancer treatment	Advanced solid tumors	NCT02588105
AZD6738	ATR	Phase II	Combination with durvalumab/other anticancer treatment	NSCLC	NCT03334617
		Phase II	Combination with olaparib	Metastatic triple-negative breast cancer	NCT03330847
		Phase I/II	Combination with acalabrutinib	Relapsed or refractory chronic lymphocytic leukemia	NCT03328273
		Phase I/II	Combination with carboplatin, olaparib, and/or MEDI4736	Advanced/metastatic solid malignancies	NCT02264678
		Phase I	Combination with paclitaxel	Refractory cancer	NCT02630199
		Phase I	Combination with radiotherapy	Solid tumor	NCT02223923
		Phase I	Alone	Relapsed/refractory chronic lymphocytic leukemia, prolymphocytic leukemia, or B-cell lymphoma	NCT01955668
		Phase I	Biomarker study, combination with olaparib	HNSCC	NCT03022409
BAY1895344	ATR	Phase I	Alone	Advanced solid tumors and lymphomas	NCT03188965
VX-970 (M6620, VE-822)	ATR	Phase II	Combination with gemcitabine	Recurrent ovarian, primary peritoneal, or fallopian tube cancer	NCT02595892
		Phase II	Combination with cisplatin and gemcitabine	Metastatic urothelial cancer	NCT02567409
		Phase I/II	Combination with carboplatin and gemcitabine	Recurrent and metastatic ovarian, primary peritoneal, or fallopian tube cancer	NCT02627443
		Phase I/II	Combination with topotecan	Small cell cancers	NCT02487095
		Phase I	Combination with veliparib and cisplatin	Refractory solid tumors	NCT02723864
		Phase I	Combination with irinotecan	Metastatic and unresectable solid tumors	NCT02595931
		Phase I	Combination with whole-brain radiation therapy	Brain metastases from NSCLC, small cell lung cancer, or neuroendocrine tumors	NCT02589522
		Phase I	Combination with cisplatin and radiotherapy	Locally advanced human papillomavirus-negative HNSCC	NCT02567422
		Phase I	Combination with cytotoxic chemotherapy	Advanced solid tumor	NCT02157792

ATM, ataxia-telangiectasia mutated; ATR, ATM-Rad3 related; Chk1/2, checkpoint kinase-1/2; HNSCC, head and neck squamous cell carcinoma; NSCLC, non-small cell lung cancer.

^aCurrently, there are many clinical trials with AZD1775; therefore, only trials in HNSCCs are listed.

HDAC Inhibitors

Modulation of the acetylation status of histones and transcription factors is an essential mechanism for regulating gene expression (Roth et al. 2001; Gillenwater et al. 2007). Histone acetylation is generally associated with elevated transcription, whereas deacetylated histones are often linked to repressed transcription (Struhl 1998). HDACs act enzymatically to remove the acetyl group from histones and silence gene

expression (Struhl 1998). Increased activities of HDACs are observed in several human cancers, including OSCC, and associated with poorer outcome in OSCC (Roth et al. 2001; Chang et al. 2009; Weichert 2009). These findings indicate that HDACs may serve as therapeutic targets in OSCC. In fact, HDAC inhibitors induce growth arrest, differentiation, and apoptosis in various cancer cell lines in vitro and suppress tumor growth in animal xenograft models, including OSCC (Roth et al. 2001; Prystowsky et al. 2009; Iglesias-Linares

Figure 4. Current approaches to target mutant p53 in oral squamous cell carcinoma (OSCC) cells. The proposed strategies to target mutant p53 in OSCC include reactivation of p53 by small molecules and peptides.

et al. 2010). The small-molecule HDAC inhibitor vorinostat (formerly known as suberoylanilide hydroxamic acid [SAHA]), displayed preferential cytotoxicity in vitro and in vivo in cancer cells harboring *TP53* mutations (Blagosklonny et al. 2005; Li et al. 2011; Yan et al. 2013). Despite the absence of single-agent activity of the HDAC inhibitors, they are now emerging as attractive classes of antitumor agents being tested clinically in combination with conventional chemotherapeutics or molecularly targeted agents (Lane and Chabner 2009). Recent data demonstrated that the HDAC inhibitor, trichostatin A (TSA), completely ablated cisplatin resistance in HNSCC (Almeida et al. 2014). Another report also demonstrated that treatment with pan-HDAC inhibitor SAHA synergized with cisplatin in vitro and significantly decreased tumor growth and metastasis in vivo in HNSCC (Kumar et al. 2015). Taken together with the ongoing clinical trials, we were motivated to evaluate the combination of Wee1 and HDAC inhibitors in OSCC with mutant p53. Our study has revealed that vorinostat synergizes with AZD1775 in OSCC cells with mutant p53 in vitro and in vivo through induction of replication stress associated with decreased Rad51-mediated homologous recombination, resulting in apoptotic cell death within the S-phase of the cell cycle (Tanaka et al. 2017). Thus, our study provides a pre-clinical foundation for initiation of clinical trials using combination of HDAC and Wee1 inhibitors, particularly for patients with advanced, recurrent, and/or metastatic OSCC.

Restoration of Full p53 Functionality in OSCC as a Novel Therapeutic Strategy

Since wtp53 is a known potent inducer of apoptosis and senescence when expressed in tumor cells, reactivation of wild-type function in mutant p53 is an attractive therapeutic approach. However, attempts have been seriously hindered by the fact that wtp53 has no known enzymatic activities and rather operates primarily as a sequence-specific transcription factor. Furthermore, restoring the activity of a faulty tumor suppressor protein is enormously more difficult than abrogating the activity of hyperactive

oncoprotein (Oren et al. 2016). Nevertheless, a variety of compounds that can restore wtp53 function have been developed in recent years (Fig. 4). A small-molecule PRIMA-1, which is capable of reactivating mutant p53 (Bykov et al. 2005), was subsequently modified to a new derivative, PRIMA-1-met (also known as APR-246). PRIMA-1 and its derivative, PRIMA-1-met, specifically bind to several mutant p53 proteins and interact with the DNA-binding domain, thereby promoting proper folding of mutant proteins and restoration of some wtp53 functions (Bykov et al. 2005). Recently, PRIMA-1-met has entered a phase II clinical trial (Bykov et al. 2016). While metal zinc (Zn^{2+}) ions are also crucial for stabilizing the correct folding of the DNA-binding domain of wtp53, many cancer-associated mutant p53 have lower binding affinity to zinc than that of wtp53 and therefore tend to misfold and aggregate. The small molecule ZMC1 (NSC319726), one of the family of the thiosemicarbazone metal ion chelators, was found to deliver Zn^{2+} to the DNA-binding domain of mutant p53, which facilitates its correct folding and restores wtp53 function in a small subset of mutant p53-expressing tumor cell lines. Similar to PRIMA-1 and APR-246, ZMC1 can cause unpredictable side effects in patients since its chemical activity is not p53 specific (Yu et al. 2012). COTI-2 is a novel third-generation thiosemicarbazone that was discovered using a prediction system through a proprietary computational platform named CHEMSAS. COTI-2 showed anticancer effects in vitro by inhibiting proliferation, leading to death through apoptosis in various cancer cells (Salim et al. 2016). Moreover, COTI-2 appeared to act not only by restoring wild-type-like p53 function but also by negatively modulating the PI3K/AKT/mTOR pathway, which is a prototypic survival pathway constitutively activated in many types of cancer (Salim et al. 2016). In xenograft mouse models, COTI-2 inhibits tumor growth and is well tolerated (Salim et al. 2016). We have found that COTI-2 displays single-agent activity and markedly decreases cell growth in vitro at a nanomolar concentration, particularly in OSCC cells with mutant p53, and inhibits tumor growth in vivo in orthotopic mouse models of OSCC (unpublished data). In addition, COTI-2 synergizes with cisplatin and radiotherapy in vitro and in vivo in OSCC harboring high-risk p53 mutations (unpublished data). Mechanistically, COTI-2 appears to reactivate p53 downstream target genes. This compound holds great promise and is currently tested under a phase I clinical trial of patients with refractory gynecologic and head and neck malignancies at the University of Texas MD Anderson Cancer Center (NCT02433626).

Recently, using a rational design approach, Soragni and colleagues identified a peptide dubbed ReACp53, which can block the amyloid-like aggregation of mutant p53 proteins containing either R248Q or R175H mutations (Soragni et al. 2016). ReACp53 inhibited aggregation and rescued wtp53 properties determined by induction of its target genes and apoptosis. In addition, administration of conjugated ReACp53 to mice bearing ovarian carcinomas expressing aggregation-prone p53 mutations resulted in decreased cell proliferation and tumor shrinkage (Soragni et al. 2016). The spectrum of *TP53* mutations targeted by this peptide remains to be

determined. Despite its greater specificity, lack of efficient delivery route of the *TP53* reactivation peptides into the tumor cells could potentially limit their clinical applications.

Summary and Concluding Remarks

OSCC remains one of the most deadly type of head and neck cancer worldwide and in the United States. Comprehensive integrative genomic analysis of HNSCCs by TCGA confirmed that *TP53* is the most frequently mutated gene in OSCC. Clinically, *TP53* mutations are significantly associated with poor survival and tumor resistance to radiotherapy and chemotherapy in OSCC patients. This suggests that *TP53* mutations can be used as a marker that is prognostic and predictive of clinical response. Also, the development of effective and long-lasting therapeutic approaches for OSCC patients with tumors bearing *TP53* mutations is greatly needed. Recent studies reveal that *TP53* mutations can lead to not only loss of *wtp53* function but also gain-of-function activities. In addition, *TP53* mutations are heterogeneous and work through a complex and intricate network of multiple proteins. Therefore, discovering novel drug-based strategies to safely and efficiently target mutant p53 in OSCC is highly challenging and will require better understanding of the biology of mutant p53, such as its interaction partners and identification of downstream signaling pathways. Since p53 activation leads to cell cycle arrest and initiation of DNA repair in response to DNA damage, inhibitors of critical proteins that regulate DNA damage response and induction of replicative stress during cell cycle progression have potential to sensitize OSCC cells with mutant p53 to chemotherapy and radiation. Recently, this therapeutic approach has been exploited by us and others to target the cell cycle using small-molecule inhibitors of Chk1/2, Wee1, and HDAC. With better understanding of DNA repair and replication, we anticipate that the emergence of novel cancer therapies in this field will have great impact on OSCC patients. Reactivation of mutant p53 in OSCC cells with specific small molecules and synthetic peptides is another therapeutic approach that should be considered, and substantial research has been initiated in the past several years in different cancer types using this novel strategy. Although difficulties in restoring the activity of a faulty p53 and delivering the *TP53* reactivation peptides into the tumor cells do exist, this approach will probably become impactful on the treatment of cancer patients with tumor-expressing mutant p53. In conclusion, identifying reliable biomarkers and novel molecular targets is vital to stratify OSCC patients for precision and personalized treatment plans.

Author Contributions

A. Lindemann, A.A. Osman, contributed to conception, design, data acquisition, analysis, and interpretation, drafted and critically revised the manuscript; H. Takahashi, A.A. Patel, contributed to design, critically revised the manuscript; J.N. Myers, contributed to conception, design, data acquisition, analysis, and interpretation, critically revised the manuscript. All authors gave final approval and agree to be accountable for all aspects of the work.

Acknowledgments

This work was supported by the National Institutes of Health/ National Institute of Dental and Craniofacial Research R01DE024601 (A.A. Osman and J.N. Myers) and the University of Texas MD Anderson Cancer Center Stiefel Oropharyngeal Cancer Program (A.A. Osman and J.N. Myers). The authors declare no potential conflicts of interest with respect to the authorship and/or publication of this article.

References

- Agrawal N, Frederick MJ, Pickering CR, Bettgowda C, Chang K, Li RJ, Fakhry C, Xie TX, Zhang J, Wang J, et al. 2011. Exome sequencing of head and neck squamous cell carcinoma reveals inactivating mutations in notch1. *Science*. 333(6046):1154–1157.
- Allen C, Ashley AK, Hromas R, Nickoloff JA. 2011. More forks on the road to replication stress recovery. *J Mol Cell Biol*. 3(1):4–12.
- Almeida LO, Abrahao AC, Rosselli-Murai LK, Giudice FS, Zagni C, Leopoldino AM, Squarize CH, Castilho RM. 2014. NFκB mediates cisplatin resistance through histone modifications in head and neck squamous cell carcinoma (HNSCC). *FEBS Open Bio*. 4:96–104.
- Argiris A, Karamouzis MV, Raben D, Ferris RL. 2008. Head and neck cancer. *Lancet*. 371(9625):1695–1709.
- Bartek J, Lukas J. 2003. Chk1 and Chk2 kinases in checkpoint control and cancer. *Cancer Cell*. 3(5):421–429.
- Blagosklonny MV, Trostel S, Kayastha G, Demidenko ZN, Vassilev LT, Romanova LY, Bates S, Fojo T. 2005. Depletion of mutant p53 and cytotoxicity of histone deacetylase inhibitors. *Cancer Res*. 65(16):7386–7392.
- Bridges KA, Hirai H, Buser CA, Brooks C, Liu H, Buchholz TA, Molkentine JM, Mason KA, Meyn RE. 2011. Mk-1775, a novel wee1 kinase inhibitor, radiosensitizes p53-defective human tumor cells. *Clin Cancer Res*. 17(17):5638–5648.
- Brosh R, Rotter V. 2009. When mutants gain new powers: news from the mutant p53 field. *Nat Rev Cancer*. 9(10):701–713.
- Bykov VJ, Zache N, Stridh H, Westman J, Bergman J, Selivanova G, Wiman KG. 2005. Prima-1(met) synergizes with cisplatin to induce tumor cell apoptosis. *Oncogene*. 24(21):3484–3491.
- Bykov VJ, Zhang Q, Zhang M, Ceder S, Abrahamsen L, Wiman KG. 2016. Targeting of mutant p53 and the cellular redox balance by APR-246 as a strategy for efficient cancer therapy. *Front Oncol*. 6:21.
- Cancer Genome Atlas N. 2015. Comprehensive genomic characterization of head and neck squamous cell carcinomas. *Nature*. 517(7536):576–582.
- Chang HH, Chiang CP, Hung HC, Lin CY, Deng YT, Kuo MY. 2009. Histone deacetylase 2 expression predicts poorer prognosis in oral cancer patients. *Oral Oncol*. 45(7):610–614.
- Chen CC, Kennedy RD, Sidi S, Look AT, D'Andrea A. 2009. Chk1 inhibition as a strategy for targeting fanconi anemia (FA) DNA repair pathway deficient tumors. *Mol Cancer*. 8:24.
- Coutts AS, La Thangue N. 2006. The p53 response during DNA damage: impact of transcriptional cofactors. *Biochem Soc Symp*. 2006(73):181–189.
- De Witt Hamer PC, Mir SE, Noske D, Van Noorden CJ, Wurdinger T. 2011. Wee1 kinase targeting combined with DNA-damaging cancer therapy catalyzes mitotic catastrophe. *Clin Cancer Res*. 17(13):4200–4207.
- DeSantis C, Naishadham D, Jemal A. 2013. Cancer statistics for African Americans, 2013. *CA Cancer J Clin*. 63(3):151–166.
- Dobbelstein M, Sorensen CS. 2015. Exploiting replicative stress to treat cancer. *Nat Rev Drug Discov*. 14(6):405–423.
- Ferlay J, Shin HR, Bray F, Forman D, Mathers C, Parkin DM. 2010. Estimates of worldwide burden of cancer in 2008: GLOBOCAN 2008. *Int J Cancer*. 127(12):2893–2917.
- Ferraro PT, Bukczynska EP, Johnstone RW, McArthur GA. 2012. Efficacy of CHK inhibitors as single agents in MYC-driven lymphoma cells. *Oncogene*. 31(13):1661–1672.
- Gadhikar MA, Sciuto MR, Alves MV, Pickering CR, Osman AA, Neskey DM, Zhao M, Fitzgerald AL, Myers JN, Frederick MJ. 2013. Chk1/2 inhibition overcomes the cisplatin resistance of head and neck cancer cells secondary to the loss of functional p53. *Mol Cancer Ther*. 12(9):1860–1873.
- Gadhikar MA, Zhang J, Shen L, Rao X, Wang J, Zhao M, Kalu NN, Johnson F, Byers LA, Heymach J, et al. 2018. CDKN2A/p16 deletion in head and neck cancer cells is associated with Cdk2 activation, replication stress, and vulnerability to Chk1 inhibition. *Cancer Res*. 78(3):781–797.
- Gillenwater AM, Zhong M, Lotan R. 2007. Histone deacetylase inhibitor suberoylanilide hydroxamic acid induces apoptosis through both mitochondrial

- and Fas (Cd95) signaling in head and neck squamous carcinoma cells. *Mol Cancer Ther.* 6(11):2967–2975.
- Guertin AD, Martin MM, Roberts B, Hurd M, Qu X, Miselis NR, Liu Y, Li J, Feldman I, Benita Y, et al. 2012. Unique functions of CHK1 and WEE1 underlie synergistic anti-tumor activity upon pharmacologic inhibition. *Cancer Cell Int.* 12(1):45.
- Haddad RI, Shin DM. 2008. Recent advances in head and neck cancer. *N Engl J Med.* 359(11):1143–1154.
- Hashibe M, Brennan P, Chuang SC, Boccia S, Castellsague X, Chen C, Curado MP, Dal Maso L, Daudt AW, Fabianova E, et al. 2009. Interaction between tobacco and alcohol use and the risk of head and neck cancer: pooled analysis in the international head and neck cancer epidemiology consortium. *Cancer Epidemiol Biomarkers Prev.* 18(2):541–550.
- Hirai H, Arai T, Okada M, Nishibata T, Kobayashi M, Sakai N, Imagaki K, Ohtani J, Sakai T, Yoshizumi T, et al. 2010. MK-1775, a small molecule Wee1 inhibitor, enhances anti-tumor efficacy of various DNA-damaging agents, including 5-fluorouracil. *Cancer Biol Ther.* 9(7):514–522.
- Hirai H, Iwasawa Y, Okada M, Arai T, Nishibata T, Kobayashi M, Kimura T, Kaneko N, Ohtani J, Yamanaka K, et al. 2009. Small-molecule inhibition of Wee1 kinase by MK-1775 selectively sensitizes p53-deficient tumor cells to DNA-damaging agents. *Mol Cancer Ther.* 8(11):2992–3000.
- Iglesias-Linares A, Yañez-Vico RM, González-Moles MA. 2010. Potential role of HDAC inhibitors in cancer therapy: insights into oral squamous cell carcinoma. *Oral Oncol.* 46(5):323–329.
- Kumar B, Yadav A, Lang JC, Teknos TN, Kumar P. 2015. Suberoylanilide hydroxamic acid (SAHA) reverses chemoresistance in head and neck cancer cells by targeting cancer stem cells via the downregulation of nanog. *Genes Cancer.* 6(3–4):169–181.
- Lane AA, Chabner BA. 2009. Histone deacetylase inhibitors in cancer therapy. *J Clin Oncol.* 27(32):5459–5468.
- Leijen S, Schellens JH, Shapiro G, Pavlick AC, Tibes R, Demuth T, Viscusi J, Cheng JD, Xu Y, Oza AM. 2010. A phase I pharmacological and pharmacodynamic study of MK-1775, a Wee1 tyrosine kinase inhibitor, in monotherapy and combination with gemcitabine, cisplatin, or carboplatin in patients with advanced solid tumors. *J Clin Oncol.* 28(15):3067–3067.
- Leijen S, van Geel RM, Sonke GS, de Jong D, Rosenberg EH, Marchetti S, Pluim D, van Werkhoven E, Rose S, Lee MA, et al. 2016. Phase II study of WEE1 inhibitor AZD1775 plus carboplatin in patients with TP53-mutated ovarian cancer refractory or resistant to first-line therapy within 3 months. *J Clin Oncol.* 34(36):4354–4361.
- Li D, Marchenko ND, Moll UM. 2011. SAHA shows preferential cytotoxicity in mutant p53 cancer cells by destabilizing mutant p53 through inhibition of the HDAC6-Hsp90 chaperone axis. *Cell Death Differ.* 18(12):1904–1913.
- Lindenbergh-van der Plas M, Brakenhoff RH, Kuik DJ, Buijze M, Bloemena E, Snijders PJ, Leemans CR, Braakhuis BJ. 2011. Prognostic significance of truncating TP53 mutations in head and neck squamous cell carcinoma. *Clin Cancer Res.* 17(11):3733–3741.
- Muller PA, Vousden KH. 2013. p53 mutations in cancer. *Nat Cell Biol.* 15(1):2–8.
- Neskey DM, Osman AA, Ow TJ, Katsonis P, McDonald T, Hicks SC, Hsu TK, Pickering CR, Ward A, Patel AA, et al. 2015. Evolutionary action score of TP53 identifies high-risk mutations associated with decreased survival and increased distant metastases in head and neck cancer. *Cancer Res.* 75(7):1527–1536.
- Oren M, Tal P, Rotter V. 2016. Targeting mutant p53 for cancer therapy. *Aging (Albany NY).* 8(6):1159–1160.
- Osman AA, Monroe MM, Ortega Alves MV, Patel AA, Katsonis P, Fitzgerald AL, Neskey DM, Frederick MJ, Woo SH, Caulin C, et al. 2015. Wee-1 kinase inhibition overcomes cisplatin resistance associated with high-risk TP53 mutations in head and neck cancer through mitotic arrest followed by senescence. *Mol Cancer Ther.* 14(2):608–619.
- Osman AA, Neskey DM, Katsonis P, Patel AA, Ward AM, Hsu TK, Hicks SC, McDonald TO, Ow TJ, Alves MO, et al. 2015. Evolutionary action score of TP53 coding variants is predictive of platinum response in head and neck cancer patients. *Cancer Res.* 75(7):1205–1215.
- Pickering CR, Zhang J, Yoo SY, Bengtsson L, Moorthy S, Neskey DM, Zhao M, Ortega Alves MV, Chang K, Drummond J, et al. 2013. Integrative genomic characterization of oral squamous cell carcinoma identifies frequent somatic drivers. *Cancer Discov.* 3(7):770–781.
- Pfister SX, Markkanen E, Jiang Y, Sarkar S, Woodcock M, Orlando G, Mavrommati I, Pai CC, Zalmas LP, Drobinitzky N, et al. 2015. Inhibiting WEE1 selectively kills histone H3K36me3-deficient cancers by dNTP starvation. *Cancer Cell.* 28(5):557–568.
- Poeta ML, Manola J, Goldwasser MA, Forastiere A, Benoit N, Califano JA, Ridge JA, Goodwin J, Kenady D, Saunders J, et al. 2007. TP53 mutations and survival in squamous-cell carcinoma of the head and neck. *N Engl J Med.* 357(25):2552–2561.
- Portugal J, Mansilla S, Bataller M. 2010. Mechanisms of drug-induced mitotic catastrophe in cancer cells. *Curr Pharm Des.* 16(1):69–78.
- Prystowsky MB, Adomako A, Smith RV, Kawachi N, McKimpson W, Atadja P, Chen Q, Schlecht NF, Parish JL, Childs G, et al. 2009. The histone deacetylase inhibitor LBH589 inhibits expression of mitotic genes causing G2/M arrest and cell death in head and neck squamous cell carcinoma cell lines. *J Pathol.* 218(4):467–477.
- Rajeshkumar NV, De Oliveira E, Ottenhof N, Watters J, Brooks D, Demuth T, Shumway SD, Mizuarai S, Hirai H, Maitra A, et al. 2011. MK-1775, a potent Wee1 inhibitor, synergizes with gemcitabine to achieve tumor regressions, selectively in p53-deficient pancreatic cancer xenografts. *Clin Cancer Res.* 17(9):2799–2806.
- Roth SY, Denu JM, Allis CD. 2001. Histone acetyltransferases. *Annu Rev Biochem.* 70:81–120.
- Salim KY, Maleki Vareki S, Danter WR, Koropatnick J. 2016. Coti-2, a novel small molecule that is active against multiple human cancer cell lines in vitro and in vivo. *Oncotarget.* 7(27):41363–41379.
- Seiwert TY, Zuo Z, Keck MK, Khattri A, Pedamallu CS, Stricker T, Brown C, Pugh TJ, Stojanov P, Cho J, et al. 2015. Integrative and comparative genomic analysis of HPV-positive and HPV-negative head and neck squamous cell carcinomas. *Clin Cancer Res.* 21(3):632–641.
- Shechter D, Costanzo V, Gautier J. 2004. ATR and ATM regulate the timing of DNA replication origin firing. *Nat Cell Biol.* 6(7):648–655.
- Siegel RL, Miller KD, Jemal A. 2016. Cancer statistics, 2016. *CA Cancer J Clin.* 66(1):7–30.
- Soragni A, Janzen DM, Johnson LM, Lindgren AG, Thai-Quynh Nguyen A, Tiourin E, Soriaga AB, Lu J, Jiang L, Faull KF, et al. 2016. A designed inhibitor of p53 aggregation rescues p53 tumor suppression in ovarian carcinomas. *Cancer Cell.* 29(1):90–103.
- Squire CJ, Dickson JM, Ivanovic I, Baker EN. 2005. Structure and inhibition of the human cell cycle checkpoint kinase, Wee1A kinase: an atypical tyrosine kinase with a key role in CDK1 regulation. *Structure.* 13(4):541–550.
- Struhl K. 1998. Histone acetylation and transcriptional regulatory mechanisms. *Genes Dev.* 12(5):599–606.
- Suram A, Herbig U. 2014. The replicometer is broken: telomeres activate cellular senescence in response to genotoxic stresses. *Aging Cell.* 13(5):780–786.
- Syljuasen RG, Sorensen CS, Hansen LT, Fugger K, Lundin C, Johansson F, Helleday T, Sehested M, Lukas J, Bartek J. 2005. Inhibition of human Chk1 causes increased initiation of DNA replication, phosphorylation of ATR targets, and DNA breakage. *Mol Cell Biol.* 25(9):3553–3562.
- Tanaka N, Patel AA, Tang L, Silver NL, Lindemann A, Takahashi H, Jaksik R, Rao X, Kalu NN, Chen TC, et al. 2017. Replication stress leading to apoptosis within the S-phase contributes to synergism between vorinostat and AZD1775 in HNSCC harboring high-risk TP53 mutation. *Clin Cancer Res.* 23(21):6541–6554.
- Tanaka N, Patel AA, Wang J, Frederick MJ, Kalu NN, Zhao M, Fitzgerald AL, Xie TX, Silver NL, Caulin C, et al. 2015. Wee-1 kinase inhibition sensitizes high-risk HPV+ HNSCC to apoptosis accompanied by down-regulation of MCL-1 and XIAP antiapoptotic proteins. *Clin Cancer Res.* 21(21):4831–4844.
- Vermorken JB, Mesia R, Rivera F, Remenar E, Kaweck A, Rottey S, Erfan J, Zabolotnyy D, Kienzer HR, Cupissol D, et al. 2008. Platinum-based chemotherapy plus cetuximab in head and neck cancer. *N Engl J Med.* 359(11):1116–1127.
- Vigneswaran N, Williams MD. 2014. Epidemiologic trends in head and neck cancer and aids in diagnosis. *Oral Maxillofac Surg Clin North Am.* 26(2):123–141.
- Wang Y, Decker SJ, Sebolt-Leopold J. 2004. Knockdown of Chk1, Wee1 and Myt1 by RNA interference abrogates G2 checkpoint and induces apoptosis. *Cancer Biol Ther.* 3(3):305–313.
- Weichert W. 2009. HDAC expression and clinical prognosis in human malignancies. *Cancer Lett.* 280(2):168–176.
- Xie TX, Zhou G, Zhao M, Sano D, Jasser SA, Brennan RG, Myers JN. 2013. Serine substitution of proline at codon 151 of TP53 confers gain of function activity leading to anoikis resistance and tumor progression of head and neck cancer cells. *Laryngoscope.* 123(6):1416–1423.
- Yan W, Liu S, Xu E, Zhang J, Zhang Y, Chen X, Chen X. 2013. Histone deacetylase inhibitors suppress mutant p53 transcription via histone deacetylase 8. *Oncogene.* 32(5):599–609.
- Yu X, Vazquez A, Levine AJ, Carpizo DR. 2012. Allele-specific p53 mutant reactivation. *Cancer Cell.* 21(5):614–625.