Review article

Pharmacological targeting of mutant p53

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Abstract: TP53 is the most commonly mutated gene in cancer, with over half of all human cancers harboring a mutation in the gene. The p53 protein is a transcription factor that functions as a tumor suppressor, and a subset of its numerous roles include the arrest of proliferation, promotion of DNA repair, and induction of apoptosis in cells with severe DNA damage or stress. The vast majority of p53 mutations are single amino acid substitutions within the DNA binding domain, which either directly impede the protein's ability to bind DNA or destabilize the structure, resulting in misfolding. These missense mutant proteins are found at high levels due to loss of the MDM2 mediated regulation, and consequently serve as potential drug targets. Numerous pharmacological approaches have been investigated to restore wild type p53 function to these mutants (so-called reactivating mutant p53) with some entering in clinical trials while most have failed in early development. Recently, the field of cancer drug development has produced a number of new compounds that continue to advance this field, each with a different mechanism of action. Here we sought to review these compounds and approaches to reactivating mutant p53. Given the large number of patients with missense mutant p53 mutations, reactivating mutant p53 remains a highly sought after goal in developmental therapeutics.

Keywords: Mutant p53; mutant p53 reactivation; zinc-metallochaperone

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Introduction

More than half of all cancers harbor a mutation in p53, abrogating the protein's tumor suppressor function (1). Wild type p53 plays crucial roles in cell cycle arrest, apoptosis, and senescence (2,3), and while these mechanisms are believed to be important for its tumor suppressor functions, it is clear that other mechanisms can be involved as mutant p53 mice engineered to be deficient in the regulation of cell cycle arrest, apoptosis, and senescence still do not form tumors (4).

The majority of mutations in other tumor suppressors like APC, Rb, and PTEN are deletions or frameshift mutations that do not produce a protein (5). In contrast, p53 mutations are predominantly missense in one allele with loss of the second allele by loss of heterozygosity (6). Additionally, it has been discovered that mutant p53 not only loses wild type function, but the mutated protein may exert a dominant-negative effect on any remaining wild-type p53, further preventing the anti-tumor effects of p53 (7-9). There is now a large body of evidence that indicates mutant p53 proteins acquire gain-of-function (GOF) activity including the promotion of invasion, migration, proliferation, angiogenesis, and chemo-resistance which supports the hypothesis that these mutations are selected for during tumorigenesis (10). A therapeutic approach to restore the wild type structure and function of mutant p53 would also conceivably abrogate this GOF activity.

The different classes of mutant p53

The majority of mutations (>70%) are missense mutations in which a single amino acid change generates a defective protein (3,11). Ninety-five percent of missense mutations occur in the DNA binding domain (DBD = amino acids 94–312), affecting its function as a transcription factor. Six of these codon sites occur substantially more frequently than others and are referred to as "hotspot" codons (R273, R248, R175, G245, G249, and R282) (6,12). Tumorigenic p53 mutations fall into three broad categories: destabilizing, zinc-binding, and DNA contact (12,13). Destabilizing mutations are often found in the beta-sandwich core of DBD distant from the zinc and DNA-binding sites. They act by lowering the melting temperature of p53 to where it partially unfolds at 37 ℃. Zinc binding mutants are classified by their proximity to the loops involved in coordinating the zinc ion (14). The most well characterized zinc-binding mutant is R175H, which is the most frequently found missense mutation is cancer (6). When the R175H DBD loses its zinc ion it misfolds, and loses its ability to discriminate between consensus and non-consensus DNA sequences (15). In contrast, DNA contact mutations such as R248W and R273H typically diminish DNA affinity while having little effect on stability or zinc-binding affinity and hence resemble the WT structure.

MDM2 is the primary E3 ubiquitin ligase that targets both wild type and mutant p53 for proteasomal degradation (6,10). MDM2 is a p53-target gene, and thus it exists in a negative feedback loop, keeping p53 levels low under non-stressed conditions (6). In wild type cells, inhibition of MDM2 in response to stress allows for the activation of p53 (6). While it was long believed that the loss of p53-mediated transcription of MDM2 leads to overexpression of p53, this only partially explains the hyperstability of mutant p53. Unlike wild type p53, the unfolded and aggregated mutant p53 proteins are bound by HSP90, which blocks the interaction of endogenous MDM2 and CHIP (carboxy-terminus of Hsp70-interacting protein) with mutant p53. Interference of the HSP90-mutant p53 interaction, knockdown of Hsp90 protein, or pharmacological inhibition of Hsp90 activity with 17AAG, destroys this complex, releasing mutant p53, and allowing MDM2 and CHIP to degrade mutant p53 (16). Overexpression and amplification of MDM2 is seen in many cancers that lack p53 mutations, which has led some to hypothesize that this is an alternative mechanism of inactivating wild type p53 activity (17).

Several research strategies have been pursued in attempts to either restore the wild type structure and function of mutant p53 or to enhance the activity of wild type p53 (10,18,19). Enhancing the activity of wild type p53 comes with its own concerns, as the activation of wild type p53 in normal tissue may potentially be harmful to benign tissue. While several small molecules capable of reactivating the wild type activity of mutant p53 have shown promise in pre-clinical studies, most have failed in early phase development. PRIMA-1 and its metabolite APR-246 demonstrated the ability to reactivate mutant p53 in pre-clinical studies and went on to become the first in-human clinical trial of a mutant p53-targeting drug (20,21). PRIMA-1 has completed Phase I/IIa trials for refractory hematological malignancies and prostate cancer, which showed that the drug is relatively well tolerated, with mild and transient side effects (NCT00900614). This trial involved 22 patients with hematological malignancies or prostate cancer, who received 2-hour intravenous infusions once per day for 2 consecutive days. While 113 adverse events were recorded in 18 of the 22 patients, only 38 adverse events in 12 patients were judged as being related to the drug, whereas all others were deemed to be a result of the primary malignancy (21). PRIMA-1 has now entered Phase Ib/II trials and is being tested in combination with carboplatin and pegylated doxorubicin in high-grade serous ovarian cancer, a malignancy with 95% frequency of p53 mutations (21) (NCT02098343). Despite the clear indications for targeting mutant p53, developing drugs that restore the wild type structure and function of mutant p53 have proven exceedingly difficult.

Here we offer a focused review of the latest research in the pharmacological reactivation of mutant p53 organized by the different mechanisms of action.

Small peptide conformational stabilization of mutant p53

Tal et. al developed a system using phage display to select mutant p53 reactivating peptides (22). Briefly, their system involved the incubation of PAb1620 (WT p53-specific antibody) cross-linked beads in a solution with a phage library and purified recombinant mutant p53 (p53 R175H, R249S, V143A). While mutant p53 fails to be recognized by PAb1620, if the mutant protein were to bind to a peptide that stabilized its wild type conformation, mutant-p53 would then be expected to be detected by PAb1620 staining (22). Binding of PAb1620 cross-linked beads to the phage displaying mutant p53 would result in an immobilized PAb1620-mutant p53-phage complex, which could be

extracted for identification of the particular phage particle. An inherent downside of the phage display system is the relatively high rate of false positive phage bindings to non-specific elements within the experimental system (23). To overcome this hurdle, the researchers replaced PAb1620 with immobilized p53 response elements (p53RE, short segments of DNA specifically bound by WT p53) or SV40 large T antigen (LT-Ag) which also binds preferentially to WT p53 (22). The researchers alternated between all three selection processes to increase the likelihood of identifying a biologically relevant peptide.

Three hundred and fifty p53 conformation activating peptides (pCAPs) were advanced to the next stage of semi high-throughput functional screening. Cell-free ELISA assays were used to evaluate the effect of the peptides on mutant p53 DNA binding and WT conformation reactivation *in vitro*. A subset of peptides were capable of shifting PAb240 (mutant p53-specific) to PAb1620 (WT p53-specific) staining as well as restore sequence-specific DNA binding to mutant p53 (22). To evaluate whether the peptides had reactivating effects in live cells, the researchers employed a crystal-violet based viability assay using H1299 cells expressing R175H. 30 of the most promising lead peptides were identified through this next screen.

In a cell-based model, the peptides were able to induce p53 target gene activation including *p21*, *PUMA*, and *Mdm2*. Furthermore, chromatin immunoprecipitation (ChIP) analysis revealed the ability of the peptides to restore the binding of mutant p53 to p53REs within living cells (22). Likewise, using Annexin V and propidium iodide (PI) stains, the authors were able to demonstrate that the peptides were able to induce apoptosis in mutant p53 cells, providing more evidence of reactivation of p53-dependent apoptosis.

To evaluate the anti-tumoral effects of the peptides, subcutaneous human xenograft models in nude mice were injected intratumorally with either active or control peptides. Intratumoral injection of 3 p53 reactivating peptides, but not control peptides, resulted in a decrease in tumor cells, as inferred by luciferase intensity. Notably, 12 days after treatment the average tumor luminescence decreased by 93%, with 11/18 tumors showing a complete response. Additionally, analysis of excised tumors revealed that those treated with p53 reactivating peptides were smaller than control peptide-treated tumors, as well as displaying significantly higher levels of the p53 targets p21 and MDM2. In total, the researchers examined the peptides' *in vivo* activity in 3 mouse cancer models—breast (R280K and R175H), ovarian (R241H), and colon (R273H/P3092)—each showing significant anti-tumoral activity (22). The R280K and R273H are reported as contact mutants, making it unclear why these mutants would provide hits with peptides in this assay, as this assay was a screen for conformational mutant reactivation.

Fungal extract (CTM) reactivates mutant p53-R175H

Hiraki *et al.* screened a chemical library containing 20,000 compounds and 36,256 natural extracts from the National Cancer Institute Natural Products repository through the use of a luciferase report system expressing the p53 DNA binding site of the *PUMA* promoter in a stable cell line with mutant p53 R175H (24). Compounds were evaluated for their ability to restore WT activity to mutant p53-R175H as measured by an increase in the luciferase activity of the *PUMA* promoter. The initial screen identified five hits that displayed a greater than 2.5-fold increase in luciferase activity compared with DMSO control. All of the top five compounds were from the fungal extract library. High-performance liquid chromatography (HPLC) was performed to identify the active molecules(s) from the natural extracts. Nuclear magnetic resonance spectroscopy was then used to further identify the compounds within the extracts. Chetomin (CTM), was identified as the fungal-derived compound which produced the largest increase in luciferase activity in their system, suggesting CTM is capable of restoring WT p53 activity to mutant p53-R175H (24).

The anticancer activity of CTM was evaluated by treating human cancer cell lines with p53 R175H, R273H, WT p53, p53 null, and also normal cells. CTM was most cytotoxic to p53 R175H cell lines, and p53 target genes (*p21*, *PUMA*, and *MDM2*) were significantly induced upon CTM treatment. Furthermore, CTM induced the protein expression of p21 and PUMA in a dose-dependent manner in a variety of p53 R175H cell lines, whereas minimal or no induction was measured in cancer cell lines with other p53 mutations, WT p53, or p53 null cells. siRNA knockdown of p53 R175H resulted in the loss of induction of the proteins of the p53 target genes *p21*, *PUMA*, and *NOXA* Furthermore, in p53-R175H cells, CTM increased the p53 promoter occupancy at *p21*, *PUMA*, and *MDM2* promoters, indicating CTM restores DNA binding activity of mutant p53-R175H. Together, these results suggest CTM functions through a p53-R175H-dependent manner (24).

The antitumor effects of CTM were investigated using

mouse xenografts of a variety of tumor cell lines carrying mutant p53 R175H, R273H, or p53 null cells. In p53-R175H tumors, CTM treatment significantly decreased tumor volume and weight; however, CTM failed to inhibit p53 R273H or p53 null tumor growth, providing further evidence of the allele-specific effects of CTM.

The authors next set out to decipher the mechanism of CTM reactivation of mutant p53 R175H. CTM failed to bind p53 directly, so they explored whether CTM binds to p53 binding partners through a co-immunoprecipitation-coupled mass spectrometry analysis. This assay revealed that upon CTM treatment, some p53 binding partners displayed increased p53 binding—among these proteins was Hsp40. Heat-shock proteins have been known to be p53 binding partners, and also to function as chaperones to regulate protein conformation and stability (25,26). Hsp40, but not Hsp90, showed increased binding to p53 R175H upon CTM treatment. Hsp40 did not show increased p53 R175H binding in response to DNA-damaging agents (ETO and camptothecin) or mutant p53 reactivators (MIRA-1 and PRIMA-1) (24). siRNA knockdown of Hsp40 resulted in impaired protein level induction of p53 target genes after CTM treatment. The Biocore assay, which measures surface plasmon resonance, showed CTM binds to Hsp40 with a K_D value of 3.7 μM. Using the same assay, the binding of CTM to mutant p53 R175H was analyzed, which failed to detect a significant interaction. The authors conclude that CTM reactivated mutant p53 R175H without directly binding p53, but rather by increasing the binding of Hsp40 to p53 R175H. The CTM-Hsp40-p53 R175H complex can be recognized by the WT p53-specific antibody PAb1620 (24).

Dietary extract phenethyl isothiocyanate (PEITC)

The dietary extract PEITC, found in watercress and cruciferous vegetables, has long been observed to have cancer chemopreventive effects in animal models, and epidemiological studies have supported the consumption of isothiocyanates in protection against human cancers. Despite PEITC having been studied in both phase 1 and phase 2 clinical trials (http://www.clinicaltrials. gov/ct2/results?term=PEITC), its mechanism of action is still unknown.

To begin to explore the mechanism of PEITC, Aggarwal *et al.* screened PEITC against a panel of cancer cell lines harboring the p53 hotspot codons 175, 248, and 273. PEITC had the largest anti-proliferation effect in cells expressing p53-R175H, with IC_{50} values approximately 2.5-5-fold lower than in cells with the 248 and 273 mutations. siRNA knockdown of p53-R175H resulted in reduced sensitivity to growth inhibition by PEITC. WT-p53 cells treated with PEITC had no significant difference in proliferation whether transfected with p53 siRNA or nonspecific siRNA. Taken together, this data suggests that the anti-proliferation activity of PEITC is at least partially dependent on p53-R175H (27).

After observing that PEITC induced apoptosis most strongly in p53-R175H cancer cells, the authors sought to determine whether restoration of WT structure and function triggered the induction of apoptosis. Conformation specific antibodies are commonly used to evaluate the ability of compounds to restore the WT structure to mutant p53 (24,28,29). In both an ELISA and cell-based assay, PEITC increased the PAb1620 (WT) staining and decreased the PAb240 (mutant) staining of mutant p53-R175H, demonstrating that PEITC induces reactivation of WT p53 structure (27).

To evaluate whether the WT function of p53 is restored to p53-R175H by PEITC, the researchers measured whether the molecule could increase the amount of chromatin-bound p53-R175H. PEITC increased the chromatin-bound fraction of p53-R175H in SK-BR-3 cells, as well as enhanced the expression of the p53-target genes *p21*, *MDM2*, *PUMA*, *NOXA*, *BCL2* and *BAX* (27).

High doses of PEITC (>10 μM) have been shown to selectively deplete levels of mutant p53 protein, but not WT p53; however, this mechanism is not yet understood (30). SK-BR-3 cells treated with both PEITC and MG132 (proteasome inhibitor) displayed a significant accumulation of p53 in both the insoluble fraction, as well as the whole-cell lysate, as compared to cells treated with either drug alone. Further experimentation showed that doses of PEITC ≥8 μM resulted in aggregation of p53-R175H. Protein aggregates are typically cleared by autophagy, so the authors investigated whether p53-R175H protein aggregates undergo autophagy. SK-BR-3 cells treated with 8 μM PEITC and 50 μM chloroquine (inhibitor of autophagy) displayed an increase in p53 in the whole cell lysate, compared to cells treated with either drug alone. Taken together, the data suggests that PEITC reduces mutant p53-R175H protein levels by both the MDM2-mediated proteasomal degradation pathways and autophagy (27).

Using an SK-BR-3 xenograft mouse model, the researchers investigated the anti-tumor effects of PEITC *in vivo*. Mice fed a diet containing PEITC (5 μmol/g) had a statistically significant inhibition of tumor growth

($P<0.05$) compared to mice on a control diet. However, tumor volumes decreased in the control mice after week 6, which the authors believe to be due to the relatively non-aggressive phenotype of SK-BR-3 cells. Tumors from mice in the PEITC group had markedly fewer Ki67- and p53-mutant staining cells. Further, the mRNA and protein expression of p53-target genes p21 and Bax were increased in PEITC-fed mice compared to controls.

Peptide inhibitors of mutant p53 aggregation

Recent discoveries have revealed that p53 aggregates *in vitro* (31), and several p53 mutants form amyloid aggregates in both cancer cell lines (32) and breast cancer biopsies (33). Structural mutations of p53 uncover the highly adhesive residues 252–258, resulting in the highly favorable process of protein aggregation, where the exposed residues adhere to one another on different mutant p53 proteins(12,34). Aggregation of p53 depletes the cell of the functional protein, resulting in loss of its transcriptional capacity and apoptosis-promoting activity (32).

While many of the approaches to reactivate mutant p53 have focused on stabilizing the native folding, Soragni *et al.* developed a peptide (ReACp53) to halt aggregation of p53 in cells (35). Previously, it has been reported that the residues 252–258 are the most aggregation-prone in the DNA binding domain (DBD) of p53 (36). The authors first synthesized the aggregation-region and observed formation of amyloid-like fibrils and microcrystals, which allowed for their structure determination. Using a rational-design approach, the investigators synthesized peptide inhibitors of p53 aggregation, with the p53 sequence as a backbone, and incorporating aggregation-inhibiting sequences (35). To improve entrance into cells, they fused an N-terminal poly-arginine cell-penetrating tag, followed by a three-amino acid linker to the peptide—this candidate peptide was termed ReACp53.

Primary cells were isolated from high-grade serous ovarian carcinoma (HGSOC) patients harboring a variety of p53 mutations. After confirming that ReACp53 could penetrate the primary cells, they tested its ability to prevent aggregation of mutant p53. Cells from patients with the R248Q mutation all exhibited p53 aggregation in the cytosol, and 16–24 hours after ReACp53 treatment, cytosolic aggregation of p53 was markedly reduced, and p53 could be detected in the nucleus of 70–100% of cells (35).

Mutations in p53 abrogate the protein's transcriptional functions, resulting in loss of p53-dependent gene expression, including pathways involved in apoptosis, cell-cycle arrest, and negative regulation of p53 expression. Mutant p53 accumulates to high levels in cells, due to the loss of MDM2-mediated negative feedback. The ReACp53 peptide was able to reduce cell viability in a dose-dependent manner, while neither a control scrambled ReACp53 nor the poly-arginine tag had any effect on cell viability (35). Similarly, the ReACp53 peptide increased the percentage of apoptotic cells, while the scrambled peptide did not. Further, ReACp53 allowed the cancer cells to enter cell-cycle arrest, while untreated and scrambled-treated cells had fewer cells in G0/G1 and more in the G2/M phase. ReACp53 treatment resulted in lower levels of mutant p53 in cells, while co-treatment with Nutlin-3 (an MDM2-p53 interaction inhibitor) resulted in higher levels of p53, suggesting that ReACp53 allows for the refolding of mutant p53 into a WT conformation capable of interacting with p53. Notably, co-treatment of ReACp53 and Nutlin-3 lowered the EC_{50} of the peptide, suggesting synergy, most likely due to increased levels of properly folded p53 (35).

To more closely recapitulate the conditions of a tumor, the researchers developed organoids from a panel of mutant p53, WT p53, and p53 null cancer cell lines, as well as patient derived cells and treated them with ReACp53. When treated with ReACp53, organoids bearing aggregation-prone p53 mutations had reduced viability, loss of organoid morphology, and increase in apoptosis. Organoids derived from cell lines with WT p53 or p53 null cells did not display the same sensitivity to ReACp53, suggesting the activity of the peptide is specific for p53 aggregation (35). RNAseq was performed on ReACp53-treated organoids harboring either mutant p53 or WT p53. Only the mutant-p53 organoids treated with ReACp53 showed an increase in expression p53-target genes (*p21*, *GADD45B*, *PUMA*, *THBS1*, *NOXA*, and *DRAM1*)—no such increase was seen in the treated WT organoids. Taken together, this suggests that ReACp53 specifically acts on aggregation of mutant p53 and restores structure and function, while having no effect on WT p53.

To test the *in vivo* efficacy of ReACp53, a xenograft mouse model was used in which one flank was injected with OVCAR3 (mutant p53) cells and the contralateral flank injected with MCF7 (WT p53 cells), which served as an internal control. Treatment consisted of 3 weeks of daily intraperitoneal injections of ReACp53, sequence-scramble control peptide, or vehicle alone. Only OVCAR (mutant p53) tumors treated with ReACp53 decreased in size, while the vehicle and scramble-control tumors more

than doubled in size. MCF7 xenografts (WT p53) did not respond to ReACp53 treatment, further providing evidence the ReACp53 activity is specific for mutant p53. As in the *in vitro* experiments, ReACp53 treatment increased expression of the p53 target genes *p21* and *MDM2* in mutant p53 bearing tumors, but not WT tumors (35).

Zinc metallochaperones

Proper folding of the p53 protein is dependent on its binding of a single zinc ion, and there is substantial evidence showing that manipulation of intracellular zinc concentrations can alter the structure and function of wild type p53 (37-39). This concept extends to a subset of mutant p53 proteins in which the missense mutation impairs the protein's ability to coordinate zinc, resulting in misfolding and loss of wild type structure and function. Supplemental zinc in culture media has been shown to restore the wildtype structure to some mutant p53 cells, as well as restoring expression of wild type p53-dependent gene expression (40-42). Recently, zinc metallochaperones (ZMCs) have been identified as a novel approach to targeting p53 mutants that have lost the ability to bind zinc (28). These small molecules are termed zinc metallochaperones (ZMC) and have the ability to bind zinc outside the cell, transport the zinc into the cytoplasm, and donate the zinc ion to the mutant p53 to enable proper folding (43,44). ZMC1 is capable of restoring the wild type structure and function to p53-R175H, the most commonly occurring mutation of p53. The R175H mutation in p53 results in inactivation of p53, as the mutated protein has reduced affinity for zinc, resulting in loss of zinc binding in the low-zinc concentration of the cell. Additionally, *in vitro* and *in vivo* experiments with ZMC1 have shown that the small molecule is capable of mutant-p53 specific cell killing and xenograft tumor regression (28,43,44).

ZMC are defined by two properties—they must increase the free concentration of zinc within the cell, and buffer the zinc concentration to the range most appropriate to donate zinc to the target protein (29,44). Furthermore, a ZMC does not bind the target protein; rather, it acts solely through the binding and release of free zinc ion (44). ZMCs function as ionophores, transporting extracellular zinc across the plasma membrane into the cell (44).

The anti-cancer mechanism of ZMCs is actually two folds: (I) restoration of WT structure to mutant p53 by providing the optimal free zinc concentration for proper folding of p53-R175 and (II) increasing reactive oxygen species (ROS) via Fenton chemistry, which induces the DNA damage response through ATM, which results in the post-translational phosphorylation (serine-15 and serine-46) and acetylation (lysine-120) that activate the transcription of genes in the apoptotic program (28,29,43,44). p21, PUMA, and BAX are among the p53-dependent genes that are expressed in both cells and tumors bearing the p53-R175H treatment after ZMC1 treatment.

Table 1 provides a brief summary of the compounds discussed in this review.

Conclusions

TP53 is the most commonly mutated gene in cancer, as such it is an attractive pharmacological target. Numerous studies in murine cancer models have shown that restoration of wild type p53 function in tumors is highly therapeutic (45,46). Given the preponderance of tumors expressing missense mutant p53, there remains a large unmet need in cancer drug development for mutant p53 reactivators. Despite decades of research, unfortunately there is still not one approved mutant p53 drug. Early strategies were specific for wild type p53 and included reintroduction of wild type protein into cancer cells using viral vectors (47) and inhibition of the p53-MDM2 interaction (48). Debate remains on the potential negative consequences of systemic activation of WT p53, thus therapies that specifically reactivate mutant p53 within malignant tissue are hypothesized to have fewer widespread toxicities.

Within the past few years there has been a surge of interest in the development of mutant-p53 reactivating molecules. While the mechanisms of action of these new therapies vary, as a whole they tend to target a specific subset of missense mutations in p53, restore WT structure and function to the mutant proteins, increase p53-mediated gene expression, and result in tumor regression. Notably, these molecules lack activity for WT p53, thus display specificity for malignant tissue.

An important area of future research is the application of combinatorial therapy with p53 reactivating drugs. Reactivation of mutant p53 restores WT structure and function, but supplementary induction of p53 may provide additional anti-tumoral effects. Traditional chemotherapeutic agents that further stress the cells by increasing reactive oxygen species, inhibiting cell division, damaging DNA, or impairing DNA repair should be evaluated alongside p53 reactivating molecules, as combination therapy likely result in greater death of the

Table 1 Compounds targeting mutant p53

Molecule/compound	Mechanism of action	Targeted p53 missense mutation	References
Small peptide mutant p53 conformation stabilizers (pCAPs)	Believed to preferentially stabilize the transient WT conformation of the mutant protein	R175H; R280K; R241H; R273H/P309S	Tal *et al*. (22)
CTM	Binds Hsp40, enhancing its binding to mutant p53, and restoring WT-structure	R175H	Hiraki *et al*. (24)
PEITC	Not yet known. Reduces levels of R175H through induction of proteasomal and autophagy degradation	R175H	Aggarwal *et al*. (27)
ReACp53	Cell-penetrating peptide designed to inhibit aggregation of mutant p53	R248Q; R175H	Soragni *et al*. (35)
ZMC1	Increases intracellular free zinc, allowing donation to R175H; modulation of cellular ROS to transactivate the reactivated p53	R175H	Yu *et al*. (28,43); Blanden *et al*. (44)

malignant cells. The adverse effects of these conventional cancer therapies may potentially be significantly reduced with the lower doses used in dual therapy.

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Footnote

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