Cancer Chemotherapy and Pharmacology (2019) 83:1–15
https://doi.org/10.1007/s00280-018-3701-x

REVIEW ARTICLE

# Awakening the "guardian of genome": reactivation of mutant p53

Akshay Binayke[1] · Sarthak Mishra[1] · Prabhat Suman[1] · Suman Das[1] · Harish Chander[1]

Received: 29 March 2018 / Accepted: 10 September 2018 / Published online: 15 October 2018


**Abstract**
The role of tumor suppressor protein p53 is undeniable in the suppression of cancer upon oncogenic stress. It induces diverse conditions such as cell-cycle arrest, cell death, and senescence to protect the cell from carcinogenesis. The rate of mutations in p53 gene nearly accounts for 50% of the human cancers. Upon mutations, the conformation gets altered and becomes non-native. Mutant p53 displays long half-life and accumulates in the nucleus and interacts with oncoproteins to promote carcinogenesis and these interactions present a formidable challenge for clinicians in therapy of the disease. Variety of approaches have been developed, through which native-like function of p53 can be restored, such as restoration of the native-like structure of p53, activating the p53 family members, etc. Modern scientific techniques have led to the discovery of a variety of molecules to reactivate mutant p53 and restore its transcriptional activity. These compounds include small molecules, various peptides, and phytochemicals. In this review article, we comprehensively discuss these molecules to reactivate mutant p53 to restore the normal function with a particular focus on molecular mechanisms.

**Keywords** Mutant p53 · Reactivation · Gain-of-function · Cancer therapy · Drug target

## Introduction

One of the most studied and altered gene in cancer is tumor suppressor p53. p53 is regarded as "Guardian of the Genome" because of its role in the prevention of cancer [1]. Several missense mutations are often found to alter tumor protein p53 in its DNA-binding domain; and these mutations nearly account for 70% of the alterations and results in its wide range of cellular effects which controls diverse group of biological activities [2, 3]. TP53 functions as a transcription factor that guards the cells against different stress signals through activation of cellular mechanisms like cell-cycle arrest, apoptosis, and senescence, therefore, which serves as a crucial tumor suppressor [4]. During tumor development, TP53 gene is often mutated, and this mutation may inactivate the protein and thereby lead to the loss of its tumor suppressor functions. Upon mutations and loss-of-function in most of the cancers, TP53 gene, the "Guardian of the Genome" is transformed into "Rebel Angel" [5]. In several other cancers, where p53 is not mutated, the wild-type function is, however, compromised through various inhibitory mechanisms [1, 2]. One example of such inhibitory mechanisms is the overexpression of E3 ubiquitin ligase Mouse double minute 2 homolog (MDM2), which upon binding to p53 causes inhibition of its transcriptional activity and promotes its proteasomal degradation, another reason is the lack of its chaperones or activators [5, 6]. Either because of mutations or lack of chaperones, the loss-of-function of tumor suppressor p53 plays a significant role in initiation and evolution of carcinoma. The strong association of p53 mutations and carcinogenesis suggests that cancer cells equip themselves with a survival strategy [7, 8]. Moreover, it is well understood that the expression of mutant p53 does not equal p53 loss, and the mutated protein gains new functions that promote carcinogenesis [2, 3]. The clustering of mutations shows that vital functions like DNA-binding activity are altered—suggesting that the alteration in transcriptional targets could be a key to the activity of mutant p53. However, it is important to note that mutations in the structural core of p53 gene can also have significant consequences on the folding of the protein [7]. These mutations ultimately result in the non-native conformation of the protein, which could arise not only because of mutation but also due to the

✉ Harish Chander
harish.chander@cup.edu.in

[1] Laboratory of Molecular Medicine, Department of Human Genetics and Molecular Medicine, School of Health Sciences, Central University of Punjab, Bathinda 151001, India

lack of chaperones [5]. Mutant p53 accumulates in a cell and MDM2 is no longer able to degrade it efficiently. As a result, mutant p53 displays extended half-life due to the non-induction of MDM2 in the absence of wild-type p53 [5]. A mutant form of p53 not just accumulates in the nucleus of the cell, but also interacts with many other proteins. These novel protein–protein interactions either lead to the disabling of other tumor suppressors such as p63 and p73 or responsible for the enabling of oncogenes such as electron transport system (ETS) family members, Necrosis Factor kappaB (NF-KappaB), etc. [9–11]. This kind of cooperation between mutant p53 and oncogenes furnish the cancer cell with a variety of strategies to promote carcinogenesis.

Given the fact that p53 is most frequently mutated in cancer and its characteristic gain-of-function tactic to promote carcinogenesis, mutant p53 becomes a promising therapeutic target for cancer treatment [2, 9, 12, 13]. The strategies that are commonly employed for targeting mutant p53 includes (a) reactivation or restoration of wild-type p53 activity and elimination of mutant p53; (b) destabilization of the oncogenic mutant p53 protein; and (c) inhibiting the downstream signaling resulting from mutant p53 gain-of-function and initiating synthetic lethality in the cells expressing mutant p53. The burden of mutant p53 in cancer cells is much higher, and due to its high burden, it can serve as a better substrate for reactivation and can be very effective in stimulating cell death pathways [14]. Reactivation seems to be the only strategy so far to counteract the effects of mutant p53, and this might question the possibility that is the restoration of wild-type p53 function sufficient for tumor eradication? In addition, in advanced cancers, where multiple oncogenic mutations like those in Ras, Myc, phosphatase and tensin homolog (PTEN), or phosphoinositide-3 (PI-3) kinase gene has taken place? The answer to these questions is yes, and reactivation of mutant p53 alone is sufficient for elimination of tumor-associated genetic alterations. These findings have been supported by several in vivo studies which have concluded that the restoration of wild-type p53 activity in mice results in rapid regression of the tumor [15–17]. These results are as a consequence of the central role of p53 in pro-apoptotic or pro-senescence response to oncogenic stress. The presence of the other oncogenic mutations would further enhance the stress and p53-mediated cell death upon p53 reconstitution in a tumor cell [15–17]. Another set of studies also suggested that the knockdown of mutant p53 is also a powerful strategy in reducing the oncogenic potential of cancer cells, expressing only mutant p53 [18–21], since malignant properties are supported by the gain-of-function transcriptional activity of mutant p53. In this review, we highlighted the recent information about the compounds, peptides, small molecules, etc. that were studied as mutant p53-reactivating compounds.

## Approaches to reactivate mutant p53

p53 is involved in a variety of cellular responses that can efficiently reduce the chances of the normal cell from taking a turn towards carcinogenesis [22]. Since mutant p53 displays longer half-life and interact with a variety of proteins to promote oncogenesis, reactivation strategy is the most influential for the treatment of cancers expressing high levels of the mutant form [14]. Several strategies have been employed to restore the transcriptional activity of the mutant p53 which are summarized as follows (Fig. 1): (a) exploitation of temperature-sensitive characteristic for the possible restoration of the p53 function at the permissive temperature [23, 24]; (b) small peptides which restore wild-type p53 transcriptional activity [25, 26]; (c) incorporation of secondary mutations or an N-terminal deletion to restore p53 function [27–29]; (d) small molecules may disrupt the complex association between p53 family members (p63 and p73) with mutant p53, thus restoring the tumor suppressor function of p63 and p73 [30]; and (e) binding of small molecules directly to the appropriate sites of mutant p53 to stabilize the core domain and promote wild-type folding [30].

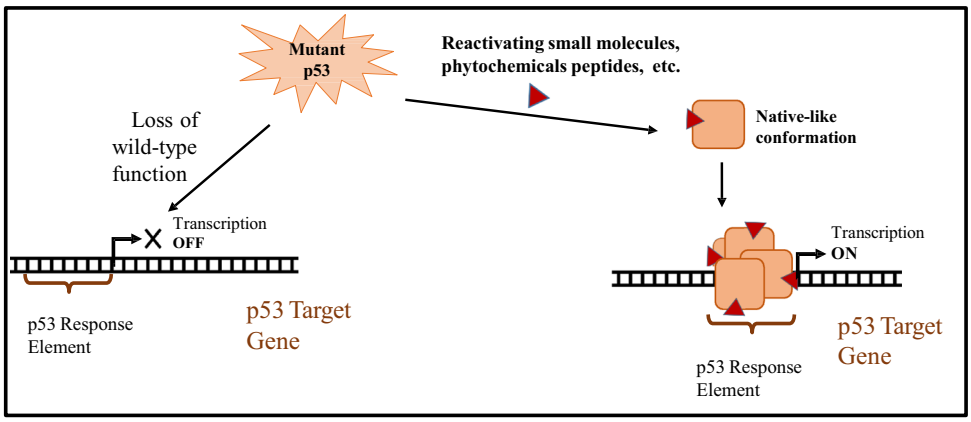



**Fig. 1** Effect of reactivating molecules on mutant p53. Upon mutation, wild-type p53 loses its function and transcription of target genes gets repressed. The treatment of mutant p53 reactivating molecules leads to native-like conformational restoration of transcriptional activity

## Small molecules

Small molecules are the chemical entities with low molecular weight that includes metabolites, monosaccharides, lipids, second messengers, various natural products as well as a variety of drugs and xenobiotics with low molecular weight. From the compendious categories, variety of small molecules are known to act on mutant p53 to restore its wild-type activity through different mechanisms which have been studied are shown in Fig. 2 and summarized below.

### CP-31398

A styrylquinazole was discovered through high-throughput screening and proved to restore the DNA-binding ability of mutant form of p53 using conformation-specific antibody PAb1620 as a probe [31, 32]. Treatment of this drug on Saos-2 (p53 null) cells expressing $p53^{V173A}$ and $p53^{R249S}$ mutants resulted in increased expression of p21 mRNA [31]. CP-31398 treatment resulted in reduction in tumor growth in urothelial cancer and induced apoptosis of tumor cells along with elevated levels of p21 in $APC^{min}$ colon cancer mouse model [33, 34]. In another study on pancreatic adenocarcinoma cell lines, CP-31398 induced cell growth inhibition, apoptosis, and autophagy by activating p53 phosphorylation (S15) and p53-DNA binding, without affecting the total p53 amount [35].

### STIMA-1 (SH group-targeting compound that induces massive apoptosis)

STIMA-1, a CP-31398 derivative is a small molecule which shares the feature of CP-31398 and MIRA, in reacting with *N*-acetylcysteine and alkylate thiols in p53 restore its redox status which is crucial for the tumor suppressor activity of p53 [36]. It showed a significant mutant p53-dependent growth-inhibitory effect in H1299 (transfected with mutant $p53^{R175H}$) lung carcinoma and Saos-2 (mutant $p53^{R273H}$) cells [37]. STIMA-1 has also been shown to induce the expression of various downstream targets of p53 by increasing the DNA-binding ability of mutant p53 [37].

### PRIMA-1 and APR-246 (PRIMA-$1^{MET}$)

A cell-based assay screening of over 2000 compounds from national cancer institute found the compounds PRIMA-1 (p53 reactivation and induction of massive apoptosis) and APR-246 [30]. PRIMA-1 [2,2-bis(hydroxymethyl)-1-azabicyclo(2,2,2)octan-3-one] restored active conformation of mutant p53 resulted in a native state conformation of mutant p53. This restoration ultimately led to the DNA-binding triggering of apoptosis [38]. Further investigations revealed that PRIMA-1 as well as APR-246 (PRIMA-$1^{MET}$), a more active methylated derivative of PRIMA-1, inhibited mutant p53 expressing cells as well as xenograft tumors in animal models [39]. With IC-50 value ranging

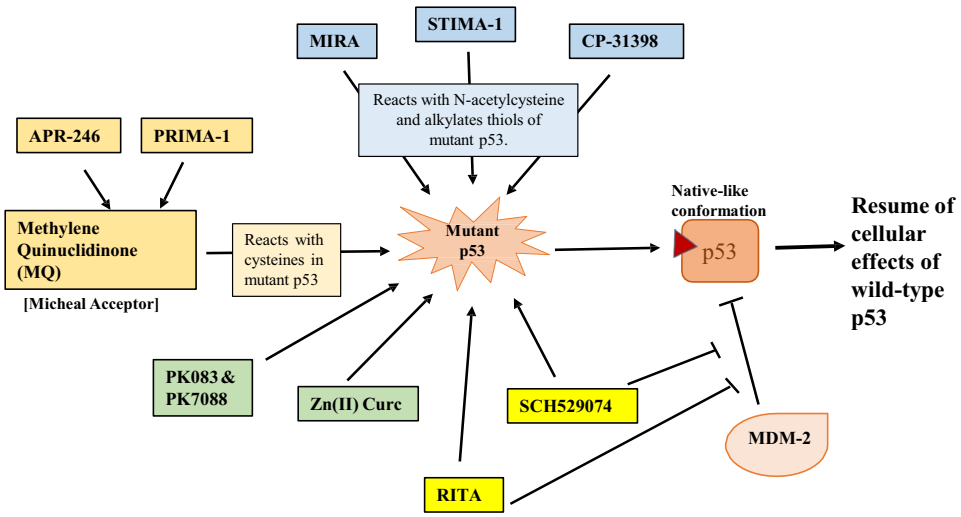



**Fig. 2** Summary of mechanism of action for drugs that target mutant p53. Redox status is crucial for tumor suppressor activity of p53. By reduction of mutant p53 at specific sites, native-like conform can be achieved. MIRA, STIMA-1, and CP-31398 reacts with *N*-acetylcysteine and alkylate thiols of mutant p53 resulting in its conformational restoration. Alternatively, PRIMA-1 and APR-246 are both converted into methylene quinuclidinone (MQ), and it is a Micheal acceptor that reacts covalently with the cysteines in mutant p53 and thus leads to an oxidative environment in the tumor cells, thereby restoring the reactive oxygen species (ROS)—inducing capacity to mutant p53. PK083, PK7088, Zn(II) Curc, RITA, and SCH529074 bring about restoration of native structure by directly interacting with the mutant p53. Along with the reactivation of mutant p53, RITA and SCH529074 upon binding inhibit the ubiquitination of p53 by MDM2, thus increasing the half-life of the native-like mutant p53 protein

below 20 µM in a broad variety of mutant p53 expressing cell lines, insignificant toxicity was evident in animal models [38, 40]. PRIMA-1 and its analog altered several target genes which were induced by classical wild-type p53. A strong synergy has been observed between APR-246 and DNA-damaging drugs when treated on primary cancer cells from patients of ovarian cancer with mutant p53 and the use of this compound also displayed antitumor activity in other cancers [41].

PRIMA-1 and APR-246 are both converted into methylene quinuclidinone (MQ), and it is a Micheal acceptor that reacts covalently with the cysteines in mutant p53 and thus creates an oxidative environment in the tumor cells [42]. Recently, the synergistic effect of APR-246 with $^{V600E/K}$BRAF inhibitor vemurafenib in inducing apoptosis, arresting proliferation and tumor growth, has been demonstrated both in vitro and in vivo [43]. Currently, APR-246 is the only mutant p53-targeting compound in clinical trials that showed positive signs and promising results regarding clinical effects in ovarian, prostate, and hematological malignancies [44–46]. Evidences that showed APR-246 acted via p53 reactivation includes initiation of cell-cycle arrest, increased apoptosis, and upregulation of several p53 target genes in numerous subjects [47]. This study, therefore, validates that APR-246 is safe at expected therapeutic plasma levels, can induce biological effects in tumor cells in vivo, and has a positive pharmacokinetic profile.

## MIRA-1 (mutant p53 reactivation and induction of rapid apotosis 1)

A maleimide derivative compound MIRA-1 (NSC19630) showed the preferential killing of tumor cells expressing mutant p53 and the potency of MIRA-1 for inducing cell death in a mutant p53-dependent manner is even higher than that of PRIMA-1 [27]. MIRA-1 and its analogs (MIRA-2 and MIRA-3) tend to shift the unfolded conformation of p53 towards the native structure, leading to restoration of transcriptional activity. Therefore, induction of p53-dependent apoptosis of cancer cells expressing p53R175H, p53R248Q, and p53R273H has been demonstrated [27]. The study also reported in vivo antitumor activity of MIRA-3 against human mutant p53-carrying tumor xenografts in SCID mice. However, the compound was toxic at higher levels and, therefore, has narrow therapeutic window [27]. Moreover, MIRA-1 treatment along with other anti-myeloma agents in multiple myeloma cells displayed endoplasmic reticulum stress mediated apoptosis in a p53-independent fashion [48]. MIRA-1 unaided or in synergy with dexamethasone arrested tumor growth and extended survival without exhibiting any toxicity in the mice-bearing multiple myeloma tumor [48].

## PK083 and PK7088

One of the most common mutations in cancers is $p53^{Y220C}$ which occurs in about 75,000 cases every year in the structural domain of p53 and causes the formation of a cavity that destabilizes its structure [49]. PK083 is a carbazole derived compound that binds to the cavity of the protein $p53^{Y220C}$ and stabilizes its structure by supporting the S7/S8 loop of mutant p53 [50], thus increasing its half-life from 3.8 to 15.7 min [49]. It also raises the melting temperature by 2 °C, thereby increasing its stability [49]. Since Y220C occurs at the non-DNA-binding site, it is an attractive site for the screening of small molecules targeting p53. Similarly, PK7088 increases the $p53^{Y220C}$ stability by increasing the melting temperature and causes cell-cycle arrest and apoptosis in tumor cells expressing $p53^{Y220C}$ [51]. PK7088 induces p53-mediated expression of p21 and the NOXA protein. PK7088 works synergistically with Nutlin-3 on upregulation of p21 expression [51]. PK7088 also restores non-transcriptional apoptotic functions of p53 by inducing nuclear export of pro-apoptotic Bcl2 Associated X (BAX) protein to the mitochondria [51]. X-ray crystallography studies of p53 core domain led to the design of PK083 and PK7088 [30].

## RITA

A small molecule RITA (reactivation of p53 and induction of tumor cell apoptosis) was identified from the screening of chemical library followed by cell proliferation assay on isogenic cell lines of HCT116 (p53 wild type and p53 null). RITA displayed its ability to suppress the growth of p53 wild-type cells but not p53 null cells [52]. Various studies have suggested the role of RITA regarding inhibition of p53 and HDM2 both in vitro and in vivo models [53–57]. HDM-2-mediated proteasomal degradation of p53 is often deregulated in several tumors with wild-type p53 [5]. RITA was shown to restore the transcriptional activity of p53, therefore, upregulating its targets p21, NOXA, GADD45, and PUMA, along with other pro-apoptotic proteins like BAX, therefore, inducing apoptosis in these mutant cell lines [53, 54, 56]. RITA has also been shown to display activity against mutant p53. It has also demonstrated the ability to suppress the growth of different cancers such as breast, colon, and lung, carcinoma as well as Burkitt lymphoma carrying various p53 mutants ($p53^{R175H}$, $p53^{R213Q/Y234H}$, $p53^{R248W}$, $p53^{R248Q}$, $p53^{I254D}$, $p53^{R273H}$, and $p53^{R280K}$) [53–57].

In vivo studies of this drug successfully demonstrated its potent antitumor activity against HCT116-derived tumors [52] and neuroblastoma-derived tumors [53] in a mouse xenograft model exclusive of any systemic toxicity (Table 1). Nevertheless, on using RITA along with other drugs such as cisplatin, it enhanced cisplatin cytotoxicity synergistically through activation of p53 apoptotic activity in head and neck

**Table 1** Mechanistic action of compounds in reactivation of mutant p53

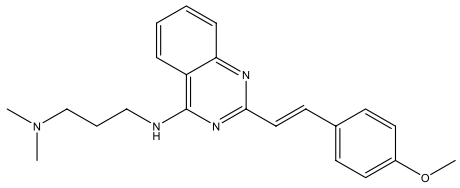
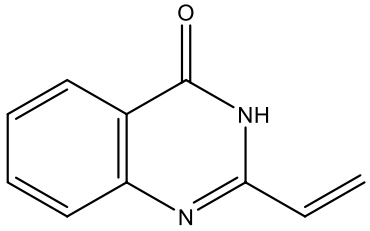
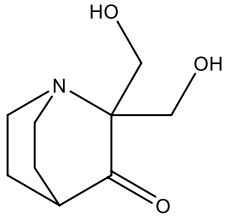
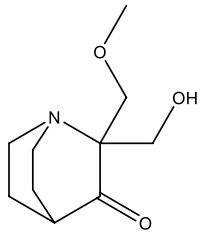
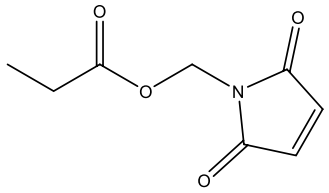
| Compounds | Structures | Mutant form of p53 | Mechanism | References |
|---|---|---|---|---|
| Small molecules | | | | |
| CP-31398 |  | $P53^{V173A}$ and $p53^{R249S}$ | Restoration of the DNA-binding ability | [31] |
| STIMA-1 |  | $p53^{R175H}$ and $p53^{R273H}$ | Restoration of the redox status and DNA-binding ability | [36] |
| PRIMA-1 and APR-246 |   | $p53^{V173M}$ | Restoration of the native conformation | [27] |
| MIRA-1 |  | $p53^{R175H}$, $p53^{R273H}$ and $p53^{R248Q}$ | Restoration of transcriptional activity | [30] |

**Table 1** (continued)

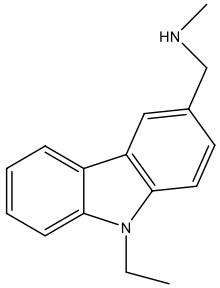
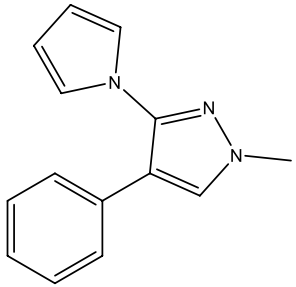
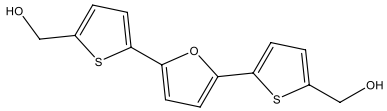
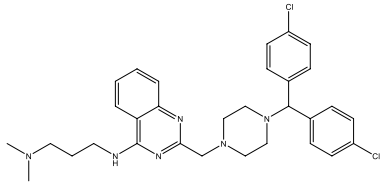
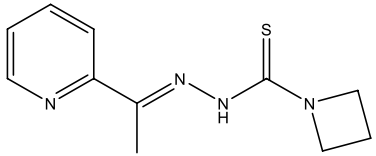
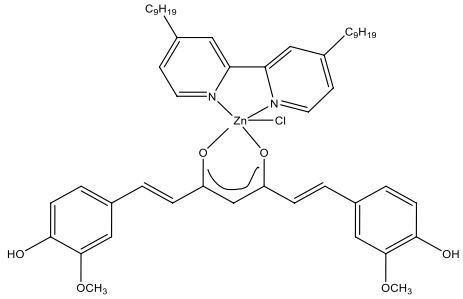
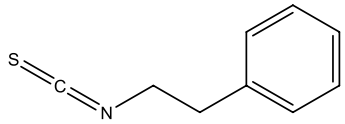
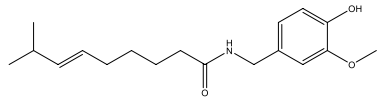
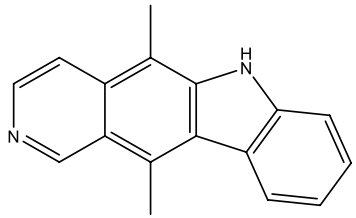
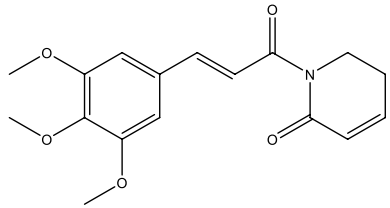
| Compounds | Structures | Mutant form of p53 | Mechanism | References |
|---|---|---|---|---|
| PK083 and PK7088 |   | $p53^{Y220C}$ | Restored stability by supporting the S7/S8 loop and raising its melting point and half-life | [49, 50] |
| RITA |  | $p53^{R175H}$ $p53^{R248Q}$ $p53^{R273H}$ $p53^{R280K}$ $p53^{R248W}$ | Restoration of transcriptional activity | [53–57] |
| SCH529074 |  | $p53^{R175H}$ $p53^{R273H}$ and $p53^{R249S}$ | Binds to DBD and restore its wild-type functional activity | [59] |
| ZMC 1 |  | $p53^{R175H}$ and $p53^{R273H}$ | Restoration of the DNA-binding ability | [62] |

**Table 1** (continued)

| Compounds | Structures | Mutant form of p53 | Mechanism | References |
|---|---|---|---|---|
| Zn(II) Curc |  | $p53^{R175H}$ and $p53^{R273H}$ | Restoration of the native conformation | [66] |
| Phytochemicals | | | | |
| PEITC |  | $p53^{R175H}$ | Restoration of p53 wild-type conformation and transactivation functions | [72] |
| Capsaicin |  | $p53^{R175H}$ and $p53^{R273H}$ | Mutant protein degradation and restore WT function | [77] |
| Ellipticine |  | $p53^{R175H}$, $p53^{R273H}$, $p53^{R248W}$ $p53^{D281G}$ | Structural restoration | [79] |
| Piperlongumine |  | $p53^{R175H}$ and $p53^{R273H}$ | Structural restoration via protein glutathionylation | [73] |

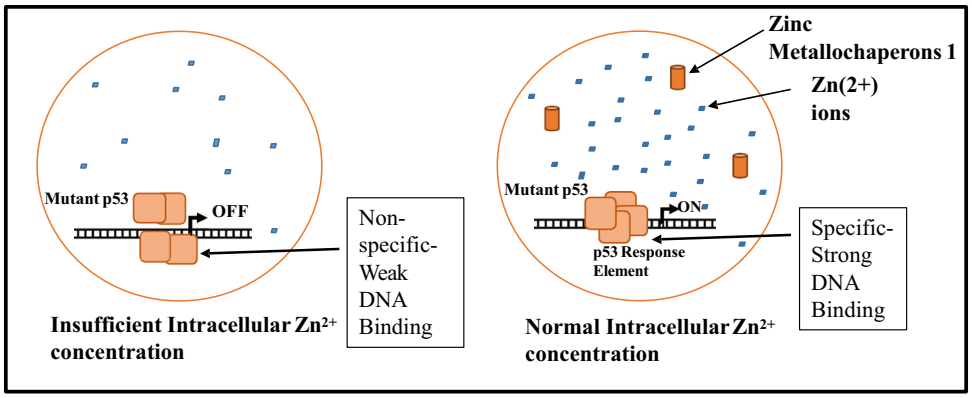



**Fig. 3** Mechanism of action Zinc Metallochaperons 1 (ZMC 1). Some mutations in p53 cause inactivation by a reduction in its affinity for zinc ion. This reduced affinity leaves the mutant protein unable to bind the metal at such a limited free [Zn(2+)] ion concentration inside the cell. ZMC1 reactivate p53R175H binding Zn(2+) and buffering the free Zn(2+) ion concentration in the environment to a level, such that these ions can repopulate the impaired binding site and restore the DNA-binding ability of p53

cancer cells and cervical cancer cells with defective p53 expression [55]. Many studies warrant further investigation to explore the mechanism of action of RITA on both wild type and mutant p53 [53].

## SCH529074

A quinazoline-based small molecule which restores mutant p53 structure through its action as a molecular chaperone, via a mechanism similar to that of the peptide CDB3 [58]. SCH529074 binds specifically to the DNA-binding domain of p53 in a saturable manner, such that its binding restores the wild-type functional activity of several mutant forms of p53 ($p53^{R175H}$, $p53^{R273H}$, and $p53^{R249S}$) [59]. Restoration of the Pab1620 epitope of the mutant p53 results in upregulation of several transcriptional targets of wild-type p53 like p21, NOXA, PUMA, Cyclin G1, and BAX [59].

## ZMC 1 (zinc metallochaperone-1) or NSC319726

Zn(2+) ions are essential for tumor suppressor p53 at its central core domain to remain in the native state [38, 60, 61], as described in Fig. 3. In vivo studies on mice-carrying tumor with mutant p53($p53^{R175H}$ and $p53^{R273H}$) showed regression in tumor growth upon administration of zinc via improvement in DNA-binding activity of p53 protein [62]. Notably, administration of ZMC1 produces preferentially greater toxicity in $p53^{R172H/R172H}$ (homolog to human $p53^{R175H}$) mice than in wild-type mice in a dose-dependent manner [63]. ZMC1 might serve as an attractive therapeutic lead for drug development because of its exceptional nontoxicity to wild-type animal models and impressive pharmacokinetic value when administered intravenously [63]. Another report also suggests that ZMC1 increases cellular ROS that further triggers the newly conformed $p53^{R175H}$ (via post-translational modifications), thereby inducing an apoptotic program [64].

The cancer drug zinc metallochaperone-1 (ZMC1) or NSC319726, a thiosemicarbazone family compound, was recognized by in silico screening methodology to selectively target cancer cells expressing mutant $p53^{R175H}$. This drug reactivates $p53^{R175H}$ binding to Zn(2+) and buffering the free Zn(2+) ion concentration in the environment to a level, such that these ions can repopulate the impaired binding site and restore the DNA-binding ability of p53. However, the exact mechanism is still unresolved; one report suggests that ZMC1 functions as a Zn(2+) ionophore that binds to the Zn(2+) in the extracellular environment, passes through the plasma membrane, and releases it intracellularly [65].

## Zn (II) Curc

A fluorescent curcumin-based Zn(II)-complex (Zn-curc) was tested to reactivate mutant p53 in cancer cells [66]. Zn-curc treatment reinstated wild-type p53-DNA binding and transactivation properties and also induced apoptotic cell death in cell lines with mutant p53 ($p53^{R175H}$ and $p53^{R273H}$) through a conformational change in the mutant protein [66]. In vivo studies by immunofluorescence analysis of glioblastoma tissues of an orthotopic mice model showed that the Zn-curc complex was formed upon drug treatment, highlighting its ability to cross the blood-tumor barrier [66]. The mechanism through which Zn(II)-curc reactivates mutant $p53^{R175H}$ involves at least in part, induction of mutant p53 degradation via wild-type p53-mediated autophagy [67]. When tested on thyroid cancer cells, Zn(II)-curc caused reactivation of mutant $p53^{R273H}$ and elicited p53 targeted gene expression in wild-type p53-carrying cells [68].

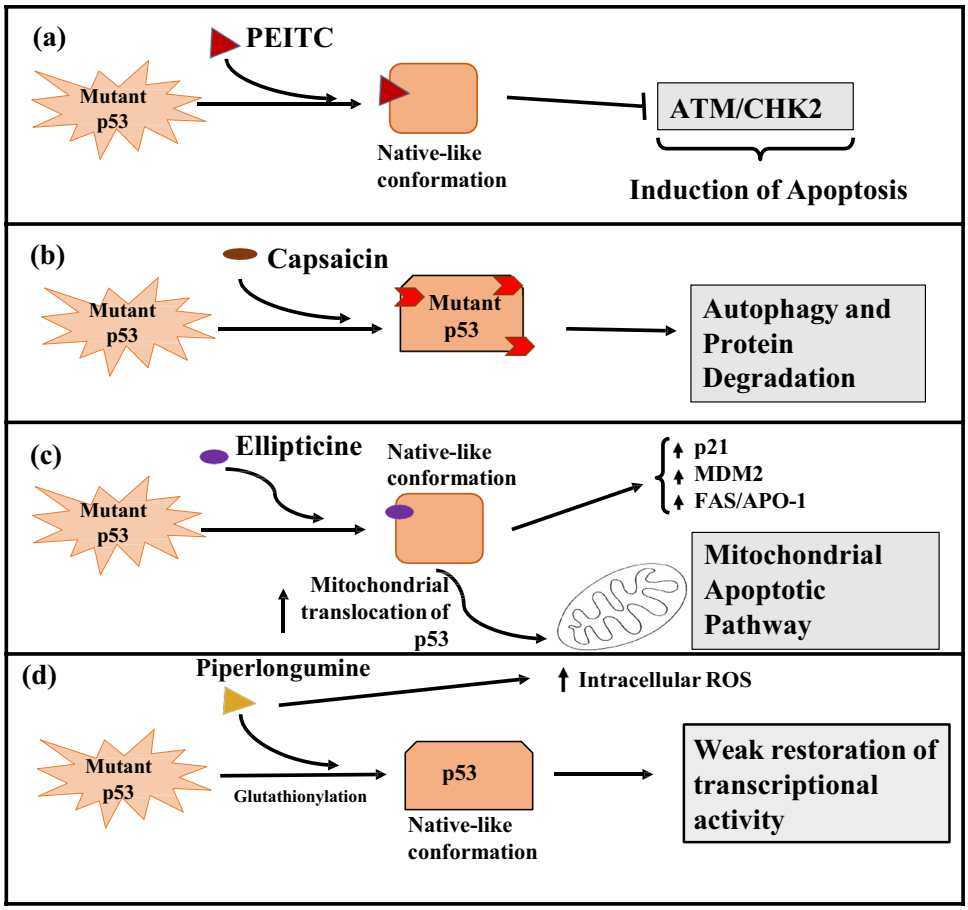



**Fig. 4** Summary of phytochemicals that reactivate mutant p53. **a** In PEITC (phenethyl isothiocyanate) treated cells the restored wild-type p53 induces apoptosis by phosphorylation of ATM/CHK2 and by causing a delay in S and G2/M phase. **b** Capsaicin induces autophagy mediated mutant p53 protein degradation. Retraction of mutant p53 by capsaicin restores wild-type p53 activities leading to cancer cell death. **c** Ellipticine along with restoring the native-like structure of mutant p53 leading to increased expression of downstream transcriptional targets of p53, it also enhances p53 mitochondrial translocation and initiates mitochondrial apoptotic pathways. **d** Piperlongumine (PL) increases the level of intracellular ROS which affects the structure and function of the redox-sensitive mutant p53 protein which gets activated and promotes cell death. PL induced oxidative milieu assists a weak functional restoration of mutant p53 via protein glutathionylation

## Phytochemicals

Phytochemicals are non-nutritive bioactive components from natural plants that have protective or disease-preventive properties. Various review articles have` summarized natural phytochemicals and their anti-cancer effects [69–71]. In recent years, in exploring the mechanism of action of phytochemicals, many of it have been found to act on mutant p53 and restore its wild-type functions [72–74]. These phytochemicals and their mechanism are summarized in Fig. 4.

### PEITC

Phenethyl isothiocyanate (PEITC), a phytochemical derived from cruciferous vegetables. PEITC has been shown to act on mutant p53 and its restoration both in vitro and in vivo conditions [72, 75]. PEITC exhibits growth-inhibitory activity in p53 mutants cells with preferential activity towards $p53^{R175H}$ by restoring p53 wild-type conformation and transactivation functions [72]. The treatment of cancer cells with PEITC restored wild-type p53 induces apoptosis by phosphorylation of ATM/CHK2 and by causing a delay in S and G2/M phases [72]. In vivo studies on xenograft mouse model using breast cancer, SKBR3 cells, showed that dietary supplementation of PEITC caused significant inhibition of tumor growth [72]. The phase II clinical trials using PEITC have been completed and the use of this compound evidenced the reduction in the mutated p53 oral cancer cells [76].

### Capsaicin

A major bioactive phytochemical capsaicin found in pepper showed antitumor activity by targeting several molecular pathways [74, 77]. Assessment of the effect of capsaicin on mutant p53 and its reactivation demonstrated that capsaicin-induced autophagy that was responsible for mutant p53 protein degradation [77]. Retraction of mutant p53 by capsaicin and overexpression of $p53^{R175H}$ and $p53^{R273H}$ mutants in H1299 (p53 null) cells restored wild-type p53 activities leading to cell death [77].

### Ellipticine

Ellipticine (5,11-dimethyl-6H-pyrido[4,3-b]carbazole) is a phyto-alkaloid isolated from the leaves of *Ochrosia elliptica Labill* (Apocynaceae). Ellipticine showed restoration of the transactivation function of several p53 mutants ($p53^{R175H}$,

p538$^{R248W}$, p53$^{R249S}$, p53$^{R273H}$, p53$^{D281G}$, etc.), leading to upregulation of p53-downstream target genes p21(WAF1) and MDM2 [78, 79]. Conformation-specific antibodies Pab240 and Pab1620 confirmed the wild-type structural restoration of mutant p53 [79]. In vivo studies confirmed the induction of p21(WAF1) and MDM2 expression [79]. Exposure of ellipticine to the cells led to the upregulation of p53, death receptor Fas/APO-1, and Fas ligand [80]. Ellipticine was also reported to prevent the growth of human hepatocellular carcinoma cell line HepG2 in a dose- and time-dependent manner followed by induction of apoptosis [78]. Moreover, ellipticine treatment also initiated mitochondrial apoptotic pathway via activation of caspases and altered mitochondrial membrane potential through regulation of Bcl-2 family proteins [78] as well as enhanced mitochondrial p53 [81].

### Piperlongumine

Piperlongumine (PL) is an alkaloid phytochemical obtained from black pepper (*Piper longum*). It increases the level of intracellular ROS which affects the structure and function of the redox-sensitive mutant p53 protein which gets activated and promotes cell death [82]. Testing of PL on mutant p53 (p53R273H) cancer cell lines showed a significant increase in ROS production and protein glutathionylation with an associated increase in Nrf-2 expression and an increase in the wild-type-like p53 conformation of mutant p53 [73]. These findings advocate that Piperlongumine-induced oxidative milieu assists a weak functional restoration of mutant p53 via protein glutathionylation [73]. In an attempt to develop hybrid anti-cancer drugs with multiple targets, a molecule was generated having PL derivatives with an aryl group introduced at the C-7 position [83]. This composite structure exhibited potent antiproliferative properties against a variety of mutant p53 cell lines and particularly the ones harboring R175H mutation. Further studies revealed that the drug restores the wild-type biological activity and structure, identified by conformation-specific antibodies along with induction of abundant ROS generation and protein glutathionylation [83].

## Peptides

### CDB3 (core domain binding 3)

CDB3 is a nine-residue small peptide derived from a p53-binding protein. CDB3 binds to p53 core domain at the edge of DNA-binding site and stabilizes its structure in vitro. CDB3 is proposed to act as a molecular chaperone that restores existing or newly synthesized denatured p53 mutants (p53$^{I195T}$, p53$^{R173H}$, p53$^{R273H}$, and p53$^{R249S}$) into its native wild-type conformation and thus retaining its DNA-binding activity [25, 58, 84]. In all cases, p53 target genes like p21, GADD45, and MDM2 were induced along with the trigger of p53 dependent apoptosis pathway. Fl-CDB3 is a fluorescently labeled CDB3 peptide which upon treatment on cancer cells, induced wild-type p53 to mediate apoptosis induced by gamma-radiation. Fl-CDB3 actively binds to and rescues p53 activity in cell and, thus, can serve as a strong lead for the advancement of novel anti-cancer therapy [84].

### Peptide-46

Peptide-46 is a synthetic 22-mer, which corresponds to the C-terminal amino acid residues 361–382 of p53. In vitro studies of this peptide has shown reactivation of and

**Table 2** Function of peptides in the reactivation of mutant p53

| Peptides | Sequence | Forms of mutant p53 | Mechanism of reactivation | References |
|---|---|---|---|---|
| CDB3 | REDEDEIEW | p53$^{R175H}$, p53$^{R273H}$, p53$^{R249S}$, p53$^{I195T}$ | Binds to DBD and stabilizes its structure | [25, 58, 83] |
| Peptide-46 | GSRAHSSHLKSKKGQSTSRHKK | p53$^{R273H}$ | Restored transcriptional activity | [26] |
| Small peptides | | | | |
| pCAP-250 | myr-RRHSTPHPD | p53$^{R280H}$,p53$^{R273H}$ | Restored transcriptional activity | [85] |
| pCAP-60R | SFILFIRRGRLGRRRRRRRRR | p53$^{R280H}$ | | |
| pCAP-54 | IRGRIIR | p53$^{R280H}$ | | |
| pCAP-325 | myr-RRIRDPRILLLHFD | p53$^{R273H}$ | | |
| Peptide aptamers | | | | |
| A3 | AKYCQCAAKVRVTAAM | p53$^{R175H}$, p53$^{R273H}$ | Inhibited the gain-of-function transactivation activity of mutant protein | [86] |
| A29 | GPVVPRTQYMSLAFGW | | | |
| A60 | IQITLTGWSARVTTSG | | | |
| A79 | VWAESCDDCGEYWRYV | | | |
| sA79 | DVADWESCGEYWCYRV | | | |

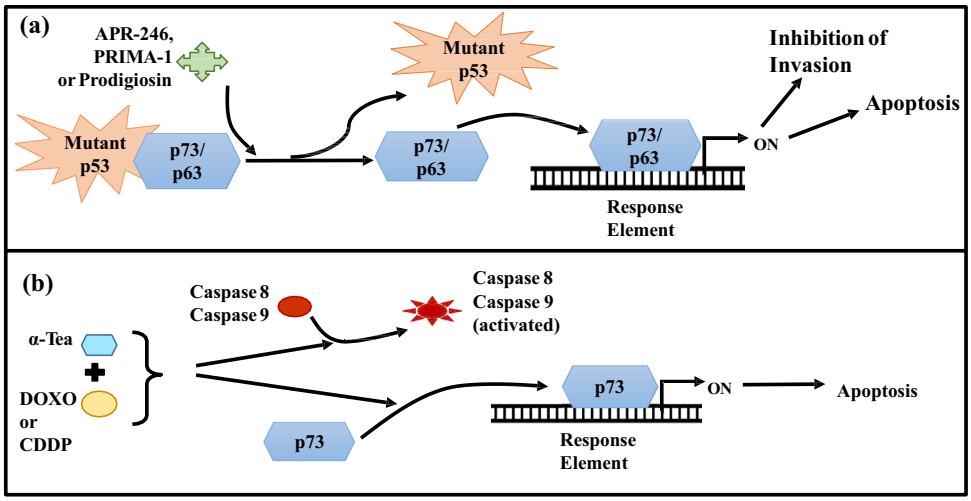



**Fig. 5** Activating the p53 family members for p53 pathway restoration. **a** APR-246, PRIMA-1, and Prodigiosin induce the expression of p73 and disrupt the interaction of p73 with mutant p53. **b** α-TEA upon combinatorial treatment with DOXO (doxorubicin) or CDDP (cisplatin), reconstitutes the p53 tumor suppressor pathway via p73. This combinatorial treatment induces apoptosis, cleavage of caspase-8 and caspase-9, and induction of expression of p73

restoration of transcriptional activity in some mutant p53 ($p53^{R273H}$) [26]. The introduction of peptide 46 in Saos-2 cells with a Tet-regulatable mutant $p53^{R273H}$ construct produced mutant p53-dependant cell-cycle inhibition and apoptosis, endorsing that the peptide mediates the mutant p53 reactivation mechanism to bring about induction of cell death pathways. Furthermore, peptide 46 showed similar effects on different human cancer cell lines carrying mutant as well as wild-type p53. However, no effect was observed against p53 null tumor cells indicating the specificity of the peptide [26].

### Small peptides

A vast library of random peptide sequences was selected and validated for the purpose of mutant p53 reactivation. These sequences were allowed to interact with mutant p53 using phage display technology with an aim to identify peptides that favor correctly folded conformation of p53. The sequence of these peptides used in the studies for reactivation of mutant p53 is given in Table 2. After screening out a pool of small peptides with a potential for p53 restoration, lead peptides were synthesized and tested [85]. These lead peptides were studied for the restoration of mutant p53 in cell lines as well as in mouse xenograft assays. Cell line studies have suggested the effect on the conformation of the protein, binding to response elements (RE) of p53 and increased cell death upon the use of the small peptides. The tumor xenograft assays using the small peptides suggested the reduction in tumor size, and in some cases, the use of small peptides led to the complete suppression of the tumor. The application of these peptides in pre-clinical studies raised the hope of novel agents for anti-cancer therapy [85].

### Peptide aptamers

Inhibiting the gain-of-function activity of mutant p53 is an attractive approach for blocking tumor growth and development of aggressive phenotypes. Peptide aptamers (PA) are a class of peptide molecules which upon binding to specific proteins can modulate its interaction and activities [86]. Molecular modeling techniques were used for the construction and characterization of PAs for their interaction with mutant p53. Transient expression of PAs was able to lessen the gain-of-function transactivation activity of mutant p53 and to trigger apoptosis particularly in cells expressing mutant p53. Five PAs were able to interact preferentially with p53 conformational mutants ($p53^{R175H}$, $p53^{D281G}$) comparing with contact mutants ($p53^{R273H}$, $p53^{R248W}$) [86]. Furthermore, binding with wild-type p53 was less efficient, signifying that the altered protein conformation is responsible for enhanced interactions of mutant p53. These PAs could provide a novel strategy to constrain the oncogenic gain-of-function activity of mutant p53 and thus serve as a promising candidate for mutant p53-targeted cancer therapies [86].

## Activating the p53 family members for p53 pathway restoration

The p53 family members p63 and p73 hold the ability to trigger regression of tumor upon activation. Therefore, molecular approach to activate the p53 family members is an attractive approach for restoration of the p53 pathway in cells expressing mutant p53 [87]. The mechanism for the activation of p53 family members by various compounds is summarized below and is shown in Fig. 5.

### Prodigiosin

Prodigiosin is a red pigmented secondary metabolite obtained from different bacterial sources which have been documented for its broad bioactive potential [88].

Prodigiosin treatment on p53-deficient cancer cell lines induced cell-cycle arrest and apoptosis by induction of the expression of downstream target genes of p53 [89]. The compound showed similar results in cancer cells harboring mutant p53 by restoration of the p53 signaling pathway. Prodigiosin maintained the p53 signaling in mutant p53 cell lines by inducing the expression of p73 and disrupting the interaction of p73 with mutant p53 [89]. In vitro and in vivo studies on colorectal cancer stem cells reconfirmed the effect of prodigiosin on the upregulation of p73 and restoration of p53-mediated apoptotic pathway [87].

### α-TEA (RRR-α-tocopherol ether-linked acetic acid analog)

α-TEA is a small bioactive lipid that upon combinatorial treatment with doxorubicin (DOXO) or cisplatin (CDDB), reconstitutes the p53 tumor suppressor pathway via p73 mediated, p53-independent manner [90]. This combinatorial treatment induced apoptosis, cleavage of caspase-8 and caspase-9, and induction of expression of p73, phospho-c-Ab1, and phospho-JNK protein in triple-negative breast cancer cells (TNBC). p53 downstream targets like CD95/APO-1 (Fas), death receptor-5, Nova, and Bax, as well as Yap, were also induced because of the combinatorial treatment [90]. siRNA-mediated knockdown of p73, c-Abl, JNK, or Yap shows that p73 is a critical player in the induction of apoptosis and related mechanism upon combination treatment [90].

## Conclusions and future prosectives

Given the aggressive role of mutant p53 protein in promoting carcinogenesis and resistance to the existing drugs, the goal to reactivate mutant p53 has become requisite. Its been more than three decades of discovery of p53 and has been a long journey to understand the gain-of-function approach of mutant p53 to promote oncogenesis. Only recently, the concept of targeting mutant p53 have gained the velocity. The scientific literature now evident the application of various compounds, molecules and natural products to restore mutant p53 and few of them have reached clinical trials. These studies have suggested the sincere efforts of scientists and clinicians to curtail the notorious behaviour of mutant p53 by reactivation strategies. The heterogeneity of tumors and mutant p53 poses a greater challenge for the development of efficient, specific and safe compounds.

It is the time to dust off various mechanisms and the targets of mutant p53 through which it functions as an oncogene. Elucidation of exact mechanisms of mutant p53 interactions and downstream signaling would provide new insights for the discovery of therapeutics. Further investigations would be required to assess the advantages of these compounds over conventional chemotherapy in beating cancer cell's strategies for proliferation and survival.

**Acknowledgements** We acknowledge Department of Science and Technology-Science and Engineering Research Board (DST-SERB), Government of India for extramural Research Grant (EMR/2015/000761) to H. C. and Central University of Punjab, Bathinda, India for additional support.

**Funding** This study was funded by the Department of Science and Technology-Science and Engineering Research Board, (DST-SERB), Government of India (EMR/2015/000761).

### Compliance with ethical standards

**Conflict of interest** Author AB declares that he has no conflict of interest. Author SM declares that he has no conflict of interest. Author PS declares that he has no conflict of interest. Author SD declares that he has no conflict of interest. Author HC declares that he has no conflict of interest.

**Ethical approval** This article does not contain any studies with human participants or animals performed by any of the authors.

## References

1. Menendez D, Inga A, Resnick MA (2009) The expanding universe of p53 targets. Nat Rev Cancer 9(10):724–737
2. Goldstein I, Marcel V, Olivier M, Oren M, Rotter V, Hainaut P (2011) Understanding wild-type and mutant p53 activities in human cancer: new landmarks on the way to targeted therapies. Cancer Gene Ther 18(1):2–11. https://doi.org/10.1038/cgt.2010.63
3. Muller PAJ, Vousden KH, Norman JC (2011) p53 and its mutants in tumor cell migration and invasion. J Cell Biol 192(2):209–218. https://doi.org/10.1083/jcb.201009059
4. Brown CJ, Cheok CF, Verma CS, Lane DP Reactivation of p53: from peptides to small molecules. Trends Pharmacol Sci 32(1):53–62. https://doi.org/10.1016/j.tips.2010.11.004
5. Stindt MH, Muller PA, Ludwig RL, Kehrloesser S, Dotsch V, Vousden KH (2015) Functional interplay between MDM2, p63/p73 and mutant p53. Oncogene 34(33):4300–4310. https://doi.org/10.1038/onc.2014.359
6. Sherman M, Gabai V, O'Callaghan C, Yaglom J (2007) Molecular chaperones regulate p53 and suppress senescence programs. FEBS Lett 581(19):3711–3715. https://doi.org/10.1016/j.febslet.2007.05.036
7. Muller PAJ, Vousden KH (2013) p53 mutations in cancer. Nat Cell Biol 15(1):2–8
8. Hollstein M, Sidransky D, Vogelstein B, Harris CC (1991) p53 mutations in human cancers. Science 253(5015):49–53

9. Brosh R, Rotter V (2009) When mutants gain new powers: news from the mutant p53 field. Nat Rev Cancer 9(10):701–713. https://doi.org/10.1038/nrc2693
10. Cooks T, Pateras IS, Tarcic O, Solomon H, Schetter AJ, Wilder S, Lozano G, Pikarsky E, Forshew T, Rozenfeld N (2013) Mutant p53 prolongs NF-κB activation and promotes chronic inflammation and inflammation-associated colorectal cancer. Cancer Cell 23(5):634–646
11. Sampath J, Sun D, Kidd VJ, Grenet J, Gandhi A, Shapiro LH, Wang Q, Zambetti GP, Schuetz JD (2001) Mutant p53 cooperates with ETS and selectively up-regulates human MDR1 not MRP1. J Biol Chem 276(42):39359–39367
12. Lang GA, Iwakuma T, Suh YA, Liu G, Rao VA, Parant JM, Valentin-Vega YA, Terzian T, Caldwell LC, Strong LC, El-Naggar AK, Lozano G (2004) Gain of function of a p53 hot spot mutation in a mouse model of Li-Fraumeni syndrome. Cell 119(6):861–872. https://doi.org/10.1016/j.cell.2004.11.006
13. Terzian T, Suh YA, Iwakuma T, Post SM, Neumann M, Lang GA, Van Pelt CS, Lozano G (2008) The inherent instability of mutant p53 is alleviated by Mdm2 or p16INK4a loss. Genes Dev 22(10):1337–1344. https://doi.org/10.1101/gad.1662908
14. Parrales A, Iwakuma T (2015) Targeting oncogenic mutant p53 for cancer therapy. Front Oncol 5:288. https://doi.org/10.3389/fonc.2015.00288
15. Martins CP, Brown-Swigart L, Evan GI (2006) Modeling the therapeutic efficacy of p53 restoration in tumors. Cell 127(7):1323–1334. https://doi.org/10.1016/j.cell.2006.12.007
16. Ventura A, Kirsch DG, McLaughlin ME, Tuveson DA, Grimm J, Lintault L, Newman J, Reczek EE, Weissleder R, Jacks T (2007) Restoration of p53 function leads to tumour regression in vivo. Nature 445(7128):661–665. https://doi.org/10.1038/nature05541
17. Xue W, Zender L, Miething C, Dickins RA, Hernando E, Krizhanovsky V, Cordon-Cardo C, Lowe SW (2007) Senescence and tumour clearance is triggered by p53 restoration in murine liver carcinomas. Nature 445(7128):656–660. https://doi.org/10.1038/nature05529
18. Alexandrova EM, Yallowitz AR, Li D, Xu S, Schulz R, Proia DA, Lozano G, Dobbelstein M, Moll UM (2015) Improving survival by exploiting tumour dependence on stabilized mutant p53 for treatment. Nature 523(7560):352–356. https://doi.org/10.1038/nature14430
19. Freed-Pastor WA, Mizuno H, Zhao X, Langerod A, Moon SH, Rodriguez-Barrueco R, Barsotti A, Chicas A, Li W, Polotskaia A, Bissell MJ, Osborne TF, Tian B, Lowe SW, Silva JM, Borresen-Dale AL, Levine AJ, Bargonetti J, Prives C (2012) Mutant p53 disrupts mammary tissue architecture via the mevalonate pathway. Cell 148(1–2):244–258. https://doi.org/10.1016/j.cell.2011.12.017
20. Masciarelli S, Fontemaggi G, Di Agostino S, Donzelli S, Carcarino E, Strano S, Blandino G (2014) Gain-of-function mutant p53 downregulates miR-223 contributing to chemoresistance of cultured tumor cells. Oncogene 33(12):1601–1608. https://doi.org/10.1038/onc.2013.106
21. Weisz L, Oren M, Rotter V (2007) Transcription regulation by mutant p53. Oncogene 26(15):2202–2211. https://doi.org/10.1038/sj.onc.1210294
22. Hanahan D, Weinberg RA (2000) The hallmarks of cancer. Cell 100(1):57–70
23. Selivanova G, Kawasaki T, Ryabchenko L, Wiman KG (1998) Reactivation of mutant p53: a new strategy for cancer therapy. Semin Cancer Biol 8(5):369–378
24. Zhang W, Guo XY, Hu GY, Liu WB, Shay JW, Deisseroth AB (1994) A temperature-sensitive mutant of human p53. EMBO J 13(11):2535–2544
25. Friedler A, Hansson LO, Veprintsev DB, Freund SM, Rippin TM, Nikolova PV, Proctor MR, Rudiger S, Fersht AR (2002) A peptide that binds and stabilizes p53 core domain: chaperone strategy for rescue of oncogenic mutants. Proc Natl Acad Sci USA 99(2):937–942. https://doi.org/10.1073/pnas.241629998
26. Selivanova G, Iotsova V, Okan I, Fritsche M, Strom M, Groner B, Grafstrom RC, Wiman KG (1997) Restoration of the growth suppression function of mutant p53 by a synthetic peptide derived from the p53 C-terminal domain. Nat Med 3(6):632–638
27. Bykov VJN, Issaeva N, Zache N, Shilov A, Hultcrantz M, Bergman J, Selivanova G, Wiman KG (2005) Reactivation of mutant p53 and induction of apoptosis in human tumor cells by maleimide analogs. J Biol Chem 280(34):30384–30391. https://doi.org/10.1074/jbc.M501664200
28. Liu WL, Midgley C, Stephen C, Saville M, Lane DP (2001) Biological significance of a small highly conserved region in the N terminus of the p53 tumour suppressor protein. J Mol Biol 313(4):711–731. https://doi.org/10.1006/jmbi.2001.5082
29. Nikolova PV, Wong KB, DeDecker B, Henckel J, Fersht AR (2000) Mechanism of rescue of common p53 cancer mutations by second-site suppressor mutations. EMBO J 19(3):370–378. https://doi.org/10.1093/emboj/19.3.370
30. Bykov VJ, Wiman KG (2014) Mutant p53 reactivation by small molecules makes its way to the clinic. FEBS Lett 588(16):2622–2627. https://doi.org/10.1016/j.febslet.2014.04.017
31. Foster BA, Coffey HA, Morin MJ, Rastinejad F (1999) Pharmacological rescue of mutant p53 conformation and function. Science 286(5449):2507–2510
32. Wang W, Takimoto R, Rastinejad F, El-Deiry WS (2003) Stabilization of p53 by CP-31398 inhibits ubiquitination without altering phosphorylation at serine 15 or 20 or MDM2 binding. Mol Cell Biol 23(6):2171–2181
33. Rao CV, Swamy MV, Patlolla JMR, Kopelovich L (2008) Suppression of familial adenomatous polyposis by CP-31398, a TP53 modulator in $APC^{min/+}$ mice. Cancer Res 68(18):7670–7675. https://doi.org/10.1158/0008-5472.can-08-1610
34. Tang X, Zhu Y, Han L, Kim AL, Kopelovich L, Bickers DR, Athar M (2007) CP-31398 restores mutant p53 tumor suppressor function and inhibits UVB-induced skin carcinogenesis in mice. J Clin Investig 117(12):3753–3764. https://doi.org/10.1172/jci32481
35. Fiorini C, Menegazzi M, Padroni C, Dando I, Dalla Pozza E, Gregorelli A, Costanzo C, Palmieri M, Donadelli M (2013) Autophagy induced by p53-reactivating molecules protects pancreatic cancer cells from apoptosis. Apoptosis 18(3):337–346. https://doi.org/10.1007/s10495-012-0790-6
36. Hainaut P, Milner J (1993) Redox modulation of p53 conformation and sequence-specific DNA binding in vitro. Can Res 53(19):4469–4473
37. Zache N, Lambert JMR, Rökaeus N, Shen J, Hainaut P, Bergman J, Wiman KG, Bykov VJN (2008) Mutant p53 targeting by the low molecular weight compound STIMA-1. Mol Oncol 2(1):70–80. https://doi.org/10.1016/j.molonc.2008.02.004
38. Bykov VJ, Issaeva N, Shilov A, Hultcrantz M, Pugacheva E, Chumakov P, Bergman J, Wiman KG, Selivanova G (2002) Restoration of the tumor suppressor function to mutant p53 by a low-molecular-weight compound. Nat Med 8(3):282–288. https://doi.org/10.1038/nm0302-282
39. Duffy MJ, Synnott NC, McGowan PM, Crown J, O'Connor D, Gallagher WM (2014) p53 as a target for the treatment of cancer. Cancer Treat Rev 40(10):1153–1160. https://doi.org/10.1016/j.ctrv.2014.10.004
40. Zandi R, Selivanova G, Christensen CL, Gerds TA, Willumsen BM, Poulsen HS (2011) PRIMA-1Met/APR-246 induces apoptosis and tumor growth delay in small cell lung cancer expressing mutant p53. Clin Cancer Res 17(9):2830–2841. https://doi.org/10.1158/1078-0432.ccr-10-3168
41. Fransson A, Glaessgen D, Alfredsson J, Wiman KG, Bajalica-Lagercrantz S, Mohell N (2016) Strong synergy with APR-246

and DNA-damaging drugs in primary cancer cells from patients with TP53 mutant high-grade serous ovarian cancer. J Ovarian Res 9(1):27. https://doi.org/10.1186/s13048-016-0239-6
42. Lambert JM, Gorzov P, Veprintsev DB, Söderqvist M, Segerbäck D, Bergman J, Fersht AR, Hainaut P, Wiman KG, Bykov VJ (2009) PRIMA-1 reactivates mutant p53 by covalent binding to the core domain. Cancer cell 15(5):376–388
43. Krayem M, Journe F, Wiedig M, Morandini R, Najem A, Sales F, van Kempen LC, Sibille C, Awada A, Marine JC, Ghanem G (2016) p53 Reactivation by PRIMA-1(Met) (APR-246) sensitises (V600E/K)BRAF melanoma to vemurafenib. Eur J Cancer (Oxford England: 1990) 55:98–110. https://doi.org/10.1016/j.ejca.2015.12.002
44. Rao CV, Patlolla JM, Qian L, Zhang Y, Brewer M, Mohammed A, Desai D, Amin S, Lightfoot S, Kopelovich L (2013) Chemopreventive effects of the p53-modulating agents CP-31398 and Prima-1 in tobacco carcinogen-induced lung tumorigenesis in A/J mice. Neoplasia (New York, NY) 15(9):1018–1027
45. Bykov VJ, Zhang Q, Zhang M, Ceder S, Abrahmsen 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. https://doi.org/10.3389/fonc.2016.00021
46. Nahi H, Merup M, Lehmann S, Bengtzen S, Möllgård L, Selivanova G, Wiman K, Paul C (2006) PRIMA-1 induces apoptosis in acute myeloid leukaemia cells with p53 gene deletion. Br J Haematol 132(2):230–236
47. Lehmann S, Bykov VJN, Ali D, Andrén O, Cherif H, Tidefelt U, Uggla B, Yachnin J, Juliusson G, Moshfegh A, Paul C, Wiman KG, Andersson P-O (2012) Targeting p53 in vivo: a first-in-human study with p53-targeting compound APR-246 in refractory hematologic malignancies and prostate cancer. J Clin Oncol 30(29):3633–3639. https://doi.org/10.1200/jco.2011.40.7783
48. Saha MN, Chen Y, Chen MH, Chen G, Chang H (2014) Small molecule MIRA-1 induces in vitro and in vivo anti-myeloma activity and synergizes with current anti-myeloma agents. Br J Cancer 110(9):2224–2231. https://doi.org/10.1038/bjc.2014.164
49. Boeckler FM, Joerger AC, Jaggi G, Rutherford TJ, Veprintsev DB, Fersht AR (2008) Targeted rescue of a destabilized mutant of p53 by an in silico screened drug. Proc Natl Acad Sci USA 105(30):10360–10365. https://doi.org/10.1073/pnas.0805326105
50. Rauf SMA, Endou A, Takaba H, Miyamoto A (2013) Effect of Y220C mutation on p53 and its rescue mechanism: a computer chemistry approach. Protein J 32(1):68–74. https://doi.org/10.1007/s10930-012-9458-x
51. Liu X, Wilcken R, Joerger AC, Chuckowree IS, Amin J, Spencer J, Fersht AR (2013) Small molecule induced reactivation of mutant p53 in cancer cells. Nucleic Acids Res 41(12):6034–6044. https://doi.org/10.1093/nar/gkt305
52. Issaeva N, Bozko P, Enge M, Protopopova M, Verhoef LG, Masucci M, Pramanik A, Selivanova G (2004) Small molecule RITA binds to p53, blocks p53-HDM-2 interaction and activates p53 function in tumors. Nat Med 10(12):1321–1328. https://doi.org/10.1038/nm1146
53. Burmakin M, Shi Y, Hedstrom E, Kogner P, Selivanova G (2013) Dual targeting of wild-type and mutant p53 by small molecule RITA results in the inhibition of N-Myc and key survival oncogenes and kills neuroblastoma cells in vivo and in vitro. Clin Cancer Res 19(18):5092–5103. https://doi.org/10.1158/1078-0432.ccr-12-2211
54. Grinkevich VV, Nikulenkov F, Shi Y, Enge M, Bao W, Maljukova A, Gluch A, Kel A, Sangfelt O, Selivanova G (2009) Ablation of key oncogenic pathways by RITA-reactivated p53 is required for efficient apoptosis. Cancer Cell 15(5):441–453. https://doi.org/10.1016/j.ccr.2009.03.021
55. Roh JL, Ko JH, Moon SJ, Ryu CH, Choi JY, Koch WM (2012) The p53-reactivating small-molecule RITA enhances cisplatin-induced cytotoxicity and apoptosis in head and neck cancer. Cancer Lett 325(1):35–41. https://doi.org/10.1016/j.canlet.2012.05.020
56. Zhao CY, Grinkevich V, Nikulenkov F, Bao W, Selivanova G (2010) Rescue of the apoptotic-inducing function of mutant p53 by small molecule RITA. Cell Cycle 9(9):1847–1855. https://doi.org/10.4161/cc.9.9.11545
57. Zhu H, Abulimiti M, Liu H, Su XJ, Liu CH, Pei HP (2015) RITA enhances irradiation-induced apoptosis in p53-defective cervical cancer cells via upregulation of IRE1alpha/XBP1 signaling. Oncol Rep 34(3):1279–1288. https://doi.org/10.3892/or.2015.4083
58. Friedler A, DeDecker BS, Freund SM, Blair C, Rudiger S, Fersht AR (2004) Structural distortion of p53 by the mutation R249S and its rescue by a designed peptide: implications for "mutant conformation". J Mol Biol 336(1):187–196
59. Demma M, Maxwell E, Ramos R, Liang L, Li C, Hesk D, Rossman R, Mallams A, Doll R, Liu M, Seidel-Dugan C, Bishop WR, Dasmahapatra B (2010) SCH529074, a small molecule activator of mutant p53, which binds p53 DNA Binding domain (DBD), restores growth-suppressive function to mutant p53 and interrupts HDM2-mediated ubiquitination of wild type p53. J Biol Chem 285(14):10198–10212. https://doi.org/10.1074/jbc.M109.083469
60. Margalit O, Simon AJ, Yakubov E, Puca R, Yosepovich A, Avivi C, Jacob-Hirsch J, Gelernter I, Harmelin A, Barshack I, Rechavi G, D'Orazi G, Givol D, Amariglio N (2012) Zinc supplementation augments in vivo antitumor effect of chemotherapy by restoring p53 function. Int J Cancer 131(4):E562–E568. https://doi.org/10.1002/ijc.26441
61. Pintus SS, Ivanisenko NV, Demenkov PS, Ivanisenko TV, Ramachandran S, Kolchanov NA, Ivanisenko VA (2013) The substitutions G245C and G245D in the Zn(2+)-binding pocket of the p53 protein result in differences of conformational flexibility of the DNA-binding domain. J Biomol Struct Dyn 31(1):78–86. https://doi.org/10.1080/07391102.2012.691364
62. Puca R, Nardinocchi L, Porru M, Simon AJ, Rechavi G, Leonetti C, Givol D, D'Orazi G (2011) Restoring p53 active conformation by zinc increases the response of mutant p53 tumor cells to anticancer drugs. Cell Cycle 10(10):1679–1689. https://doi.org/10.4161/cc.10.10.15642
63. Yu X, Vazquez A, Levine AJ, Carpizo DR (2012) Allele-specific p53 mutant reactivation. Cancer Cell 21(5):614–625. https://doi.org/10.1016/j.ccr.2012.03.042
64. Yu X, Blanden AR, Narayanan S, Jayakumar L, Lubin D, Augeri D, Kimball SD, Loh SN, Carpizo DR (2014) Small molecule restoration of wildtype structure and function of mutant p53 using a novel zinc-metallochaperone based mechanism. Oncotarget 5(19):8879
65. Blanden AR, Yu X, Wolfe AJ, Gilleran JA, Augeri DJ, O'Dell RS, Olson EC, Kimball SD, Emge TJ, Movileanu L, Carpizo DR, Loh SN (2015) Synthetic metallochaperone ZMC1 rescues mutant p53 conformation by transporting zinc into cells as an ionophore. Mol Pharmacol 87(5):825–831. https://doi.org/10.1124/mol.114.097550
66. Garufi A, Trisciuoglio D, Porru M, Leonetti C, Stoppacciaro A, D'Orazi V, Avantaggiati M, Crispini A, Pucci D, D'Orazi G (2013) A fluorescent curcumin-based Zn(II)-complex reactivates mutant (R175H and R273H) p53 in cancer cells. J Exp Clin Cancer Res CR 32:72. https://doi.org/10.1186/1756-9966-32-72
67. Garufi A, Pucci D, D'Orazi V, Cirone M, Bossi G, Avantaggiati ML, D'Orazi G (2014) Degradation of mutant p53H175 protein by Zn(II) through autophagy. Cell Death Dis 5:e1271. https://doi.org/10.1038/cddis.2014.217
68. Garufi A, D'Orazi V, Crispini A, D'Orazi G (2015) Zn(II)-curc targets p53 in thyroid cancer cells. Int J Oncol 47(4):1241–1248. https://doi.org/10.3892/ijo.2015.3125
69. Lee KW, Bode AM, Dong Z (2011) Molecular targets of phytochemicals for cancer prevention. Nat Rev Cancer 11(3):211–218. http://www.nature.com/nrc/journal/v11/n3/suppinfo/nrc3017_S1.html

70. Singh S, Sharma B, Kanwar SS, Kumar A (2016) Lead phytochemicals for anticancer drug development. Front Plant Sci 7:1667. https://doi.org/10.3389/fpls.2016.01667
71. Wang H, Khor TO, Shu L, Su Z, Fuentes F, Lee J-H, Kong A-NT (2012) Plants against cancer: a review on natural phytochemicals in preventing and treating cancers and their druggability. Anti Cancer Agents Med Chem 12(10):1281–1305
72. Aggarwal M, Saxena R, Sinclair E, Fu Y, Jacobs A, Dyba M, Wang X, Cruz I, Berry D, Kallakury B, Mueller SC, Agostino SD, Blandino G, Avantaggiati ML, Chung FL (2016) Reactivation of mutant p53 by a dietary-related compound phenethyl isothiocyanate inhibits tumor growth. Cell Death Differ. https://doi.org/10.1038/cdd.2016.48
73. Basak D, Punganuru SR, Srivenugopal KS (2016) Piperlongumine exerts cytotoxic effects against cancer cells with mutant p53 proteins at least in part by restoring the biological functions of the tumor suppressor. Int J Oncol 48(4):1426–1436. https://doi.org/10.3892/ijo.2016.3372
74. Mori A, Lehmann S, Kelly J, Kumagai T, Desmond JC, Pervan M, McBride WH, Kizaki M, Koeffler HP (2006) Capsaicin, a component of red peppers, inhibits the growth of androgen-independent, p53 mutant prostate cancer cells. Can Res 66(6):3222
75. Wang X, Di Pasqua AJ, Govind S, McCracken E, Hong C, Mi L, Mao Y, Wu JY, Tomita Y, Woodrick JC, Fine RL, Chung FL (2011) Selective depletion of mutant p53 by cancer chemopreventive isothiocyanates and their structure-activity relationships. J Med Chem 54(3):809–816. https://doi.org/10.1021/jm101199t
76. A study of the effects of PEITC on oral cells with mutant p53. https://ClinicalTrials.gov/show/NCT01790204
77. Clark R, Lee S-H (2016) Anticancer properties of capsaicin against human cancer. Anticancer Res 36(3):837–843
78. Kuo YC, Kuo PL, Hsu YL, Cho CY, Lin CC (2006) Ellipticine induces apoptosis through p53-dependent pathway in human hepatocellular carcinoma HepG2 cells. Life Sci 78(22):2550–2557. https://doi.org/10.1016/j.lfs.2005.10.041
79. Peng Y, Li C, Chen L, Sebti S, Chen J (2003) Rescue of mutant p53 transcription function by ellipticine. Oncogene 22(29):4478–4487. https://doi.org/10.1038/sj.onc.1206777
80. Kuo P-L, Hsu Y-L, Chang C-H, Lin C-C (2005) The mechanism of ellipticine-induced apoptosis and cell cycle arrest in human breast MCF-7 cancer cells. Cancer Lett 223(2):293–301
81. Wang F, Liu J, Robbins D, Morris K, Sit A, Liu Y-Y, Zhao Y (2011) Mutant p53 exhibits trivial effects on mitochondrial functions which can be reactivated by ellipticine in lymphoma cells. Apoptosis 16(3):301–310. https://doi.org/10.1007/s10495-010-0559-8
82. Raj L, Ide T, Gurkar AU, Foley M, Schenone M, Li X, Tolliday NJ, Golub TR, Carr SA, Shamji AF, Stern AM, Mandinova A, Schreiber SL, Lee SW (2011) Selective killing of cancer cells by a small molecule targeting the stress response to ROS. Nature 475(7355):231–234. https://doi.org/10.1038/nature10167
83. Punganuru SR, Madala HR, Venugopal SN, Samala R, Mikelis C, Srivenugopal KS (2016) Design and synthesis of a C7-aryl piperlongumine derivative with potent antimicrotubule and mutant p53-reactivating properties. Eur J Med Chem 107:233–244. https://doi.org/10.1016/j.ejmech.2015.10.052
84. Issaeva N, Friedler A, Bozko P, Wiman KG, Fersht AR, Selivanova G (2003) Rescue of mutants of the tumor suppressor p53 in cancer cells by a designed peptide. Proc Natl Acad Sci USA 100(23):13303–13307. https://doi.org/10.1073/pnas.1835733100
85. Tal P, Eizenberger S, Cohen E, Goldfinger N, Pietrokovski S, Oren M, Rotter V (2016) Cancer therapeutic approach based on conformational stabilization of mutant p53 protein by small peptides. Oncotarget 7(11):11817–11837. https://doi.org/10.18632/oncotarget.7857
86. Guida E, Bisso A, Fenollar-Ferrer C, Napoli M, Anselmi C, Girardini JE, Carloni P, Del Sal G (2008) Peptide aptamers targeting mutant p53 induce apoptosis in tumor cells. Cancer Res 68(16):6550–6558. https://doi.org/10.1158/0008-5472.can-08-0137
87. Prabhu V, Hong B, Allen J, Zhang S, Lulla A, Dicker D, El-Deiry W (2016) Small molecule prodigiosin restores p53 tumor suppressor activity in chemoresistant colorectal cancer stem cells via c-Jun-mediated ΔNp73 inhibition and p73 activation. Cancer Res 76(7):1989–1999. https://doi.org/10.1158/0008-5472.CAN-14-2430
88. Darshan N, Manonmani HK (2015) Prodigiosin and its potential applications. J Food Sci Technol 52(9):5393–5407. https://doi.org/10.1007/s13197-015-1740-4
89. Hong B, Prabhu VV, Zhang S, van den Heuvel AP, Dicker DT, Kopelovich L, El-Deiry WS (2014) Prodigiosin rescues deficient p53 signaling and antitumor effects via upregulating p73 and disrupting its interaction with mutant p53. Cancer Res 74(4):1153–1165. https://doi.org/10.1158/0008-5472.can-13-0955
90. Tiwary R, Yu W, Sanders BG, Kline K (2011) alpha-TEA cooperates with chemotherapeutic agents to induce apoptosis of p53 mutant, triple-negative human breast cancer cells via activating p73. Breast Cancer Res BCR 13(1):R1. https://doi.org/10.1186/bcr2801