Published in final edited form as:
Curr Pharm Des. 2011 ; 17(6): 631–639.

Pharmacological Activation of p53 in Cancer Cells

Mohammad Athar[1,*], **Craig A. Elmets**[1], and **Levy Kopelovich**[2]

[1]Department of Dermatology and Skin Diseases Research Center, University of Alabama at Birmingham, Birmingham, Alabama, USA. 35294

[2]Division of Cancer Prevention, National Cancer Institute, Bethesda, Maryland, USA 20892

Abstract

Tumor suppressor p53 is a transcription factor that regulates a large number of genes and guards against genomic instability. Under multiple cellular stress conditions, p53 functions to block cell cycle progression transiently unless proper DNA repair occurs. Failure of DNA repair mechanisms leads to p53-mediated induction of cell death programs. p53 also induces permanent cell cycle arrest known as cellular senescence. During neoplastic progression, p53 is often mutated and fails to efficiently perform these functions. It has been observed that cancers carrying a wild-type p53 may also have interrupted downstream p53 regulatory signaling leading to disruption in p53 functions. Therefore, strategies to reactivate p53 provide an attractive approach for blocking tumor pathogenesis and its progression. p53 activation may also lead to regression of existing early neoplastic lesions and therefore may be important in developing cancer chemoprevention protocols. A large number of small molecules capable of reactivating p53 have been developed and some are progressing through clinical trials for prospective human applications. However, several questions remain to be answered at this stage. For example, it is not certain if pharmacological activation of p53 will restore all of its multifaceted biological responses, assuming that the targeted cell is not killed following p53 activation. It remains to be demonstrated whether the distinct biological effects regulated by specific post-transnationally modified p53 can effectively be restored by refolding mutant p53. Mutant p53 can be classified as a loss of function or gain of function protein depending on the type of mutation. It is also unclear whether reactivation of mutant p53 has similar consequences in cells carrying gain-of-function and loss-of-function p53 mutants. This review provides a description of various pharmacological approaches tested to activate p53 (both wild-type and mutant) and to assess the effects of activated p53 on neoplastic progression

Keywords

p53; cancer therapeutics; mutant p53 refolding; restoration of tumor suppressor functions; mdm2

I. INTRODUCTION

p53 is a major tumor suppressor discovered as a cellular SV40 large T-antigen binding protein in 1979 [1]. Since then, there has been tremendous interest in discovering its role in diverse cellular functions, particularly under stress conditions. The importance of this tumor suppressor became more evident when approximately 50% of all cancers were discovered to have mutant p53 and the remaining tumors showed disrupted downstream pathways known to be involved in the activation and homeostasis of this protein [2]. Defects in p53-

[*]To whom correspondence should be addressed: Mohammad Athar, Ph.D., Department of Dermatology, University of Alabama at Birmingham, 1530 3rd Avenue South, VH 509, Birmingham, AL 35294-0019, USA. Tel: (205) 975-2616 mathar@uab.edu.

dependent functions allow cancer cells to escape normal stress responses. The ability of p53 to manifest growth arrest or induce apoptosis in response to diverse cellular stresses, such as DNA damage, oncogenic activation, hypoxia, etc., are considered as key functions for its tumor suppressor activities [3]. Interestingly, recent studies highlighted its role in various other diverse cellular functions in addition to balancing cell growth and death. These special functions include but are not limited to autophagy, cellular metabolism, and extracellular effects that regulate tumor stroma, angiogenesis, metastasis, and escape from innate immune responses [4]. However, current pharmacological approaches de-signed to activate wild-type p53 or revert mutant p53 to perform wild-type functions focus primarily on cell cycle regulation, DNA repair, cellular redox state, and anti-apoptotic activities [5,6]. Table **1** summarizes the majority of the therapeutic approaches related to p53. However, this review provides a description of a sample of important small molecules which have been tested in a variety of cancer models. Finally, a recent study provided a basis for a thera-peutic approach that may selectively inhibit tumors in p53 deficient patients using small molecules [120].

II. DEVELOPMENT OF SMALL MOLECULES FOR p53 ACTIVATION

i. Activating Wild-type p53

Since 50% of tumors carry wild-type p53 which is nonfunctional as a result of disruption in downstream signaling regulating its cellular levels, this therapeutic strategy focuses on rectifying these downstream signaling regulating proteins [7]. In this regard, the emphasis is on developing small molecule inhibitors of E3 ligase activity of MDM2 or on developing agents that can bind the hydrophobic p53 pocket in MDM2 [8]. One concern that must be addressed before these molecules can be taken to the clinic is their toxicity in normal tissues. It is likely that these agents may induce p53 in normal cells, causing their non-specific toxicity. However, potential toxicity in normal cells depends on the ability of these chemicals to induce differential p53 protein concentrations between normal and cancer cells.

A. Targeting Mdm2-p53 Axis—Based on the fact that in normal cells p53 levels are kept low through the activity of its negative regulator degrading enzymes, these proteins have been considered as therapeutic targets in cancers carrying wild-type p53. The most extensively studied p53 degrading enzyme is an E3 ubiquitin ligase, MDM2 (designated as HDM2 in humans), which binds to the N-terminal transcriptional activation domain of p53. The binding of MDM2 with p53 inhibits its transcriptional act ivit y and targets it for proteosomal degradation. It is known that interruption of MDM2/p53 interactions leads to the cellular accumulation of p53 protein following various stress conditions including DNA damage. p53 accumulation may have severe consequences ranging between a reversible cell cycle arrest, induction of DNA repair machinery, senescence, or cell death [5,7,8]. The exact molecular mechanism for regulating this range of effects is not well characterized. However, several p53 partner proteins or enzymes important in its post-translational modifications can dictate the manifestations between cell cycle arrest and apoptosis [9]. Pharmacological reactivation of p53 by small molecules that target MDM2 is considered as an important approach to therapy of cancers retaining wild-type p53 gene but carrying compromised functional components due to dysregulation of MDM2.

a. RITA, (reactivation of p53 and induction of tumor cell apoptosis) is a small molecule which was identified in a cell-based screen for wild-type p53-reactivating compounds. RITA prevents p53-HDM2 interaction both *in vitro* and *in vivo*. It has also affected p53 interactions with its several negative regulators Treatment of wild-type p53 carrying tumor cells with RITA induced p53 target genes and manifested massive apoptosis [10]. RITA also suppressed the growth of human fibroblasts and lymphoblasts only upon oncogene expression and showed substantial p53-dependent antitumor effects *in vivo*. It has also been shown that MDM2 released from p53 by RITA promotes degradation of p21 and the p53

cofactor hnRNP K, required for the transcription of p21. These studies also uncovered that MDM2-dependent inhibition of p21 acts as a switch capable of regulating cell fate decisions upon p53 reactivation. In an independent study it was shown that RITA-mediated p53 activation [11] unleashes the transcriptional repression of anti-apoptotic proteins, Mcl-1, Bcl-2, MAP4, and survivin. In addition, it blocks the Akt signaling pathway on various levels by down regulating c-Myc, cyclin E, and j3-catenin [12]. Interestingly, these studies show that the threshold for p53-mediated repression of survival genes is higher than for transactivation of pro-apoptotic target genes [13]. Furthermore, these studies demonstrate that inhibition of oncogenes by p53 reduces the cell's ability to buffer pro-apoptotic signals leading to robust apoptosis [14].

b. Nutlins, cis-imidazoline analogs, are another category of very well studied inhibitors of MDM2-p53 complex. These agents strongly bind with and selectively inhibit MDM2. Among this class of compounds nutlin-3 is known to displace p53 from MDM2-p53 complex by blocking the MDM2 binding pocket in p53 [15]. As learned from murine models, deletion of the MDM2 gene is highly toxic. However, pharmacological antagonism does not manifest major toxicity [16]. Treatment of tumor cells with MDM2 inhibitors induces apoptosis both *in vitro* and in murine xenograft models. However, normal non-malignant cells and tissues remain largely unaffected [17]. In different tumor types, nutlin treatment has been shown to induce apoptosis or senescence [18]. It also inhibits autophagy and affects tumor tissue differentiation programming [19], however the mechanisms by which these changes are regulated are not clearly understood. Nutlin-3 was found to be effective against a variety of tumor-types including acute myelogeneous leukemia, myeloma, and acute lymphoblastic leukemia, B-cell chronic lymphocytic leukemia, neuroblastoma, in addition to some other solid malignancies [20]. Additional studies conducted with a combinatorial approach using nutlin-3 and other established anti-tumor agents, for example vinblastine or roscovitine, etc., showed a synergistic tumor inhibitory action [21]. In contrast to these positive effects, tumor resistance to nutlin-3 treatment has also been reported. It has been shown that nutlin-3 treatment results in a p53-dependent activation of NOTCH1 which, in turn, limits the apoptosis inducing effects of nutlin-3. Support for this notion is provided by studies in which treatment of tumor cells with nutlin-3 and y-secretase inhibitors DAPT and L-685458, the known blockers of NOTCH signaling pathway were found effective in overcoming tumor resistance against nutlin-3 [22]. In a murine model of prostate carcinogenesis, nutlin-3 was found to enhance PTEN-loss-induced cellular senescence (PICS) leading to tumor regression [23].

c. Other Approaches to Induce Wild-type p53—A number of other approaches to induce wild-type p53 have proved successful in cell culture and xenograft murine model systems. In this regard, tenovin-1 and tenovin-6 were found to reversibly increase p53 and p21CIP/WAF1 expression and decrease cellular growth. These agents were found to be potent inhibitors of SIRT1 and SIRT2. SIRT1 is an important known negative regulator of p53 functions [24]. Other agents that are included in this category are MDM4/MDMX inhibitors and nuclear export signal inhibitors [25–27]. Although these agents have promise for development as important therapeutic agents, studies are required to under-stand their exact mechanisms of action, off-target effects and to define their utility as either single agents or a part of a combinatorial protocol with other p53 regulating or chemotherapeutic drugs. It is likely that these agents may be helpful in the restoration of chemical sensitivity against chemo-resistance in cancer cells.

ii. REACTIVATING MUTANT p53

The high frequency of p53 mutations in human cancers and increased resistance of mutant p53 expressing tumors to conventional chemotherapy and radiation therapy makes mutant

p53 an appealing cancer therapy target [28]. Further support for this concept comes from studies demonstrating that restoration of p53 expression in p53 deficient murine tumors triggers efficient removal of tumor cells. However, mutant p53 reactivation remains a challenge since a range of p53 mutations occur in human tumors. These mutations may give rise to unique structural alterations in the p53 protein [29] and therefore a single small molecule-mediated reversion of mutant p53 to wild-type conformation may not prove very efficient.

a. Various strategies were adopted for the discovery of mutant p53 reactivating chemical compounds. In this regard, the use of reverse chemicogenetic approaches led to identification of CP-31398, an agent found to refold mutant p53 into its wild-type conformation. This restoration of wild-type epitope (the 1620 epitope) to mutant p53 protein was associated with the transcriptional activation of p53 target genes. CP-31398 manifested anti-tumor effects in murine xenograft models [30]. Interestingly, this small molecule was also found to stabilize wild-type p53 protein through its reduced ubiquitination [31]. CP-31398 was found to restore wild-type p53 functions to multiple p53 mutants although there is a lack of evidence that any stable physical association occurs between CP-31398 and mutant p53 proteins [32]. Although there is a controversy about the exact mechanism of action of this Pfizer compound [33], it is assumed that it intercalates with DNA to manifest the observed tumor growth blocking effects [34].

Recently, we demonstrated the chemopreventive and chemotherapeutic effects of CP-31398 in a SKH-1 murine skin neoplasm [35]. Similar to humans who develop epidermal hyperplasia, actinic keratoses, and SCCs, these animals manifest pathogenesis of skin hyperplasia, benign squamous papillomas, and SCCs following chronic UVB irradiation [36]. The molecular changes-associated with the pathobiology of these lesions in SKH-1 mice closely resembles that of sun-exposed human skin. These studies showed that CP-31398 restores p53-dependent cell cycle arrest and apoptosis in UVB-irradiated $p53^{+/+}$ skin but not in UVB-irradiated $p53^{-/-}$ skin. Employing a standard UVB cancer chemoprevention protocol, we demonstrated that CP-31398 is a potent chemopreventive agent in skin. It reduces the growth of UVB-induced cutaneous SCCs and affords protection against cutaneous photo carcinogenesis. In addition, in human epidermoid carcinoma A431 cells carrying a mutation in codon 273, R273H (Arg to His) of the p53 gene that abolishes the ability of p53 to transactivate its downstream target genes, CP-31398 induces p53-dependent cell cycle arrest and apoptosis. CP-31398 also leads to mitochondrial translocation of p53, as well as p53-dependent mitochondrial opening of membrane permeability pore (MOMP), thereby signaling the release of cytochrome *c* and activation of caspase-3 [35]. This p53 translocation can be inhibited by cyclosporine A (CsA), pointing to a possible molecular crosstalk between the CsA inhibitable mitochondrial permeability transition pore and the p53 system [37]. In an independent study, we also demonstrated that CP-31398 blocks the growth of rhabdomyosar-coma (RMS) cells irrespective of their p53 status (wild-type or mutant). RMS is a commonly occurring soft-tissue sarcoma of childhood for which effective therapeutic options are lacking [38]. The mechanism by which CP-31398 blocks RMS growth involves cell cycle arrest followed by apoptosis in a p53-dependent manner. CP-31398 blocks proliferation in these cells through the co-induction of SOX9 and p21. It also reduces the invasiveness of these tumors by inducing mesenchymal-epithelial transition, ultimately leading to enhanced tumor-free survival of the host animals [38]. These studies suggest that reactivation of p53 may play a role in reversing the aggressiveness of neoplasm. In another study employing intestinal cancer prone APC^{MIN} mice, CP-31398 was found to reduce the growth of spontaneous colon tumors. In this study the authors found that CP-31398 was more effective in the preventative rather than therapeutic mode of action [39]. In an independent study, these authors also showed a

positive tumor growth blocking response in the presence of a specific COX-2 inhibitor, celecoxib [40].

b. Ellipticine is another such compound which can enhance sequence specific DNA binding and transcriptional activity of mutant p53 leading to p53-dependent cell cycle arrest and cell death [41]. Ellipticine was initially discovered by analysis of drug sensitive profiles of the panel of NCI's tumor cell lines, showing specificity for p53 mutant carrying cell lines [42]. In a recent study, this small molecule was found to enhance the ability of 5-fluorouracil to deplete cancer stem cell populations [43].

c. In a phenotypic screen of a chemical library of compounds which can preferentially kill cancer cells expressing mutant p53 and have no effects on isogenic cells lacking p53, Bykov *et. al.* discovered **PRIMA-1**. This chemical agent was found to restore transcriptional activity of numerous p53 mutant proteins and induce apoptosis. Parenteral administration of this compound was found to suppress the growth of human tumor xenografts [44]. The mechanism of action of PRIMA-1 is not quite clear. However, it involves transcriptional activation of mutant p53 and p53-dependent genes such as PUMA, Noxa and Bax in addition to apoptosis induction [45]. Caspase-2 induction was found to be involved in PUMA-mediated apoptotic cell death in lung, ovarian, and osteosarcoma cells, all carrying a mutant p53 [46]. In addition, JNK survival pathway inhibition by PRIMA-1 has also been demonstrated to be the underlying mechanism of the reduction of breast and colon cell survival [47]. PRIMA-1 was found efficient in killing tumor cells from patients with acute myeloid leukemia (AML) and chronic lymphocytic leukemia (CLL). Its more effective analog PRIMA-1 (also known as APR-246) was found to have a suitable pharmacokinetics and absence of toxic manifestations in animal models suggesting its potential for clinical applications [48].

d. The p53 family member's p63 and p73 contribute to the tumor suppressor functions of p53. These isoforms share many common transcriptional targets with p53 and, as a result, p63 and p73 affect p53-dependent apoptosis and chemosensitivity to cancer cells [49]. It is also known that activation of p73 in human cancer cells lacking p53 produces substantial cytotoxic effects providing a logic for employing p73 as an anti-cancer molecular target [50]. In this regard, a small molecule **RETRA** was identified as an activator of p73. RETRA activates a number of p53-regulated genes and suppresses the growth of tumor cells carrying mutant p53 [50]. The mechanism of action of RETRA involves release of p73 from its blocking complex with mutant p53 [50]. Interestingly, this agent does not affect normal cells. In this regard, Bell *et al* described a peptide-dependent pharmacological approach to directly activate p73. This peptide binds with iASPP, a common inhibitor of p53 family proteins, and disrupts the interaction between iASPP and p73. This in turn results in derepression of endogenous p73, which induces p73-dependent cell death and tumor regression irrespective of the presence or absence of wild-type p53 [51].

iii. TARGETING p53 TO REGULATE ENERGY METABOLISM

Investigated long ago by Warburg (1956), the rate of anaerobic lactic acid production in cancer cells is much higher than in normal cells. However, it has recently been linked to a p53-dependent pathway [52]. It has been shown that p53 is involved in the modulation of glycolysis. Pyruvate, the major metabolic product of glycolysis, induces p53 expression [52]. Another link between p53 and aerobic respiration was provided by Maoba *et. al.* who showed that $p53^{+/+}$ mice consumed significantly more oxygen and produced more ATP than their $p53^{-/-}$ littermates [53]. Various enzymes involved in glycolysis are also regulated by p53. Type 2 hexokinase, an enzyme responsible for the conversion of glucose to glucose-6-phosphate during the initial reaction of glycolysis, is up regulated by p53 [54]. It has also been shown that this enzyme possesses a p53 response element in the promoter region of its

gene [55]. Hypoxia which induces p53 expression also induces type 2 hexokinase [56]. Phosphoglyceratemutase, another enzyme involved in the glycolysis which converts 3-phosphoglycerate to 2-phosphoglycerate during the late stage of glycolysis, is negatively regulated by p53. TP53-induced glycolysis and apoptosis regulator (TIGAR) has recently been discovered to connect p53 with glucose metabolism [57]. TIGAR is similar to proteins of the phosphoglyceratemutase family and shares similarity with bisphosphatase domain of 6-phosphofructo-2-kinase/fructose-2, 6-bisphosphatase [58]. An increase in TIGAR expression results in a decrease in fructose-2, 6-bisphosphatase levels to blunt glycolytic flux. TIGAR also decreases the cellular levels of reactive oxygen species (ROS) during mild cellular stress. Interestingly, ROS can upregulate p53 transcriptional activity thus providing a feedback loop. This mechanism is significant during hypoxic conditions where ROS are required to partially mediate the effects of radiation and anti-cancer drugs [59]. In addition to regulating the proteins involved in mitochondrial apoptotic pathway (intransic) such as Bac, Bcl-2 and PUMA, p53 is considered important in regulating the translocation of proteins localized to the outer surface of mitochondria thus regulating mitochondrial membrane potential [60]. These differences in the energy metabolism of p53 deficient and proficient cells provide a therapeutic opportunity to target specifically p53 deficient cells. However, it remains to be demonstrated whether some of the target proteins in this pathway represent an effective druggable target.

iv. TARGETING P53 AND NUCLEAR REPROGRAMMING

Recently, the presence of non-functional p53 or its absence was shown to facilitate nuclear reprogramming. In this regard, Takaha-shi and Yamanaka reported that overexpression of four transcription factors, Oct4, Sox2, Klf4, and c-Myc, is required to attain the *de novo* pluripotency of murine and human differentiated cells [61]. This reprogramming of somatic cells highlights a dedifferentiation process resembling tumor formation. It has been shown that p53 acts as a barrier to somatic cell reprogramming [62]. Recently, Sarig *et al* showed that a mutant form of p53 increases the efficiency of the reprogramming process beyond that facilitated by the absence of p53 alone. In this regard, mouse embryonic fibroblasts (MEFs) derived from p53 R172H-knockin mice were reprogrammed more efficiently than MEFs derived from either wild-type or p53 knockout mice [63]. Hypothetically, this provides a window for gene therapy dependent dedifferentiation of tumor cells into normal or normal appearing cells that may ultimately differentiate and then be eliminated.

iii. CONCLUSIONS AND PROSPECTS

In summary, the major strategies for translating p53 research into the clinic to treat cancer have been focused on developing small molecules that may target mutant p53 and refold it to its wild-type conformation or target molecules that eventually may lead to enhanced accumulation of wild-type p53 [64]. Nonetheless, development of vaccines against p53 if successful in human trials is considered a more versatile approach than the development of small molecules. Both approaches are very appealing but the final outcome in terms of successful cancer therapy is not certain at this stage. According to a recent report, a least 151 trials exploiting the p53 pathway have been conducted [65]. p53 activation in cancers occurring at different anatomical sites does not produce identical therapeutic effects [66]. Studies utilizing genetic approaches revealed that the responses of p53 activation are also dependent on tumor microenvironment that, in part, is controlled by innate immunity [67]. In addition, activation of aberrant oncogenic signaling pathways that drive tumor growth may interfere with p53 activation signaling leading to alterations in final outcome or even in some cases override the tumor growth-halting effect of p53 activation. These effects, in turn, may result in tumor resistance against p53 activating drugs. The success of various strategies for vaccine development has been partial as a significant percentage of patients do not show the desired tumor regression [68]. Current approaches to improve p53 vaccine-based cancer

treatment exploit stronger and more correctly polarized responses for the development of both DNA-based and dendritic cell-delivered p53 vaccines. These novel approaches are likely to overcome the existing obstacles in the development of p53 vaccines.

The future prospect for development of p53-based therapeutics lies in identifying novel p53-dependent drugable targets. Most current investigations related to p53-activating small molecules have been conducted either in cell culture systems and/or employing highly immunodeficient murine xenograft models. Use of immune competent murine models that faithfully recapitulate human pathology will be more informative in predicting clinical results. Current genetic models in this regard demonstrate that the outcome of p53 activation is tissue context dependent. However, pharmacological activation of p53 has not been tested from this perspective. Investigations are also needed to unravel the unique mechanisms associated with p53 activation and their biological consequences in terms of invoking crosstalk with the host tissue and regulating tumor microenvironment.

Acknowledgments

Grant Support from the following awards, R01 ES015323, R21ES017494, NCI NO1-CN-43300 261200433001C-521958, P30AR050948.

REFERENCES

[1]. DeLeo AB, Appella JG, Dubois GC, Law LW, Old LJ. Detection of a transformation-related antigen in chemically induced sarcomas and other transformed cells of the mouse. Proc Natl Acad Sci USA. 1979; 76(5):2420–4. [PubMed: 221923]

[2]. Zilfou JT, Lowe SW. Tumor suppressive functions of p53. Cold Spring Harb Perspect Biol. 2009; 1(5):a001883. [PubMed: 20066118]

[3]. Beckerman R, Prives C. Transcriptional regulation by p53. Cold Spring Harb Perspect Biol. 2010; 2(8):a000935. [PubMed: 20679336]

[4]. Gottlieb E, Vousden KH. P53 regulation of metabolic pathways. Cold Spring Harb Perspect Biol. 2010; 2(4):a001040. [PubMed: 20452943]

[5]. Cheok CF, Verma CS, Baselga J, Lane DP. Translating p53 into the clinic. Nat Rev Clin Oncol. 2011; 8(1):25–37. [PubMed: 20975744]

[6]. Mandinova A, Lee SW. The p53 pathway as a target in cancer therapeutics: obstacles and promise. Sci Transl Med. 2011; 3(64):64rv1.

[7]. Haupt S, Haupt Y. Improving cancer therapy through p53 man-agement. Cell Cycle. 2004; 2(7): 912–6. [PubMed: 15254405]

[8]. Allende-Vega N, Saville MK. Targeting the ubiquitin-proteasome system to activate wild-type p53 for cancer therapy. Semin Cancer Biol. 2010; 20(1):29–39. [PubMed: 19897040]

[9]. Kruse JP, Gu W. Modes of p53 regulation. Cell. 2009; 137(4):609–22. [PubMed: 19450511]

[10]. Issaeva N, Bozko P, Enge M, et al. Small molecule RITA binds to p53, blocks p53-HDM-2 interaction and activates p53 function in tumors. Nat Med. 2004; 10(12):1321–8. [PubMed: 15558054]

[11]. Yang J, Ahmed A, Poon E, et al. Small-molecule activation of p53 blocks hypoxia-inducible factor 1 alpha and vascular endothelial growth factor expression *in vivo* and leads to tumor cell apoptosis in normoxia and hypoxia. Mol Cell Biol. 2009; 29(8):2243–53. [PubMed: 19223463]

[12]. Saha MN, Jiang H, Mukai A, Chang H. RITA inhibits multiple myeloma cell growth through induction of p53-mediated caspase-dependent apoptosis and synergistically enhances nutlin-induced cytotoxic responses. Mol Cancer Ther. 2010l; 9(11):3041–51. [PubMed: 21062913]

[13]. Grinkevich VV, Nikulenkov F, Shi Y, et al. Ablation of key onco-genic pathways by RITA-reactivated p53 is required for efficient apoptosis. Cancer Cell. 2009; 15(5):441–53. 5. [PubMed: 19411072]

[14]. Zhao CY, Grinkevich VV, Nikulenkov F, Bao W, Selivanova G. Rescue of the apoptotic-inducing function of mutant p53 by small molecule RITA. Cell Cycle. 2010; 9(9):1847–55. [PubMed: 20436301]

[15]. Vassilev LT, Vu BT, Graves B, et al. *In vivo* activation of the p53 pathway by small-molecule antagonists of MDM2. Science. 2004; 303(5659):844–8. [PubMed: 14704432]

[16]. Verma R, Rigatti MJ, Belinsky GS, Godman CA, Giardina C. DNA damage response to the Mdm2 inhibitor nutlin-3. Biochem Phar-macol. 2010; 79(4):565–74.

[17]. Shangary S, Wang S. Small molecule inhibitors of the MDM2-p53 protein-protein interaction to reactivate p53 function: a novel ap-proach for cancer therapy. Annu Rev Pharmacol Toxicol. 2009; 49:223–41. [PubMed: 18834305]

[18]. Leonitieva OV, Gudkov AV, Blagosklonny MV. Weak p53 permits senescence during cell cycle arrest. Cell Cycle. 2010; 9(21):4323–7. [PubMed: 21051933]

[19]. Zauli G, Fimondi E, Corallini F, Fadda R, Capitani S, Secchiero P. MDM2 antagonist Nutlin-3 suppresses the proliferation and differ-entiation of human pre-osteoclasts through a p53-dependent path-way. J Bone Miner Res. 2007; 22(10):1621–30. [PubMed: 17592964]

[20]. Lauria A, Tutone M, Ippolito M, Pantano L, Almerico AM. Mo-lecular modeling approaches in the discovery of new drugs for anti-cancer therapy: the investigation of p53-MDM2 interaction and its inhibition by small molecules. Curr Med Chem. 2010; 17(28):3142–54. [PubMed: 20666726]

[21]. Cheok CF, Dey A, Land DP. Cyclin-dependent kinase inhibitors sensitize tumor cells to nutlin-induced apoptosis: a potent drug combination. Mol Cancer Res. 2007; 5(11):1133–45. [PubMed: 18025259]

[22]. Secchiero P, Melloni E, di lasio MG, et al. Nutlin-3 up-regulates the expression of Notch1 in both myeloid and lymphoid leukemic cells, as part of a negative feedback antiapoptotic mechanism. Blood. 2009; 113(18):4300–8. [PubMed: 19190243]

[23]. Alimonti A, Nardella C, Chen Z, et al. A novel type of cellular senescence that can be enhanced in mouse models and human tu-mor xenografts to suppress prostate tumorigenesis. J Clin Invest. 2010; 120(3):681–93. [PubMed: 20197621]

[24]. Lain S, Hollick JJ, Campbell J, et al. Discovery, *in vivo* activity, and mechanism of action of a small-molecule p53 activator. Cancer Cell. 2008; 13(5):454–63. [PubMed: 18455128]

[25]. Reed D, Shen Y, shelat AA, et al. Identification and characteriza-tion of the first small molecule inhibitor of MDMX. J Biol Chem. 2010; 285(14):10786–96. [PubMed: 20080970]

[26]. Macchiarulo A, Pellicciari R. MDM2/MDMX inhibitor peptide: W02008106507. Expert Opin Ther Pat. 2009; 19(5):721–6. [PubMed: 19441944]

[27]. Mancini F, Di Conza G, Monti O, Macchiarulo A, Pellicciari R, Pontecorvi A, Moretti F. Puzzling over MDM4-p53 network. Int J Biochem Cell Biol. 2010; 42(7):1080–3. [PubMed: 20417304]

[28]. Smith ML, Fornace AJ Jr. Chemotherapeutic targeting of p53. Cancer Biology & Therapy. 2002; 1(1):56–7. [PubMed: 12197484]

[29]. Joerger AC, Fersht AR. The tumor suppressor p53: From structures to drug discovery. Cold Spring Harb Perspect Biol. 2010; 2:a000919. [PubMed: 20516128]

[30]. Foster BA, Coffey HA, Morin MJ, Rastinejad F. Pharmacological rescue of mutant p53 conformation and function. Science. 1999; 286(5449):2507–10. [PubMed: 10617466]

[31]. Wang W, Takimoto R, Rastinejad F, el-Deiry WS. Stabilization of p53 by CP-31398 inhibits ubiquitination without altering phos-phorylation at serine 15 or 20 or MDM2 binding. Mol Cell Biol. 2003; 23(6):2171–81. [PubMed: 12612087]

[32]. Tanner S, Barberis A. CP-31398, a putative p53-stabilizing mole-cule tested in mammalian cells and in yeast for its effects on p53 transcriptional activity. J Negat Results Biomed. 2004; 3(5)

[33]. Rippin TM, Bykov VJ, Freund SM, Selivanova G, Wiman KG, Fersht AR. Characterization of the p53-rescue drug CP-31398 in vitro and in living cells. Oncogene. 2002; 21(14):2119–29. [PubMed: 11948395]

[34]. Takimoto R, Wang W, Dicker DR, Rastinejad F, Lyssikatos J, Rastinejad F. The mutant p53-conformation modifying drug, CP-31398, can induce apoptosis of human cancer cells and can stabi-lize wild-type p53 protein. Cancer Biol Ther. 2002; 1(1):56–7. [PubMed: 12197484]

[35]. Tang X, Zhu Y, Han L, Kim AL, Kopelovich L, Bickers DR, Athar M. CP-31398 restores mutant p53 tumor suppressor function and inhibits UVB-induced skin carcinogenesis in mice. J Clin Invest. 2007; 117(12):3658–60. [PubMed: 18060027]

[36]. Bickers DR, Athar M. Oxidative stress in the pathogenesis of skin disease. J Invest Dermatol. 2006; 126(12):2565–75. [PubMed: 17108903]

[37]. Ow YP, Green DR, Hao Z, Mak TW. Cytochrome c: functions beyond respiration. Nat Rev Mol Cell Biol. 2008; 9(7):532–42. [PubMed: 18568041]

[38]. Xu J, Timares L, Heilpern C, Weng Z, Li C, Xu H, Pressey JG, Elmets CA, Kopelovich L, Athar M. Targeting wild-type and mu-tant p53 with small molecule CP-31398 blocks the growth of rhabdomyosarcoma by inducing reactive oxygen species-dependent apoptosis. Cancer Res. 2010; 70(16):6566–76. [PubMed: 20682800]

[39]. Rao CV, Swamy MV, Patiolla JM, Kopelovich L. Suppression of familial adenomatous polyposis by CP-31398, a TP53 modulator, in APCmin/+ mice. Cancer Res. 2008; 68(18):7670–75. [PubMed: 18794156]

[40]. Rao CV, Steele VE, Swamy MV, Patiolla JM, Guruswarmy S, Kopelovich L. Inhibition of azoxymethane-induced colorectal can-cer by CP-31398, a TP53 modulator, alone or in combination with low doses of celecoxib in male F344 rats. Cancer Res. 2009; 69(20):8175–82. [PubMed: 19826045]

[41]. Peng Y, Li C, Chen L, Sebti S, Chen J. Rescue of mutant p53 tran-scription function by ellipticine. Oncogene. 2003; 22(29):4478–87. [PubMed: 12881704]

[42]. Shi LM, Myers TG, Fan Y, O'Connor PM, Paull KD, Friend SH, Weinstein JN. Mining the National Cancer Institute aniticancer drug discovery database: cluster analysis of ellipticine analogs with p53-inverse and central nervous system-selective patterns of activ-ity. Mol Pharmacol. 1998; 53(2):241–51. [PubMed: 9463482]

[43]. Huang C, Zhang XM, Tavaluc RT, Hart LS, Dicker DT, Wang W, El-Deiry WS. The combination of 5-fluorouracil plus p53 pathway restoration is associated with depletion of p53-deficient or mutant p53-expressing putative colon cancer stem cells. Cancer Biol Ther. 2009; 8(22):2186–93. [PubMed: 19923910]

[44]. Bykov VJ, Issaeva N, Shilov A, Hultcrantz M, Pugacheva E, Chu-makov P, Bergman J, Wiman KG, Selivanova G. Restoration of the tumor suppressor function to mutant p53 by a low-molecular-weight compound. Nat Med. 2002; 8(3):282–8. [PubMed: 11875500]

[45]. Lambert JM, Moshfegh A, Hainaut P, Wiman KG, Bykov VJ. Mutant p53 reactivation by PRIMA-1MET induces multiple signal-ing pathways converging on apoptosis. Oncogene. 2010; 29(9):1329–38. [PubMed: 19946333]

[46]. Wang T, Lee K, Rehman A, Daoud SS. PRIMA-1 induces apopto-sis by inhibiting JNK signaling but promoting the activation of Bax. Biochem Biophys Res Commun. 2007; 352(1):203–12. [PubMed: 17113036]

[47]. Farnebo M, Bykov VJ, Wiman KG. The p53 tumor suppressor: a master regulator of diverse cellular processes and therapeutic target in cancer. Biochem Biophys Res Commun. 2010; 396(1):85–9. [PubMed: 20494116]

[48]. Bykov VJ, Zache N, Stridh H, Westman J, Bergman J, Selivanova G, Wiman KG. PRIMA-1(MET) synergizes with cisplatin to in-duce tumor cell apoptosis. Oncogene. 2005; 24(21):3484–91. [PubMed: 15735745]

[49]. Collavin L, Lunardi A, Del Sal G. p53-family proteins and their regulators: hubs and spokes in tumor suppression. Cell Death Dif-fer. 2010; 17(6):901–11.

[50]. Kravchenko JE, Ilyinskaya GV, Komarov PG, Agapova LS, Kochetkov DV, Strom E, Frolova EI, Kovriga I, Gudkov AV, Fein-stein E, Chumakov PM. Small-molecule RETRA suppresses mutant p53-bearing cancer cells through a p73-dependent salvage pathway. Proc Natl Acad Sci U S A. Apr 29; 2008 105(17):6302–7. 42: [PubMed: 18424558]

[51]. Bell HS, Dufes C, O'Prey J, Crighton D, Bergamaschi D, Lu X, Schatzlein AG, Vousden KH, Ryan KM. A p53-derived apoptotic peptide derepresses p73 to cause tumor regression in vivo. J Clin Invest. 2007; 117(4):1008–18. [PubMed: 17347683]

[52]. Hu W, Zhang C, Wu R, Sun Y, Levine A, Feng Z. Glutaminase 2, a novel 53 target gene regulating energy metabolism and antioxidant function. Proc. Natl Acad Sci USA. 2010; 107(16):7455–60. [PubMed: 20378837]

[53]. Choo AY, Kim SG, Vander Heiden MG, Mahoney SJ, Vu H, Yoon SO, Cantley LC, Blenis J. Glucose addiction of TSC null cells is caused by failed mTORC1-dependent balancing of metabolic de-mand with supply. Mol Cell. 2010; 38(4):487–99. [PubMed: 20513425]

[54]. Cheung EC, Vousden KH. The role of p53 in glucose metabolism. Curr Opin Cell Biol. 2010; 22(2):186–91. [PubMed: 20061129]

[55]. Malthupala SP, Heese C, Pedersen PL. Glucose catabolism in can-cer cells. The type II hexokinase promoter contains functionally ac-tive response elements for the tumor suppressor p53. J Biol Chem. 1997; 272(36):22776–80. [PubMed: 9278438]

[56]. Yalcin A, Telang S, Clem B, Chesney J. Regulation of glucose metabolism by 6-phosphofructo-2-kinase/fructose-2, 6-biphosphatases in cancer. Exp Mol Pathol. 2009; 86(3): 174–9. [PubMed: 19454274]

[57]. Bensaad K, Tsuruta A, Selak MA, Vidal MN, Nakano K, Bartrons R, Gottlieb E, Vousden KH. TIGAR, a p53-inducible regulator of glycolysis and apoptosis. Cell. 2006; 126(1):107–20. [PubMed: 16839880]

[58]. Salminen A, Kaamiranta K. Glycolysis links p53 function with NF-kappaB signaling: impact on cancer and aging process. J Cell Physiol. 2010; 224(1):1–6. [PubMed: 20301205]

[59]. Bensaad K, Cheung EC, Vousden KH. Modulation of intracellular ROS levels by TIGAR controls autophagy. EMBO J. 2009; 28(19):3015–26. [PubMed: 19713938]

[60]. Han J, Goldstein LA, Hou W, Gastman BR, Rabinowich H. Regu-lation of mitochondrial apoptotic events by p63-mediated disrup-tion of complexes between antiapoptotic Bcl-2 members and Bim. J Biol Chem. 2010; 285(29):22473–83. [PubMed: 20404322]

[61]. Ho R, Chronis C, Plath K. Mechanistic insights into reprogram-ming to induced pluripotency. J Cell Physiol. 2011; 226(4):868–78. [PubMed: 20945378]

[62]. Menendez S, Camus S, Izpisua Belmonte JC. p53: guardian of reprogramming. Cell Cycle. 2010; 9(19):3887–91. [PubMed: 20948296]

[63]. Sarig R, Rivlin N, Brosh R, Bornstein C, Kamer I, Ezra O, Mol-chadsky A, Goldfinger N, Brenner O, Rotter V. Mutant p53 facili-tates somatic cell reprogramming and augments the malignant po-tential of reprogrammed cells. J Exp Med. 2010; 207(10):2127–40. [PubMed: 20696700]

[64]. Brown CJ, Lain S, Verma CS, Fersht AR, Lane DP. Awakening guardian angels: drugging the p53 pathway. Nat Rev Cancer. 2009; 9(12):862–73. [PubMed: 19935675]

[65]. Cheok CF, Verma CS, Baselga J, Lane DP. Translating p53 into the clinic. Nat Rev Clin Oncol. 2011; 8(1):25–37. [PubMed: 20975744]

[66]. Ventura A, Kirsch DG, McLaughlin ME, Tuveson DA, Grimm J, Lintault L, Newman J, Reczek EE, Weissleder R, Jacks T. Restora-tion of p53 function leads to tumour regression in vivo. Nature. 2007; 445(7128):661–5. [PubMed: 17251932]

[67]. Xue W, Zender L, Miething C, Dickins RA, Hernando E, Krizha-novsky V, Cordon-Cardo C, Lowe SW. Senescence and tumour clearance is triggered by p53 restoration in murine liver carcino-mas. Nature. 2007; 445(7128):656–60. [PubMed: 17251933]

[68]. Zeimet AG, Marth C. Why did p53 gene therapy fail in ovarian cancer? Lancet Oncol. 2003; 4(7):415–22. [PubMed: 12850192]

[69]. Roth JA. Adenovirus p53 gene therapy. Expert Opin Biol Ther. 2006; 6(1):55–61. [PubMed: 16370914]

[70]. Zaiss AK, Machado HB, Herschman HR. The influence of innate and pre-existing immunity on adenovirus therapy. J Cell Biochem. 2009; 108(4):778–90. [PubMed: 19711370]

[71]. Thacker EE, Timares L, Matthews QL. Strategies to overcome host immunity to adenovirus vectors in vaccine development. Expert Rev Vaccines. 2009; 8(6):761–77. [PubMed: 19485756]

[72]. Shirakawa T. The current status of adenovirus-based cancer gene therapy. 2008; 25(4):462–6.

[73]. Speetjens FN, Kuppen PJ, Welters MJ, Essahsah F, Boet van den Brink AM, Lantrua MG, Valentijn AR, Oostendorp J, Fathers LM, Nijman HW, Drijfhout JW, van de Velde CJ, Melief CJ, van der Burg SH. Induction of p53-specific immunity by a p53 synthetic long peptide

vaccine in patients treated for metastatic colon cancer. Clin Cancer Res. 2009; 15(3):1086–95. [PubMed: 19188184]

[74]. van der Burg SH, de Cock K, Menon AG, Franken KL, Palmen M, Redeker A, Drijfhout J, Kuppen PJ, van de Velde C, Erdile L, Tollenaar RA, Melief CJ, Offringa R. Long lasting p53-specific T cell memory responses in the absence of anti-p53 antibodies in pa-tients with resected primary colorectal cancer. Eur J Immunol. 2001; 31:146–155. [PubMed: 11169448]

[75]. van der Burg SH, Menon AG, Redeker A, Franken KL, Drijfhout JW, Tollenaar RA, Hartgrink HH, van de Velde CJ, Kuppen PJ, Melief CJ, Offringa R. Magnitude and polarization of p53-specific T-helper immunity in connection to leukocyte infiltration of colorectal tumors. Int J Cancer. 2003; 107:425–33. [PubMed: 14506743]

[76]. Nijman HW, Lambeck A, van der Burg SH, van der Zee AG, Dae-men T. Immunologic aspect of ovarian cancer and p53 as tumor antigen. J Trans Med. 2005; 3:34.

[77]. Chiappori AA, Soliman H, Janssen WE, Antonia SJ, Gabrilovich DI. INGN-225: a dendritic cell-based p53 vaccine (Ad.p53-DC) in small cell lung cancer: observed association between immune re-sponse and enhanced chemotherapy effect. Expert Opin Biol Ther. 2010; 10:983–991. [PubMed: 20420527]

[78]. Vassilev LT. Small-molecule antagonists of p53-MDM2 binding: research tools and potential therapeutics. Cell Cycle. 2004; 3(4):419–21. [PubMed: 15004525]

[79]. Dey A, Wong ET, Bist P, Tergaonkar V, Lane DP. Nutlin-3 inhib-its NFkappaB pathway in a p53-dependent manner: implications in lung cancer therapy. Cell Cycle. 2007; 6(17):2178–85. [PubMed: 17786042]

[80]. Secchiero P, di lassio MG, Gonelli A, Zauli G. The MDM2 inhibi-tor Nutlins as an innovative therapeutic tool for the treatment of haematological malignancies. Curr Pharm Des. 2008; 14(21): 2100–10. [PubMed: 18691119]

[81]. Impicciatore G, Sancillo S, Miscia S, Di Pietro R. Nutlins and ionizing radiation in cancer therapy. Curr Pharm Des. 2010; 16(12):1427–42. [PubMed: 20166982]

[82]. Mohammad RM, Wu J, Azmi AS, Aboukameel A, Sosin A, Wu S, Yang D, Wang S, Al-Katib AM. An MDM2 antagonist (MI-319) restores p53 functions and increases the life span of orally treated follicular lymphoma bearing animals. Mol Cancer. 2009; 8:115. [PubMed: 19958544]

[83]. Azmi AS, Aboukameel A, Banerjee S, Wang Z, Mohammad M, Wu J, Wang S, Yang D, Philip PA, Sarkar FH, Mohammad RM. MDM2 inhibitor MI-319 in combination with cisplatin is an effective treatment for pancreatic cancer independent of p53 function. Eur J Cancer. 2010; 46(6): 1122–31. [PubMed: 20156675]

[84]. Grasberger BL, Parks DJ, Carver TE, Koblish HK, Cummings MD, LaFrance LV, Milkiewicz KL, Calvo RR, Maguire D, Lattanze J, Franks CF, Zhao S, Ramachandren K, Bylebyl GR, Zhang M, Manthey CL, Petrella E, Pantoliano MW, Deckman IC, Spurlino JC, Maroney AC, Tomczuk BE, Molloy CJ, Bone RF. Discovery and cocrystal structure of benzodiazepinedione HDM2 antagonists that activate p53 in cells. J Med Chem. 2005:909–912. [PubMed: 15715460]

[85]. Ding K, Lu Y, Nikolovska-Coleska Z, Wang G, Qiu S, Shangary S, Gao W, Qin D, Stuckey J, Krajewski K, Roller PP, Wang S. Struc-ture-based design of spiro-oxindoles as potent, specific small-molecule inhibitors of the MDM2-p53 interaction. J Med Chem. 2006; 49(12):3432–5. [PubMed: 16759082]

[86]. Canner JA, Sobo M, Ball S, Hutzen B, DeAngelis S, Willis W, Studebaker AW, Ding K, Wang S, Yang D, Lin J. MI-63: a novel small-molecule inhibitor targets MDM2 and induces apoptosis in embryonal and alveolar rhabdomyosarcoma cells with wild-type p53. Br J Cancer. 2009; 101(5):774–81. [PubMed: 19707204]

[87]. Samudio IJ, Duvvuri S, Clise-Dwyer K, Watt JC, Mak D, Kantar-jian H, Yang D, Ruvoio V, Borthakur G. Activation of p53 signal-ing by MI-63 induces apoptosis in acute myeloid leukemia cells. Leuk Lymphoma. 2010; 51(5):911–9. [PubMed: 20423286]

[88]. Lu C, Wang W, El-Deiry WS. Non-genotoxic anti-neoplastic effects of ellipticine derivative NSCI176327 in p53-deficient human colon carcinoma cells involve stimulation of p73. Cancer Biol Ther. 2008; 7(12):2039–46. [PubMed: 19106635]

[89]. Dar AA, Belkhiri A, Ecsedy J, Zaika A, El-Rifai W. Aurora kinase A inhibition leads to p73-dependent apoptosis in p53-deficient can-cer cells. 2008; 68(21):8998–9004.

[90]. Kojima K, Burks JK, Arts J, Andreeff. The novel tryptamine de-rivative JNJ-26854165 induces wild-type p53- and E2F1-mediated apoptosis in acute myeloid and lymphoid leukemias. Mol Cancer Ther. 2010; 9(9):2545–57. [PubMed: 20736344]

[91]. Lee S-H, Shen G-N, Jung YS, Lee S-J, Chung J-Y, Kim H-S, Xu Y, Choi Y, Lee J-W, Ha N-C, ong GY, Park B-J. Antitumor effect of novel small chemical inhibitors of Snail-p53 binding in K-Ras-mutated cancer cells. Oncogene. 2010; 29(32):4576–87. [PubMed: 20531295]

[92]. Luu Y, Li G. The p53-stabilizing compound CP-31398 enhances ultraviolet-B-induced apoptosis in a human melanoma cell line MMRU. J Invest Dermatol. 2002; 119(5):1207–9. [PubMed: 12445215]

[93]. Demma MJ, Wong S, Maxwell E, Dasmahapatra B. CP-31398 restores DNA-binding activity to mutant p53 in vitro but does not affect p53 homologs p63 and p73. J Biol Chem. 2004; 279(44): 4587–96.

[94]. Herbert BS, Pearce VP, Hynan LS, LaRue DM, Wright WE, Kope-lovich L, Shay JW. A peroxisome proliferator-activated receptor-gamma agonist and the p53 rescue drug CP-31398 inhibit the spon-taneous immortalization of breast epithelial cells. Cancer Res. 2003; 63(8):1914–9. [PubMed: 12702583]

[95]. Roh JL, Kang SK, Minn I, Califano JA, Sidransky D, Koch WM. P53-Reactivating small molecules induce apoptosis and enhance chemotherapeutic cytotoxicity in head and neck squamous cell car-cinoma. Oral Oncol. 2011; 47(1):8–15. [PubMed: 21109480]

[96]. Chipuk JE, Maurer U, Green DR, Schuler M. Pharmacologic acti-vation of p53 elicits Bax-dependent apoptosis in the absence of transcription. Cancer Cell. 2003; 4(5):371–81. [PubMed: 14667504]

[97]. Nahi H, Lehmann S, Mollgard L, Bengtzen S, Selivanova G, Wi-man KG, Paul C, Merup M. Effects of PRIMA-1 on chronic lym-phocytic leukaemia cells with and without hemizygous p53 dele-tion. Br J Haematol. 2004; 127(3):285–91. [PubMed: 15491287]

[98]. Russo D, Ottaggio L, Penna I, Foggetti G, Fronza G, Inga A, Men-ichini P. PRIMA-1 cytotoxicity correlates with nucleolar localiza-tion and degradation of mutant p53 in breast cancer cells. Biochem Biophys Res Commun. 2010; 402(2):345–50. [PubMed: 20946886]

[99]. Liang Y, Besch-Williford C, Benakanakere I, Thorpe PE, Hyder SM. Targeting mutant p53 protein and the tumor vasculature: an ef-fective combination therapy for advanced breast tumors. Breast Cancer Res Treat. 2011; 125(2):407–20. [PubMed: 20349129]

[100]. Duan W, Gao L, Wu X, Wang L, Nana-Sinkam SP, Otterson GA, Villalona-Calero MA. MicroRNA-34a is an important component of PRIMA-1-induced apoptotic network in human lung cancer cells. Int J Cancer. 2010; 27(2):313–20. [PubMed: 19921694]

[101]. Lambert JM, Gorzov P, Veprintsev DB, Söderqvist M, Segerbäck D, Bergman J, Fersht AR, Hainaut P, Wiman KG, Bykov VJ. PRIMA-1 reactivates mutant p53 by covalent binding to the core domain. Cancer Cell. 2009; 15(5):376–88. [PubMed: 19411067]

[102]. Magrini R, Russo D, Ottaggio L, Fronza G, Inga A, Menichini P. PRIMA-1 synergizes with adriamycin to induce cell death in non-small cell lung cancer cells. J Cell Biochem. 2008; 104(6):2363–73. [PubMed: 18442053]

[103]. Shi H, Lambert JM, Hautefeuille A, Bykov VJ, Wiman KG, Hain-aut P, de Fromentel CC. In vitro and in vivo cytotoxic effects of PRIMA-1 on hepatocellular carcinoma cells expressing mutant p53ser249. Carcinogenesis. 2008; 29(7):1428–34. [PubMed: 18048389]

[104]. Mehta SA, Christopherson KW, Bhat-Nakshatri P, Goulet RJ Jr, Broxmeyer HE, Kopelovich L, Nakshatri H. Negative regulation of chemokine receptor CXCR4 by tumor suppressor p53 in breast cancer cells: implications of p53 mutation or isoform expression on breast cancer cell invasion. Oncogene. 2007; 26(23):3329–37. [PubMed: 17130833]

[105]. Charlot JF, Nicolier M, Prétet JL, Mougin C. Modulation of p53 transcriptional activity by PRIMA-1 and Pifithrin-alpha on staurosporine-induced apoptosis of wild-type and mutated p53 epithelial cells. Apoptosis. 2006; 11(5):813–27. [PubMed: 16554962]

[106]. Nahi H, Merup M, Lehmann S, Bengtzen S, Möllgård L, Seli-vanova G, Wiman KG, Paul C. PRIMA-1 induces apoptosis in acute myeloid leukaemia cells with p53 gene deletion. Br J Haema tol. 2006; 132(2):230–6.

[107]. Rehman A, Chahal MS, Tang X, Bruce JE, Pommier Y, Daoud SS. Proteomic identification of heat shock protein 90 as a candidate target for p53 mutation reactivation by PRIMA-1 in breast cancer cells. Breast Cancer Res. 2005; 7(5):R765–74. [PubMed: 16168122]

[108]. Li Y, Mao Y, Brandt-Rauf PW, Williams AC, Fine RL. Selective induction of apoptosis in mutant p53 premalignant and malignant cancer cells by PRIMA-1 through the c-Jun-NH2-kinase pathway. Mol Cancer Ther. 2005; 4(6):901–9. [PubMed: 15956247]

[109]. Zache N, Lambert JM, Wiman KG, Bykov VJ. PRIMA-1MET inhibits growth of mouse tumors carrying mutant p53. Cell Oncol. 2008; 30(5):411–8. [PubMed: 18791272]

[110]. Shen J, Vakifahmetoglu H, Stridh H, Zhivotovsky B, Wiman KG. PRIMA-1MET induces mitochondrial apoptosis through activation of caspase-2. Oncogene. 2008; 27(51):6571–80. [PubMed: 18663359]

[111]. Rökaeus N, Klein G, Wiman KG, Szekely L, Mattsson K. PRIMA-1(MET) induces nucleolar accumulation of mutant p53 and PML nuclear body-associated proteins. Oncogene. 2007; 26(7): 982–92. [PubMed: 16909106]

[112]. Lambert JM, Moshfegh A, Hainaut P, Wiman KG, Bykov VJ. Mutant p53 reactivation by PRIMA-1MET induces multiple signal-ing pathways converging on apoptosis. Oncogene. 2010; 29(9):1329–38. [PubMed: 19946333]

[113]. Bykov VJ, Isaeva N, Zache N, Shilov A, Hultcrantz M, Bergman J, Selivanova G, Wiman KG. Reactivation of mutant p53 and induc-tion of apoptosis in human tumor cells by maleimide analogs. J Biol Chem. 2005; 280(34):30384–91. [PubMed: 15998635]

[114]. Wang W, Ho WC, Dicker DT, MacKinnon C, Winkler JD, Mar-morstein R, El-Deiry WS. Acridine derivatives activate p53 and in-duce tumor cell death through Bax. Cancer Biol Ther. 2005; 4(8):893–8. [PubMed: 16177561]

[115]. Mann K, Hainaut P. Aminothiol WR1065 induces differential gene expression in the presence of wild-type p53. Oncogene. 2005; 24(24):3964–75. [PubMed: 15750621]

[116]. Boeckler FM, Joerger AC, Jaggi G, Rutherford TJ, Veprintsev DB, Fersht AR. Targeted rescue of a destabilized mutant of p53 by an in silico screened drug. Proc Natl Acad Sci USA. 2008; 105(30):10360–5. [PubMed: 18650397]

[117]. 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, Das-mahapatra B. 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 ubiquination of wild type p53. J Biol Chem. 2010; 285(14):10198–212. [PubMed: 20124408]

[118]. Zache N, Lambert J, Rokaeus N, Shen J, Hainaut P, Bergman J, Wiman KG, Bykov K. Mutant p53 targeting by the low molecular weight compound STIMA-1. Molecular Oncology. 2008; 2(1):70–80. [PubMed: 19383329]

[119]. Weinmann L, Wischhusen J, Demma MJ, Naumann U, Roth P, DasMahapatra, Weller M. A novel p53 rescue compound induces p53-dependent growth arrest and sensitizes glioma cells to Apo2L/TRAIL-induced apoptosis. Cell Death and Differentiation. 2008; 15:7189729.

[120]. Herbert BS, Chanoux RA, Liu Y, Baenziger PH, Goswami CP, McClintick JN, Edenberg HJ, Pennington RE, Lipkin SM, Kope-lovich L. A molecular signature of normal breast epithelial and stromal cells from Li-Fraumeni syndrome mutation carriers. Onco-target. 2010; 1(6):405–22.

Table 1

Summary of p53-based Therapeutic Approaches

Approaches	Remarks	Citations
Adenovirus-based gene therapy	Adenovirus-based gene therapy involving the introduction of a functional copy of p53 into tu-mors was effective but was associated with strong bystander effects	69 – 72
p53 vaccines	P53 is considered an important candidate tumor antigen leading to a number of active clinical trials using immunization with large peptides derived from p53. Recently a vaccine was developed using a synthetic peptide mixture from p53 (consisting of 10 overlapping peptides). Current approaches also involve development of vaccine using both DNA-based and dendritic cell-delivered p53 vaccines.	73 – 77
Small molecules		
I. Wild-type p53 activator		
Nutlins	• These are cis-imidazoline analogs which inhibit MDM2-p53 complex. • Some similar agents are progressing to the clinic.	78 – 81
MI-319	Similar to Nutlins, it targets the MDM2-p53 axis and holds promise for the treatment of follicu-lar lymphoma. This was also found effective for the treatment of pancreatic cancer in combination with cys-platin. • Cancer cells carrying both wild-type and mutant p53 were killed by this combination.	82 – 83
TDP66-5759	TDP66-5759 is a benzodiazepine that targets p53 MDM2 interaction but also induces a low level of p53 in normal cells.	84
RITA	It blocks p53-HDM2 interaction and also affects p53 interaction with its several negative regula-tors. It shows substantial p53-dependent anti-tumor effects by inducing massive apoptosis in various wild-type p53 carrying tumor cells. It has also been shown to inhibit growth of various tumor cell lines carrying mutant p53 protein by inducing apoptosis. It was shown to restore transcriptional transactivation and transrepression functions of several hot spot p53 mutants. It blocks hypoxia inducible factor1**a** and vascular endothelial growth factor expression in a p53-dependent manner.	10 – 14
SJ172550	It is an MDMX inhibitor which binds reversibly to MDMX and effectively kills MDM amplified retinoblastoma cells. • This agent binds the p53-binding pocket of MDMX thereby displacing p53.	25 – 27
MI-63	This compound is highly effective in activating p53 functions and inhibiting cell growth in can-cer cells carrying wild-type p53. This agent was found effective in blocking the growth of em-bryonic and alveolar rhabdomyosarcoma cells with wild-type p53. • It was also an effective anti-leukemic agent when tested in AML cells overexpressing MDM2.	85 – 89
WO2008106507	This is a duel MDM2/MDMX inhibitor peptide that selectively inhibits neoplastic growth and induces apoptosis in tumor cells in a p53-dependent manner.	27
Tenovins	• Tenovin-1 and its water soluble Tenovin-6 act by inhibiting protein deacetylating activities of SirT1 and SirT2 ultimately leading to tumor inhibition by p53 activation.	24

Approaches	Remarks	Citations
RETRA	• It blocks the inhibitory effects of mutant p53 on its family member p73. • NSC 176327, a derivative of ellipticine, was found to act in a similar fashion. • These agents induce apoptosis in mutant p53 carrying cancer cells in a p73-dependent manner.	50,51
NSC176327	• As described earlier, this acts in a p73-dependent manner.	88
MLN8054	This small molecule inhibits activation of Aurora Kinase A and induces p73 transcriptional activity and apoptosis.	89
JNJ-26854165	This agent acts to induce p53-dependent apoptosis in AML cells in an E2F1-dependent manner and it preferentially acts on S-phase cells.	90
GN25 and GN29	• These agents function by inhibiting p53-SNAI binding leading to p53 activation.	91
II. Mutant p53 reactivators		
CP-31398	This is the first small synthetic molecule which was found to not only enhance the stability of wild-type p53 but also allowed mutant p53 to maintain an active conformation in the presence of mutant p53. It was found to block the growth of xenograft tumors (colon, melanoma, nonmelanoma skin cancer, rhabdomyosarcoma, head and neck, breast, lymphoma, and others).	92 – 95
PRIMA1	PRIMA-1 acts on mutant p53 by restoring its sequence specific DNA binding. It was also shown to act in a transcription-independent manner by inducing Bax translocation and cytochrome c re-lease. MicroRNA-34a was shown to be an important component of PRIMA-1-induced apoptosis net-work. It is effective against a variety of cancer types including breast, lung, and liver cancers in addi-tion to liquid malignancies. • It enhances the effects of chemotherapeutic agents.	96 – 108
PRIMA-MET (APR-246)	• It reactivates mutant p53 and induces apoptosis in mutant p53-carrying human tumor cells. • It induces multiple transcription-dependent and independent pathways.	109 – 112
Ellipticine	• Ellipticine enhances sequence specific DNA binding and transcriptional activity of mutant p53. It was initially discovered by analysis of drug sensitive profiles of the panel of NCI's tumor cell lines, showing specificity for 53 mutant carrying cells. • It enhances the ability of 5-fluorouracil to deplete cancer stem cell populations.	41 – 43
MIRA-1	This is a maleimide-derived molecule which can reactivate DNA binding of mutant p53 protein thereby restoring transcriptional activation functions of p53. • Its analog MIRA-3 showed anti-tumor activity in a tumor xenograft model.	113
Acridine derivatives	These compounds act by restoring the tumor suppressor functions of mutant p53 via DNA inter-calating and inducing p53 stabilization.	114
WR1065	• It acts in a p53-dependent manner to elicit apoptosis and cell cycle arrest. It also manifests cytoprotective effects in normal cells and has shown clinical benefit for protect-ing normal cells in cancer patients receiving radio- or chemotherapy.	115
PhiKan 083	• A carbazole derivative found in an in silico drug screen. It rescues Y220C mutant p53.	116

Approaches	Remarks	Citations
SCH529074	It binds specifically to the p53 DNA binding domain with an affinity of 1–2 μm restoring wild-type function to many oncogenic mutant p53 forms. • It also inhibits HDM2-mediated ubiquitination.	117
STIMA-1	Similar to CP-31398, it can stimulate mutant p53 DNA binding and induce expression of p53 target proteins triggering apoptosis in mutant expressing human tumor cells.	118
P53R3	It enhances recruitment of endogenous p53 to several target promoters resulting in the induction of p53 target genes in a p53-dependent manner.	119