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# p53 and MDM2 antagonists: As novel targets for human cancer therapy

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## Abstract

p53 is an attractive target for therapeutic design because of its involvement as a mediator of growth arrest and apoptosis after exposure to chemoradiotherapy and /or radiotherapy. p53 is activated in response to oncogenic and other cellular stress and induces up or downregulation of a variety of genes involved in cell cycle arrest, DNA repair, senescence or apoptosis. In a tightly controlled feedback loop p53 also induces expression of its downregulators, such as E3 ubiquitin ligases such as MDM2 (murine double minute 2) which binds to p53 and promotes its ubiquitination followed by nuclear export and proteosomal degradation. This process together with other ubiquitin ligases keeps cellular p53 levels constitutively low. MDM2 is highly overexpressed in many tumors which effectively abolishes p53 functions. MDM2 antagonists are therefore, attractive anticancer drugs. Nutilins disrupts p53-MDM2 interaction by competing with p53 for MDM2 by binding to hydrophobic p53 binding pocket in the N-terminal domain of MDM2. Blocking the MDM2-p53 interaction to reactivate the p53 function is a promising cancer therapeutic strategy. Restoration of p53 transcriptional responses in p53 deficient cells may provide a functional means to develop anticancer therapeutics. This review will highlight the role of small-molecule inhibitors of the MDM2-p53 interaction as a cancer therapeutic approach.

**Keywords:** p53/ MDM2 antagonists, Cancer

## Introduction

p53 (also known as *protein 53* or *tumor protein 53*), is a tumor suppressor protein that in humans is encoded by the *TP53* gene located on short arm of chromosome 17 [1]. The p53 protein was identified in 1979, and its gene, called *TP53*, was cloned in 1983 [2]. Due to the "exhilarating possibilities for prevention and cure of cancer," p53 was crowned as the "Molecule of the Year" in 1993 [3]. As such, p53 has been described as "the guardian of the genome", "the guardian angel gene," and "the master watchman," referring to its role in conserving stability by preventing genome mutation [1]. Homozygous loss of the p53 gene is found in virtually every type of cancer, including the carcinomas of the lung, breast and colon-the three leading causes of cancer deaths. In the remaining human cancers, p53 retains wild type status but its function is inhibited by its primary cellular inhibitor-the murine double minute 2 (MDM2; HDM2 in humans). MDM2 is an essential regulator of p53 in normal cells, but its deregulated expression provides growth advantage to cells [2]. Overexpression of MDM2 due to the amplification of the *MDM2* gene was first found in sarcomas retaining wild-type p53 [4], and this amplification was later observed in several other human cancers [5].So, in this review, we wanted to emphasize on the role of MDM2 antagonists as potential anticancer drugs.

### Domain structure of full- length p53

The functional complexity of p53 can be seen in its structure. The tertrameric p53 has a modular domain structure, consisting of an N-terminal transactivation domain (TAD), followed by a proline rich region (PRR), the central independently folded DNA–binding domain (p53C), the tetramerization domain (TET), and the extreme C terminus (CT). p53C is the domain where most cancer-associated p53 mutations are located (Figure 1) [6].

| TAD (1-61) | PRR (62-94) | p53C (95-292) | (293-325) | TET (326-356) | CT (357-393) |
|---|---|---|---|---|---|

**Figure 1.** Domain structure of full-length p53.[Redrawn from: Joerger AC, Fersht AR. Structural Biology of The Tumor Suppressor p53. Annu Rev Biochem 2008, 77:557-82]

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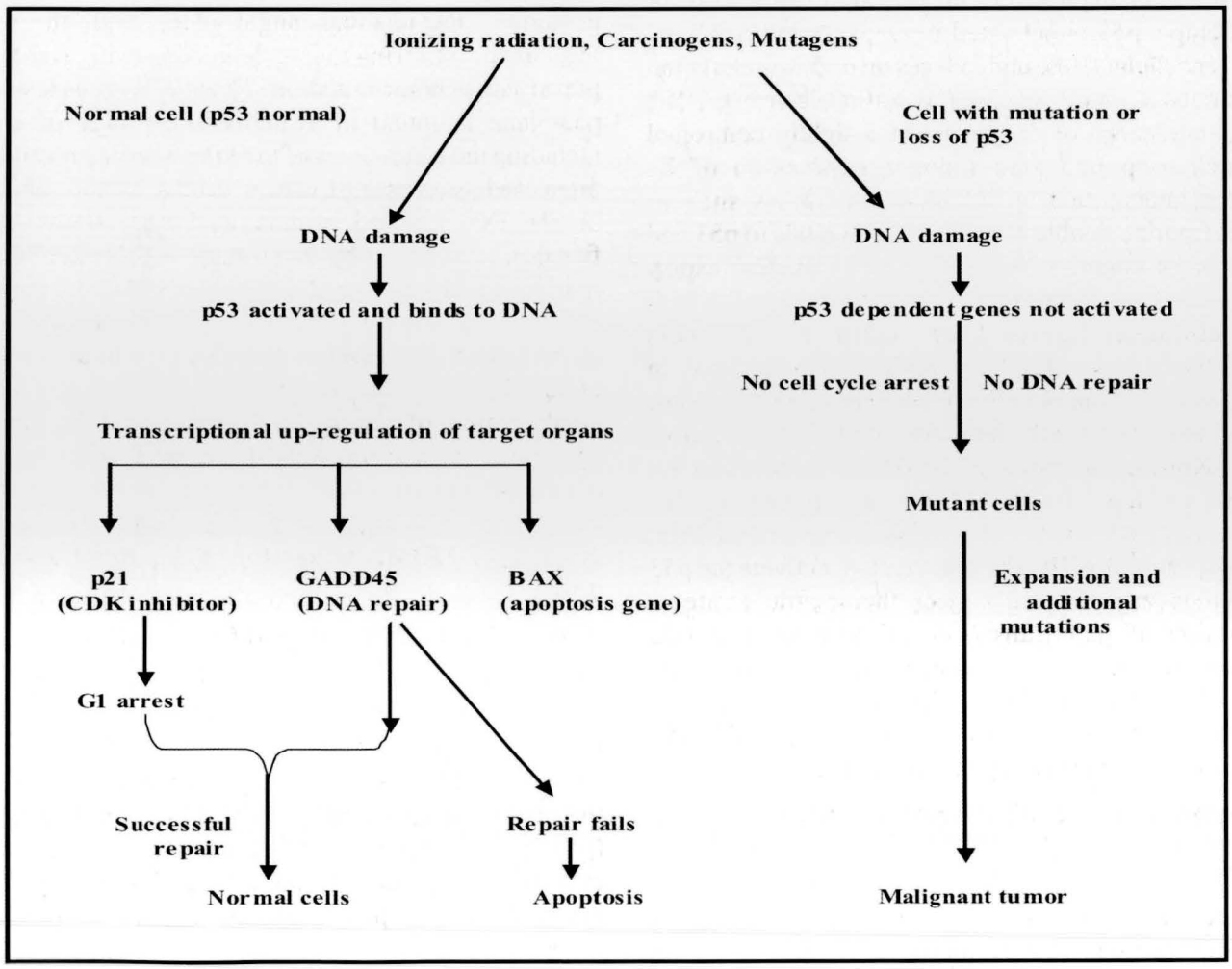
### p53 Gene: Role in maintaining the integrity of the genome

p53 does not seem to police the normal cell but is called in to apply emergency brakes when the DNA is damaged by exposure to mutagenic chemicals or ionizing radiations. With such an assault on genetic material, the normal p53 protein rapidly accumulates in the nucleus and causes the cells to arrest in G1 phase by causing transcription of an inhibitor of cyclin-dependent kinase called p21. A pause in cell cycle allows the cell to repair the DNA damage inflicted by mutagens. Indeed, p53 also helps this process directly by inducing transcription of some DNA repair enzymes. If the damaged DNA is repaired, the cell is allowed to complete the cycle. However, for some reason the repair mechanisms fail, normal p53 stops the mutant cell from dividing and activates cell-suicide genes. Thus, a cell with damaged DNA that cannot be repaired is directed by the p53 gene to undergo apoptosis. In view of these activities, p53 has been rightfully called as a "guardian of the genome". With homozygous loss of p53, DNA damage goes unrepaired, mutations become fixed in dividing cells leading to malignant transformation. (figure2) [1].

**Figure 2.** Role of p53 gene in maintaining the integrity of the genome.[ Redrawn from: Neoplasia. In: Kumar V, Cotran RS, Robbins SL, editors. Basic Pathology. 6th edition. India: HRCOURT SIA PTE.LTD, Inc; 2001. p. 132-174.]

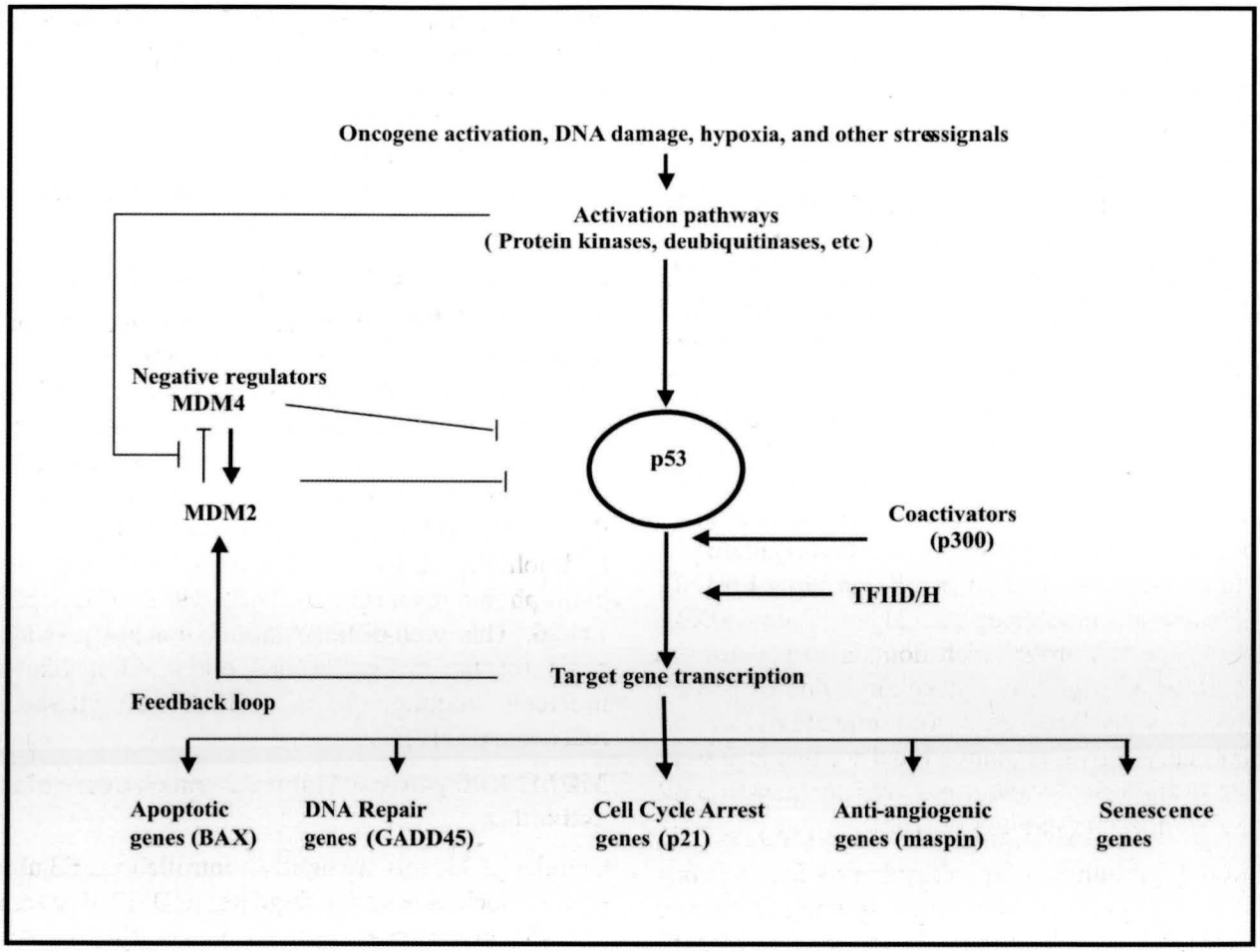



### Scheme of the core regulatory network of the p53 pathway

In response to oncogenic or other cellular stress, p53 is activated and induces up or downregulation of a variety of genes involved in cell cycle arrest, DNA repair, senescence, or apoptosis. In response to stress signals, p53 is activated through various activation pathways like protein kinases. In combination with the coactivating acyltransferaes p300/CBP and components of the transcription machinery (TFIID/H), p53 regulates the transcription of a variety of target genes, which determines the cellular response (figure 3) [6]. Thus, p53 is core molecule in the regulation of variety of genes.

**Figure 3.** Scheme of the core regulatory network of the p53 pathway

[Redrawn from: Joerger AC, Fersht AR. Structural Biology of The Tumor Suppressor p53. Annu Rev Biochem 2008;77:557-82]




## Restoration of p53 function

p53 is very effective in tumor suppression and is inactivated in virtually all human cancers. In about 50% of the human cancers, p53 is inactivated directly by mutation, whereas in others, p53 activity is suppressed because of perturbation of its associated pathways. Three strategies to restore p53 function in tumors can be: (a) Design of antagonists for negative regulators of p53 in tumors carrying wild-type p53 like MDM2 antagonists, (b) Reactivation of mutant p53, (c) Exogenous p53 expression, e.g., via adenovirus mediated gene transfer. Recent studies have shown that restoration of p53 function can lead to tumor regression in vivo [6]. So, we can say that restoring p53 function can be a promising therapeutic strategy

## Role of MDM2 in regulation of p53

The *MDM2* gene is a cellular proto-oncogene. It is often amplified in 7% of all human cancers, but is more frequently observed in soft-tissue sarcomas [7-9]. Over-expression of MDM2 protein can occur by increased transcription or enhancedtranslation [10]. In combination with a p53 mutation, the prognosis is worse than either event alone [11, 12]. Experiments have revealed that deletion of the *MDM2* gene results in embryonic lethality in mice, which can be rescued by the deletion of the p53 gene [13, 14]. Inhibition of cell growth and marked cell death are seen in the absence of p53 regulation by MDM2. So, p53–MDM2 auto-regulatory loop is important in the control of cell growth and death [15].

The transcriptional activity of the p53 is tightly controlled by complex feedback via the negative regulator MDM2 (murine double minute 2) in concert with its homolog MDM4 [6]. On activation, p53 transcribes the *MDM2* gene and, in turn, MDM2 protein inhibits p53 activity (in a tightly controlled feedback loop) by three mechanisms: (a) MDM2 inhibits transcriptional activity of p53 by binding to p53 transactivation domain. (b) Promotes p53 degradation and renders it inaccessible to the target genes

by exporting it out of the nucleus. (c) Functions as an E3 ubiquitin ligase, promoting proteasome-mediated degradation of p53. In this way MDM2 keeps cellular p53 levels constitutively low [2]. Further, MDM2 also competes with p300/CBP for binding to the p53 N terminus. p53 activity is also regulated by MDM2 homolog MDM4, which acts in both distinct and synergistic ways (figure 3) [6].

The ability of MDM2 to target p53 degradation depends highly on the phosphorylation status of p53 and on the association of p53 with other cellular proteins. For example, MDM2 binding can be competed by TAFII31 (member of the basal transcriptional machinery), which associates with p53 in the same region as that utilized by MDM2 for binding, within the amino terminal domain of p53 [16]. In response to stress and damage, when p53 phosphorylation takes place on multiple residues, including those spanning the MDM2 binding sites, MDM2 no longer associates with p53 [17,18]. MDM2 is a major regulator of p53 stability. Other proteins that regulate p53 stability are: JNK [19], human papilloma virus E6 [20], and COP9 signalosome complex [21]. All non-MDM2 regulators act on the proline rich domain of p53 for the ability to affect p53 stability. Phosphorylation of p53 by JNK, which stabilizes p53 and enables it to be transcriptionally active, is mapped to T81, that is present within the proline rich domain required for non-MDM2 basedregulation of p53 stability [22]

In addition to regulation by phosphorylation, various MDM2 forms are products of alternate splicing or caspase cleavage [23, 24]. Each of these forms renders MDM2 inactive, either due to loss of the amino terminal domain that is required for association with p53, or due to deletion of the RING domain, which is required for its activity as an E3 ligase. Truncated forms of MDM2 efficiently inhibit the activity of full-length MDM2, thereby serving to inhibit MDM2 targeted ubiquitination and degradation of p53, resulting in elevated levels of p53 [25, 26]. Truncated forms of MDM2 found in human tumors further support the notion that MDM2 may play a role in tumorigenesis, independent of p53. MDM2 would regulate proteins independent of p53 [15].

**MDM4: A modultor of the activity of MDM2 inhibitor**

MDM4 also called as MDMX is a key modulator of the activity of MDM2 inhibitors. MDM4 is a homologue of MDM2. MDM4 also binds to p53 directly and inhibits its transcriptional activity, but does not induce p53 degradation. Nutlin-3 and MI-219 bind to MDM2 with a much higher affinity than to MDM4. In the presence of MDM4, MDM2 inhibitors may not be able to fully activate p53, thus attenuating the activity of MDM2 inhibitors. Studies using ectopic expression of MDM4 and/or its down-regulation by RNAi have shown that MDM4 attenuates the p53 activation by MDM2 inhibitors and inhibits the cellular activity of MDM2 inhibitors [27-30]. So, the antitumor activity of the MDM2 inhibitors could be compromised in certain human tumors which overexpress MDM4 [2]. So, we can conclude that small molecule inhibitors targeting both MDM2 and MDM4 could be more efficacious than those that are specific for either MDM2 or MDM4

**p53- MDM2 Interaction: The structural basis**

The MDM2-p53 interaction is confined to the $NH_2$-terminal of both MDM2 and transactivation domain of p53 [31, 32]. Atomic details of the interaction of the $NH_2$-terminal domains of human MDM2 complexed with short p53 peptides (residues 15–29) has been provided by the high-resolution crystal structures[33], which show that the MDM2-p53 interaction is mediated by a well-defined hydrophobic surface pocket in MDM2 and four key hydrophobic residues in p53: Phe19, Leu22, Trp23, and Leu26. This well-defined interaction has provided the basis for the design of nonpeptide, drug-like small-molecule inhibitors of the MDM2-p53 interaction to reactivate p53 [2].

**MDM2 Antagonists: Molecular mechanism of p53 activation**

Cellular p53 levels are tightly controlled via E3 ubiquitin ligases, such as negative regulator MDM2, that sequester p53 for proteosomal degradation via ubiquitination. In many tumors, MDM2 is highly over expressed, which effectively abolishes p53 function regardless of the p53 mutation state. MDM2 antagonists are, therefore, attractive anticancer drugs for tumors carrying wild type p53. Drugs that disrupt the p53-MDM2 interaction are: (a) Benzodiazepinediones and spiro-oxindoles like MI-63 and MI-219 [2]. (b) A series of cis-imidazoline analogs (nutlins) disrupt the p53-MDM2 interaction by competing with p53 for MDM2. Nutlins bind to the hydrophobic p53-binding pocket in the N-terminal domain of MDM2 and block the intracellular MDM2-p53 interaction as it mimics the binding mode of a short peptide derived from the N terminus of p53. Nutilin-3, a highly potent and selective inhibitor of MDM2-p53 interaction in vivo in various cell lines expresses wild type p53, showed no reactivation of p53 function in cell lines expressing mutant p53, consistent with its proposed mechanism of action. More recently combined treatment of human tumor cells with nutilins and adenovirus mediated p53 gene therapy has been highly effective in killing both p53 wild type and p53 negative cancer cells. Other approaches

include downregulation of MDM2 expression with antisense oligonucleotides and design of small-molecule compounds that specifically target the E3 ubiquitin ligase activity of MDM2. [6]. Conventional genotoxic anticancer agents and radiation also induce the accumulation and activation of p53, but they do so by posttranslational modifications of p53, such as phosphorylation. In contrast, Nutlin-3 induces neither DNA damage nor p53 phosphorylation in cells [34]. So, small-molecule MDM2 inhibitors are a new class of nongenotoxic agents that can reactivate the p53 function.

### Less toxicity to normal tissues

The effect of p53 activation by an MDM2 inhibitor in normal tissues is of immense interest from a therapeutic perspective. Radio-sensitive tissues, such as small-intestine crypts and thymus are extremely susceptible to p53-induced apoptosis [35, 36]. Restoration of p53 by a genetic approach in the absence of *MDM2* results in severe pathologic damage to radio-sensitive mouse tissues and the death of all animals within five days [37]. In contrast, both Nutlin-3 [38] and MI-219 [39] show little toxicity to animals at therapeutically efficacious dose-schedules. Whereas both radiotherapy and chemotherapy induce profound apoptosis in small-intestine crypts and thymus, MI-219, in either single or repeated doses, does not cause apoptosis or damage in either radio-sensitive or radio-resistant normal mouse tissues, indicating that MDM2 inhibitors display a therapeutic window [27, 2]. The precise mechanism for the lack of toxicity of MDM2 inhibitors to normal tissues is unclear.

### Angiogenesis inhibition by MDM2 inhibitors

There is evidence that p53activation may effectively inhibit angiogenesis. Therefore, in addition to the direct effect of targeting tumor cells, MDM2 inhibitors may inhibit angiogenesis [39, 40]. Activation of p53 can up-regulate several antiangiogenic factors, including thrombospondin-1 (TSP1), and brain-specific angiogenesis inhibitor 1 (BAI1), and down-regulate several proangiogenic factors, such as vascular endothelial growth factor (VEGF), basic fibroblast growth factor (bFGF), basic fibroblast growth factor binding protein, and cyclooxygenase-2 (COX-2) [41,2]. So, we can say that MDM2 inhibitors could also be used in the treatment of tumors lacking functional p53 by inhibiting angiogenesis.

### Combined use of MDM2 inhibitors and anticancer drugs

. Two desired outcomes of the combination regimens are enhanced antitumor activity and the protection of normal healthy tissues. Many traditional genotoxic anticancer drugs that induce p53 also cause collateral damage to normal cells. A rationale and novel strategy to minimize the toxic effects of these drugs is to combine them with nongenotoxic agents of p53 activation, such as MDM2 inhibitors, which may also yield better antitumor efficacy. Studies using Nutlin-3 in cell lines show that the cell cycle arrest function of MDM2 inhibitors can be exploited to protect normal cells from the toxic effects of chemotherapy. MDM2 inhibitors halt cell cycle progression at the $G_1$-S and $G_2$-M phases, and can thus abolish the activity of S and M phase specific drugs [2]. As p53 activation is critical for the antitumor activity of MDM2 inhibitors, persistent exposure to MDM2 inhibitors may select for tumors that are defective in p53 function. Defects in p53 can arise due to deletion or mutations of the *p53* gene or other impairments in the p53 pathway. A genetic study using a mouse model, which recapitulates human Burkitt's lymphoma/leukemia, has shown that although the restoration of p53 is therapeutically effective, it selects for secondary resistant tumors, due to loss of p53 [42, 43]. Therefore, the use of the MDM2 inhibitor as a single agent in the clinic may also result in similar tumor resistance [2]. That is why, in the clinics, anticancer drugs are mostly used in combination.

### Conclusion

p53 is a key control in the cell cycle and determines the fate of the cell in response to oncogenic and other stresses. Its activity and cellular levels are tightly controlled by a multitude of regulatory proteins, involving diverse post-translational modifications. Restoration of p53 transcriptional responses in p53 deficient cells may provide a functional means to develop anticancer therapeutics. Structure based modulation continues to hold promise for development of peptides or small molecules capable of modulation of either wild type or mutant p53 proteins. MDM2 is the primary cellular inhibitor of p53 in cancers retaining wild-type p53 and targeting the MDM2-p53 protein-protein interaction is an attractive cancer therapeutic strategy. Highly potent and specific small-molecule inhibitors such as Nutlin-3 and MI-219 targeting the MDM2-p53 interaction are a promising cancer therapeutic approach. Thus, MDM2 antagonists are attractive anticancer drugs for tumors carrying wild type p53.

So, we can make use of inhibitors targeting MDM2-p53 protein-protein interaction as novel potential anticancer agents in combination with the ongoing treatment strategies so as to minimize the genotoxicity of cells.

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