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Review Article

Therapeutic opportunities in cancer therapy: targeting the p53-MDM2/MDMX interactions

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Abstract: Ubiquitination is a key enzymatic post-translational modification that influences p53 stability and function. p53 protein regulates the expression of MDM2 (mouse double-minute 2 protein) E3 ligase and MDMX (double-minute 4 protein), through proteasome-based degradation. Exploration of targeting the ubiquitination pathway offers a potentially promising strategy for precision therapy in a variety of cancers. The p53-MDM2-MDMX pathway provides multiple molecular targets for small molecule screening as potential therapies for wild-type p53. As a result of its effect on molecular carcinogenesis, a personalized therapeutic approach based on the wild-type and mutant p53 protein is desirable. We highlighted the implications of p53 mutations in cancer, p53 ubiquitination mechanistic details, targeting p53-MDM2/MDMX interactions, significant discoveries related to MDM2 inhibitor drug development, MDM2 and MDMX dual target inhibitors, and clinical trials with p53-MDM2/MDMX-targeted drugs. We also investigated potential therapeutic repurposing of selective estrogen receptor modulators (SERMs) in targeting p53-MDM2/MDMX interactions. Molecular docking studies of SERMs were performed utilizing the solved structures of the p53/MDM2/MDMX proteins. These studies identified ormeloxifene as a potential dual inhibitor of p53/MDM2/MDMX interaction, suggesting that repurposing SERMs for dual targeting of p53/MDM2 and p53/MDMX interactions is an attractive strategy for targeting wild-type p53 tumors and warrants further preclinical research.

Keywords: Ubiquitination, p53, MDM2, MDMX, drug repurposing, selective estrogen receptor modulators

Introduction

The incidence and mortality associated with cancer worldwide, were estimated to be 18.1 million new cases and 9.6 million cancer deaths respectively in 2018 [1]. Tumor heterogeneity, acquired resistance, lack of specific molecular targeted drugs, difficulties in targeting cancer stem cells, the dearth of information on the epigenetic profile of cancers, as well as lack of specificity of existing chemotherapeutic drugs, and their toxicities, represent the major challenges [2-4]. Further, a large proportion of investigational cancer drugs fail during phase III clinical trials and the lack of a biomarker-driven strategy, has been identified to be one the prime factors for such late-stage drug development failures [5]. Precision medicine has marked a new revolution in cancer management by focusing on developing innovative drug candidates and novel biomarkers to treat patients, primarily by delineation of molecular signaling pathways involved in tumor biology [6, 7]. As the ubiquitin system plays an important role in the coordination of various cellular processes via regulation of both protein degradation and nonproteolytic signaling functions, the enzymes involved in this system are implicated as either oncogenes or tumor suppressors in numerous cancers [8]. With increased understanding of ubiquitination mechanisms over the past decades, exploration of

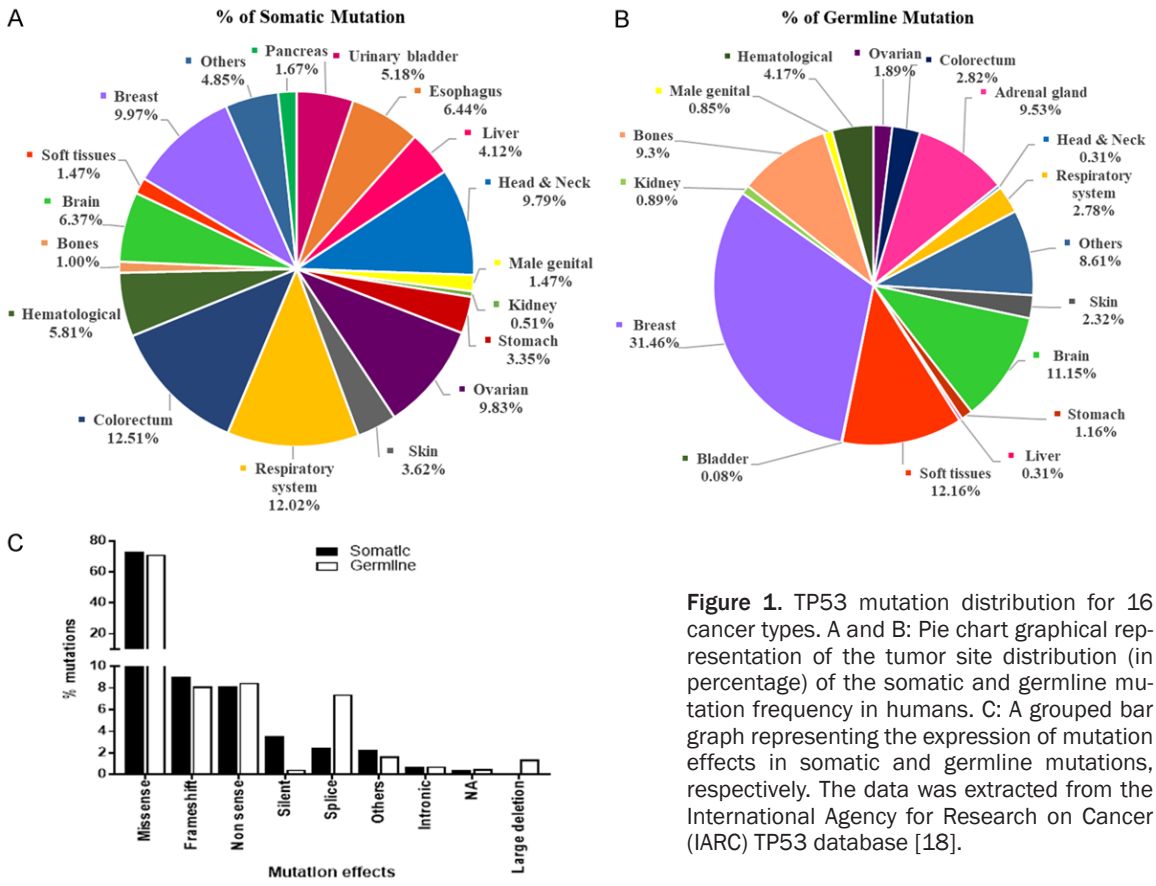


Figure 1. TP53 mutation distribution for 16 cancer types. A and B: Pie chart graphical representation of the tumor site distribution (in percentage) of the somatic and germline mutation frequency in humans. C: A grouped bar graph representing the expression of mutation effects in somatic and germline mutations, respectively. The data was extracted from the International Agency for Research on Cancer (IARC) TP53 database [18].

targeting this pathway offers a new promising strategy for precision medicine in numerous cancers [9-12].

Role of p53 mutation in cancer

The TP53 gene located on chromosome 17p13.1, encodes the p53 tumor suppressor protein. TP53 is the most commonly mutated gene in human cancer. The p53 protein is a transcriptional factor regulating the expression through proteasome based degradation by MDM2 (mouse double-minute 2 protein) E3 ligase and MDMX (Double minute 4 protein), also known as MDM4 [13]. The structure of p53 consists of multiple domains such as the N-terminal domain [transactivation domain (TA), proline-rich domain (TAD-II/PRD)], core domain [central sequence-specific DNA binding domain (DBD)] and C-terminal domain [nuclear localization sequences (NLS), tetramerization domain, nuclear export signal (NES) area, basic domain], that enable its transcriptional activities [14-16]. Upon activation as a tetramer, it binds to p53 response elements (TP53 RE) on the promoter region to transactivate the canonical target gene. TP53 known as "guardian of the genome", modulates an array of cellular processes-angiogenesis, apoptosis, autophagy, differentiation, DNA repair, ferroptosis, metabolism, proliferation, and senescence [1, 13]. Acquisition of TP53 mutation leads to early-onset breast cancer, soft tissue, and bone sarcoma, adrenocortical carcinoma, brain tumors, and Li-Fraumeni and Li-Fraumeni like syndromes [17]. Data extracted from the International Agency for Research on Cancer (IARC) TP53 database on TP53 mutation distribution for 16 cancer types and the expression of mutation effects in somatic and germline mutations [18] are depicted in **Figure 1**.

TP53 mutations

Tumor Suppressor Genes (TSGs) exhibit loss of protein expression by frameshift mutations (the majority are insertion mutations) or nonsense mutations in the DNA-binding domain (DBD). Unlike other TSGs, missense mutations comprise more than 80% of somatic and germline TP53 alterations promoting the accumulation of a stable mutant protein in the nucleus

of tumor cells. These mutations are predominantly clustered around 4-9 exons of p53 with 86% of the mutations clustered between codons 125 and 300 [13]. These mutations cause single residue modifications in the DNA-binding core domain of the protein leading to dominant, gain-of-function activities generating genomic instability. Thus, the progression of cancer cells by TP53 mutation occurs by the absence of tumor suppressor activities and the presence of activity promoting genomic instability [19].

Mechanisms of mutant p53 functions

Alterations in DNA binding ability, enhancement/aversion of transcription factors, and direct change of function of certain proteins takes place through several molecular mechanism models. Mutant p53 couples with various regions of DNA (centrosome/p53 site/matrix attachment region) using mutant p53 binding elements to regulate transcription involving proteins (PML, EGR1, TOP1) and transcriptional cofactors (p300). It enhances transcription by complexing with TFs (ETS1, p63, p73, NF-kB, SP1, SREBP, ETS2, NF-Y, E2F1), which can involve proteins (EGR1, TopBP1, PIN1, VDR) and transcriptional cofactors (p300, HDAC, CBP). A stimulus can activate target gene expression when mutant p53 interacts with transcription regulatory complex-transcription factors (NF-Y, SP1), cofactor (VDR, PLK2) and proteins (p300). Mutant p53 decreases transcription by preventing the binding of transcription regulatory complex transcription factors (p63, p73, SP1), cofactors (p300), proteins (TopBP1, ANKRD11, VDR, SMAD2) to DNA. It also interacts with proteins (NRD1, EFEMP2, TOP1, BTG2, MRE11) which are not involved in transcriptional regulation resulting in the blocking of their function [16].

Gain-of-functions and dominant negative effect

Expression of mutant p53 occurs when a mutation in one TP53 allele in cancer cells occurs followed by loss of second wild type TP53 allele leading to loss of heterozygosity [13]. The mutation leads to three different phenotypes: loss of function (LOF), gain of function (GOF) and separation of function (SOF) mutations. Loss of functions is one of the primary outcomes, where the mutant p53 results in loss of wild type p53 function. However, the stability of the p53 protein is controlled by MDM2 and MDMX interaction. Loss of suppressor functions of wild-type p53 occurs on protein accumulation in the nucleus of tumor cells. Uniform p53 accumulation is not found in all cancerous cells throughout the body. Unlike cancerous pancreatic tissues, liver metastasis and lymph node metastasis documented accumulation of p53. Hence, p53 immunostaining may be used as a biomarker for identifying pre-cancerous cells, since p53 accumulate in unstressed cells. The hotspot mutant codons 175, 245, 248, 249, 273 and 282 often acquire novel oncogenic functions generating GOF. The oncogenic property arises due to: (i) induction of chromosomal instability by transactivating an isolated group of target genes in synergism, with transcription cofactors such as peptidyl-prolyl cis-trans iso-merase (PIN1) and promyelocytic leukemia protein (PML) proteins, leading to tumor progression (ii) inhibition of DNA repair and epigenetic pathways: NF-kB, PDGFRβ, mevalonate, proteasomal integrins and (iii) stimulation of the Warburg Effect. Therapeutically, inhibition of GOF, reduces cancer cell survival and metastasis [7, 19].

Alternatively, mutant p53 is a dominant-negative inhibitor of wild-type p53. Mediated by p21, wild-type p53 controls G1 checkpoint in cells lacking functional p53. Simultaneous expression of p53 in biochemical and cell-culture studies demonstrate that p53, binds to DNA as a tetramer comprising of the dimer of dimers [19]. Studies have concluded that a minimum of 3:1 ratio of DBD-mutant to wild type p53 is required for expression of p53 activity [13]. Additionally, p63 and p73 are the family members of p53. These transcription factors have a similar functional organization as p53 comprising of (a) an N-terminal (b) a core domain (c) a C-terminal. The p63 and p73 are found to induce senescence and maintain genetic stability. Therefore, a reduction in the activity of these proteins promotes cell proliferation by inhibiting cell cycle checkpoints and apoptosis [19].

An experiment in mice with lung adenocarcinoma (p53R172H/+) showed that advanced tumors express elevated levels of p53. On determining its effects on tumorigenesis in mice, it was found that the allele p53R172H_g encodes R172H mutation. This mutation is

caused by a single nucleotide G deletion at the splice acceptor site resulting in decreased p53172H protein levels. The alteration further results in increased carcinoma and decreased lymphoma formation. On the contrary, allele p53R172H/R172H generates higher levels of p53 protein. This variant develops increased lymphomas followed by sarcomas, rather than carcinomas. Although experiments on mice concluded that the wild type and mutant p53 function in a similar way but the mechanism by which it is stabilized in human tumors is unknown [19]. New therapeutic approaches have opened up due to their predominant expression in several cancers as a therapeutic target, especially after the advent of precision medicine. Therefore, a tailored therapeutic approach based upon the wild-type and mutant p53 protein, due to its effect on molecular carcinogenesis is desirable.

p53 ubiquitination mechanistic details

Various post-translational modifications have a profound influence on the regulatory functions of a cell via their critical roles in stabilization of p53 and activation as a transcription factor. Ubiquitination is an important enzymatic post-translational modification that regulates the stability and functions of p53 [20]. Protein ubiquitination involves the ligation of polypeptide-ubiquitin protein (~8500 Da), as monomers or polymers to the lysine residue of the substrate protein [21, 22]. Protein ubiquitination is carried out by a three-step enzymatic cascade involving 3 enzymes: ubiquitin-activating enzymes (E1), ubiquitin-conjugating enzymes (E2), and ubiquitin ligases (E3). E3 enzymes play a decisive role in the selection of the target lysine for ubiquitin attachment and determining the type of conjugation (lysine specificity) and ubiquitination (mono- and poly-ubiquitination) [22-28]. The MDM2 gene is comprised of 12 exons and two p53 responsive elements in intron 1 and encodes for the 489 amino acid oncoprotein MDM2. MDM2 belongs to the RING family of E3 ubiquitin ligases [29].

MDM2 overexpression was reported in a wide range of cancers and is also associated with decreased level and function of p53 protein, leading to an increased risk of cancer development and/or progression of tumors [30]. MDM2 can abrogate the apoptotic functions of p53 by targeting p53 for ubiquitin-mediated degradation, p53 export from the nucleus to the cytoplasm and negatively regulating p53 transcriptional activity via binding of MDM2 to p53 transactivation domain [30-37]. MDM2 ubiquitinates p53 at six major lysine residues-K370, K372, K373, K381, K382, and K386, located in the C-terminus of the p53 protein [38]. Genetic amplification and inheritance of the single nucleotide polymorphism (SNP) found in the MDM2 promoter have been associated with up-regulated MDM2 activity. MDM2 gene amplification elevated MDM2 expression leading to MDM2 pathway attenuation and consequent promotion of tumor progression. SNP309 found in the MDM2 promoter increased MDM2 RNA and protein levels and subsequently led to p53 pathway attenuation. The MDM2 SNP309 was associated with accelerated tumor formation in hereditary as well as sporadic cancers [39, 40]. The antagonistic action of MDM2 toward p53, sets up a negative regulatory feed-back loop, where p53 binds to p53-responsive elements located within the P2 promoter of MDM2 gene as a response to stress signals, to promote its transcriptional activation that leads to p53 degradation. This feedback loop explains a possible mechanism for maintaining the activity of p53 activity in normal cells, in absence of any stress [41, 42]. There is an elevated production of MDM2 with an increase in p53 levels and p53 transcriptional activity. A decrease in the interaction between MDM2 and p53 with a decrease in MDM2 protein levels and/or its activity in response to stress, stabilizes the p53 protein [43]. MDM2 and p53 levels oscillate in this p53-MDM2 feedback loop, especially in response to stress [44, 45].

MDMX, is a structural homolog of MDM2 [46, 47]. The MDMX gene is located on chromosome 1q32 encodes for the 490 amino acid MDMX [48, 49]. MDMX is a critical regulator of the expression and functions of the MDM2. MDMX hetero-oligomerizes with MDM2 via their C-terminal RING finger domains [50]. The interactions between these proteins can increase the MDM2 levels by interfering with MDM2 degradation [51]. Similar to MDM2, MDMX can bind to the p53 transactivation domain located in N-terminal region and cause inhibition of p53 transcriptional activity [46]. The MDMX protein, structurally homologous to MDM2, does not target p53 for degradation,

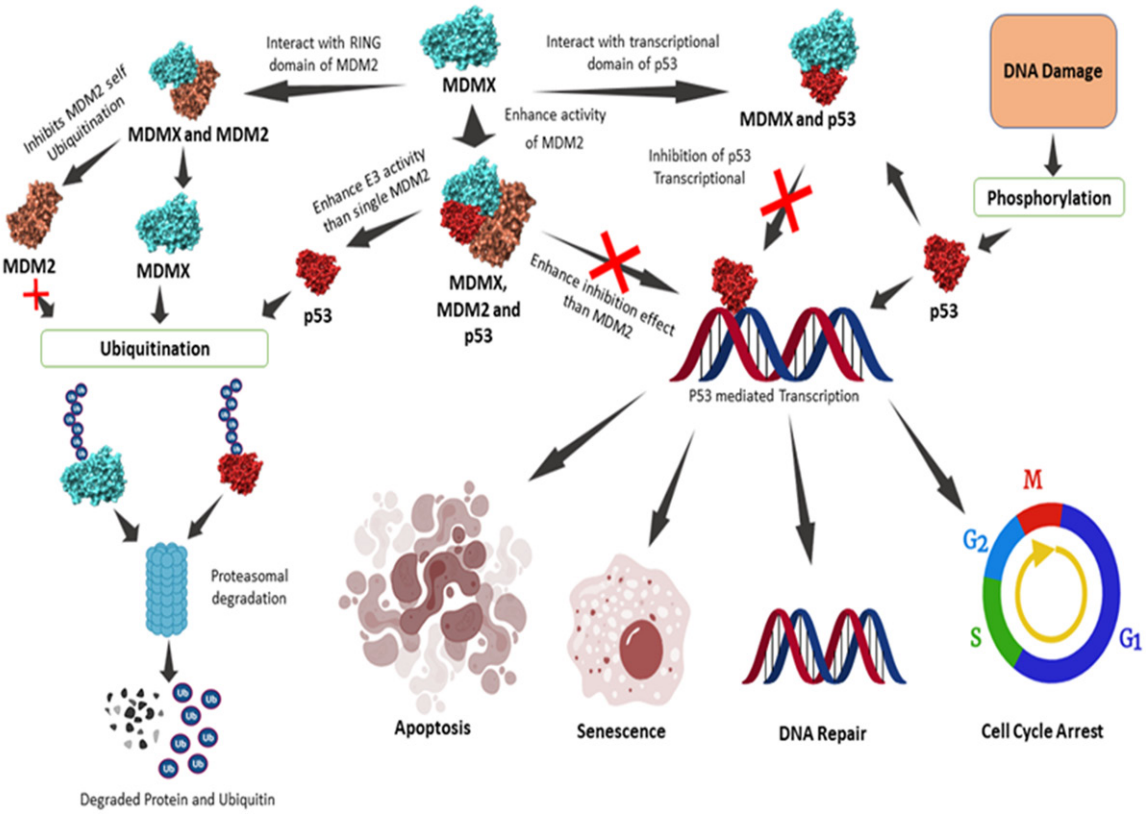


Figure 2. Schematic diagram representing MDMX interaction and transcriptional inhibition of p53.

but stabilizes both p53 and MDM2 [52]. MDMX along with MDM2, synergistically inhibited the transactivation activity of p53 [50].

Therefore, drugs that target MDM2 and MDMX could be employed as a potential direct approach for activating the wild-type p53 through the following mechanisms:

1. Reducing the levels of MDM2 and MDMX in cancer cells.

2. MDM2 E3 ubiquitin ligase activity inhibition.

3. Selectively disrupting p53-MDM2 or p53-MDMX N-terminal interaction [53].

A schematic representation of the MDMX interaction and transcriptional inhibition of p53 is shown in **Figure 2**.

Targeting p53-MDM2/MDMX interaction

The tumor suppressor gene TP53, is mutated in approximately 50% of all human cancers [54]. Under stress and physiological conditions, p53s tumor suppressor function is controlled by physical interaction with MDM2 and MDMX that are negative modulators that weakens or inhibits p53 [55]. MDM2 and MDMX oncoproteins exert their negative tumor suppressor activity on p53, by various pathways: (i) reduced p53 transcriptional function through physical interaction with the p53 NH_2-terminal domain [56]; (ii) MDM2 facilitates translocation of p53 from the nucleus to the cytoplasm through the MDM2 RING domain, inhibiting p53 transcription activity [57]; (iii) MDM2 ubiquitin E3 activity facilitates p53 ubiquitination and proteasomal degradation [58]; (iv) the autoregulatory feedback loop between MDM2 and p53 plays an important role in reducing the presence of physiological p53 and suppressing cumulative p53 on stress stimuli [59]; (v) MDMX is strongly homologous to MDM2 [46, 47] and a negative p53 regulator owing to its sequence similarity to MDM2 and its ability to inhibit overexpressed p53-induced transcription [60]. Therefore, MDM2 and MDMX are critical targets for the development of potential cancer therapy agents that reactivate p53. About half of all cancers have mutated TP53 types and 50% of

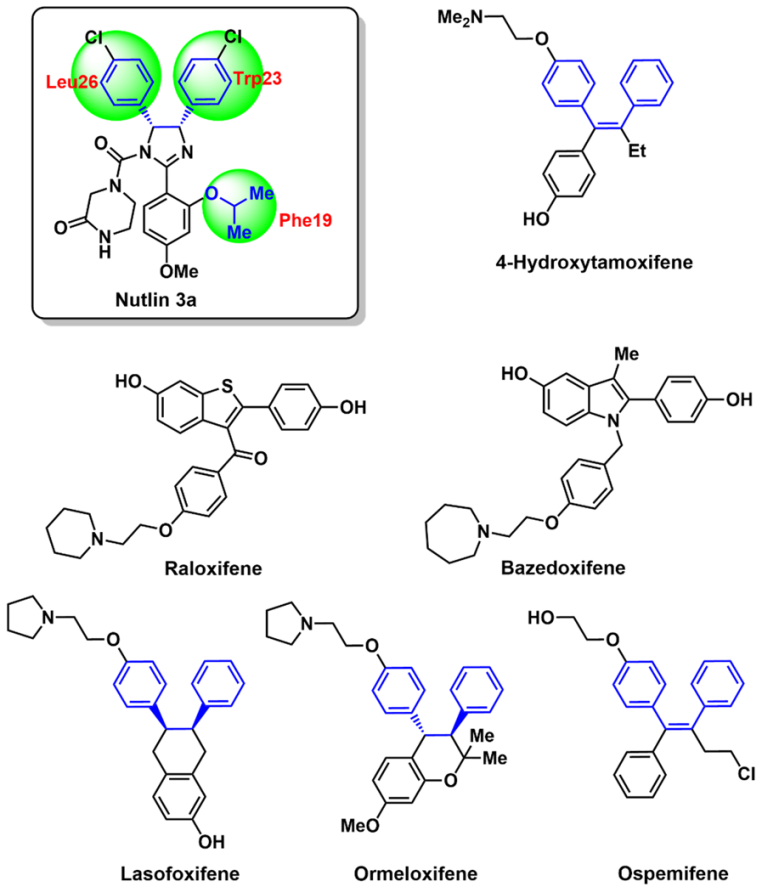


Figure 3. Chemical structure of nutlin-3a highlighting its p53 mimicking chemical features and the chemical structures of SERMs. Vicinal diphenyl structure of some SERMs is highlighted in blue.

tumors expressing wild-type p53 are potential candidates for p53 reactivation therapies. The most direct way to accomplish these goals is to develop potent inhibitors against MDM2 and MDMX's p53-binding pockets to avoid interaction with p53. There are currently several MDM2-p53 interaction small-molecule inhibitors in clinic. Studies reported that mutant reconfiguration to normal, i.e. active wild-type p53 conformation restores apoptosis and enhances tumor regression [61-63]. Identifying strategies to restore the homeostasis of MDM2 and MDMX functions in tumorigenesis can improve diagnostic and prognostic approaches in treating certain cancers.

Past literature or important discoveries related to drug development

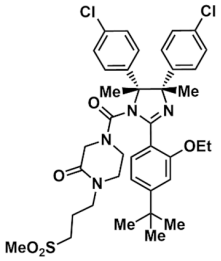
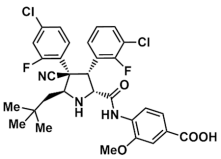
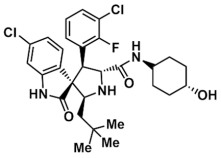
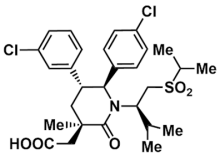
Because of the p53-MDM2-MDMX feedback loop function in initiating and developing wild-type p53-containing tumors, substantial research has been carried over the past decade to identify small molecules or peptides that could precisely target individual protein molecules of this pathway to enhance anticancer therapy. The p53-MDM2-MDMX pathway provides numerous molecular targets for screening small molecules as potential therapies for wild type p53-harboring cancers [64]. Lately, many compounds, targeting MDM2 and MDMX proteins have been identified to reactivate the wild-type p53 form. Some of the identified molecules with promising preclinical results have entered clinical trials [65-67].

MDM2 inhibitors

MDM2 oncogene is the principal downstream target of p53. It binds to the active transcriptional sites of p53, which leads to p53 repressive function. MDM2 inhibitors are aimed at either blocking the expression of MDM2 or by hindering the physical interaction between MDM2 and p53. Other molecular strategies of MDM2 inhibition aim at controlling the E3 ubiquitin ligase activity of MDM2 or by targeting the MDM2-p53 protein complex. Indeed, several molecules have been developed to target the MDM2-p53 axis in order to free p53 from MDM2 regulation and restore its onco-suppressor property [68-70].

A non-peptide small-molecule inhibitor with a 1,2,4,5-tetrasubstituted-4,5-imidazoline structure known as nutlins, was reported by Vassilev and coworkers in 2004 [71]. The nutlins (Nutlin-1, -2, and -3) were the first selective and potent MDM2 inhibitors, capable of interrupting p53-MDM2 binding. Nutlin compounds induce stabilization of p53, stimulate p21 target genes, cell cycle arrest, and apoptosis [72]. Nutlin-3 (1, **Figure 3**), the most potent first-generation cis-imidazolines, is considered as the proof-of-concept molecule for demonstrating the pharmacological reactivation of p53 by antagonizing MDM2 protein. Further optimization of the nutlin-3 structure led to the discov-

Table 1. p53-MDM2 and p53-MDMX inhibitors in clinical trials

Class	Compounds Nature	Compound	Status	NCT Identifier
Small molecule MDM2 inhibitors	Cis-imidazoline Cis-pyrrolidine	RG7112 	Phase I study in advanced solid, hematological cancers, and liposarcoma (completed)	NCT00559533
		RG7388 (or RO5503781 or idasanutlin) 	Phase I study in advanced solid cancers (completed)	NCT01462175
		RG7388 (or RO5503781 idasanutlin)	Phase I study in polycythemia vera and essential thrombocythemia (completed)	NCT02407080
		RG7388 (or RO5503781 idasanutlin) with cytarabine	Phase I study in acute myelogenous leukemia (completed)	NCT01773408
	Spiro-oxindole	SAR405838 (or MI-77301) 	Phase I study neoplasm malignant (completed)	NCT01636479
	Piperidinone	AMG232 	Phase I study in advanced solid cancers and multiple myeloma (completed)	NCT01723020
		AMG-232 (KRT-232)	Phase I study in radiation therapy in treating patients with soft tissue sarcoma (recruiting)	NCT03217266
MDM2/X inhibitors	Peptide	ALRN-6924	Phase I study in advanced solid tumors and lymphoma (completed)	NCT02264613
Dual target MDM2/MDMX Inhibitors	Peptide	ALRN-6924 with cytarabine	Phase I study-resistant (refractory) solid tumor, brain tumor, lymphoma or leukemia (recruiting)	NCT03654716
		ALRN-6924 with paclitaxel	A Phase Ib study in wild-type TP53 advanced or metastatic solid tumors including estrogen-receptor positive breast cancer (recruiting)	NCT03725436
		ALRN-6924 with topotecan	A Phase Ib/2 study in small cell lung cancer (recruiting)	NCT04022876

Source: http://www.clinicaltrials.gov, accessed 2nd May 2021.

ery of RG7112, **Table 1**), [73]. Nutlin-3a shows efficacy in many tumor models including acute myeloid leukemia [74], chemoresistant neuroblastoma [75], and multiple myeloma [76]. RG7112, a potent and selective p53-MDM2 interaction inhibitor with promising oral bioavailability, was identified from the nutlin family [77]. The first MDM2 inhibitor RG7112 (or RO5045337), entered human clinical trials and showed superior cellular potency, pharmacokinetic parameters, chemical stability and MDM2 affinity compared to nutlin-3a [77].

The findings on the therapeutic potential of RG7112 in patients with MDM2-amplified liposarcoma were obtained in a trial of the European Community (EudraCT number: 2009-015522-10). Although the study found that the drug was effective in inhibiting MDM2 and p53 activation in this form of tumor in vivo, many clinical adverse drug-related events, including haematological toxicity, were highlighted in the study, rendering long-term treatment with RG7112 a major challenge [78].

Furthermore, 4-benzodiazepine-2,5-dione (BDP) are another class of antagonists that block p53-MDM2 interaction [79]. For example, TDP-521252 and TDP665759 benzodi-azepinedione derivatives, have been reported to stabilize and increase p53 transcriptional function, leading to a decrease in the proliferation of wild-type p53 expressing tumor cells [80]. Other compounds include spirooxindoles (or (MI compounds) such as MI-63 and MI-219 that inhibit the p53-MDM2 interaction [81-84]. MI-219 has a high binding affinity, good oral bioavailability, and better pharmacokinetic parameters than MI-63 compound [83]. Spirooxindole MI-77301 (SAR405838, **Table 1**), from Sanofi exhibited good anti-tumor activity [85]. The isoquinolinones and piperidone based small molecules are also reported to target MDM2 and MDMX [86-90]. Idasanutlin (RG7388, **Table 1**) was developed as a second generation MDM2 inhibitor through analyzing the structure of RG7112 [91]. In wild-type p53 cell lines, RG7388 activates the p53 pathway which enhanced apoptosis of cells. Moreover, tumor proliferation was suppressed in xenograft assays. Currently, RG7388 and cytarabine in combination have entered a phase III clinical trial for patients with acute myeloid leukemia [73].

MDM2 and MDMX dual target inhibitor

Many compounds are in the drug discovery pipeline which simultaneously target both MDM2 and MDMX by disrupting p53-MDM2 and p53-MDMX interactions. RO-5963 is a dual inhibitor which interferes with p53-MDM2-MDMX interaction by heterodimerizing the target genes [92]. Studies showed IC_{50} value of 17 nM and 25 nM for MDM2 and MDMX respectively [93]. However, very few dual inhibitors have been identified so far. The pyrrolopyrimidine-based compound reported [94], binds to both MDMX and MDM2. The compound OXAZ-1 (a tryptophanol-derived oxazolopiperidone lactam) and the compound DIMP53-1 (a tryptophanol-derived oxazoloisoindolinone) [95, 96] revealed in vitro, to have p53-dependent antitumor activity and DIMP53-1 showed in vivo antiproliferative, pro-apoptotic and antiangiogenic p53-dependent properties.

Currently, there are three classes of MDM2 small molecule inhibitors that have high (nM) affinity and specificity in disrupting MDM2-p53 binding [71]. However, these compounds are weak inhibitors of the MDMX-p53 interaction (Ki values of 30-70 μM) [97]. Nutlin-3 is the first and most well studied compound in the p53-MDM2 domain [98]. Spiro-oxindole derivatives constitute a second family of potent and highly selective inhibitors of the p53-MDM2 interaction [99]. MI-219 and MI-63, the most optimised spirooxindoles derivatives, bind to MDM2 with >1,000-fold affinity than the p53 wild-type peptide (Ki value of 5-6 nM). MI-219/MI-63 and Nutlin-3 derivatives have demonstrated necessary cellular downstream effects and are now in advanced preclinical development or early phase clinical trials [100]. The third group of MDM2-p53 antagonists relies on a benzodiazepinedione core scaffold [79]. The optimized compounds in this series were able to suppress the development of wild-type p53 cells with IC_{50}s in the 7-30 μM range and 3-9-fold selectivity for cells with functional p53 [80]. Su et al., 2019, 2021 [101, 102] investigated the inhibitory effect of nine bicyclic-β-proline homo-oligomer derivatives on the p53-MDM2/MDMX protein-protein interaction. Among these compounds, C1-substituted bicyclic β-proline trimer C-3 and tetramer C-6, which take trans-helix conformations, significantly inhibited p53-MDM2/MDMX binding and also been demonstrated that non-naturally occurring, stable helical trimers of bicyclic-amino acids (Abh) with all-trans amide bonds can inhibit the p53-MDM2/MDMX-helix-helix interaction, which regulates p53 function.

Recently Li et al. have reported on dual inhibition of p53-MDM2/MDMX inter-actions by peptides, resulting in the activation of p53 in vitro and in vivo [103]. Philippe et al. reported that angler peptides which are obtained by conjugating KD3, a noncell permeable, potent, and specific peptide dual inhibitor of p53-MDM2/

MDMX interaction were able to activate the p53 pathway in cancer cells [104]. A series of D-amino acid mutational PMI analogues as potent dual peptide inhibitors of p53-MDM2/MDMX interactions have been reported [105]. Pairawan et al. reported enhanced antitumor efficacy of ALRN-6924, a dual inhibitor of MDM2 and MDMX, in hormone receptor-positive (ER+) breast cancer cell line model [106]. In wild-type p53 multidrug-resistant breast cancer, Fan et al. demonstrated that a recombinant dual-target MDM2/MDMX inhibitor could reverse doxorubicin resistance by activating the TAB1/TAK1/p38 MAPK pathway [107].

Clinical trials with compounds targeting p53-MDM2/MDMX

Numerous small molecule drugs, targeting p53-MDM2/MDMX pathways have entered into clinical trials. APR-246 is a small molecule that has shown the ability to reactivate mutated and inactivated p53 protein by restoring wild-type p53 conformation and function in many types of cancer clinical trials [108]. Recently it received breakthrough therapy designation by the FDA in combination with azacitidine for the treatment of TP53 Mutant Myelodysplastic Syndromes (MDS), ClinicalTrials.gov Identifier: (NCT03745716). An overview of small molecules that inhibit p53-MDM2 and p53-MDMX interactions undergoing clinical trials are shown in the **Table 1**.

Drug repurposing strategy to target p53-MDM2/MDMX interactions

In the last decade, several reports have appeared in the literature on the discovery and development of novel molecules as inhibitors of p53-MDM2 or p53-MDMX interactions or dual inhibitors of p53-MDM2/MDMX interactions [53, 109-117]. Few promising candidates are also in various phases of clinical trials as therapeutics for cancer treatment (**Table 1**). Drug repurposing where new therapeutic indications for known marketed drugs are investigated, is another viable strategy to discover anticancer agents that can lead to significant decrease in the time and cost involved in the drug discovery process [118, 119]. In early studies on drug repurposing strategy for p53/MDM2 interaction inhibitors, Warner and coworkers used computational modelling to dock >3000 US FDA approved drugs to determine their potential to inhibit p53-MDM2 interaction [120]. These studies identified 15 FDA approved drugs as inhibitors of p53-MDM2 interaction. In a recent development, the repurposing potential of the small molecule protoporphyrin IX (PpIX) was demonstrated in vitro and it was shown to be a dual inhibitor of p53/MDM2 and p53/MDMX interactions [121-123]. Studies on the drug repurposing potential of another related FDA approved drug verteporfin is ongoing [117]. The antimalarial drug amodiaquine is also known to prevent p53/MDM2 interactions [123].

Drug repurposing potential of selective estrogen receptor modulators (SERMs)

SERMs are a class of agents that are used to treat a number of conditions in women including osteoporosis, breast cancer and postmenopausal symptoms [124, 125]. Both tamoxifen and raloxifene are used to treat breast cancer [126]. Interestingly, other SERMs such as lasofoxifene which is used to treat osteoporosis and vaginal atrophy, and ormeloxifene which is currently used as an oral contraceptive, are reported to exhibit anticancer activity in treating breast cancer [127-131]. While their therapeutic activities are attributed to estrogen receptor (ER) binding, SERMs are also known to exhibit other beneficial effects such as anti-inflammatory activity [132] and inhibition of cell proliferation via ER-independent mechanisms [133] which suggests their ability to bind to multiple molecular targets in vivo. Furthermore, we were intrigued by the anticancer activity of one of the SERMs lasofoxifene, which recently received a fast-track designation from US FDA to treat ER-positive/HER2-negative breast cancers in women, and its phase II clinical trials are in progress (NCT03781063). The exact mechanisms of SERMs in cancer therapy is not clear. Therefore, we were interested in investigating the drug repurposing potential of lasofoxifene and other related SERMs in targeting p53-MDM2/MDMX interactions. It should be noted that similar to the dual p53/MDM2 and p53/MDMX inhibitor nutlin-3a, lasofoxifene also possess a vicinal diphenyl ring substituents linked to sp3 carbons (**Figure 3**). The protein p53 is known to undergo interaction with the N-terminal segment of MDM2 through hydrophobic contacts via three critical amino acids Phe19, Trp23 and Leu26 respectively

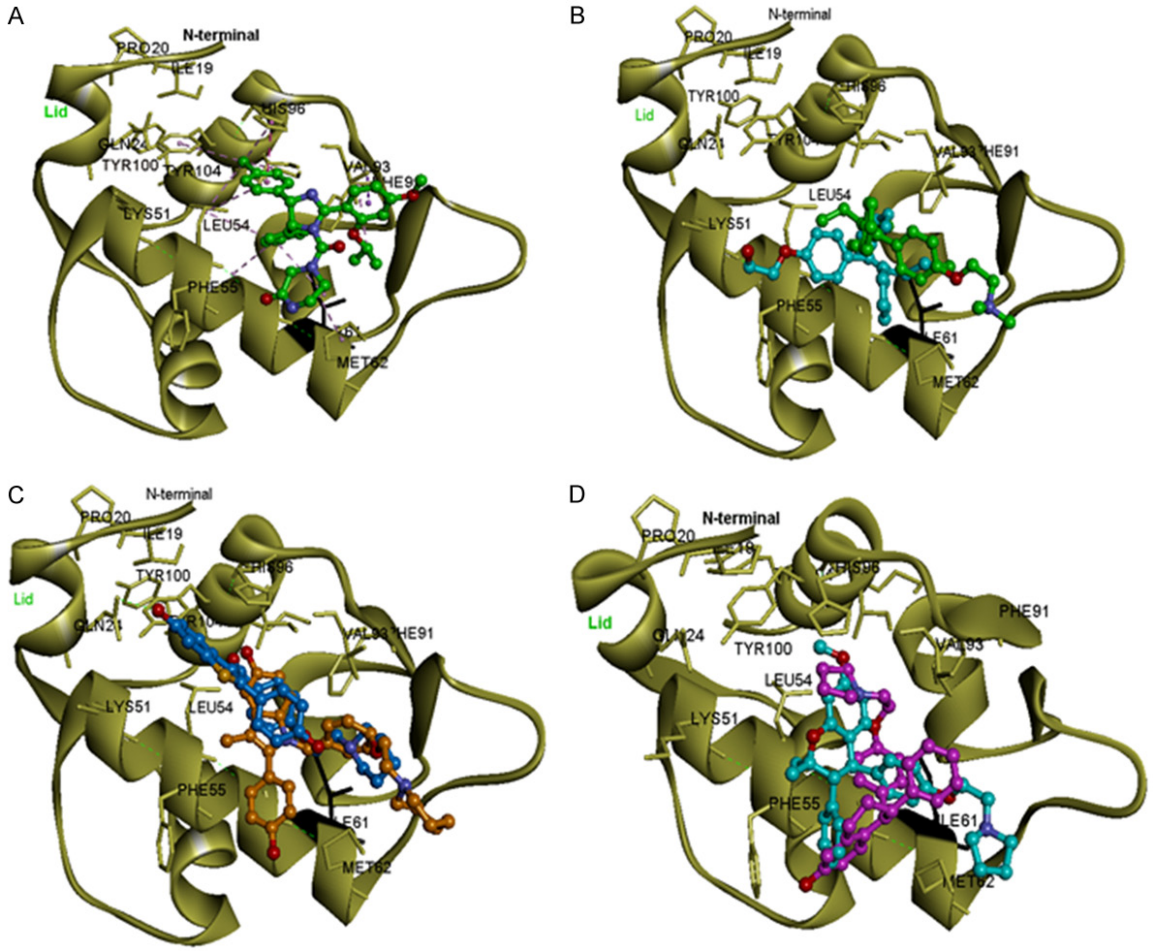


Figure 4. Binding modes of (A) nutlin-3a (green ball and stick cartoon), (B) 4-hydroxytamoxifene (green ball and stick cartoon) and ospemifene (light blue ball and stick cartoon), (C) raloxifene (orange ball and stick cartoon) and bazedoxifene (dark blue ball and stick cartoon), (D) lasofoxifene (purple ball and stick cartoon) and ormeloxifene (light blue ball and stick cartoon) in the p53 binding domain of MDM2 (pdb id: 4HG7).

[53, 109, 110, 134]. The X-ray crystal structure data of nutlin-3a and other small molecules with p53/MDM2 has demonstrated a 3-point pharmacophore model known as the "thumb-index-middle" fingers, as the minimum structural requirement to design p53/MDM2 interaction inhibitors [53, 110, 111, 134, 135]. The 2-isopropoxy substituent of nutlin-3a mimics Phe19 of p53 whereas the two chlorophenyl substituents mimic Trp23 and Leu26 of p53 protein (**Figure 3**).

Structural analysis of SERMs 4-hydroxytamoxifen (the active metabolite of tamoxifen) and other marketed SERMs raloxifene, bazedoxifene, lasofoxifene, ormeloxifene and ospemifene (**Figure 3**) shows that they satisfy the 3-point pharmacophore structural requirement for inhibiting p53/MDM2 interactions.

Modelling studies of SERMs as inhibitors of p53/MDM2 interactions

Molecular docking studies of SERMs (**Figure 4**) were carried out using the solved structure of p53/MDM2 [136]. The CDOCKER algorithm was used (Supplementary Materials) and the molecular docking protocol was validated first by docking the p53/MDM2 interaction inhibitor nutlin-3a. The top ranked binding pose of nutlin3a showed similar binding mode (**Figure 4A**) as per the solved structure (all heavy atom RMSD =1.13 Å). Figure S1 (Supplementary Materials), shows the comparison of nutlin-3a crystal structure binding mode with the docked model. Nutlin-3a interacts at 3-key hydrophobic sites in the p53 binding domain of MDM2 which includes the interactions of two 4-chlorophenyl substituents in the hydrophobic pock-

Table 2. Ligand-receptor complex binding energy ($E_{binding}$)[1] for SERMs docked in MDM2

Compound Name	$E_{binding}$ in kcal/mol^{-1}
4-Hydroxytamoxifen	-8.37
Ospemifene	-22.40
Raloxifene	-12.32
Bazedoxifene	-17.71
Lasofoxifene	-11.23
Ormeloxifene	-17.06
Nutlin-3a	-32.38

[1]The binding energy was calculated using the equation $E_{binding}$ = Energy of complex ($E_{ligand\text{-}receptor}$) - Energy of ligand ($E_{ligand}$) - Energy of receptor ($E_{receptor}$) for the top ranked pose obtained using the CDOCKER algorithm. The computational software *Discovery Studio Structure-Based-Design*, BIOVIA Inc., USA was used.

ets lined by Leu54, His96, Ile99, Tyr100 and Leu54, Leu57, Ile61 respectively via π-π stacked and π-alkyl interactions (distance <5 Å). In addition, the 2-isopropoxy-4-methoxyphenyl substituent also undergoes hydrophobic interactions with Val93 (distance <5 Å). Comparing the binding modes of 4-hydroxytamoxifen (**Figure 4B**) shows that the C5 4-chlorodiphenyl and C2 ethyl substituents were oriented in the hydrophobic region comprised of Leu54 and Ile61, which was similar to nutlin-3a. The C2 dimethylaminoethoxyphenyl substituent was in contact with Val93 and the dimethylamino group underwent cation-π interaction with Tyr67. Unlike nutlin-3a, 4-hydroxytamoxifen was not close to His96, Ile99 and Tyr100 and failed to make any contacts. The related (*Z*)-diphenyl derivative ospemifine, underwent superior interactions in the p53 binding pockets of MDM2 compared to 4-hydroxytamoxifen with its three phenyl rings and the ethylchloro substituent in contact with hydrophobic amino acids Leu54, Ile61, Met62, Val93 and Ile99 (**Figure 4B**). Interestingly, the terminal hydroxyl group of 4-phenoxyethanol substituent underwent hydrogen bonding interaction with Lys51. These predicted binding modes suggest that 4-hydroxytamoxifen is able to interact with at least two hydrophobic sites in MDM2 binding whereas ospemifine is able to interact with three hydrophobic sites in MDM2. Comparing the predicted binding modes of SERM derivatives raloxifene and bazedoxifene (**Figure 4C**) shows that both the benzothiophene and indole rings underwent π-π stacked interactions with His96 and π-alkyl interactions with Leu54 (distance <5 Å). Bazedoxifene C2 phenolic substituent underwent additional interactions in the MDM2 binding pockets unlike raloxifene and was in contact with Met62 and Val93. In addition, the C2 phenolic substituent underwent hydrogen bonding interaction with Gln59 (**Figure 4C**). Bazedoxifene exhibits linear conformation and was able to interact with all the three key hydrophobic regions in the MDM2 binding pockets whereas raloxifene was able to interact in only one of the MDM2 binding sites. Comparing the predicted binding modes of pyrrolidine containing bicyclic SERMs lasofoxifene and ormeloxifene, shows that they were able to interact with at least two key hydrophobic regions of MDM2 binding pocket (**Figure 4D**). Ormeloxifene exhibited better interaction in the MDM2 binding site with the C7 methoxy substituent, undergoing π-alkyl interactions with His96, Ile99 and Tyr100 (distance <5 Å) which was not observed for lasofoxifene. Figure S2 (Supplementary Materials) shows a 2D-interaction map of ormeloxifene interacting in the p53 domain of MDM2 highlighting the key amino acids involved in ligand binding. These molecular docking studies suggest that SERMs have the potential to bind to p53 binding sites in MDM2 and have the potential to be repurposed as inhibitors of p53/MDM2 interactions in cancer therapy.

In order to assess the binding of SERMs with MDM2 further, we also calculated the ligand-receptor binding energy using the equation $E_{binding}$ = Energy of complex ($E_{ligand\text{-}receptor}$) - Energy of ligand ($E_{ligand}$) - Energy of receptor ($E_{receptor}$), with negative values indicating stable ligand-receptor complex and positive values indicating high energy complex (**Table 2**). These studies show that among the SERMs evaluated, the (*Z*)-diphenyl derivative ospemifine exhibits greater binding to MDM2 ($E_{binding}$ =-22.40 kcal/mol) followed by bazedoxifene ($E_{binding}$ =-17.71 kcal/mol) and ormeloxifene ($E_{binding}$ =-17.06 kcal/mol). None of the SERMs were able to exhibit similar binding energy as the reference compound nutlin-3a ($E_{binding}$ =-32.38 kcal/mol) which suggests that SERMs are likely to exhibit weaker binding affinity toward MDM2 compared to nutlin-3a. This also suggests that molecules that are able to interact with the 3-key hydrophobic sites of MDM2 can exhibit better inhibition of p53/MDM2 in-

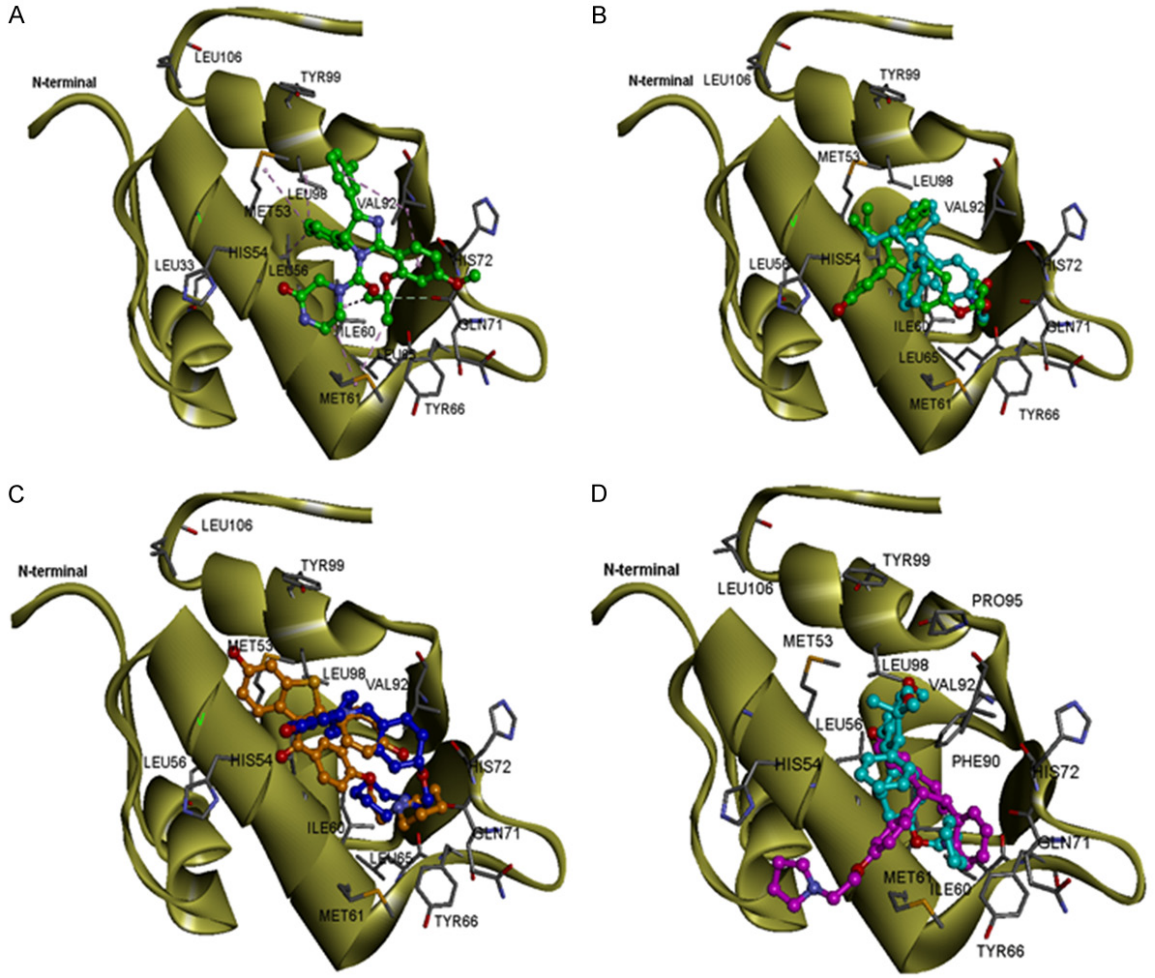


Figure 5. Binding modes of (A) nutlin-3a (green ball and stick cartoon), (B) 4-hydroxytamoxifene (green ball and stick cartoon) and ospemifene (light blue ball and stick cartoon), (C) raloxifene (orange ball and stick cartoon) and bazedoxifene (dark blue ball and stick cartoon), (D) lasofoxifene (purple ball and stick cartoon) and ormeloxifene (light blue ball and stick cartoon) in the p53 binding domain of MDMX (pdb id: 2N14).

teractions. It should be noted that small molecules that are able to undergo efficient interactions in the hydrophobic site closer to the lid region of MDM2 at the N-terminal, and are able to keep Tyr100 in open conformation, exhibit better inhibition [110].

Modelling studies of SERMs as inhibitors of p53/MDMX interactions

The binding interactions of SERMs (**Figure 5**) in the p53 binding domain of MDMX were investigated using the solved structure of MDMX [137]. Initially, molecular docking protocol was validated by docking the reference compound nutlin-3a in the p53 binding domain (**Figure 5A**). The binding mode of nutlin-3a was similar to the solved structure (all heavy atom RMSD =2.05 Å). Figure S3 (Supplementary Materials), shows the comparison of nutlin-3a crystal structure binding mode with the docked model. The interaction of nutlin-3a in the p53 binding domain is dominated by hydrophobic contacts with Met53, Leu56, Ile60, Met61 and Val92 (distance <5 Å). Nutlin-3a is a known inhibitor of p53/MDMX interaction although it is more selective and potent inhibitor of p53/MDM2 interaction [114, 138]. Furthermore, inhibiting p53/MDMX interaction is more challenging due to subtle differences in the amino acids that line the p53 binding domain. For example, MDMX has a bulky Met53 instead of smaller Leu54 in MDM2. This decreases the size of the binding pocket in MDMX and prevents the deeper interactions of ligands in the p53 binding domain [53, 114].

Table 3. Ligand-receptor complex binding energy ($E_{binding}$)[1] for SERMs docked in MDMX

Compound Name	$E_{binding}$ in kcal/mol^{-1}
4-Hydroxytamoxifen	-9.28
Ospemifene	-15.59
Raloxifene	-11.12
Bazedoxifene	-14.25
Lasofoxifene	-14.55
Ormeloxifene	-15.36
Nutlin-3a	-27.18

[1]The binding energy was calculated using the equation $E_{binding}$ = Energy of complex ($E_{ligand\text{-}receptor}$) - Energy of ligand ($E_{ligand}$) - Energy of receptor ($E_{receptor}$) for the top ranked pose obtained using the CDOCKER algorithm. The computational software *Discovery Studio Structure-Based-Design*, BIOVIA Inc., USA was used.

Predicted binding modes of SERMs show that the (*Z*)-diphenyl derivatives 4-hydroxytamoxifene and ospemifene were able to undergo interactions in the p53 binding domain of MDMX (**Figure 5B**). Ospemifene exhibited superior hydrophobic interactions and was in contact with Met53, Leu56, Ile60, Leu65, Val92 and Leu98 (distance <5 Å). The terminal hydroxyl group of 4-phenoxyethanol substituent underwent hydrogen bonding interaction with Gln71 backbone (distance =1.78 Å). Investigating the binding modes of raloxifene and bazedoxifene (**Figure 5C**) shows that the aromatic heterocycles benzothiophene and indole rings of raloxifene and bazedoxifene underwent π-alky interactions with Met53 (distance <5 Å), whereas the phenolic rings were in contact with Val92 (distance <5 Å). Bazedoxifene in general, was in close contact with amino acids in the p53 binding domain compared to raloxifene. Furthermore, top binding modes of lasofoxifene and ormeloxifene shows that they also underwent mostly hydrophobic interactions (**Figure 5D**), with ormeloxifene undergoing several π-π and π-alkyl interactions with Met53, Met61, Met65, Phe90, Val92, Pro95 and Leu98 (distance <5 Å). Figure S4 (Supplementary Materials) shows a 2D-interaction map of ormeloxifene interacting in the p53 domain of MDMX, highlighting the key amino acids involved in ligand binding. Binding energy calculations (**Table 3**) identified both ospemifene ($E_{binding}$ =-15.59 kcal/mol) and ormeloxifene ($E_{binding}$ =-15.36 kcal/mol) as promising drugs with potential to inhibit p53/MDMX interactions. The reference compound nutlin-3a exhibited superior binding compared to the SERMs studied ($E_{binding}$ =-27.18 kcal/mol).

These studies show that SERMs have the potential to exhibit dual inhibition of p53/MDM2/MDMX interactions. However, they are more likely to exhibit weaker inhibition of p53/MDMX interactions compared to their inhibition activity toward p53/MDM2 (**Tables 2** and **3**). A recent study on developing dual MDM2/MDMX inhibitors, proposes a 5-point pharmacophore model instead of the 3-point pharmacophore model to design novel inhibitors [114]. This also suggests that to obtain greater inhibition of p53/MDMX interaction, ligands should be able to target additional hydrophobic area consisting of Leu33, Val52 and Leu106. None of the SERMs studied here can reach this site, which explains their weaker binding affinity toward MDMX binding site. Yet, SERMs have the potential to exhibit dual inhibition p53/MDM2/MDMX interactions, which suggests their repurposing to treat a wide range of cancers. Interestingly, our computational studies identified ormeloxifene as a promising dual inhibitor of p53/MDM2/MDMX interaction. This drug is marketed as a popular contraceptive in India with demonstrated safety and efficacy [130]. Ormeloxifene can also be considered as a potential treatment option for menorrhagia [139-141]. Ormeloxifene has been reported to be a SERM with good therapeutic and less toxicity profile, and as a potential cost-effective treatment option for breast, cervical, ovarian, prostate, chronic myeloid leukemia and head and neck cancers by modulation of multiple pathways [142-148]. Many chemotherapeutic agents have been reported to exhibit ovarian toxicity, hormone disturbances and menorrhagia that may lead to significant patient discomfort, necessitate the administration of blood products to account for the blood loss, delay or interrupt chemotherapeutic treatments, and consequently result in poor treatment outcomes [149-151]. Hence ormeloxifene can be potentially used to explore its role in dual management of cancer and chemotherapy induced menorrhagia in female cancer patients of reproductive age. The potential cancer targets for the p53/MDM2/MDMX inhibitor therapy should possess wild-type TP53, MDM2 and MDMX gene. The likely proportion of patients with various types of cancer who are eligible for the p53/MDM2/MDMX inhibitor therapy are shown in the Table S1. Table S2 summarizes the potential cancer histologies which has p53

mutational status and overexpression of MDM2 and MDMX that could be targeted by SERMs. Therefore, our studies warrant further research to investigate the repurposing potential of ormeloxifene and other SERMs in cancer therapy as dual p53/MDM2/MDMX inhibitors.

Conclusion

Recent advances in cancer biology have shown that targeting the p53/MDM2/MDMX axis is a feasible approach to discover novel anticancer agents, to treat a wide variety of cancers. In this regard, several small molecules have been successfully designed to prevent the p53/MDM2 and p53/MDMX interactions and some of these inhibitors are at various stages of clinical trials. Developing dual inhibitors of p53/MDM2/MDMX is more challenging and recent research efforts are aimed in this direction. Drug repurposing is an attractive approach to identify dual p53/MDM2/MDMX inhibitors. Our computational modelling studies have shown that novel repurposing of SERMs for dual targeting of the p53/MDM2 and p53/MDMX interactions might be a potential alternative to treat wild-type p53 tumors. Further studies using preclinical models are required to evaluate the therapeutic potential of SERMs in cancer therapy.

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Disclosure of conflict of interest

None.

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Supplementary Materials

Materials and methods

Preparation of nutlin-3a and SERMs

The molecular docking studies were carried out using the computational software Discovery Studio (DS) *Structure-Based-Design* (SBD) from Dassault Systemes Biovia Corp. USA (v20.1.0.19295). The crystal structures of SERMs nutlin-3a, 4-hydroxytamoxifene (pdb id: 3ERT), raloxifene (pdb id: 1ERR), bazedoxifene (pdb id: 4XI3) and lasofoxifene (pdb id: 2OUZ), were extracted from the RCSB protein data bank (rcsb.org) [31]. These structures were prepared using the Small Molecules module in DS using CHARMm force field at pH 7.4. The structures of ormeloxifene, and ospemifene were built in 2D using ChemDraw Ultra 19.1 (PerkinElmer Informatics Inc. USA) and were converted to 3D using the Small Molecules module in DS using CHARMm force field and were minimized using the Smart Minimizer with 2000 minimization steps (RMS gradient =0.01 kcal/mol) and distance dependent dielectric constant at pH 7.4.

Preparation of MDM2 and MDMX proteins for molecular docking

The solved structures of MDM2 and MDMX bound to nutlin-3a were obtained from RCSB data bank (pdb id's 4HG7 and 2N14 respectively) [39]. The NMR solution structure of MDMX (pdb id: 2N14) reports 20-atomic coordinates/models. We used the first model (2N14_model_1) for small molecule docking studies. The proteins were prepared after removing water using the *Prepare Proteins* option under the *Macromolecules* module in DS using CHARMm force field. A binding sphere of 10 Å was prepared using the *Receptor-Ligand Interactions* module in DS after selecting the bound ligand nutlin-3a. The ligand was deleted and molecular docking studies were carried out using the 3D structures of nutlin-3a and the SERMs 4-hydroxytamoxifene, raloxifene, bazedoxifene, lasofoxifene, ormeloxifne and ospemifene prepared earlier in DS. The CDOCKER algorithm [40, 41] in the *Receptor-Ligand Interactions* module, was used for molecular docking studies. The CDOCKER algorithm is based on a simulated annealing protocol [40] and included 2000 heating steps to reach 700 K target temperature followed by 5000 cooling steps and a target temperature of 300 K. The molecular docking protocol for MDM2 and MDMX was validated by docking the known ligand nutlin-3a and by comparing the root-mean-square deviation (RMSD) for all the heavy atoms in Å units with the solved binding mode of nutlin-3a. The RMSD was calculated using the *Structure* module in DS. The top 10 docked binding modes of SERMs were evaluated based on the CDOCKER energy and CDOCKER interaction energy scores. The ligand-protein interactions for the top ranked binding modes were evaluated by investigating the type of polar and nonpolar interactions and their distance parameters.

Binding energy calculation of nutlin-3a and SERM complex with MDM2 and MDMX proteins

The top ranked binding modes of nutlin-3a and SERMs 4-hydroxytamoxifene, raloxifene, bazedoxifene, lasofoxifene, ormeloxifne and ospemifene obtained from the CDOCKER algorithm were further evaluated by calculating their binding energies (in kcal/mol) using the *Calculate binding energies* option in the *Receptor-Ligand Interactions* module in DS. The equation $E_{binding}$ = Energy of complex ($E_{ligand\text{-}receptor}$) - Energy of ligand ($E_{ligand}$) - Energy of receptor ($E_{receptor}$), the implicit solvent model Generalized Born with a simple Switching (GBSW) function, CHARMm force field and *Smart Minimizer* with 1000 steps of ligand minimization (RMS gradient =0.001 kcal/mol) were used to calculate ligand-receptor binding energy [41, 42].

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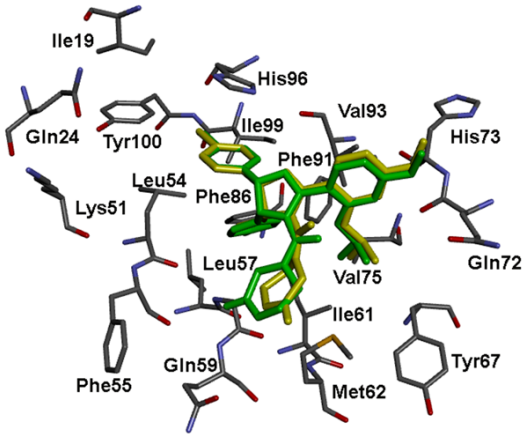

Figure S1. Comparison of the binding mode of nutlin-3a (stick cartoon, blue color) obtained using the CDOCKER docking algorithm, with the crystal structure of nutlin-3a (stick cartoon, yellow color) in the p53 binding domain of MDM2 (PDB ID: 4HG7). Hydrogen atoms are not shown to enhance clarity.

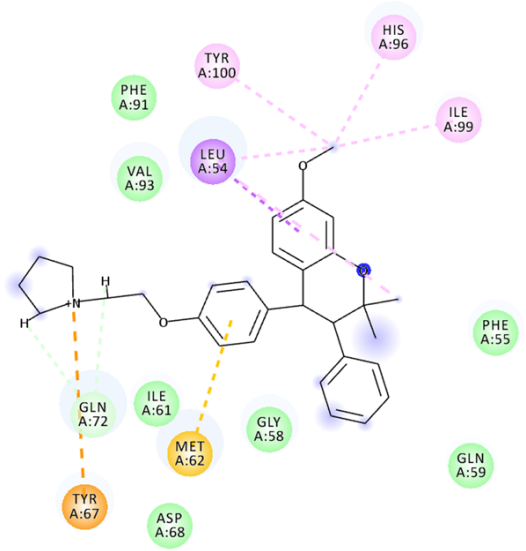

Figure S2. 2D Interaction map of ormeloxifene in the p53 binding domain of MDM2 (PDB ID: 4HG7). Hydrogen atoms are not shown to enhance clarity.

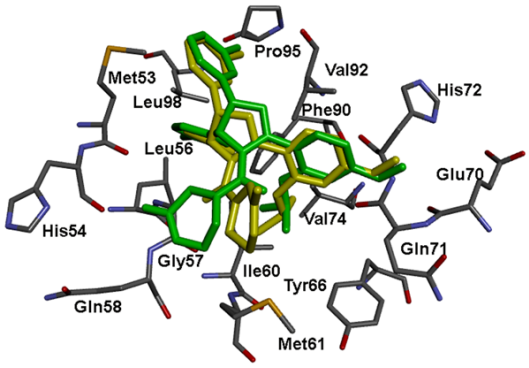

Figure S3. Comparison of the binding mode of nutlin-3a (stick cartoon, blue color) obtained using the CDOCKER docking algorithm, with the crystal structure of nutlin-3a (stick cartoon, yellow color) in the p53 binding domain of MDMX (PDB ID: 2N14). Hydrogen atoms are not shown to enhance clarity.

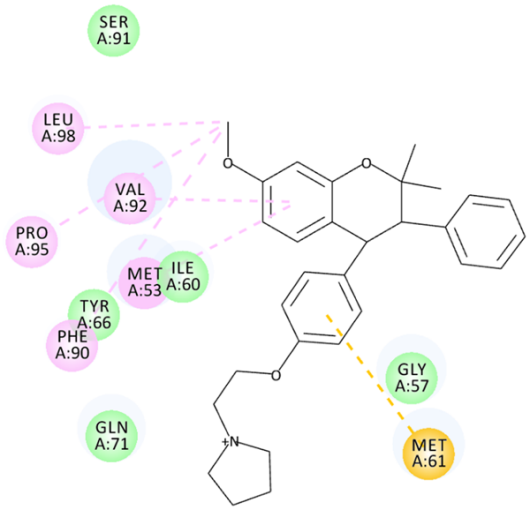

Figure S4. 2D Interaction map of ormeloxifene in the p53 binding domain of MDMX (PDB ID: 2N14). Hydrogen atoms are not shown to enhance clarity.

Table S1. Percentage breakdown of various cancer types having unaltered *p53*, *MDM2* and *MDMX* gene mutations, which are candidates for p53/MDM2/MDMX inhibitor therapy

S. No.	Cancer type	% of unaltered p53	% of unaltered MDM2	% of unaltered MDMX	Sample size	Reference
1	Pilocytic Astrocytoma	100	100	100	96	[1]
2	Pediatric Neuroblastoma	100	100	100	1089	[2]
3	Uveal Melanoma	100	100	100	80	[3]
4	Adenoid Cystic Carcinoma	100	100	100	65	[4]
5	Thyroid Carcinoma	99.6	100	100	490	[3]
6	Pheochromocytoma and Paraganglioma	99.4	100	100	178	[3]
7	Testicular Germ cell cancer	98.6	100	N/A	145	[3]
8	Myeloproliferative Neoplasm	98	100	99.3	151	[5]
9	Pediatric Wilms' tumor	97.7	100	100	657	[6]
10	Renal clear cell carcinoma	97.3	100	100	402	[3]
11	Thymoma	97	100	100	123	[3]
12	Medulloblastoma	96	100	100	125	[7]
13	Anaplastic Oligodendroglioma and anaplastic oligoastrocytoma	95	100	100	22	[8]
14	Cystic tumor of Pancreas	94	100	100	32	[9]
15	Histiocytosis cobemtinib	93	100	100	33	[10]
16	Chronic lymphocytic leukemia	93	100	99.8	537	[11]
17	Ewing sarcoma	93	100	100	112	[12]
18	Adenoid cystic carcinoma project	93	99.7	99.4	1049	[13]
19	Cervical squamous cell carcinoma	92	99.3	100	291	[3]
20	Myelodysplastic syndrome	91	99.9	99.9	1049	[14]
21	Diffuse large B cell lymphoma	91	100	100	1001	[15]
22	Primary CNS Lymphoma	90	100	100	19	[16]
23	Malignant peripheral nerve sheath tumor	87	100	100	15	[17]
24	Metastatic melanoma	86	98.2	99.1	110	[18]
25	Mesothelioma	84	100	100	86	[3]
26	Cholangiocarcinoma	77	99.5	100	195	[19]
27	Prostate adenocarcinoma	72	99.9	99.7	1465	[20]
28	Liver hepatocellular carcinoma	70	99.7	100	366	[3]
29	Glioblastoma Multiforme	69	98.7	99.2	397	[3]
30	Squamous cell carcinoma of vulva	67	100	100	15	[21]
31	Breast cancer	66	N/P	N/P	2509	[22, 23]
32	Uterine corpus endometrial carcinoma	63	98.7	98.1	517	[3]
33	Glioma	60	99.8	98.8	812	[24]
34	Bladder cancer	52	98.8	99.5	412	[25]
35	Stomach adenocarcinoma	51	98.6	98.4	436	[3]
36	Non-Small cell lung cancer	46	99.3	99	915	[26]
37	Esophageal carcinoma	40	98.8	99.2	518	[27]
38	Ampullary carcinoma	38	100	98.7	160	[28]
39	Head and neck squamous cell carcinoma	31	99.4	99.8	523	[29]
40	Metastatic colorectal cancer	27	99.5	99.6	1134	[30]

Table S2. Summary of TP53/MDM2/MDMX landscape analysis

S. No.	Cancer type	Sample size (all)	TP53 mutation	mRNA expression (high)		Study
				MDM2	MDMX	
1	Uveal melanoma	80	0 (80)	7 (80)	4 (80)	[31]
2	Pediatric Neuroblastoma	1089	0 (1089)	6 (143)	7 (143)	[2]
3	Thyroid carcinoma	500	2 (490)	25 (498)	16 (498)	[32]
4	Pheochromocytoma and Paraganglioma	178	1 (178)	7 (178)	14 (178)	[33]
5	Testicular Germ Cell Tumors	149	2 (145)	18 (149)	12 (149)	[34]
6	Pediatric Wilms' tumor	657	15 (657)	13 (130)	35 (130)	[6]
7	Kidney Renal clear cell carcinoma	512	11 (402)	34 (510)	28 (510)	[32]
8	Thymoma	123	2 (123)	4 (119)	2 (119)	[33]
9	Cervical squamous cell carcinoma	297	23 (291)	19 (294)	30 (294)	[35]
10	Cholangiocarcinoma	36	4 (36)	3 (36)	8 (36)	[32]
11	Diffuse Large B-Cell Lymphoma	48	5 (41)	2 (48)	2 (48)	[32]
12	Metastatic melanoma	110	15 (110)	2 (40)	1 (40)	[18]
13	Mesothelioma	87	14 (86)	3 (87)	8 (87)	[35]
14	Uterine Corpus Endometrial Carcinoma	373	69 (248)	3 (333)	4 (333)	[36]
15	Liver hepatocellular carcinoma	372	110 (366)	7 (366)	20 (366)	[34]
16	Glioblastoma multiforme	592	125 (397)	18 (160)	20 (160)	[35]
17	The Metastatic breast cancer project	237	76 (237)	5 (146)	4 (146)	[37]
18	Metastatic prostate cancer	150	70 (150)	3 (118)	15 (118)	[38]
19	Stomach adenocarcinoma	440	200 (436)	34 (412)	18 (412)	[35]
20	Bladder Urothelial Carcinoma	411	200 (410)	46 (407)	28 (407)	[35]
21	Lung adenocarcinoma	566	263 (566)	45 (510)	62 (510)	[35]
22	Colorectal adenocarcinoma	594	314 (534)	44 (592)	46 (592)	[32]
23	Head and Neck Squamous Cell Carcinoma	523	357 (515)	41 (515)	28 (515)	[35]
24	Lung squamous cell carcinoma	487	402 (484)	53 (484)	35 (484)	[33]
25	Esophageal adenocarcinoma	182	158 (182)	10 (181)	16 (181)	[33]

Note: Listed for each cancer type is the number of sample alterations in *TP53* mutation with its corresponding mRNA expression for MDM2 and MDMX. The number in the brackets indicates the number of samples available per cancer study for the particular data type.