



Nutlin-3, A p53-Mdm2 Antagonist for Nasopharyngeal Carcinoma Treatment



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Abstract: Nasopharyngeal carcinoma (NPC) is a form of head and neck cancer of multifactorial etiologies that is highly prevalent among men in the population of Southern China and Southeast Asia. NPC has claimed many thousands of lives worldwide; but the low awareness of NPC remains a hindrance in early diagnosis and prevention of the disease. NPC is highly responsive to radiotherapy and chemotherapy, but radiocurable NPC is still dependent on concurrent treatment of megavoltage radiotherapy with chemotherapy. Despite a significant reduction in loco-regional and distant metastases, radiotherapy alone has failed to provide a significant improvement in the overall survival rate of NPC, compared to chemotherapy. In addition, chemo-resistance persists as the major challenge in the management of metastatic NPC although the survival rate of advanced metastatic NPC has significantly improved with the administration of chemotherapy adjunctive to radiotherapy. In this regard, targeted molecular therapy could be explored for the discovery of alternative NPC therapies. Nutlin-3, a small molecule inhibitor that specifically targets p53-Mdm2 interaction offers new therapeutic opportunities by enhancing cancer cell growth arrest and apoptosis through the restoration of the p53-mediated tumor suppression pathway while producing minimal cytotoxicity and side effects. This review discusses the potential use of Nutlin-3 as a p53-activating drug and the future directions of its clinical research for NPC treatment.

Keywords: Apoptosis, Epstein-Barr virus (EBV), carcinoma, cisplatin, small molecule inhibitor, targeted molecular therapy.

1. INTRODUCTION

Nasopharyngeal carcinoma (NPC) is a common epithelial squamous cell head and neck carcinoma that originates from the nasopharyngeal mucosa layering the upper part of the throat. It is the most common type of nasopharyngeal tumor [1, 2]. NPC has an unusual racial distribution that is strongly associated with multifactorial etiologies, such as gamma herpes Epstein-Barr virus (EBV) infection [3], the intake of salted fish [2], cigarette smoking [4], occupational exposures [5], environmental risk factors and genetic susceptibility [2, 6, 7].

NPC is strongly associated with EBV infection and intake of salted fish. In 1966, Old *et al.* discovered that NPC is an EBV-associated cancer [8] and almost 95% of NPC tumors were found associated with EBV infection [9]. Salted fish is rich in carcinogenic volatile nitrosamine, an EBV

activating agent. The polymorphic nitrosamine metabolizing genes, cytochrome CYP2E1 and CYP2A6 are responsible for NPC susceptibility [5, 10]. Several commonly used herbal plant extracts have the ability to reactivate EBV as well as to increase the titers of anti-EBV antibody in EBV-infected host [5]. A study conducted among Chinese populations revealed that consumption of Chinese medicine is another dietary-related factor for NPC. A significant correlation between traditional herbal medicine consumption and increased NPC risk has been linked with NPC pathogenesis [11]. Alcohol consumption, a common lifestyle in the West and other parts of the world, has been identified as another important risk factor for the development of NPC [12].

2. EPIDEMIOLOGY

NPC is fairly rare in most parts of the world, indicating distinct racial and geographical distribution. Although it is considered as rare, NPC incidence remains significantly high in endemic regions of Southern China [13], Hong Kong, Taiwan, Northern Africa [2] and Southeast Asia. According to the global scale statistics [14], NPC is the 23rd most com-

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mon cancer with nearly 80,000 new cases diagnosed annually and a mortality rate that exceeds 50,000. NPC is more prevalent in males than females, with the male to female ratio of 2.3:1.0 [15]. High-risk rates of ASR 25-30 (per 100,000 populations) were seen in Southern China notably among the Cantonese in the area of Guangzhou; high incidence rates are also noted in Southeast Asia. In contrast, low incidence rates of ASR < 1 were observed in Europe and North America [14].

NPC is more prevalent among the Chinese populations and this could be attributed to their lifestyles, such as consumption of large amount of carcinogen-contaminated salted fish. Consumption of salted fish is also reported to have a strong correlation with NPC [16]. In addition, early childhood exposure to diet that is high in preserved foods and salted fish is shown to have significant effects on higher NPC risk [13, 17]. Approximately 90% NPC cases from Hong Kong [16], 60% from Malaysian Chinese [18] and 50% from Guangzhou [19] are attributed to frequent consumption of salted fish in childhood. Several case-control studies observed that high-risk NPC populations frequently have high intake of preserved food, pickled vegetables and fermented products, such as beans, bean pastes, eggs and seafood pastes [20, 21].

3. THERAPY COMPLICATIONS

Nasopharyngeal tissue consists of several types of cells, whereby, each type has the ability to transform into different types of tumors. These differences are significant for the classification of the disease subtypes, severity as well as the efficacy of treatment. The selection of treatment option for NPC is dependent on the tumor location and disease extent [22]. Surgery is rarely the main therapeutic option for NPC due to its complex anatomical proximity to critical structures. Traditional surgery leads to transposition of obstructing bone and soft tissue, and has risk of nerves and blood vessels damages, weakness in the arm or lower lip and numbness of the ear [23, 24]. Endoscopic nasopharyngectomy causes significant postoperative complications including surgical pain, headache, nasopharyngeal necrosis, and crusting of the otitis media [25, 26].

NPC is mainly treated with radiotherapy, often in combination with chemotherapy based on the different stages of the disease [27, 28]. The major concerns of radiotherapy are specific organ damage in the head and neck regions due to exposure to harmful radiation. Radiotherapy contributed to radiation-induced sensory neural hearing loss (SNHL) with an incidence rate of 24.2% [29]. Intensity-modulated radiotherapy (IMRT) improves the loco-regional control of NPC, but distant metastasis and recurrence remain the main causes of treatment failure [27, 30, 31].

The most active chemotherapy agents used in NPC are cisplatin and 5-fluorouracil (5-FU) [27, 28]. These drugs are highly toxic yet not cancer cell specific as they are cytotoxic to actively dividing cells during mitotic phase [32, 33]. Cisplatin is extensively used mainly due to its effectiveness in treating difficult to cure cancer, but is associated with nephrotoxicity, neurotoxicity, ototoxicity, alopecia, myelotoxicity, nausea and vomiting [29, 34, 35]. Among the severe ad-

verse effects of 5-FU are chest pain associated with heart disease, fatigue, nausea, mouth sores, photophobia (light sensitivity) and diarrhea.

NPC patients are often adversely affected by post-chemoradiotherapy effects and tend to experience therapy failure mainly due to local recurrence [36]. In an effort to improve the prognosis and efficacy of NPC therapy, a combination of definitive radiotherapy plus cisplatin-based chemotherapy is recommended [37]. Concurrent advanced combined chemoradiotherapy (CCRT) technique is the main treatment modality for advanced-stage NPC [28]. However, the use of chemotherapy either before or after radiotherapy for patients with advanced NPC has shown to yield no improvement on the overall survival or relapse-free survival [38, 39]. Severe chemo-radio adverse effects, post-treatment late complications such as osteoradionecrosis and xerostomia, therapy-resistance, loco-regional recurrence and distant metastases remain as major causes of mortality and morbidity in NPC [40-42]. Nearly 50% NPC patients experience cancer recurrence; 80% reported cases of incurring auxiliary late complications of long-term toxicity effect [40] and 43% treated-patients showing distant metastases within four years [37, 43]. The addition of cisplatin to CCRT increased the risk of SNHL, severe hearing impairment or deafness [29]. Furthermore, the emergence of chemo-resistance remains as major obstacle for cisplatin-based chemotherapy which impaired cisplatin-induced apoptosis [44].

The anti-epidermal growth factor receptor (EGFR), cetuximab, is the first monoclonal antibody specifically designed to inhibit EGFR in both normal and cancer cells. Cetuximab enhances the sensitivity of cancer cells to chemoradiotherapy. It has been approved for use during chemotherapy with cisplatin or along with radiotherapy [45, 46]. Cetuximab therapy rarely has side effects, but patients may develop severe skin allergy, infusion reaction, anemia, cardiac toxicity and lung disease [45].

In addition, early stage NPC may be asymptomatic or can be presented with trivial symptoms, thus likely to escape early diagnosis, which results in undetected progression to stages III or IV of the disease [47, 48]. Late stage NPC is associated with poor prognosis and treatment failures [28, 36]. Although the initial response and local control rates achieved with advance radiotherapy and/or chemotherapy in advanced stage III and IV NPC are high, NPC remains incurable with unacceptable 5- and 10-year survival rates [49]. The 5-year overall survival rate of stage III and IV NPC patients were reported to be 56.2% for radiotherapy and 47.2% for chemotherapy [49]. In fact, recurrence, distant metastases, drug resistance and adverse effects of treatments remain as major challenges in the clinical scenario [40, 42].

Therefore, reducing undesirable complications of chemotherapy drugs is a major goal in pharmaceutical research for NPC treatment. In view of these issues and challenges, the design of modern cancer therapeutic must evolve from non-specific targeting that affects both normal and cancer cells to specifically targeting unique molecular signature of cancer cells and to produce greater antitumor efficacy with less toxicity effects, such as p53-based targeted therapy.

4. p53-Mdm2 INTERACTION: A THERAPEUTIC TARGET

4.1. Tumor Suppressor p53

The p53 gene located on chromosome 17 (17p13.1), contains 11 exons, which encode for a 2.8 kb mRNA, translated into a 53 kDa protein made up of 393 amino acids [50, 51]. The wild type (wt) p53 tumor suppressor gene has been termed as the “guardian of the genome” [52], or “cellular gatekeeper of growth and division” [53] due to its important biological roles in protecting cells from becoming cancerous by regulating the transition of G₁-S phase in renewable functional tissue and preventing gene aberration for genome stability by eradicating DNA-damaged cells in a natural way [52].

4.2. Antagonizing Mdm2 to Reactivate p53

The murine double-minute type 2 protein (Mdm2), known in human as Hdm2, is encoded by an oncogene located on the acrocentric extra chromosomal nuclear bodies of chromosome 12 (12q14.3 – q15). Mdm2 acts as an inhibitor of the tumor-suppressive effects of p53. In a normal cell, Mdm2 protein is the main regulator in mastering the stability and activity of p53 protein by E3-ubiquitin ligase activity [54, 55]. The wt p53 is a potent pro-apoptotic protein with a short half-life of 6-20 minutes (Fig. 1). In the absence of stress, p53 which retards inappropriate growth arrest and

apoptosis is almost undetectable in proliferating cells. Dissociation of p53-Mdm2 complex occurs in response to cellular stress, DNA damage or activation of oncogene which leads to the activation of p53 [56] and free p53 protein from Mdm2 binding (Fig. 2). The degradation of p53 is inhibited resulting in prolonged p53 half-life to hours and accumulation of p53 in the cell [57]. As a DNA damage-inducible nuclear transcription factor, p53 determines whether the damaged cells are repaired or undergo self-destruction [58]. Reactivation of p53 promotes DNA repair, cell cycle arrest, senescence or cell death by apoptosis [59]; thereby protecting the cell from malignant transformation, suppressing tumor development and eradicating tumor in a natural way. Its tumor suppressing activities are recognized in human cancers [60]; and Mills confirmed that wt p53 contributes tumor-suppressive capabilities compared to mutated p53, which is oncogenic [61].

4.3. Nutlin-3: Inhibitor of the p53-Mdm2 Interaction

Nutlin-3 (C₃₀H₃₀Cl₂N₄O₄, 581.4896 g/mol) is a small molecular weight cis-imidazoline analogue that was designed to compete with Mdm2 for binding to p53 [102]. Nutlin-3 induces the regulation and activation of p53 pathway [102], and is found to be effective and non-genotoxic in stabilizing p53 using experimental models in tumors expressing wt p53 (Fig. 3). Since its discovery, *in vitro* and *in vivo* therapeutic-based studies have revealed that Nutlin-3 could

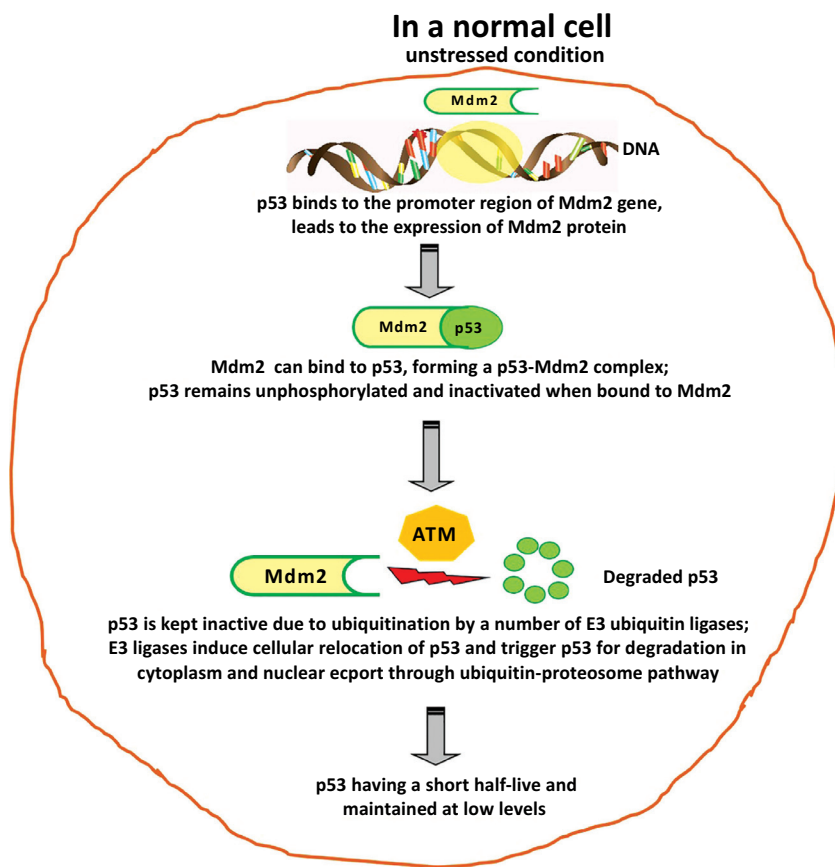


Fig. (1). The stabilization of p53 by Mdm2 in normal cell. The regulation of p53 is tightly controlled by its oncogenic E3 ubiquitin ligase negative regulator Mdm2.

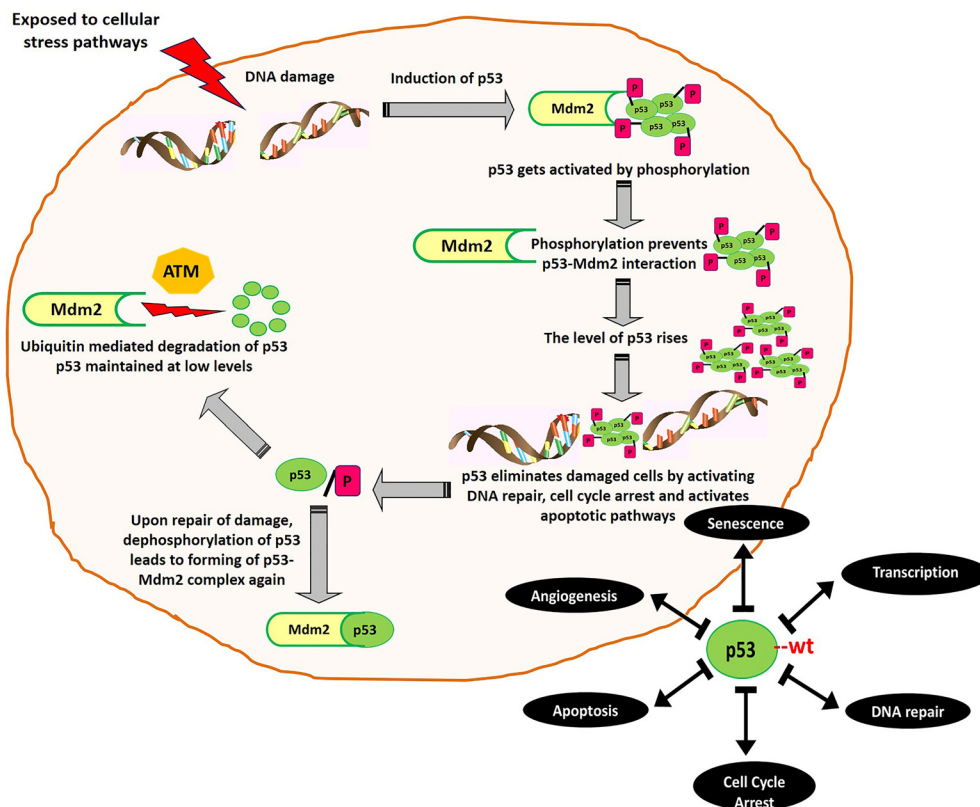


Fig. (2). The wt p53 protects cells from genome mutation, eradicates tumor cells by activating cell cycle arrest, DNA repair and apoptosis.

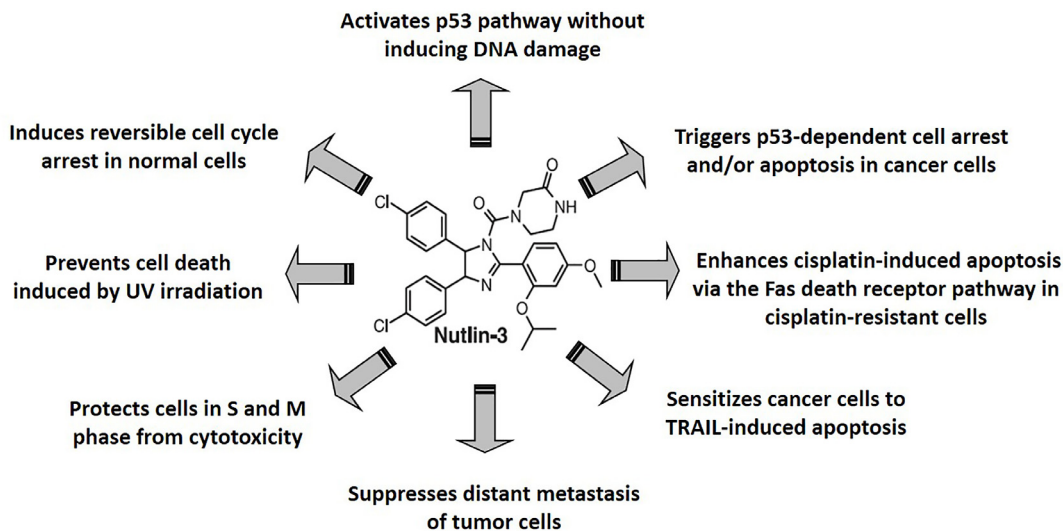


Fig. (3). p53-dependent effects of Nutlin-3.

be a potential alternative for targeted therapy in the current chemotherapy regime. Nutlin-3 has been reported to selectively enhance apoptosis in wt p53 cancer cells by activating the p53 pathway (Table 1). Nutlin-3 is non-genotoxic and protects normal cells against mitotic toxicity [103, 104] and kidney cells from the cytotoxic effect of cisplatin [105]. Apart from Nutlin-3, other small molecules that reactivate

the p53 pathway undergoing clinical trials are shown in (Table 2).

4.4. Nutlin-3: A Potential Anti-NPC Agent

Mutations in p53 alter its ability for DNA repair, thus contributing to cancer development [106-108]. In a comparative

Table 1. Summary of published experimental Nutlin-3 for human cancer therapy.

Published Research	Cancer Types	p53-dependent Actions of Nutlin-3
Kojima <i>et al.</i> , 2005 [62]	AML	Inhibits p53-Mdm2 binding-induced growth arrest and apoptosis in AML cells.
Stuhmer <i>et al.</i> , 2005 [63]	MM	Activates p53 pathway and induces apoptosis in MM cells.
Tovar <i>et al.</i> , 2006 [64]	OS	Induces G ₁ and G ₂ phase cell-cycle arrest function of the p53 pathway in OS cells.
Muller <i>et al.</i> , 2007 [65]	LS	Induces apoptosis in LS cells with high wt p53 levels.
Saddler <i>et al.</i> , 2008 [66]	CLL	p53 status is the major determinant of response to Nutlin-3 in CLL cells.
Miyachi <i>et al.</i> , 2009 [67]	RMS	Restores p53 pathway-induced apoptosis and cell cycle arrest in RMS cells.
Van Maerken <i>et al.</i> , 2009 [68]	NB	Activates p53 pathway in chemoresistant NB cells. Induces apoptosis and suppresses distant metastasis in xenograft model NB.
Hori <i>et al.</i> , 2010 [69]	CCa	Sensitizes CCa cells to TRAIL-induced mitochondrial dysfunction, and increases death receptor DR5 promoter.
Koster <i>et al.</i> , 2011 [70]	TC	Upregulates Fas membrane expression and increases Fas death receptor expression in TC cells.
Sonnemann <i>et al.</i> , 2011 [71]	ES	Induces cellular senescence, antineoplastic effects and apoptosis in ES cells.
Ye <i>et al.</i> , 2012 [72]	KS	Activates p53 pathway-induced apoptosis and inhibits “KS-like” tumor growth in nude mice.
Voon <i>et al.</i> , 2015 [73]	NPC	Activates p53 pathway and induces apoptosis in EBV-positive NPC cells. Sensitizes NPC cells to cisplatin-induced apoptosis by modulating pro-apoptotic targets <i>via</i> p53 pathway.

AML, acute myeloid leukemia; MM, multiple myeloma; OS, osteosarcoma; LS, liposarcoma; CLL, chronic lymphocytic leukemia; RMS, rhabdo-myosarcoma; NB, neuroblastoma; CCa, colon cancer; TC, testicular cancer; ES, Ewing's sarcoma; KS, Kaposi sarcoma.

study using several previously sequenced tumors, p53 is the most frequently mutated gene in epithelial malignancies [109]. p53 mutation is common in the early stage of human lung, colon, head and neck cancers [110]. Mutant p53 has a long half-life when compared to wt p53 found in over 60% of head and neck cancer. However, p53 mutations have been reported to be rare in NPC [6, 111, 112]. The findings of a genome-wide association study confirmed that NPC oncogenesis is strongly related to multiple genetic alterations, which involved point mutations of p53 gene detected in hot spots of exons 5, 7 and 8 in <10% of NPC cases [113].

Apart from these reported events, Lin and colleagues [109] recently determined nine significantly mutated genes with the two most commonly found in NPC being PIK3CA and p53. The whole-exome sequencing (WES) and single-nucleotide polymorphism (SNP) array profiles showed detectable G₁-S cell cycle transition with most frequent 9p21 and p53 genes deletions detected in 28% of NPCs [109]. However, using the most sensitive combination of WES, targeted deep sequencing and SNP array approaches, it is shown that NPC results in a relatively low level of genomic alteration, as well as rare p53 mutation frequency with <10% cases observed in EBV-associated NPCs [109]. The p53 mutation is rare even in recurrent radiotherapy refractory NPC [114], thus making NPC a potential candidate for treatment with p53 activators, like Nutlin-3. p53 is often overexpressed in NPC cells [115, 116] and overexpression of p53 using an adenoviral vector has been reported effective against NPC [117, 118] indicating that further elevating p53 levels by Nutlin-3 could provide an alternative treatment strategy against NPC.

In our previous study, we have demonstrated that Nutlin-3 could activate the p53 pathway for sensitization of cisplatin-induced apoptosis in EBV-positive NPC cells in a p53-dependent manner [73]. Our findings suggest that restoration of wt p53 with Nutlin-3 maximizes the protection of normal cells while it enhances cytotoxicity of cisplatin. Cisplatin was more cytotoxic to the normal nasopharyngeal cells compared to the NPC cells, while Nutlin-3 was more selective in inhibiting NPC cells. The combination drug treatment of cisplatin and Nutlin-3 showed stronger anti-proliferative effect against NPC cells, demonstrating that combined treatment was more effective than single drug treatment [73]. In addition, combination treatment of NPC cells with cisplatin and Nutlin-3 significantly induced the accumulation of apoptotic cells, concomitant with the upregulation of the protein expressions of apoptosis regulators, BAX and PUMA and detection of an apoptosis marker, cleaved PARP. These indicate that Nutlin-3 sensitizes NPC cells to cisplatin-induced apoptosis by modulating pro-apoptotic targets *via* the p53 pathway. In this regard, reducing the dose of cisplatin could lead to lesser adverse effects while retaining stronger cytotoxic effect against NPC cells.

Apart from these reported events, we have also determined that the extended treatment of NPC cells with Nutlin-3 did not result in the emergence of p53 mutation, albeit reduced sensitivity to Nutlin-3 was observed [73]. This stresses on the importance of treatment duration and clinical doses optimization to improve the efficacy of Nutlin-3 significantly. Collectively, these findings are important for the development and design of clinical trials of Nutlin-3 for NPC treatment in the near future.

Table 2. Small-molecule p53 activators currently in clinical trials.

	Compound	Small-Molecule	Clinical Trial Development		
			Stage of Development	Cancer Types	p53-Dependent Actions
Activate wt p53 by:					
Mdm2 binding: Mimics the p53 amino acid side-chains involved in the binding of the p53 peptide to Mdm2 that inhibits p53-Mdm2 binding	Nutlins (RG7112, RO5045337)	cis-imidazoline	Phase I/II [64, 74]	Advanced solid tumors, sarcoma, liposarcomas, neoplasms, hematological malignancies	Induces cell cycle arrest Induces apoptosis
	BDA (TDP 665759 TDP 521252)	Benzodiazepine derivatives	Preclinical [75, 76]	JAR choriocarcinoma, hepatocellular carcinoma	Inhibits cancer cell proliferation Induces apoptosis
Hdm2 binding: Inhibits p53-Hdm2 binding, prevents p53 degradation by its inhibitor Hdm2 and enables p53 activation	Spiro-oxindoles (MI-219, MI-63, MI-43)	Computational modeling	Preclinical [77-79]	Breast cancer, colon carcinoma, prostate cancer, lung cancer, hematological malignancies	Inhibits cancer cell proliferation Induces apoptosis Inhibits tumor growth
	Serdemetan (JNJ-26854165)	Tryptamine derivative	Phase I [80, 81]	B-cell lymphoma, colorectal cancer, lymphoma, melanomas, sarcomas, acute leukemia, lung cancer	Induces apoptosis Increases p53 stability Reduces the rate of DNA synthesis
MdmX binding: Mimics the p53 amino acid side-chains involved in the binding of the p53 peptide to MdmX that inhibits p53-MdmX binding	SJ-172550	Identified through a peptide-based high throughput screening	Preclinical [82]	MdmX over-expressing tumor, retinoblastoma	Induces cell cycle arrest Induces apoptosis
	RO-2443/ RO-5693	Indolyl hydantoin	Preclinical [83]	MdmX over-expressing tumors, breast cancer, osteosarcoma	Induces cell cycle arrest Induces apoptosis
	XI-011	Pseudourea derivative	Preclinical [84-86]	Breast cancer	Induces apoptosis
p53 binding: Induces conformational change that prevent p53-Mdm2 binding	RITA (NSC 652287)	2,5-bis(5-hydroxymethyl-2-thienyl)furan	Preclinical [74, 87, 88]	Multiple myeloma (mt), AML, CLL, colon carcinoma, lung cancer, breast cancer, Burkitt's lymphoma	Induces cell cycle arrest Induces apoptosis
E3 ubiquitin ligase inhibition: Enhances the autoubiquitination and degradation of Hdm2's E3 activity that acts as the predominant p53 negative regulator	HL198, HL1373	7-nitro-10-aryl-5 deazaflavins	Preclinical [89, 90]	Human retinal pigment epithelial cells, colon carcinoma, melanoma, lung carcinoma, fibrosarcoma, osteosarcoma	Activates p53-dependent transcription Induces apoptosis
Protein deacetylators inhibition: Inhibits deacetylase activity of protein deacetylators SirT1 and SirT2; and results in the stabilization of p53	Tenovin 1 Tenovin 6	Identified by screening of small molecules from Chembridge DIVERSet	Preclinical [91, 92]	Burkitt's lymphoma, melanoma, breast cancer, colon carcinoma	Negative regulation of p53 Delays tumor growth <i>in vivo</i> Induces cell cycle arrest Induces apoptosis
Restoration of wt function of mt p53 by:					
Conformational stabilization of p53 DBD: Interacts with DNA, reactive oxygen species	CP-31398 (V173A, R175S, R249S, R273H)	Styrylquinazoline synthetic	Preclinical [93]	Osteosarcoma, colon carcinoma, melanoma, lung carcinoma	Stabilizing the active conformation of wt p53 DBD
Chelation/redox modulation: Restores wt-like DNA binding properties to mt protein p53 ^{R273H} (DNA contact) or p53 ^{R175H} (conformational)	Thiosemicarbazone: Zinc	Metal ion chelators	Preclinical [94, 95]	p53 ^{R175} mt ovarian carcinoma, p53 ^{R175} mt breast cancer, p53 ^{R273H} mt glioblastoma, p53-null lung carcinoma	Induces apoptosis Induces cell cycle arrest Improves the sensitivity of mt p53-expressing cells to anti-tumor drugs

(Table 2) Contd....

	Compound	Small-Molecule	Clinical Trial Development		
			Stage of Development	Cancer Types	p53-Dependent Actions
Covalent modifications of cysteine residues: Induces wt-like conformational change and protein folding to mt protein p53 ^{R273H} or p53 ^{R175H}	PRIMA-1 ^{MET} (APR-246)	Identified by screening of NCI chemical library that inhibits Saos-2 cell proliferation	Phase I/II [96]	Hematologic malignancies, prostate cancer, lung cancer, prostate cancer	Induces cell cycle arrest Induces apoptosis
Alkylation of cysteine and lysine residues: Reactivates DNA binding and preserves the conformation of mt p53 protein	MIRA-1	Maleimide derivative	Preclinical [97, 98]	Myeloma, osteosarcoma, lung adenocarcinoma, ovarian carcinoma, colon carcinoma	Restores apoptotic activity to mt p53 Induces apoptosis
Bindings to the mutation-induced cleft: Raises T _m , slows down the thermal denaturation of mt p53-Y220C	PhiKan083	Carbazole derivative	Preclinical [99-101]	p53-Y220C mt gastric cancer, hepatoblastoma	Regains structural stability and restores wt p53 conformation of mt p53-Y220C Increases p53 half-lives by stabilizing ligands

AML, acute myeloid leukemia; BDA, benzodiazepinediones; CLL, chronic lymphocytic leukemia; DBD, DNA binding domain; Hdm2, human double minute 2; MIRA-1, mutant p53-dependent induction of rapid apoptosis; mt, mutant; PRIMA-1, p53 reactivation and induction of massive apoptosis; RITA, reactivation of p53 and induction of tumor cell apoptosis; Saos-2, osteosarcoma cells; T_m, melting temperature; wt, wild type.

5. FUTURE DIRECTIONS

The margin of success of standard therapy approach for most radiocurable NPC is still dependent on concurrent megavoltage radiotherapy [27, 30, 40]. However, disease relapse, refractory diseases and management of long-term toxicities are critical issues that remain to be addressed. In view of these issues, the National Comprehensive Cancer Network Clinical Practice Guidelines recommend the utilization of multiple alternative strategies as the current standard cancer therapies to improve the prognosis of hardly curable NPC [76]. On the other hand, the need to find new drug targets and establish novel effective therapy for NPC necessitates the focus on specific targeting of unique molecular signature of the cancer cells rather than non-specific targeting that affects both normal and cancer cells.

Despite all the advances in cancer therapies, more drugs that reactivate the p53 pathway are entering clinical trials because of the growing demand for alternative therapy to cure cancers [119]. The preliminary trials on p53-activating therapies showed that appropriate design of a clinical trial remains a crucial issue [119]. Identifying tumors with low incidence p53 mutation and highly responsive to p53-activating drugs, as well as determination of p53 status as a prerequisite for administering the drugs are vital in the design of such clinical trials. Moreover, the contribution of p53 mutation to cancer development is expected to be associated with drug resistance, hence understanding the mechanism of drug resistance induction, particularly the inhibition of p53-induced apoptosis from extended treatments with p53-activating drugs, should be considered as well. Despite the fact that NPC is an aggressive disease characterized by over-expressions of p53 [120] and several p53 gene mutations, p53 mutation is considerably rare compared to other human cancers in general [109, 121]. Hence, antagonizing Mdm2 to reactivate p53 provides an alternative strategy to the current standard cancer therapy [76].

The rationale of using Nutlin-3 to antagonize Mdm2 to reactivate p53 in NPC is formed based on the facts that: (1) Nutlin-3 has been explored as a specific p53 activator in wt-expressing cancer cells and is applicable in NPC which is rarely mutated [73]; (2) Nutlin-3 chemo-protectively conserves normal cells [73, 105]; and (3) Nutlin-3 integrates effectively with radiation, genotoxic or anti-mitotic agents in suppressing various tumors [65, 84-86]. Currently, research for the development of p53-reactivating therapy in NPC is still limited compared to other cancer types where optimized clinical doses, treatment durations and safety profiles remain out of reach for now. Moreover, the effectiveness of Nutlin-3 in preclinical animal models has yet to be established. The synergistic effects *via* combination of low doses of cisplatin and Nutlin-3, drug interaction and dose-limiting toxicity studies have to be further investigated. Signaling pathways involved in the synergistic effects of the combination could also be delineated for the identification of additional targets. In addition, the mechanism of Nutlin-3-resistant induction and emergence of p53 alteration in NPC should also be investigated to study the pathological significances in NPC to gain deeper understanding to the molecular basis of this disease.

CONCLUSION

Although Nutlin-3 is far from completion for its clinical use for NPC, it is still an attractive alternative drug that should be given attention and explored further, given the poor prognosis and limited treatment choices for NPC. It is anticipated that the mortality and morbidity rate of NPC could be reduced with the combination use of Nutlin-3 and standard chemotherapeutic drugs coupled with the determination of p53 mutation status in each individual.

CONSENT FOR PUBLICATION

Not applicable.

CONFLICT OF INTEREST

The authors declare no conflict of interest, financial or otherwise.

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