Drugs of the Future 2012, 37(4): 273-281

CCC: 0377-8282/2012
DOI: 10.1358/dof.2012.37.4.1769833

REVIEW ARTICLE

NEW PLAYERS IN CANCER THERAPEUTICS: FOCUS ON INHIBITORS OF MDM2–P53 PROTEIN–PROTEIN INTERACTION

J. Capdevila[1], A. Cervantes[2] and J. Tabernero[1]

[1]Medical Oncology Dept., Vall d'Hebron University Hospital, Barcelona, Spain; [2]Hematology and Medical Oncology Dept., INCLIVA Health Research Institute, University of Valencia, Valencia, Spain

CONTENTS

Summary 273
Introduction 273
Regulation of p53 through Mdm2 273
Translational research in Mdm2–p53 interaction inhibitors 275
Clinical development of Mdm2–p53 protein-protein inhibitors . . 276
Future directions 279
Conclusions 279
References 280

SUMMARY

The recovery of cellular tumor antigen p53 functionality has become an attractive target in cancer therapy. The transcriptional activity of p53, mainly regulating cell cycle, DNA damage repair and apoptosis induction, is deregulated in the majority of human tumors. Indeed, the TP53 gene constitutes the most frequent mutated gene in cancer. Furthermore, the upregulation of the main negative controller of p53, oncoprotein Mdm2, has also been well described in different tumor types. Recent advances in the design of small-molecule inhibitors have allowed the development of highly specific protein–protein modulators that block the interaction between p53 and Mdm2. In this review, we aim to highlight the cornerstones of the current knowledge of the Mdm2–p53 interaction and summarize the preclinical and clinical development of Mdm2–p53 inhibitors.

Key words: Serdemetan – p53 – Oncoprotein Mdm2 – RG-7112 – MI-219 – CYC-700 – Nutlins

INTRODUCTION

The tumor suppressor gene *TP53* plays a central role in the regulation of cell homeostasis, eliminating defective and malfunctioning cells from the tissues. The cellular tumor antigen p53 receives information about genetic damage or metabolic disorders and may produce cell cycle arrest (senescence), DNA repair, or if p53 learns that the genome damage is too severe to be repaired, it may decide to enter the cell in the apoptosis process (1). The process of apoptosis is controlled by two main mechanisms, the first originated extracellularly (by extrinsic inducers such as toxins, hormones, growth factors or cytokines) and the second intracellularly (by intrinsic inducers activated by several stimuli, such as nutrient deprivation, radiation, viral infection or hypoxia), the intrinsic apoptosis pathway being the one where p53 protein develops its main functions (2).

Because of the prominent role of *TP53* as a tumor suppressor, this gene has been related with cancer development from the very beginning. Indeed, among all the genes examined to date in human cancer genomes, *TP53* is the most frequently mutated, being present in mutant status in almost half of all human tumors (3). Furthermore, a germline *TP53* mutation has been described as responsible for most cases of Li-Fraumeni syndrome, an extremely rare autosomal dominant hereditary disorder that predisposes to a wide range of malignancies, particularly breast cancer, brain tumors, acute leukemia, adrenocortical carcinomas and soft tissue and bone sarcomas (4, 5).

However, not only *TP53* gene mutations or deletions are responsible for the loss of p53 function. In those tumors with wild-type *TP53* the deregulation of the main controllers of p53 activity may also induce p53 silence, avoiding the induction of the apoptotic process. In this context, the main player is the Mdm2 protein (oncoprotein Mdm2 in mouse cells), the most important negative controller of p53. A better knowledge of the complex network made up by the p53–Mdm2 union and their many interaction partners has raised the therapeutic interest of apoptosis induction in cancer cells, leading to the development of protein–protein inhibitors directed against the p53–Mdm2 interaction.

REGULATION OF P53 THROUGH MDM2

The degradation of p53 is via the ubiquitin–proteasome system, like a wide range of other cellular proteins. The proteins that are des-

Correspondence: Josep Tabernero, MD, Head, Medical Oncology Department, Vall d'Hebron University Hospital, Vall d'Hebron Institute of Oncology, P. Vall d'Hebron 119-129, 08035 Barcelona, Spain. E-mail: jtabernero@vhio.net.

tined to be degraded by this system are initially labeled by the covalent binding of polyubiquitin side chains that allows the transport to proteasomes, where they are digested into oligopeptides. In normal cells, the degradation of p53 is regulated by Mdm2 (oncoprotein Hdm2 in human cells) (Fig. 1). This protein recognizes p53 as a target and directly binds to it and forms a complex with p53, inhibiting its transactivation (6).

The p53 protein has the structure of a transcription factor with several domains (Fig. 2). After Mdm2 binding to p53, the ability of p53 to function in this role is immediately blocked. Thereafter, Mdm2 directs the attachment of a ubiquitin moiety to p53 and exports p53 from the nucleus (where p53 does its main work) to the cytoplasm for a subsequent polyubiquitination of p53 that allows rapid cytoplasmatic proteasome degradation (Fig. 3). The highly efficient and non-stop activity of Mdm2 in normal cells ensures the short half-life of p53 (20 minutes). One of the main targets of p53 as a transcription factor is the *MDM2* gene. Consequently, when p53 is active, it stimulates the synthesis of Mdm2, the main enzyme responsible for its destruction, creating a negative feedback loop that usually ensures that p53 molecules are degraded rapidly after their synthesis, keeping the levels of p53 very low and preventing an excessive shutdown of cell proliferation and apoptosis (7, 8).

Under stress circumstances, cells need to accumulate p53 to functionally significant levels, protecting it from Mdm2-mediated destruction. This protection is often achieved by p53 phosphorylation, which blocks the binding of Mdm2 and the subsequent ubiquitination. The cycle of p53 synthesis and degradation can be modulated by many other mechanisms in addition to Mdm2 blockade and p53 phosphorylation, such as the DNA damage-sensitive kinases serine-protein kinase ATM (ataxia telangiectasia mutated) and serine/threonine-protein kinase ATR (ataxia telangiectasia and Rad3-related protein), or serine/threonine-protein kinase Chk1 (checkpoint kinase-1) and Chk2 (checkpoint kinase-2). Certain survival signals collaborate in *MDM2* gene expression via the Ets and AP-1 (Fos + Jun) transcription factors, resulting in a rapid increase of Mdm2 protein levels. These survival signals, which also activate the phosphatidylinositol 3-kinase (PI3K)–serine/threonine-protein kinase Akt–mTOR pathway have the capability, at least indirectly, to phosphorylate through activated Akt the already synthesized Mdm2, leading to binding with p53 and triggering its ubiquitination and proteasome degradation (9, 10). All these effects converge on p53 protein level suppression and thereby prevent the entrance of a cell into cell cycle arrest (senescence) or into the apoptotic suicide program. Although the mechanism of action for the final downregulation of p53 is clearly different between the Mdm2-mediated process and the effect of classical pro-oncogenic genes, the final outcome is the same, favoring the increase in cell number.

After the main role that Mdm2 plays in the regulation process of p53, influencing the growth and survival of normal cells and the growth, survival, angiogenesis, metastasis and DNA repair of cancer

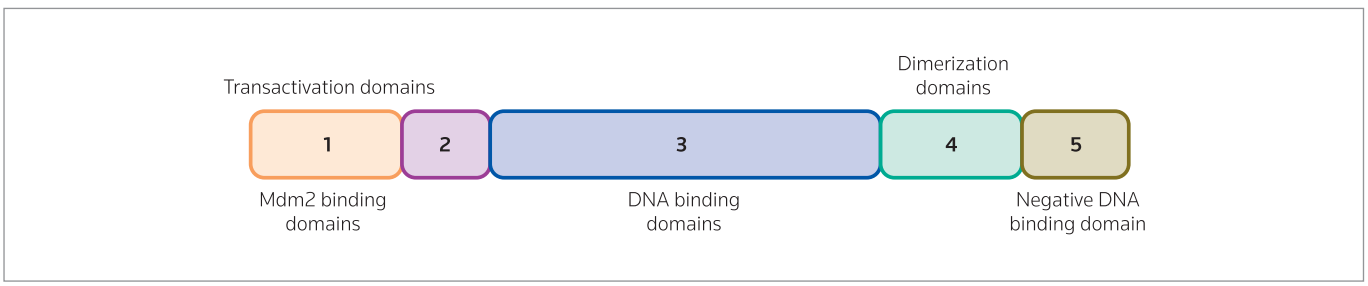


Figure 1. The p53 protein structure. P53 is divided into five domains: 1) the amino-terminal part that contains the first transactivation domain and the Mdm2 binding site; 2) and the second transactivation domain; 3) the DNA binding central region that focuses 90% of mutations in human cancer; 4) the oligomerization domain necessary for dimerization and exportation from nucleus; and 5) the carboxy-terminal part that contains the nuclear localization signals and a non-specific DNA binding domain related to negative feedback of the central DNA binding site.

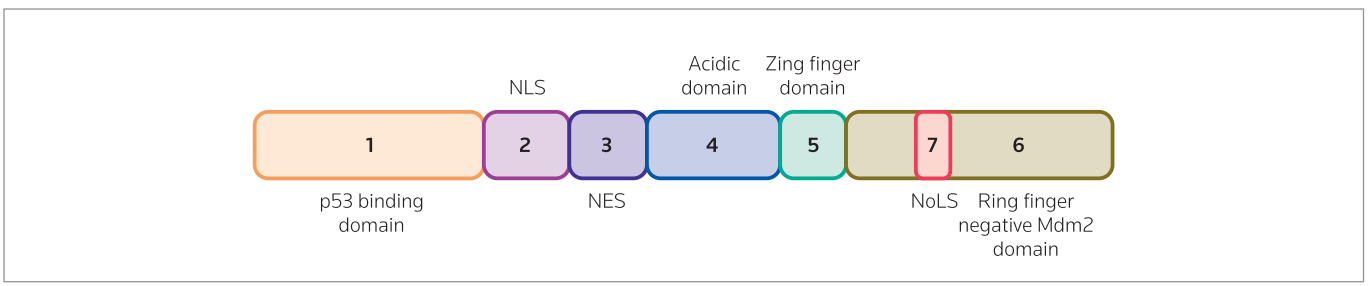


Figure 2. The Mdm2 protein structure. Mdm2 is divided into seven main domains: 1) the amino-terminal part that contains the p53 binding site; 2) a nuclear localization signal domain (NLS); 3) a nuclear exclusion signal domain (NES); 4) an acidic region; 5) the zing finger domain; 6) the carboxy-terminal ring finger domain that promotes p53 ubiquitinylation and targets Mdm2 for negative feedback; and 7) a nucleolar localization signal domain (NoLS).

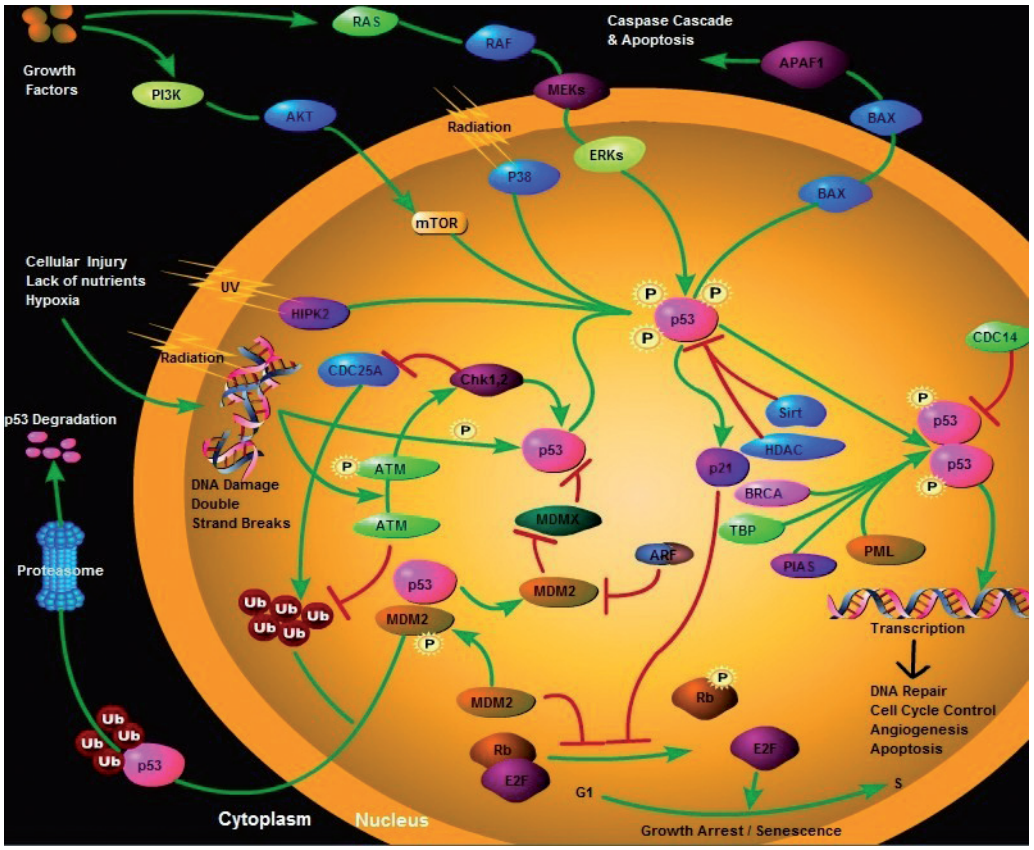


Figure 3. Schematic view of the Mdm2–p53 pathway. Several kinases can control p53 synthesis and degradation, such as DNA damage-sensitive ATM, or the final downstream of mitogenic and cell survival signals. Mdm2 directly binds the transactivation domain of p53 and inhibits its transcriptional activity. Mdm2 also causes the ubiquitination of p53 and the exportation to the cytoplasm, where p53 is degraded by the proteasome. Protein Mdm4 also recognizes the transactivation domain of p53, binding it and inhibiting the activity of p53, but does not cause p53 destruction. ARF is one of the most important inhibitors of Mdm2, binding it and facilitating the stabilization of p53. Through the activation of p21, p53 can control the cycle progression from G_1 to S, producing growth arrest and senescence. PI3K, phosphatidylinositol 3-kinase; AKT, protein kinase B; mTOR, mammalian target of rapamycin; RAS/RAF, protein kinase Ras/Raf subfamilies; MEK/ERK, mitogen-activated protein kinases; BAX, apoptosis regulator BAX; APAF1, apoptotic protease-activating factor 1; HIPK2, homeodomain-interacting protein kinase 2; CDC25A, cell division cycle 25 homolog A; Chk1/2, checkpoint protein kinase-1/2; ATM, ataxia telangiectasia mutated; MDM2, double minute 2 protein; MDMX, double minute 4 protein; Rb, retinoblastoma protein; E2F, transcription factor E2F family; Ub, ubiquitin; ARF, human p14arf protein; Sirt, NAD-dependent deacetylase sirtuin protein; CDC14, dual specificity protein phosphatase CDC14; HDAC, histone deacetylase; BRCA, breast cancer susceptibility protein; TBP, Tat-binding protein; PIAS, protein inhibitor of activated STAT protein; PML, protein PML.

cells, was determined, the next steps were to elucidate different mechanisms for the regulation of Mdm2. One of these mechanisms is Mdm2 protein phosphorylation, which can be done at multiple sites, leading to changes in protein function and stabilization of p53 after DNA damage, or accumulation of phosphorylation in certain residues within the central acidic domain, stimulating its affinity to target p53 for degradation. One of the main inhibitors of Mdm2 is the human p14arf (ARF) protein, the alternative reading frame product of the p16INK4a locus (p19arf in mice), which negatively regulates the p53–Mdm2 interaction, binding directly to Mdm2 and dragging it to the nucleus, where it would no longer be available to target p53, leading to upregulation of p53 transcriptional response. ARF keeps sequestered Mdm2 in the nucleus, resulting in inhibition of nuclear export, which is essential for adequate p53 destruction (11).

Finally, Mdm2 has been shown to interact with many other proteins involved in the main processes of cell homeostasis, proliferation, angiogenesis and survival, such as histone deacetylase 1 (HD1), guanine nucleotide-binding protein-like 3 (*GNL3*), insulin-like growth factor 1 receptor (*IGF1R*), forkhead box protein O4 (*FOXO4*), tyrosine-protein kinase ABL1 (Abelson murine leukemia viral oncogene homolog 1) or hypoxia-inducible factor 1-alpha (HIF1-alpha), among many others (12).

TRANSLATIONAL RESEARCH IN MDM2–P53 INTERACTION INHIBITORS

Restitution of full p53 activity appears to be an interesting approach for cancer therapy. The deeper knowledge of the mechanism of action of p53 inhibitors, such as Mdm2, has raised the hypothesis of

targeting the main controller of p53 to recover the functionality of this tumor suppressor protein. Four main approaches have been hypothesized to activate the p53 pathway in tumor cells. The first option would be to decrease the cellular levels of Mdm2 using antisense oligonucleotides (13). The inhibition of the ubiquitination of p53 mediated by Mmd2 is another therapeutic option that has been explored (14). The third approach investigated is the use of analogues of natural inhibitors of Mdm2, such as p14arf (15). And finally, the fourth strategy is to disrupt the binding process between Mdm2 and p53. The Mdm2–p53 interaction has been well described and is mapped into the 106-amino-acid *N*-terminus domain of Mdm2 and the *N*-terminus of the transactivation domain of p53 (16). Targeting protein–protein interactions had been a challenging approach for many years, mainly due to the large binding interface of the protein partners. The small, well-defined and specific locus of interaction between Mdm2 and p53 allows, a priori, the development of small-molecule inhibitors to target the Mdm2–p53 interaction.

The development of small molecules capable of inhibiting Mdm2 and restoring p53 pathway functionality should have some specific characteristics, such as high binding affinity and specificity for Mdm2, potent activity in tumor cells in increasing the concentration levels of active p53, and favorable pharmacokinetic and pharmacodynamic profiles. As mentioned previously, one of the main issues of the development of protein–protein interaction inhibitors is the high possibility of disrupting many other protein–protein unions, offering an unacceptable toxicity profile. Most of the developed Mdm2 inhibitors mimic the helical p53 peptide used for binding with Mdm2, and hypothetically, these inhibitors could also bind other helix-binding proteins, such as Bcl-2 family proteins. In addition, Mdm2 inhibitors should have excellent cell permeability to be employed as anticancer agents and may induce accumulation of p53 in cancer and normal cells, without inducing DNA damage, in contrast to classical chemotherapeutic drugs or radiotherapy, which may also induce an increase in p53 levels by post-translational changes, such as p53 phosphorylation (17).

The investigational program for Mdm2 inhibitors has been focused on cancers with wild-type *TP53* status, although some data for the activity of certain Mdm2 inhibitors in a mutant *TP53* population have also been reported (18, 19). In preclinical studies, Mdm2 inhibition has shown reactivation of the p53 pathway, leading to antitumor effects in colon, lymphoma, hepatocarcinoma and soft tissue sarcoma cell lines (20). Xenograft models with a retriggerable p53 knockout animal model that develops sarcomas and lymphomas have also been described. The reactivation of p53 functionality in these models has shown tumor regression in mice (21). Three main approaches have been carried out for the development of small molecules capable of blocking the Mdm2–p53 interaction, depending on the main protein target: Mdm2 protein, p53 and E3 ubiquitin-protein ligase Mdm2 (22, 23). The main Mdm2 inhibitors in clinical/preclinical development are summarized in Table I.

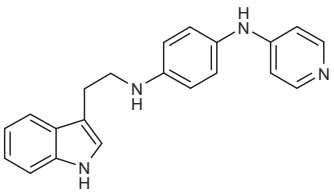
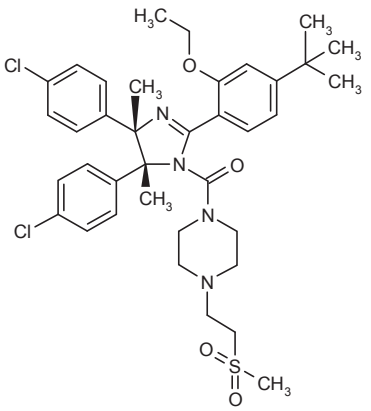
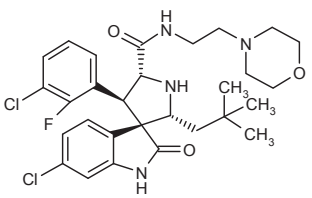
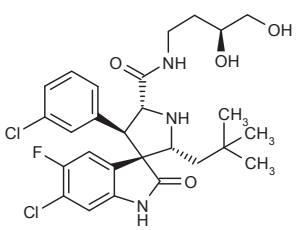
The analogues of *cis*-imidazoline, known as nutlins-1, -2 and -3, are a class of inhibitors of the Mdm2–p53 interaction via targeting Mdm2, which are far more advanced in the preclinical and clinical setting and were the first potent, nonpeptide, small-molecule Mdm2 inhibitors described. Nutlins fit in the binding pocket of p53 in Mdm2 and block the union between these proteins (22). Nutlin-3 has the most potent (K_d = 11 nmol/L) in vivo antitumor activity in xenograft models of wild-type p53 human cancer (17, 23). The activity of nutlin-3 has been described in a broad spectrum of cancers, such as neuroblastoma, osteosarcoma, lymphoma, and breast and colon carcinomas (24-26). The biological activity of nutlin-3 has been shown to be significantly different in cancer cells compared with normal cells. In both cases, nutlin-3 therapy induces cell cycle arrest, but in cancer cells it also induces cell death, indicating that the activation of p53 by Mdm2 inhibition could not be toxic for normal cells and highly specific for cytotoxicity in cancer cells (17, 23). This observation was very encouraging from a therapeutic perspective, increasing investigational efforts in this setting.

Using structure-based engineering, other more potent Mdm2 inhibitors have been developed, such as MI-219, MI-43, MI-319 and MI-63, a new class of highly specific Mdm2 inhibitors known as spiro-oxindoles. These compounds have demonstrated significant preclinical activity in various wild-type p53 tumor cell lines and xenograft models, such as rhabdomyosarcoma, follicular lymphoma or colorectal carcinoma (27-29). Additionally, five other different biochemical compounds that target Mdm2 (benzodiazepinediones and isoquinolones), p53 (thiophenes) and E3 ubiquitin-protein ligase Mdm2 inhibitors (5-deazaflavin and tryptamines), are currently in preclinical development and have demonstrated antitumor activity in several cancer cell lines (see Table I) (30-35). JNJ-26854165 (serdemetan) is a novel oral tryptamine derivative that targets the ring finger domain of Mdm2 and inhibits the interaction between p53 and Mdm2, which has demonstrated significant preclinical activity in a wide spectrum of malignancies. Interestingly, in preclinical models JNJ-26854165 demonstrated inhibition of the p53–Mdm2 interaction in both wild-type and mutant *TP53* tumors, including breast cancer, multiple myeloma and leukemia (36). Although the exact mechanism of action of JNJ-26854165 is currently under investigation, microarray analysis of wild-type *TP53* cell lines treated with this drug demonstrated a gene expression profile similar to that observed with classical DNA-damaging agents that interfere with DNA synthesis and produce S-phase arrest (19).

CLINICAL DEVELOPMENT OF MDM2–P53 PROTEIN–PROTEIN INHIBITORS

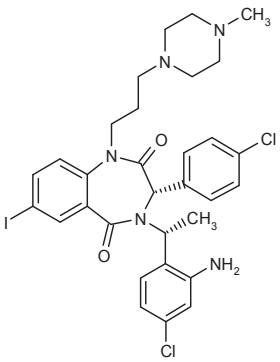
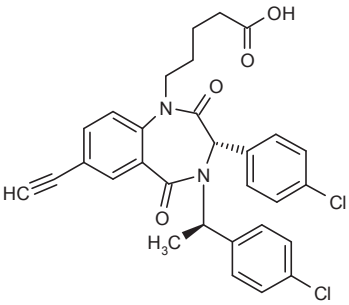
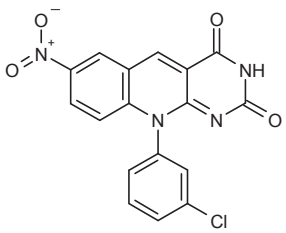
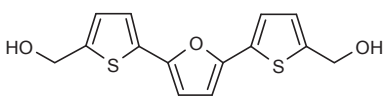
During the last years, several preclinical studies and clinical trials with Mdm2–p53 interaction inhibitors have been launched or have reported initial results. The results of the first-in-human phase I pharmacokinetic (PK) and pharmacodynamic (PD) study of serdemetan in patients with advanced solid tumors have recently been published (NCT00676910) (37). Seventy-one patients were included in the study and received 13 dose levels ranging from 4 to 400 mg once or twice daily. The main side effects observed were grade 1-2, including nausea, vomiting, anorexia, fatigue and mild liver impairment. The most frequent dose-limiting toxicity observed was grade 3 QTc prolongation, observed in four patients. Grade 2 QTc prolongation was observed in 10 additional patients. This was the major concern of serdemetan therapy and was directly correlated with plasma concentrations. Little bone marrow toxicity was observed, raising the possibility of combination with cytotoxic agents. The maximum tolerated dose was 350 mg once daily and 150 mg for the twice-daily schedule. One patient with a case of advanced breast cancer obtained a confirmed partial response and

Table I. *Main characteristics of Mdm2–p53 interaction inhibitors in preclinical/clinical development.*

Biochemical family	Name	Target	Mdm2–p53 binding area	IC_{50} (nmol/L) K_d (nmol/L)	Development stage
Tryptamine	Serdemetan (JNJ-26854165) 	Mdm2	Ring finger domain	300 NA	Phase I
cis-Imidazolines	RO-5045337 (RG-7112) 	Mdm2	*N*-terminal/ p53 binding domain	450 11	Phase I/Ib
Spiro-oxindoles	MI-63  MI-219 	Mdm2	p53 binding domain	140-160 3-5	Preclinical

Continued

Table I. *(Cont.) Main characteristics of Mdm2–p53 interaction inhibitors in preclinical/clinical development.*

Biochemical family	Name	Target	Mdm2–p53 binding area	IC_{50} (nmol/L) K_d (nmol/L)	Development stage
Benzodiazepinediones	JNJ-27291199 (TDP-665759) 	Mdm2	p53 binding domain	704 NA	Preclinical
	JNJ-27065909 (TDP-521252) 				
5-Deazaflavin	HLI-98 	Mdm2	Ring finger domain	8000-75,000 NA	Preclinical
Isoquinolinones	PXN-727 PXN-822	Mdm2	p53 binding domain	700-7400 NA	Preclinical
Thiophene	NSC-652287 (RITA) 	p53	Mdm2 binding domain	140 NA	Preclinical

NA, not available. *Differences could be observed in IC_{50} between different cell lines.

seven additional patients showed a decrease in tumor size that did not reach the criteria of partial response by RECIST criteria. Twenty-two (38%) patients had stable disease, with 4 prolonged stabilizations in patients with angiosarcoma, breast cancer, a thyroid Hürthle cell carcinoma and ependymoma. Interestingly, in this phase I study, an extensive PK/PD program was developed in order to find the minimum biologically active dose. Serdemetan showed a linear, dose-proportional PK profile and was rapidly absorbed after oral

administration. PD analyses were performed in paired skin and tumor biopsies for predictive surrogate and tumor biomarkers. The nuclear p53 levels determined by immunohistochemistry in skin samples were increased after treatment with serdemetan in an exposure-related fashion. Accordingly, reduction in antigen KI-67 levels was observed at 300 mg daily. Unfortunately, this meaningful correlation was not observed in tumor samples, where the levels of p53 were increased in only 8 samples (of a total of 13), with no decrease in KI-67. Plasma levels of macrophage inhibitory cytokine 1 (MIC-1), a transforming growth factor-beta family cytokine induced by p53 activation, were used as a biomarker for p53 activation. Although MIC-1 serum levels were increased after serdemetan therapy, the magnitude of this effect was not dose-related.

RO-5045337 (RG-7112) is an oral Mdm2 inhibitor, interesting data for which were reported during the 2011 American Society of Clinical Oncology (ASCO) meeting. Beryozkina et al. presented the results of two phase I studies of RG-7112 in patients with leukemias and solid tumors (NCT00623870 and NCT00559533) (38). Forty-nine patients with leukemia (acute myeloid leukemia [AML], acute lymphocytic leukemia [ALL], chronic myelogenous leukemia [CML] in blast phase or refractory chronic lymphocytic leukemia/small cell lymphocytic leukemia [CLL/SCLL]) and 76 patients with advanced and refractory solid tumors were included. Dose escalation ranged between 20 and 1920 mg/m^2/day for 10 days followed by 18 days of rest between cycles. The PK exposure observed was dose-proportional and tended to be higher for patients with solid tumors. Serum MIC-1 levels increased proportionally with dose levels starting from > 320 mg/m^2/day, suggesting a good relationship between p53 activation and drug exposure.

MDM2 gene amplification has been well described in liposarcomas and a phase I PD biomarker study has been carried out in this setting with RG-7112 (39). The primary objective of the study was to evaluate the proof of mechanism of RG-7112 to act as an Mdm2 inhibitor, leading to upregulation of p53-dependent pathways in tumor tissue. Twenty patients with chemoradiotherapy-naive well-differentiated or dedifferentiated liposarcomas eligible for debulking surgery were treated with up to 2 cycles of RG-7112 at 1440 mg/m^2 once daily during 10 days (28 days per cycle). Two tumor samples were obtained at baseline and after 8 days of treatment. Blood samples were also obtained for MIC-1 determination. The most frequent severe side effects (grade 3-4) were vomiting (n = 2), neutropenia (n = 3; one case of febrile neutropenia) and thrombocytopenia (n = 5). One partial response was observed, with 14 disease stabilizations and 5 cases of progressive disease. The authors reported the expected PD effects of Mdm2 inhibition by RG-7112, with increases in p53, p21 and Mdm2 RNA levels, decreases in proliferation ratio measured by KI-67 index, exposure-related increases in MIC-1 plasma levels and evidence of tumor apoptotic changes by TUNEL immunostaining. RG-7112 is currently being investigated in phase I clinical trials (ClinicalTrials.gov: NCT01462175 and NCT01143740).

FUTURE DIRECTIONS

Nowadays, many efforts are focused on increasing the knowledge of the multiple complex pathways modulated by p53 and Mdm2. The future development of Mdm2–p53 protein–protein inhibitors should run in parallel with a robust biomarker program that could allow a better selection of patients for optimal clinical use. One of the most promising biomarkers explored in the preclinical and clinical settings has been MIC-1. MIC-1 is secreted when the p53 pathway is activated and can be detected in serum, avoiding tumor biopsies required for analysis of most *TP53* target genes that are located in the cells. Analyses of CLL patients' blood samples and endothelial cells have shown upregulation of MIC-1 after p53 pathway activation by Mdm2 inhibition with nutlin-3 (40, 41). Although MIC-1 appears to be a promising biomarker candidate of p53 pathway activation, the results of the first two phase I clinical trials with two different Mdm2 inhibitors, serdemetan and RG-7112, showed contradictory data.

The activity of Mdm2 inhibitors is linked to the non-mutant status of p53. The continuous exposure to Mdm2 inhibitors may select clones with deficient p53 function and generate early resistance to these agents. In addition to *TP53* gene mutations or deletions, loss of Mdm2 controllers, like p14arf, has also been related with primary resistance to Mdm2 inhibitors (42). Mdm4 is a protein with high homology and a similar p53 binding site to Mdm2 (43). Mdm4 binds to the *N*-terminus of p53 and blocks p53 transcriptional activity, but does not produce p53 degradation (44). Mdm4 also binds with Mdm2 at the ring finger domain, forming the complex p53–Mdm2–Mdm4, which regulates p53 function. Mdm2 inhibitors, such as MI-219 or RG-7112, bind to the Mdm2 pocket with significantly higher affinity compared with the Mdm4 binding site, inhibiting the p53–Mdm2 interaction but not the p53–Mdm4 union. The overexpression of Mdm4 has been related with intrinsic resistance to nutlin-3 therapy. The incapacity of Mdm2 inhibitors to block the p53–Mdm4 interaction or produce Mdm4 degradation prevents full activation of p53 transcriptional activity, generating resistance against these drugs (45).

And last but not least, Mdm2 inhibitors are attractive drugs for combination with classical genotoxic anticancer drugs that induce p53 activation. The two main objectives of these drug combinations lie in minimizing the toxic side effects of p53 induction in normal cells and enhancing antitumor activity. Synergistic effects have been described with the combination of nutlin-3 and doxorubicin, chlorambucil, fludarabine and gemcitabine (41, 46). Furthermore, the double inhibition of the two extrinsic and intrinsic apoptotic pathways by blocking TNF-related apoptosis-inducing ligand (TRAIL) and Mdm2 is another interesting strategy for increasing apoptosis induction (47).

CONCLUSIONS

The future of cancer therapy is establishing a personalized medicine approach through the advance in the knowledge of molecular cancer biology due to the increasing specificity of new targeted therapy agents. The *TP53* gene is the most altered gene in oncology and the complex relationship between p53 protein and its partners has been the main drawback in the development of drugs directed towards this pathway. The recovery of the function of the popularly called "guardian of the genome" has been a suitable target in oncology for many years. The awareness of the relationship between Mdm2 and p53 has opened the opportunity to target one of the main inhibitors of p53 function, reactivating the apoptotic pathway in cancer cells. However, as discussed in this review, the interactions of both Mdm2 and p53 include a large number of proteins related with the main

processes of cell differentiation, growth and survival, and it appears rather difficult that the simple inhibition of Mdm2 could produce a significant effect. The first steps of Mdm2 inhibitors and p53 transcriptional function enhancers should run in parallel with predictive biomarkers and probably with multidrug combinations, both with DNA-damaging agents and other targeted therapies.

DISCLOSURES

The authors state no conflicts of interest.

REFERENCES

1. Vogelstein, B., Lane, D., Levine, A.J. *Surfing the p53 network.* Nature 2000, 408(6810): 307-10.
2. Susin, S.A., Daugas, E., Ravagnan, L. et al. *Two distinct pathways leading to nuclear apoptosis.* J Exp Med 2000, 192(4): 571-80.
3. Hainaut, P., Hollstein, M. *p53 and human cancer: The first ten thousand mutations.* Adv Cancer Res 2000, 77: 81-137.
4. Li, F.P., Fraumeni, J.F. Jr. *Soft-tissue sarcomas, breast cancer, and other neoplasms. A familial syndrome?* Ann Intern Med 1969, 71(4): 747-52.
5. Malkin, D., Li, F.P., Strong, L.C. et al. *Germ line p53 mutations in a familial syndrome of breast cancer, sarcomas, and other neoplasms.* Science 1990, 250(4985): 1233-8.
6. Momand, J., Zambetti, G.P., Olson, D.C., George, D., Levine, A.J. *The mdm-2 oncogene product forms a complex with the p53 protein and inhibits p53-mediated transactivation.* Cell 1992, 69(7): 1237-45.
7. Freedman, D.A., Wu, L., Levine, A.J. *Functions of the MDM2 oncoprotein.* Cell Mol Life Sci 1999, 55(1): 96-107.
8. Wu, X., Bayle, J.H., Olson, D., Levine, A.J. *The p53-mdm-2 autoregulatory feedback loop.* Genes Dev 1993, 7(7A): 1126-32.
9. Kussie, P.H., Gorina, S., Marechal, V. et al. *Structure of the MDM2 oncoprotein bound to the p53 tumor suppressor transactivation domain.* Science 1996, 274(5289): 948-53.
10. Haupt, Y., Maya, R., Kazaz, A., Oren, M. *Mdm2 promotes the rapid degradation of p53.* Nature 1997, 387(6630): 296-9.
11. Zhang, Y., Xiong, Y., Yarbrough, W.G. *ARF promotes MDM2 degradation and stabilizes p53: ARF-INK4a locus deletion impairs both the Rb and p53 tumor suppression pathways.* Cell 1998, 92(6): 725-34.
12. Bond, G.L., Levine, A.J. *A single nucleotide polymorphism in the p53 pathway interacts with gender, environmental stresses and tumor genetics to influence cancer in humans.* Oncogene 2007, 26(9): 1317-23.
13. Wang, H., Zeng, X., Oliver, P. et al. *MDM2 oncogene as a target for cancer therapy: An antisense approach.* Int J Oncol 1999, 15(4): 653-60.
14. Lai, Z., Yang, T., Kim, Y.B. et al. *Differentiation of Hdm2-mediated p53 ubiquitination and Hdm2 autoubiquitination activity by small molecular weight inhibitors.* Proc Natl Acad Sci U S A 2002, 99(23): 14734-9.
15. Midgley, C.A., Desterro, J.M., Saville, M.K. et al. *An N-terminal p14ARF peptide blocks Mdm2-dependent ubiquitination in vitro and can activate p53 in vivo.* Oncogene 2000, 19(19): 2312-23.
16. Picksley, S.M., Vojtesek, B., Sparks, A., Lane, D.P. *Immunochemical analysis of the interaction of p53 with MDM2; fine mapping of the MDM2 binding site on p53 using synthetic peptides.* Oncogene 1994, 9(9): 2523-9.
17. Shangary, S., Qin, D., McEachern, D. et al. *Temporal activation of p53 by a specific MDM2 inhibitor is selectively toxic to tumors and leads to complete tumor growth inhibition.* Proc Natl Acad Sci U S A 2008, 105(10): 3933-8.
18. Smith, M.A., Gorlick, R., Kolb, E.A. et al. *Initial testing of JNJ-26854165 (serdemetan) by the pediatric preclinical testing program.* Pediatr Blood Cancer 2011, Epub ahead of print.
19. Kojima, K., Burks, J.K., Arts, J., Andreeff, M. *The novel tryptamine derivative JNJ-26854165 induces wild-type p53- and E2F1-mediated apoptosis in acute myeloid and lymphoid leukemias.* Mol Cancer Ther 2010, 9(9): 2545-57.
20. Chene, P., Fuchs, J., Bohn, J., Garcia-Echeverria, C., Furet, P., Fabbro, D. *A small synthetic peptide, which inhibits the p53-hdm2 interaction, stimulates the p53 pathway in tumour cell lines.* J Mol Biol 2000, 299(1): 245-53.
21. Ventura, A., Kirsch, D.G., McLaughlin, M.E. et al. *Restoration of p53 function leads to tumour regression in vivo.* Nature 2007, 445(7128): 661-5.
22. Vassilev, L.T. *Small-molecule antagonists of p53-MDM2 binding: Research tools and potential therapeutics.* Cell Cycle 2004, 3(4): 419-21.
23. Vassilev, L.T., Vu, B.T., Graves, B. et al. *In vivo activation of the p53 pathway by small-molecule antagonists of MDM2.* Science 2004, 303(5659): 844-8.
24. Van Maerken, T., Ferdinande, L., Taildeman, J. et al. *Antitumor activity of the selective MDM2 antagonist nutlin-3 against chemoresistant neuroblastoma with wild-type p53.* J Natl Cancer Inst 2009, 101(22): 1562-74.
25. Tabe, Y., Sebasigari, D., Jin, L. et al. *MDM2 antagonist nutlin-3 displays antiproliferative and proapoptotic activity in mantle cell lymphoma*. Clin Cancer Res 2009, 15(3): 933-42.
26. Tovar, C., Rosinski, J., Filipovic, Z. et al. *Small-molecule MDM2 antagonists reveal aberrant p53 signaling in cancer: Implications for therapy.* Proc Natl Acad Sci U S A 2006, 103(6): 1888-93.
27. Canner, J.A., Sobo, M., Ball, S. et al. *MI-63: A novel small-molecule inhibitor targets MDM2 and induces apoptosis in embryonal and alveolar rhabdomyosarcoma cells with wild-type p53.* Br J Cancer 2009, 101(5): 774-81.
28. Shangary, S., Ding, K., Qiu, S. et al. *Reactivation of p53 by a specific MDM2 antagonist (MI-43) leads to p21-mediated cell cycle arrest and selective cell death in colon cancer.* Mol Cancer Ther 2008, 7(6): 1533-42.
29. Mohammad, R.M., Wu, J., Azmi, A.S. et al. *An MDM2 antagonist (MI-319) restores p53 functions and increases the life span of orally treated follicular lymphoma bearing animals.* Mol Cancer 2009, 8: 115.
30. Grasberger, B.L., Lu, T., Schubert, C. et al. *Discovery and cocrystal structure of benzodiazepinedione HDM2 antagonists that activate p53 in cells.* J Med Chem 2005, 48(4): 909-12.
31. Rothweiler, U., Czarna, A., Krajewski, M. et al. *Isoquinolin-1-one inhibitors of the MDM2-p53 interaction*. ChemMedChem 2008, 3(7): 1118-28.
32. Nieves-Neira, W., Rivera, M.I., Kohlhagen, G. et al. *DNA protein cross-links produced by NSC 652287, a novel thiophene derivative active against human renal cancer cells*. Mol Pharmacol 1999, 56(3): 478-84.
33. Wilson, J.M., Henderson, G., Black, F. et al. *Synthesis of 5-deazaflavin derivatives and their activation of p53 in cells.* Bioorg Med Chem 2007, 15(1): 77-86.
34. Issaeva, N., Bozko, P., Enge, M. et al. *Small molecule RITA binds to p53, blocks p53-HDM-2 interaction and activates p53 function in tumors.* Nat Med 2004, 10(12): 1321-8.
35. Yang, Y., Ludwig, R.L., Jensen, J.P. et al. *Small molecule inhibitors of HDM2 ubiquitin ligase activity stabilize and activate p53 in cells.* Cancer Cell 2005, 7(6): 547-59.
36. Arts, J., Page, M., Valckx, A. et al. *JNJ-26854165 - A novel hdm2 antagonist in clinical development showing broad-spectrum preclinical antitumor activity against solid malignancies*. Proc Am Assoc Cancer Res (AACR) 2008, 49: Abst 1592.

37. Tabernero, J., Dirix, L., Schoffski, P. et al. *A phase I first-in-human pharmacokinetic and pharmacodynamic study of serdemetan in patients with advanced solid tumors.* Clin Cancer Res 2011, 17(19): 6313-21.

38. Beryozkina, A., Nichols, G.L., Reckner, M. et al. *Pharmacokinetics (PK) and pharmacodynamics (PD) of RG7112, an oral murine double minute 2 (MDM2) antagonist, in patients with leukemias and solid tumors.* 47th Annu Meet Am Soc Clin Oncol (ASCO) (June 3-7, Chicago) 2011, Abst 3039.

39. Ray-Coquard, I.L., Blay, J., Italiano, A. et al. *Neoadjuvant MDM2 antagonist RG7112 for well-differentiated and dedifferentiated liposarcomas (WD/DD LPS): A pharmacodynamic (PD) biomarker study.* 47th Annu Meet Am Soc Clin Oncol (ASCO) (June 3-7, Chicago) 2011, Abst 10007b.

40. Secchiero, P., Corallini, F., Gonelli, A. et al. *Antiangiogenic activity of the MDM2 antagonist nutlin-3.* Circ Res 2007, 100(1): 61-9.

41. Secchiero, P., Barbarotto, E., Tiribelli, M. et al. *Functional integrity of the p53-mediated apoptotic pathway induced by the nongenotoxic agent nutlin-3 in B-cell chronic lymphocytic leukemia (B-CLL).* Blood 2006, 107(10): 4122-9.

42. Kastan, M.B. *Wild-type p53: Tumors can't stand it.* Cell 2007, 128(5): 837-40.

43. Bottger, V., Bottger, A., Garcia-Echeverria, C. et al. *Comparative study of the p53-mdm2 and p53-MDMX interfaces*. Oncogene 1999, 18(1): 189-99.

44. Jackson, M.W., Berberich, S.J. *MdmX protects p53 from Mdm2-mediated degradation.* Mol Cell Biol 2000, 20(3): 1001-7.

45. Patton, J.T., Mayo, L.D., Singhi, A.D., Gudkov, A.V., Stark, G.R., Jackson, M.W. *Levels of HdmX expression dictate the sensitivity of normal and transformed cells to Nutlin-3.* Cancer Res 2006, 66(6): 3169-76.

46. Jones, R.J., Baladandayuthapani, V., Neelapu, S. et al. *HDM-2 inhibition suppresses expression of ribonucleotide reductase subunit M2, and synergistically enhances gemcitabine-induced cytotoxicity in mantle cell lymphoma.* Blood 2011, 118(15): 4140-9.

47. Secchiero, P., Zerbinati, C., di Iasio, M.G. et al. *Synergistic cytotoxic activity of recombinant TRAIL plus the non-genotoxic activator of the p53 pathway nutlin-3 in acute myeloid leukemia cells.* Curr Drug Metab 2007, 8(4): 395-403.