Perspective

p53 Activation by Small Molecules: Application in Oncology

Lyubomir T. Vassilev

J. Med. Chem., **2005**, 48 (14), 4491-4499• DOI: 10.1021/jm058174k • Publication Date (Web): 18 June 2005

Journal of Medicinal Chemistry

Volume 48, Number 14 July 14, 2005

Perspective

p53 Activation by Small Molecules: Application in Oncology

Lyubomir T. Vassilev†

Discovery Oncology, Roche Research Center, Hoffmann-La Roche Inc., Nutley, New Jersey 07110

Received January 20, 2005

Introduction

Since its discovery 25 years ago, the tumor suppressor p53 has been a subject of intense study, yielding in excess of 30 000 research articles. The high interest in this molecule is largely due to the fact that p53 is the most frequently altered protein in human cancer. Approximately 50% of all human malignancies harbor mutations or deletions in the TP53 gene that disable the tumor suppressor function of the encoded protein.[1,2] This high rate of genetic alterations underscores the important cellular function of p53. The tumor suppressor controls a signal transduction pathway evolved to protect multicellular organisms from cancer development that could be initiated by diverse stresses including DNA damage. p53 is a potent transcription factor capable of activating multiple target genes, leading to cell cycle arrest, apoptosis, or senescence.[3,4]

While p53 plays a protective role in normal somatic tissues by limiting the propagation of damaged cells, its powerful growth suppressive and proapoptotic activity could be turned into a powerful weapon against cancer cells that have retained the functionality of the p53 pathway. Fortunately, half of all human tumors express wild-type protein that is capable of activating p53 target genes. However, aberrations in p53 regulation and signaling mechanisms could attenuate the tumor suppressor function of p53. One such aberration involves the product of the *m*urine *d*ouble *m*inute-2 gene (MDM2), a negative regulator of p53 activity and stability.[5−7] MDM2 is overexpressed in many human tumors and effectively impairs the function of the p53 pathway.[8] Therefore, restoration of p53 function by antagonizing MDM2 has been proposed as a novel approach for treating cancer, and studies using macromolecular tools have shown its validity in vitro.[9−11] Several classes of low molecular weight inhibitors of the p53−MDM2 interaction have been reported that can disrupt the binding between the two proteins. Recently, the first potent and selective small-molecule antagonists of MDM2 have been developed.[12] These druglike molecules, termed nutlins, have shown the ability to activate the p53 pathway in vitro and in vivo and provided a proof of concept for the therapeutic utility of MDM2 antagonists in tumors with wild-type p53. This Perspective examines the latest developments in the search for pharmacological activators of wild-type p53 with emphasis on agents that antagonize the p53−MDM2 interaction.

The p53 Tumor Suppressor Pathway

p53 regulates cellular response to stress through a complex network of proteins known as the p53 pathway.[3,4] It constantly monitors cell integrity and homeostasis and responds to diverse forms of stress by stabilization and accumulation of p53 (Figure 1). These upstream signaling events involve activation of protein kinases that modify p53 on specific residues and mediate its stabilization and activation.[13,14] Activated p53 then induces the transcription of multiple target genes containing the p53 recognition sequence in their promoter regions. Depending on the subset of activated genes, different downstream signaling events are initiated, leading to blockage of cell cycle progression, DNA repair, apoptosis, senescence, or differentiation. Although many of the components of the p53 signal transduction network have been identified, the mech-

† Phone: 973 235-8106. Fax: 973 235-6185. E-mail: lyubomir.vassilev@roche.com.

10.1021/jm058174k CCC: $30.25
Published on Web 06/18/2005

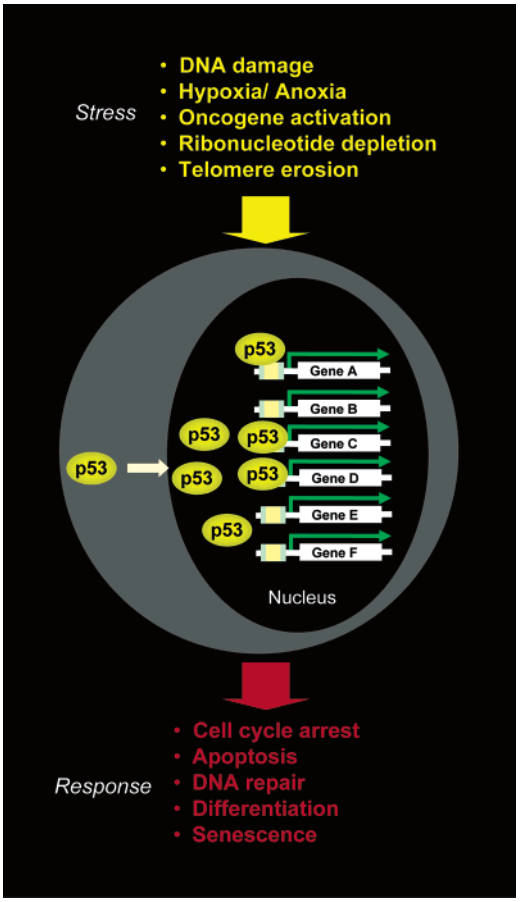

Figure 1. p53 regulates cellular response to stress. Diverse stress factors can activate p53 by a post-translational mechanism that involves stabilization and accumulation of the protein in cell nuclei. p53 then activates subsets of genes that trigger signaling events leading to cell cycle arrest, apoptosis, DNA repair, differentiation, or senescence.

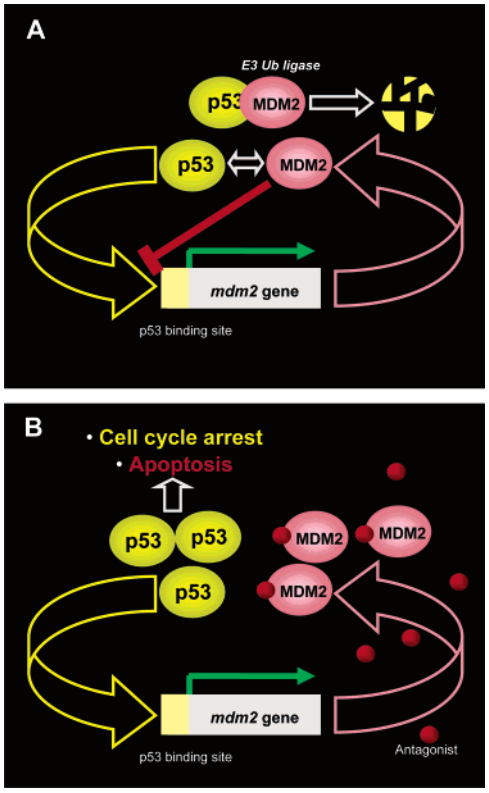

Figure 2. p53−MDM2 autoregulatory circuit. (A) MDM2 and p53 mutually regulate their levels in unstressed proliferating cells. MDM2 expression is controlled by a p53-dependent promoter and increases when the level of p53 rises. In turn, MDM2 binds p53 and inhibits its transcriptional activity by blocking the transcriptional activation domain of the transcription factor. MDM2 also serves as the E3 ubiquitin ligase for p53 and facilitates its ubiquitin-dependent degradation in the proteasome. As a result, both p53 and MDM2 are kept at very low levels in proliferating cells. (B) Small-molecule antagonists of MDM2 that can bind to the p53 pocket on the surface of the molecule will inhibit the p53−MDM2 interaction and release p53 from negative control. p53 will stabilize, accumulate in cell nuclei, and activate the p53 pathway.

anisms that control the choice of p53 response and its execution are still poorly understood.[15]

Cell cycle arrest and apoptosis are the most frequent and important cellular responses to stress. The p53 pathway utilizes G1/S and G2/M checkpoint mechanisms to arrest cell cycle progression and thus prevent propagation of DNA damage while cells attempt to repair it. However, if the damage is too severe, the p53 pathway chooses apoptotic cell death as the ultimate means of preventing possible malignant transformation of the damaged cells. Experiments in which the p53 pathway has been activated in human tissue culture cells have indicated that malignant cells undergo apoptosis much more readily than normal cells, which tend to respond to p53 activation by reversible growth arrest.[16,17] Although the molecular mechanisms underlying this phenomenon are still not well understood, these observations support the potential utility of p53 activation in cancer therapy.

MDM2, a Master Regulator of p53

p53 is a potent growth suppressive and proapoptotic molecule that could harm normal proliferating cells if left uncontrolled. Therefore, the cellular level and activity of p53 are subjected to tight control both under normal physiological conditions and during stress. p53 is a short-lived protein, and its cellular level is regulated primarily by degradation via the ubiquitin−proteasome pathway. A growing number of cellular proteins have been implicated in the regulation of p53 stability including MDM2,[18,19] Pirh2,[20] COP-1,[21] and HAUSP.[22,23] Among these, MDM2 appears to function as a master regulator of p53. Its essential role in controlling p53 stability and activity is supported by the fact that genetic disruption of the *mdm2* gene in mice is embryonic lethal but can be rescued by concomitant disruption of the p53 gene.[24,25]

MDM2 regulates p53 via an autoregulatory feedback loop in which both proteins control mutually their cellular level (Figure 2A).[26,27] MDM2 is one of the p53 target genes, and increased nuclear levels of p53 activate *mdm2* gene transcription, leading to elevated levels of MDM2 protein. In turn, MDM2 binds physically to p53 at its N-terminal domain and negatively regulates its stability and activity. The binding site for MDM2 overlaps with the transcriptional activation domain of p53, and therefore, the p53−MDM2 complex is devoid of transcriptional activity.[7] MDM2 also serves as the E3 ubiquitin ligase for p53 and targets the tumor suppressor for ubiquitin-dependent degradation in the proteasome. As a result of the MDM2-mediated inhibition and degradation, p53 is effectively disabled as a

transcription factor and its level in proliferating nonstressed cells is practically undetectable. In the absence of its transcriptional activator, MDM2 is also expressed at very low levels in proliferating cells.

Genetic and biochemical evidence has indicated that the p53−MDM2 regulatory circuit plays a central role in p53 regulation.[4,15,28] Several experimental approaches using antibodies, peptides, or antisense oligonucleotides have demonstrated that disruption of the p53−MDM2 interaction can release p53 from negative control, leading to stabilization and accumulation of transcriptionally active protein and activation of the p53 pathway.[9−11] From a clinical perspective, the most desirable way to inhibit p53−MDM2 binding is by druglike small molecules that can bind selectively to the p53 pocket on the surface of MDM2 and thus block its ability to form a complex with p53 (Figure 2B). Free of its negative regulator, p53 is expected to accumulate in cell nuclei and induce p53 responsive genes and the p53 pathway. Such small molecules should not bind physically to p53 or interfere with p53 activity. Relieved from inhibition, p53 will continue to activate MDM2 expression, leading to accumulation of MDM2 protein. Therefore, by disruption of the p53−MDM2 regulatory loop, MDM2 antagonists will elevate the cellular levels of both p53 and MDM2.

Targeting the p53−MDM2 Interaction

Protein−protein interactions have long been considered difficult targets for therapeutic intervention by small molecules. This is primarily due to the fact that their interacting surfaces are too large and flat for effective disruption by druglike chemical compounds. Nevertheless, successes in designing protein−protein inhibitors have been reported.[29,30] In these cases, the targeted interactions involved relatively well-defined pockets on the surface of one or both protein partners. Studies of the p53−MDM2 interaction have revealed structural features suggesting that it might be targetable by low molecular weight compounds.

Genetic and biochemical studies mapped p53−MDM2 binding sites to the N-terminal domain of MDM2 and the N-terminal part of the transactivation domain of p53.[31] The crystal structure of a p53-derived peptide bound to the p53 binding domain of MDM2 revealed the existence of a relatively deep cavity on the surface of the MDM2 molecule.[32] More importantly, only three amino acid residues from the p53 peptide (Phe^{19}, Trp^{23}, and Leu^{26}) appeared to play a critical role in the binding between the two proteins by projecting residues deep into the hydrophobic cavity of the p53 pocket. These structural features of the p53−MDM2 complex suggested an increased likelihood of identifying small molecules that might interfere successfully with the protein−protein binding by mimicking the key amino acid contacts between the two proteins. This assumption was supported by quantitative assessment of the p53−MDM2 binding using the CavSearch program. CavSearch is a computational tool that maps cavities in proteins using a grid-based measure of accessibility. When structural information is available, CavSearch can calculate the relative size of any hydrophobic pocket for which small-molecule ligands have been identified. When subjected to this analysis, the p53 binding pocket on MDM2 was ranked in the "medium" category among 29 known proteins with respect to their relative likelihood for targeting with small molecules.[33]

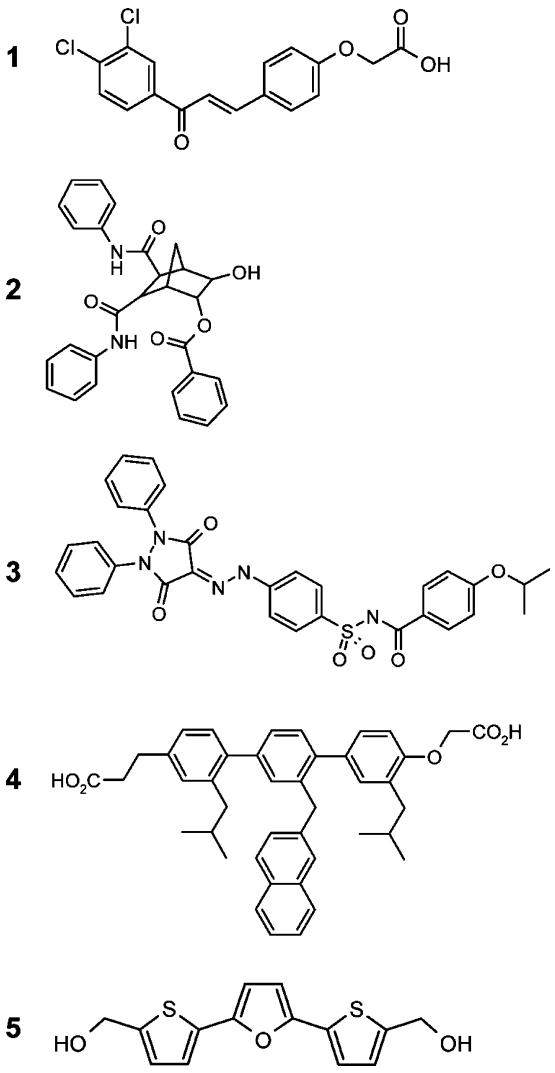

Figure 3. Small-molecule inhibitors of p53−MDM2 binding.

Small-Molecule Antagonists of p53−MDM2 Binding

The realization that p53−MDM2 binding is largely dependent on the interaction of three p53 amino acid residues with a well-defined MDM2 pocket stimulated the efforts to identify druglike inhibitors of this protein−protein interaction. Consequently, several small-molecule antagonists have been identified and described in recently published research articles. Peptide inhibitors of p53−MDM2 binding have been reviewed recently.[34,35]

The first reported low molecular weight inhibitor of the p53−MDM2 interaction **1** belongs to a class of phenoxyacetic acid and phenoxymethyltetrazole derivatives known as chalcones (Figure 3). They inhibited p53−MDM2 binding in vitro with a relatively low potency but were also shown to inhibit glutathione-*S*-transferase activity.[36] Modified boronic chalcones were reported recently that inhibited the growth of cultured tumor cells.[37] However, the report has not provided convincing evidence that this activity is derived from activation of the p53 pathway. Two compounds with the ability to disrupt p53−MDM2 binding have been identi-

fied using computer-aided design based on crystal structure data. The first one, **2**, was able to penetrate cancer cells in culture and induced p53 accumulation, but its cytotoxicity did not correlate well with the wild-type status of p53.[38] The second compound, a sulfonamide derivative **3**, showed a dose-dependent but relatively weak inhibition ($IC_{50} \approx 30$ μM) of p53−MDM2 binding that translated into a modest increase in the transcriptional activity of p53.[39] Recently, Yin and Hamilton reported the synthesis of a relatively potent MDM2 antagonist **4** based on a terphenyl scaffold.[40] This compound inhibited the p53−MDM2 interaction with submicromolar potency in vitro and induced p53 accumulation in tumor cells. However, its potential as a drug is not yet established.

Recently, another type of inhibitor of the p53−MDM2 interaction has been described.[41] This compound, **5**, termed RITA, was identified by a cell-based approach that screened small-molecule compounds from the NCI library for their ability to suppress cell growth in a p53-dependent manner using the isogenic colon cancer cell lines HCT116TP53$^{+/+}$ and HCT116TP53$^{-/-}$.[42] RITA up-regulated the p53-responsive LacZ reporter in the cell line expressing endogenous wild-type p53 (HCT116TP53$^{+/+}$) and showed selectivity between the two cell lines, indicating that its effect is dependent on the presence of wild-type p53. RITA activated several functions of the p53 pathway and showed low micromolar potency against cultured tumor cells with wild-type p53 status. The authors have subjected the molecule to a series of studies and concluded that it activates p53 by a nongenotoxic mechanism involving disruption of the p53−MDM2 interaction. However, RITA does not bind to MDM2 but instead to p53. The mechanism by which it interferes with p53−MDM2 binding and the potential effects of the p53 binding on the functions of p53 are not fully understood yet. RITA offers a new paradigm for activation of p53 by interference with MDM2. However, better understanding of its binding mode will be required for further optimization of this chemical class.

Nutlins

The first potent and selective small-molecule inhibitors of the p53−MDM2 interaction have been identified recently by high-throughput screening followed by structure-based optimization.[12] Nutlins represent a class of *cis*-imidazoline analogues that bind to the p53 pocket on the surface of MDM2 in an enantiomer-specific manner. The three reported nutlins **6**, **7**, **8** (Figure 4, nutlin-1, -2, -3) showed potency against the p53−MDM2 binding in the 100−300 nM range with approximately 150- to 200-fold difference in affinity between enantiomers. Nutlins inhibit the p53−MDM2 interaction effectively by mimicking the interaction of the three critical p53 amino acid residues with the hydrophobic cavity of MDM2 (Figure 5). The imidazolines are a nontraditional peptide mimetic in the sense that they do not attempt to duplicate the position and orientation of the Cα−Cβ bonds of the key amino acid side chains. Thus, the ethoxy group overlays the position of the Phe^{19} side chain, one bromophenyl group overlaps the position of Trp,^{23} and the other occupies the Leu^{26} location.

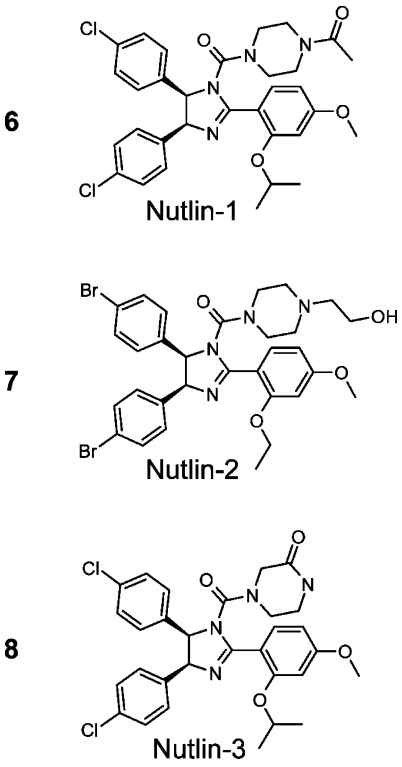

Figure 4. Nutlins.

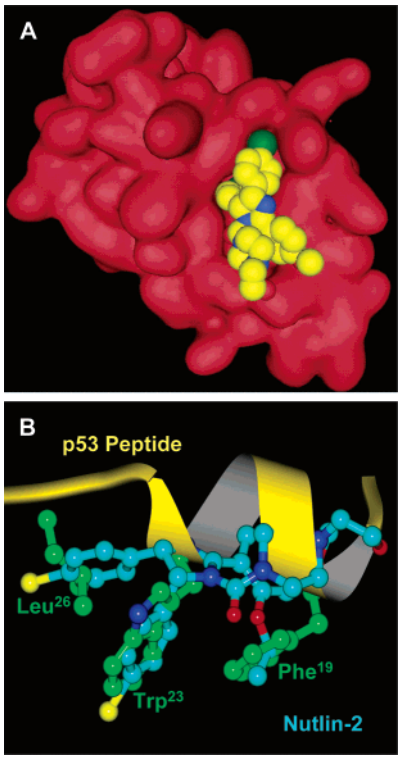

Figure 5. Nutlins bind selectively to the p53 site on MDM2. (A) **7** (nutlin-2) bound to the p53 pocket on the MDM2 molecule (a surface rendition from crystal structure data[12]). (B) Nutlin-2 (blue) mimics the interaction of the three critical amino acid residues (green) from the p53 peptide (yellow) with the hydrophobic cavity of MDM2 (an overlay).

In addition to their relatively high potency in vitro, nutlins penetrate cell membranes, activate the p53 pathway, and inhibit cell growth with potency in the 1−3 μM range. Released from negative control in the

presence of nutlins, p53 is stabilized and accumulates in cell nuclei, leading to activation of p53 target genes (e.g., $p21^{Waf1}$, MDM2) and the p53 pathway. This effect is dependent on the presence of wild-type p53 because cells in which p53 is deleted or mutated do not respond to the nutlin treatment. Moreover, the inactive enantiomer, which represents a virtually identical molecule and thus ideal control for nonmechanistic cellular activity, is inactive in the presence or absence of functional p53. Nutlins do not induce p53 phosphorylation on Ser^{15} that is seen in response to genotoxic stress. These experiments provide strong support for the notion that nutlins are highly selective molecules that derive their cellular activity from inhibition of p53−MDM2 binding and activation of the p53 pathway. Therefore, they represent valuable molecular probes for studying the consequences of p53 activation in living cells.

Nutlins can be administered to mouse xenografts models of human cancer via the desirable oral route. Treatment of established osteosarcoma SJSA-1 tumor xenografts with **8** (racemic) for 20 days inhibited tumor growth by 90% compared to the vehicle controls at a dose that is not toxic to the animals. The activity of nutlins in this model appears superior to that of doxorubicin used at the maximal tolerated dose.[12] Purification of the active enantiomer of **8** (nutlin-3a) increases the potency of the compound 2-fold and showed over 100% tumor growth inhibition in the SJSA-1 tumor model as well as the prostate xenograft model LnCaP. In both cases, multiple partial tumor regressions were observed, suggesting that activation of the p53 pathway not only blocks cell cycle progression but also induces apoptosis in the tumor cells in vivo (manuscript in preparation).

Nongenotoxic p53 Activation, a Novel Approach for Treating Cancer

Activation of p53 by intervention of its regulation has been proposed as a new therapeutic approach for treatment of tumors with wild-type p53 several years ago.[28,41] However, this strategy was largely based on theoretical considerations and limited experimental data derived with macromolecular tools for inhibiting the p53−MDM2 interaction in tissue culture cells and was not validated in vivo.[9−11] Antisense oligonucleotides against MDM2 have been used successfully for activation of the p53 pathway in human xenografts grown in nude mice and have shown antitumor activity in multiple tumor types.[44−47] However, their antitumor activity did not depend on the p53 status of xenografts. In addition, antisense inhibited MDM2 expression in the human tumor cells but not the normal tissues in the host, thus not allowing us to assess the therapeutic window of this strategy. The development of the first selective small-molecule MDM2 antagonists, the nutlins, provided tools for addressing the therapeutic utility of p53 activation in vivo.[12]

There are several major issues associated with p53 activation as a therapeutic strategy in cancer. First, it has been postulated that p53 activation requires not only stabilization and accumulation of the protein but also post-translational modification. p53 undergoes multiple modifications (e.g., phosphorylation, acetylation, sumoylation, etc.) in response to diverse stresses, and it has been speculated that post-translational modifications play a critical role in p53 activation and function.[51−53] Small-molecule antagonists of MDM2 can prevent p53 from binding to MDM2 but should not affect its post-translational modification status. Therefore, p53 that is stabilized by interference with its regulation may not be adequately activated and thus fully functional. Second, although approximately half of all human tumors have retained wild-type p53, it is not clear if other p53 signaling components are still intact and functional in these tumors. Defects in the signaling cascade upstream of p53 can be compensated for by small molecules that stabilize the protein by preventing its degradation. However, there can be no compensation for defects downstream of p53, and such deregulation would render p53 activators inefficient in these tumors. Third, small-molecule p53 modulators will activate the p53 pathway in cancer and normal tissues. Although there is abundant data regarding the growth suppressive and proapoptotic function of p53 in cancer cells, much less is known about the consequences of p53 activation in normal proliferating tissues in vivo. Overt toxicity in these tissues may significantly decrease the therapeutic margin of p53 activating drugs. With the help of the nutlins, one can start to address these issues using relevant cellular and animal models.

p53 phosphorylation on several serine residues mostly within the N-terminal domain, which includes the MDM2 binding site, has been considered critical not only for its dissociation from MDM2 but also for its activation as a transcription factor.[48−50] Since transcriptional activation is of paramount importance for p53 function, it has been speculated that unphosphorylated p53 will be inadequate for activation of the p53 pathway. However, genetic data have raised questions about the importance of p53 phosphorylation.[51,52] Recent experiments in which cancer cells with wild-type p53 were treated with **8** (nutlin-3) demonstrated that nutlin-induced p53 is free of phosphorylation on six key serine residues (Ser^6, Ser^{15}, Ser^{20}, Ser^{37}, Ser^{46}, Ser^{392}).[53] Despite the lack of detectable modification, p53 was shown to have equal or better activity as a transcription factor or apoptosis inducer compared to phosphorylated p53 activated by two different genotoxic drugs: doxorubicin and etoposide. These experiments raise a question about the importance of phosphorylation for p53 function and suggest that p53 levels rather than modification status determine the ability of p53 to activate its gene targets and the p53 pathway. They also suggest that small-molecule MDM2 antagonists can be effective as single agents in cancer therapy.

It has been generally accepted that tumors with wild-type p53 are likely to have other aberrations in the p53 pathway that might attenuate or completely disable its tumor suppressor functions.[4,17] Some of these aberrations have been well-haracterized (e.g., MDM2 overproduction), but many remain unknown. This is mainly due to the lack of specific tools for p53 modulation. Nutlins provide such tools and allow for the first time the dissection of upstream and downstream p53 signaling events. By treatment of tumor-derived cell lines with nutlins, one can narrow the defects to the upstream or downstream signaling components and examine changes in specific signaling events. Data derived from multiple

human cancer cell lines with wild-type p53 treated with nutlins indicated that the growth arrest function of the p53 pathway is preserved in all cell lines but that the apoptotic response differs significantly. Some cell lines responded to p53 activation by nutlins with intense apoptosis, while in others the apoptotic response was substantially attenuated (manuscript in preparation). These observations suggest that MDM2 antagonists may have antiproliferative activity in the majority of human tumors with wild-type p53 but their apoptotic activity may be limited to those tumors that have retained intact p53-dependent apoptotic signaling.

Patients with tumors that express wild-type p53 and high levels of MDM2 protein are expected to benefit the most from p53 activating therapy that can restore p53 function by inhibiting the p53−MDM2 interaction. Overexpression of MDM2 as a result of amplification of the *mdm2* gene locus has been shown to correlate well with wild-type p53 status.[8] This is likely due to the fact that more than one genetic alteration rarely occur in the same pathway. Thus, MDM2 amplification in cells with wild-type p53 may indicate otherwise intact p53 signaling. Consistent with this prediction, cancer cell lines with the amplified *mdm2* gene (e.g., SJSA-1 osteosarcoma) have responded very well to nutlin treatment both in vitro and in vivo.[12]

Recent genetic experiments in mice in which the expression of the *mdm2* gene has been reduced to 30% of its normal level provided important insights into p53 function and regulation in normal tissues in vivo.[54] Reduced MDM2 expression in these transgenic mice led to increased p53 levels in most tissues. However, the consequences of p53 activation depended on the tissue type. Only cells from the thymus, spleen, and the epithelium of the small intestine have shown elevated levels of apoptosis. The mice with reduced MDM2 showed a higher p53 level in many tissues but suffered only from reduced body weight and white blood cell count. No severe adverse effects were reported, and the mice had normal life expectancy. These experiments indicate that p53 regulation by MDM2 in homeostatic tissues may differ from its regulation in cancer cells and suggested that p53 activating therapy of cancer may not cause severe side effects.

Early results from treatment of nude mice bearing established human tumor xenografts with MDM2 antagonists support this expectation.[12] Mice treated for 3 weeks with nutlin doses causing effective tumor inhibition and regression did not show a significant body weight loss or pathological changes. This was not due to lower sensitivity of mouse cells to the drug because cultured mouse fibroblasts respond to nutlin treatment with cell cycle arrest at the same concentrations as human cancer cells or human fibroblasts.[55] In contrast to cancer cells, both human and mouse fibroblasts did not undergo apoptosis and partially resumed cycling after drug removal. These experiments support the therapeutic utility of p53 activators, but further studies in higher species and ultimately in the clinic are needed to assess their true safety margin. MDM2 antagonists do not damage DNA and thus offer a nongenotoxic alternative for activation of the p53 response. This is an important advantage of these drugs over the currently used cytotoxics, the majority of which are DNA damaging agents, imposing a substantial genotoxic burden in patients.

p53 Activators as Chemoprotective Agents

The eukaryotic cell cycle is a set of biochemical events that allows for faithful duplication and segregation of their genetic material between two daughter cells.[56] This process is controlled with high precision, and entry into any cell cycle phase is prohibited before completion of the previous one.[57] Therefore, in some instances, cell cycle arrest could protect cells from cytotoxic agents that require progression through the cell cycle for their activity. This phenomenon allows us to design strategies for protection of normal cells from cell cycle phase specific cytotoxic drugs such as antimitotics.[58−60] Antimitotic drugs, currently dominated by tubulin poisons (e.g., taxanes, vinca alkaloids), are widely used chemotherapeutic agents that require the cells to transition through mitosis for their activity.[61] Cell cycle block in any phase other than mitosis would decrease the sensitivity of cells and could be exploited for chemoprotection.

One of the main functions of activated p53 is induction of G1/S and G2/M checkpoints.[3,4] By halting cell cycle progression before entry into the S phase or mitosis, p53 prevents the propagation of DNA damage and facilitates its repair. Cell cycle arrest induced by p53, especially in normal proliferating tissues, is generally reversible, and cells can resume proliferation once they are free of stress. On the other hand, approximately 50% of all human tumors have lost p53 checkpoint function and will not be protected by specific inducers of the p53 pathway. These features make the p53 pathway ideally suited for utilization in chemoprotective strategies.

It has been shown that activation of the p53 pathway by genotoxic stress (e.g., doxorubicin treatment) in cancer cells with wild-type p53 can significantly decrease their sensitivity to paclitaxel.[62] However, DNA damaging agents are not specific for p53 and can induce p53-independent checkpoints, thus protecting not only normal cells but also the targeted tumor cells during antimitotic chemotherapy. MDM2 antagonists, the nutlins, are nongenotoxic p53 inducers that derive their cellular activity from selective activation of the p53 pathway. They arrest effectively cell cycle progression at the G1/S and G2/M borders but do not induce p53-independent checkpoints.[12] Therefore, nutlins are valuable tools for exploiting p53-based chemoprotective strategies.

Treatment of proliferating cancer cells and normal skin fibroblasts with nutlins prevented their entry into mitosis and protected them partially from high doses of paclitaxel.[63] Under the same treatment conditions, cancer cells with mutant p53 have retained their sensitivity to the mitotic poison. These results suggest a potential utility of MDM2 antagonists in protecting normal proliferating tissues during antimitotic chemotherapy of patients with tumors that have lost p53 function (Figure 6). Pretreatment of patients with MDM2 antagonists should activate the p53 pathway and induce cell cycle arrest in their normal proliferating tissues, which will decrease their sensitivity to antimitotic chemotherapeutic agent. The same treatment will have no consequences on tumor cells with mutant p53, which will continue to proliferate. This strategy may protect normal tissues at least partially from cytotox-

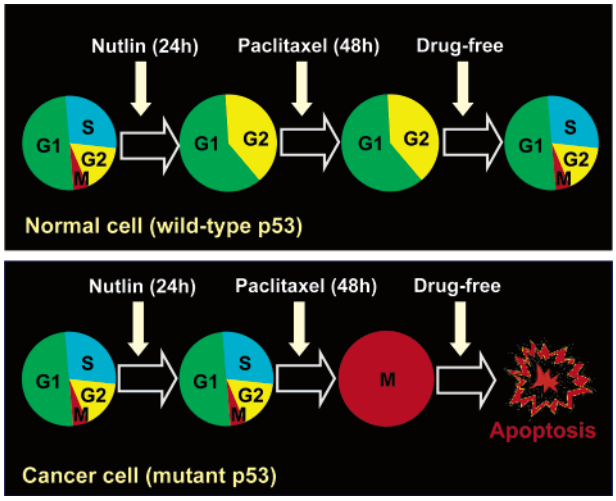

Figure 6. MDM2 antagonists may have utility in protecting normal proliferating tissues during antimitotic chemotherapy of tumors expressing mutant p53. Normal cells possess wild-type functional p53, and pretreatment with nutlins will arrest their proliferation and may protect them from the toxicity of paclitaxel. They may resume proliferation after drug removal. Cancer cells in which p53 is mutant will be insensitive to MDM2 antagonists and will be killed by paclitaxel-induced apoptosis.

icity of mitotic inhibitors and thus lower the occurrence of side effects. However, further studies are needed to assess the validity of this approach in more relevant in vivo models.

The checkpoint function of the p53 pathway has another important implication concerning combination chemotherapy. Because of their potential protective effect in cancer cells with wild-type p53, MDM2 antagonists or any p53 activating drugs should be used with caution when combined with antimitotic agents in the clinic. This also applies to many of the currently used cytotoxic agents, the majority of which are genotoxins and can induce p53-dependent checkpoint arrest.

Conclusion

In the search for more efficacious and less toxic cancer drugs, the tumor suppressor p53 has long been a desirable therapeutic target. Several independent studies have demonstrated that antitumor activity of p53 can be unleashed in cancer cells that have retained the wild-type status of the tumor suppressor by intervention in its regulation and validated the p53−MDM2 interaction as a target. Structural features of this protein−protein interaction raised the hopes for finding small-molecule antagonists and facilitated drug discovery efforts. As a result, several classes of nonpeptidic MDM2 inhibitors have been identified. The discovery of the first potent and selective small-molecule MDM2 antagonists, the nutlins, helped to validate this novel therapeutic strategy in animal models of human cancer.

Despite the great progress in understanding p53 function and regulation, there are still many biology issues that need to be addressed before one can predict the therapeutic value of p53 activators in the clinic. Early studies with the nutlins have indicated that MDM2 antagonists may be effective as single agents if tumor cells possess wild-type p53 and relatively intact downstream p53 signaling. However, it is still not clear how many human tumors will fall into this category in the clinical setting. MDM2 amplification may provide a positive clinical marker for intact p53 signaling, but this concept requires validation. The role of MDM2 overexpression that does not involve gene amplification in disabling p53 function needs further clarification. The availability of nutlins will greatly facilitate these studies.

Another issue not fully addressed yet is the therapeutic margin of p53-activating therapy. Experiments in nude mice have shown minimal adverse effects at efficacious doses but species-specific differences between tumor xenografts and host tissues could in principle enlarge the estimated therapeutic window of tested drugs. p53-based gene therapy has been approved recently in China for the treatment of head and neck cancer.[64] However, this treatment involves intratumoral delivery of p53-expressing adenoviral vectors and will not be very useful in assessing the potential for adverse effects from systemic p53 activation. Ultimately, the true therapeutic window of small-molecule p53 activators will be determined in the clinic.

Recent progress in the discovery of selective small-molecule modulators of the p53 pathway provided much needed tools to probe the validity of this novel therapeutic concept. The encouraging results obtained with these agents have increased the hope that pharmacological activation of p53 can provide a clinical benefit for cancer patients.

Acknowledgment. The author thanks Bradford Graves for help with the figures and thanks Mary Simcox and David Heimbrook for critical reading of the manuscript.

Biography

Lyubomir T. Vassilev is a Senior Research Leader in the Oncology Division of Hoffmann-La Roche Inc. in Nutley, NJ, where he has been leading small-molecule drug discovery efforts for the past 12 years. His primary research interests include signal transduction, cell cycle regulation, and their deregulation in cancer. Dr. Vassilev received his Ph.D. degree in Molecular Biology from the Institute of Molecular Biology, Bulgarian Academy of Sciences, where he worked on the structure and function of chromatin. He did his postdoctoral training at the Mount Sinai School of Medicine, New York, NY, and the Roche Institute of Molecular Biology, Nutley, NJ, studying the mechanisms of mammalian DNA replication.

References

(1) Hollstein, M.; Sidransky, D.; Vogelstein, B.; Harris, C. C. p53 mutations in human cancers. *Science* **1991**, *253*, 49−53.
(2) Hainaut, P.; Hollstein, M. p53 and human cancer: the first ten thousand mutations. *Adv. Cancer Res.* **2000**, *77*, 81−137.
(3) Levine, A. J. p53, the cellular gatekeeper for growth and division. *Cell* **1997**, *88*, 323−331.
(4) Vogelstein, B.; Lane, D.; Levine, A. J. Surfing the p53 network. *Nature* **2000**, *408*, 307−310.
(5) Fakharzadeh, S. S.; Trusko, S. P.; George, D. L. Tumorigenic potential associated with enhanced expression of a gene that is amplified in a mouse tumor cell line. *EMBO J.* **1991**, *10*, 1565−1569.
(6) Oliner, J. D.; Kinzler, K. W.; Meltzer, P. S.; George, D. L.; Vogelstein, B. Amplification of a gene encoding a p53-associated protein in human sarcomas. *Nature* **1992**, *358*, 80−83.

(7) Oliner, J. D.; Pietenpol, J. A.; Thiagalingam, S.; Gyuris, J.; Kinzler, K. W.; et al. Oncoprotein MDM2 conceals the activation domain of tumour suppressor p53. *Nature* **1993**, *362*, 857−860.
(8) Momand, J.; Jung, D.; Wilczynski, S.; Niland, J. The MDM2 gene amplification database. *Nucleic Acids Res.* **1998**, *26*, 3453−3459.
(9) Bottger, A.; Bottger, V.; Sparks, A.; Liu, W. L.; Howard, S. F.; et al. Design of a synthetic Mdm2-binding mini protein that activates the p53 response in vivo. *Curr. Biol.* **1997**, *7*, 860−869.
(10) Wasylyk, C.; Salvi, R.; Argentini, M.; Dureuil, C.; Delumeau, I.; et al. p53 mediated death of cells overexpressing MDM2 by an inhibitor of MDM2 interaction with p53. *Oncogene* **1999**, *18*, 1921−1934.
(11) Chene, P.; Fuchs, J.; Bohn, J.; Garcia-Echeverria, C.; Furet, P.; et al. A small synthetic peptide, which inhibits the p53−hdm2 interaction, stimulates the p53 pathway in tumour cell lines. *J. Mol. Biol.* **2000**, *299*, 245−253.
(12) Vassilev, L. T.; Vu, B. T.; Graves, B.; Carvajal, D.; Podlaski, F.; et al. In vivo activation of the p53 pathway by small-molecule antagonists of MDM2. *Science* **2004**, *303*, 844−848.
(13) Ljungman, M. Dial 9-1-1 for p53: mechanisms of p53 activation by cellular stress. *Neoplasia* **2000**, *2*, 208−225.
(14) Jimenez, G. S.; Khan, S. H.; Stommel, J. M.; Wahl, G. M. p53 regulation by post-translational modification and nuclear retention in response to diverse stresses. *Oncogene* **1999**, *18*, 7656−7665.
(15) Oren, M. Decision making by p53: life, death and cancer. *Cell Death Differ.* **2003**, *10*, 431−442.
(16) Smart, P.; Lane, E. B.; Lane, D. P.; Midgley, C.; Vojtesek, B.; et al. Effects on normal fibroblasts and neuroblastoma cells of the activation of the p53 response by the nuclear export inhibitor leptomycin B. *Oncogene* **1999**, *18*, 7378−7386.
(17) Vousden, K. H.; Lu, X. Live or let die: the cell's response to p53. *Nat. Rev. Cancer* **2002**, *2*, 594−604.
(18) Honda, R.; Tanaka, H.; Yasuda, H. Oncoprotein MDM2 is a ubiquitin ligase E3 for tumor suppressor p53. *FEBS Lett.* **1997**, *420*, 25−27.
(19) Haupt, Y.; Maya, R.; Kazaz, A.; Oren, M. Mdm2 promotes the rapid degradation of p53. *Nature* **1997**, *387*, 296−299.
(20) Leng, R. P.; Lin, Y.; Ma, W.; Wu, H.; Lemmers, B.; et al. Pirh2, a p53-induced ubiquitin-protein ligase, promotes p53 degradation. *Cell* **2003**, *112*, 779−791.
(21) Dornan, D.; Wertz, I.; Shimizu, H.; Arnott, D.; Frantz, G. D.; et al. The ubiquitin ligase COP1 is a critical negative regulator of p53. *Nature* **2004**, *429*, 86−92.
(22) Li, M.; Chen, D.; Shiloh, A.; Luo, J.; Nikolaev, A. Y.; et al. Deubiquitination of p53 by HAUSP is an important pathway for p53 stabilization. *Nature* **2002**, *416*, 648−653.
(23) Li, M.; Brooks, C. L.; Kon, N.; Gu, W. A dynamic role of HAUSP in the p53−Mdm2 pathway. *Mol. Cell* **2004**, *13*, 879−886.
(24) Montes de Oca Luna, R.; Wagner, D. S.; Lozano, G. Rescue of early embryonic lethality in mdm2-deficient mice by deletion of p53. *Nature* **1995**, *378*, 203−206.
(25) Jones, S. N.; Roe, A. E.; Donehower, L. A.; Bradley, A. Rescue of embryonic lethality in Mdm2-deficient mice by absence of p53. *Nature* **1995**, *378*, 206−208.
(26) Picksley, S. M.; Lane, D. P. The p53−mdm2 autoregulatory feedback loop: a paradigm for the regulation of growth control by p53? *BioEssays* **1993**, *15*, 689−690.
(27) Michael, D.; Oren, M. The p53−Mdm2 module and the ubiquitin system. *Semin. Cancer Biol.* **2003**, *13*, 49−58.
(28) Freedman, D. A.; Wu, L.; Levine, A. J. Functions of the MDM2 oncoprotein. *Cell. Mol. Life Sci.* **1999**, *55*, 96−107.
(29) Toogood, P. L. Inhibition of protein−protein association by small molecules: approaches and progress. *J. Med. Chem.* **2002**, *45*, 1543−1558.
(30) Arkin, M. R.; Wells, J. A. Small-molecule inhibitors of protein−protein interactions: progressing towards the dream. *Nat. Rev. Drug Discovery* **2004**, *3*, 301−317.
(31) Chen, J.; Marechal, V.; Levine, A. J. Mapping of the p53 and mdm-2 interaction domains. *Mol. Cell. Biol.* **1993**, *13*, 4107−4114.
(32) Kussie, P. H.; Gorina, S.; Marechal, V.; Elenbaas, B.; Moreau, J.; et al. Structure of the MDM2 oncoprotein bound to the p53 tumor suppressor transactivation domain. *Science* **1996**, *274*, 948−953.
(33) Fry, D.; Graves, B.; Vassilev, L. T. Exploiting Protein−Protein Interactions To Design an Activator of p53. In *Protein−Protein Interaction: A Molecular Cloning Manual*, 2nd ed.; Cold Spring Harbor Press: Cold Spring Harbor, NY, in press.
(34) Chene, P. Inhibition of the p53−MDM2 interaction: targeting a protein−protein interface. *Mol. Cancer Res.* **2004**, *2*, 20−28.
(35) Klein, C.; Vassilev, L. T. Targeting the p53−MDM2 interaction to treat cancer. *Br. J. Cancer* **2004**, *91*, 1415−1419.
(36) Stoll, R.; Renner, C.; Hansen, S.; Palme, S.; Klein, C.; et al. Chalcone derivatives antagonize interactions between the human oncoprotein MDM2 and p53. *Biochemistry* **2001**, *40*, 336−344.
(37) Kumar, S. K.; Hager, E.; Pettit, C.; Gurulingappa, H.; Davidson, N. E.; et al. Design, synthesis, and evaluation of novel boronic-chalcone derivatives as antitumor agents. *J. Med. Chem.* **2003**, *46*, 2813−2815.
(38) Zhao, J.; Wang, M.; Chen, J.; Luo, A.; Wang, X.; et al. The initial evaluation of non-peptidic small-molecule HDM2 inhibitors based on p53−HDM2 complex structure. *Cancer Lett.* **2002**, *183*, 69−77.
(39) Galatin, P. S.; Abraham, D. J. A nonpeptidic sulfonamide inhibits the p53−mdm2 interaction and activates p53-dependent transcription in mdm2-overexpressing cells. *J. Med. Chem.* **2004**, *47*, 4163−4165.
(40) Yin, H.; Hamilton, A. D. Synthetic inhibitors based on a terphenyl scaffolds antagonize p53/MDM2 interaction and induce p53 accumulation in vivo. *Abstracs of Papers*, 227th National Meeting of the American Chemical Society, Anaheim, CA, 2004.
(41) Issaeva, N.; Bozko, P.; Enge, M.; Protopopova, M.; Verhoef, L. G.; et al. Small molecule RITA binds to p53, blocks p53−HDM-2 interaction and activates p53 function in tumors. *Nat. Med.* **2004**, *10*, 1321−1328.
(42) Bunz, F.; Dutriaux, A.; Lengauer, C.; Waldman, T.; Zhou, S.; et al. Requirement for p53 and p21 to sustain G2 arrest after DNA damage. *Science* **1998**, *282*, 1497−1501.
(43) Lane, D. P. Exploiting the p53 pathway for cancer diagnosis and therapy. *Br. J. Cancer* **1999**, *80* (Suppl. 1), 1−5.
(44) Wang, H.; Zeng, X.; Oliver, P.; Le, L. P.; Chen, J.; et al. MDM2 oncogene as a target for cancer therapy: An antisense approach. *Int. J. Oncol.* **1999**, *15*, 653−660.
(45) Tortora, G.; Caputo, R.; Damiano, V.; Bianco, R.; Chen, J.; et al. A novel MDM2 anti-sense oligonucleotide has anti-tumor activity and potentiates cytotoxic drugs acting by different mechanisms in human colon cancer. *Int. J. Cancer* **2000**, *88*, 804−809.
(46) Zhang, Z.; Li, M.; Wang, H.; Agrawal, S.; Zhang, R. Antisense therapy targeting MDM2 oncogene in prostate cancer: Effects on proliferation, apoptosis, multiple gene expression, and chemotherapy. *Proc. Natl. Acad. Sci. U.S.A.* **2003**, *100*, 11636−11641.
(47) Zhang, R.; Wang, H. Antisense oligonucleotide inhibitors of MDM2 oncogene expression. *Methods Mol. Med.* **2003**, *85*, 205−222.
(48) Brooks, C. L.; Gu, W. Ubiquitination, phosphorylation and acetylation: the molecular basis for p53 regulation. *Curr. Opin. Cell Biol.* **2003**, *15*, 164−171.
(49) Appella, E.; Anderson, C. W. Post-translational modifications and activation of p53 by genotoxic stresses. *Eur. J. Biochem.* **2001**, *268*, 2764−2772.
(50) Kapoor, M.; Lozano, G. Functional activation of p53 via phosphorylation following DNA damage by UV but not gamma radiation. *Proc. Natl. Acad. Sci. U.S.A.* **1998**, *95*, 2834−2837.
(51) Ashcroft, M.; Kubbutat, M. H.; Vousden, K. H. Regulation of p53 function and stability by phosphorylation. *Mol. Cell Biol.* **1999**, *19*, 1751−1758.
(52) Wu, Z.; Earle, J.; Saito, S.; Anderson, C. W.; Appella, E.; et al. Mutation of mouse p53 Ser23 and the response to DNA damage. *Mol. Cell. Biol.* **2002**, *22*, 2441−2449.
(53) Thompson, T.; Tovar, C.; Yang, H.; Carvajal, D.; Vu, B. T.; et al. Phosphorylation of p53 on key serines is dispensable for transcriptional activation and apoptosis. *J. Biol. Chem.* **2004**, *279*, 53015−53022.
(54) Mendrysa, S. M.; McElwee, M. K.; Michalowski, J.; O'Leary, K. A.; Young, K. M.; et al. mdm2 is critical for inhibition of p53 during lymphopoiesis and the response to ionizing irradiation. *Mol. Cell. Biol.* **2003**, *23*, 462−472.
(55) Vassilev, L. T. Small-molecule antagonists of p53−MDM2 binding: research tools and potential therapeutics. *Cell Cycle* **2004**, *3*, 419−421.
(56) Pardee, A. B.; Dubrow, R.; Hamlin, J. L.; Kletzien, R. F. Animal cell cycle. *Annu. Rev. Biochem.* **1978**, *47*, 715−750.
(57) Morgan, D. O. Principles of CDK regulation. *Nature* **1995**, *374*, 131−134.
(58) Blagosklonny, M. V.; Pardee, A. B. Exploiting cancer cell cycling for selective protection of normal cells. *Cancer Res.* **2001**, *61*, 4301−4305.
(59) Blagosklonny, M. V.; Darzynkiewicz, Z. Cyclotherapy: protection of normal cells and unshielding of cancer cells. *Cell Cycle* **2002**, *1*, 375−382.
(60) Keyomarsi, K.; Pardee, A. B. Selective protection of normal proliferating cells against the toxic effects of chemotherapeutic agents. *Prog. Cell Cycle Res.* **2003**, *5*, 527−532.
(61) Hadfield, J. A.; Ducki, S.; Hirst, N.; McGown, A. T. Tubulin and microtubules as targets for anticancer drugs. *Prog. Cell Cycle Res.* **2003**, *5*, 309−325.

(62) Blagosklonny, M. V.; Robey, R.; Bates, S.; Fojo, T. Pretreatment with DNA-damaging agents permits selective killing of checkpoint-deficient cells by microtubule-active drugs. *J. Clin. Invest.* **2000**, *105*, 533−539.
(63) Carvajal, D.; Tovar, C.; Yang, H.; Heimbrook, D. C.; Vassilev, L. T. Activation of the p53 pathway by MDM2 antagonists can protect proliferating cells from mitotic inhibitors. *Cancer Res.* **2005**, *65*, 1918−1924.
(64) Surendran, A. The virtuous virus. *Nat. Med*. **2004**, *10*, 767−768.

JM058174K