Awakening guardian angels: drugging the p53 pathway

Christopher J. Brown, Sonia Lain‡, Chandra S. Verma§, Alan R. Fersht‖ and David P. Lane**

Abstract | Currently, around 11 million people are living with a tumour that contains an inactivating mutation of *TP53* (the human gene that encodes p53) and another 11 million have tumours in which the p53 pathway is partially abrogated through the inactivation of other signalling or effector components. The p53 pathway is therefore a prime target for new cancer drug development, and several original approaches to drug discovery that could have wide applications to drug development are being used. In one approach, molecules that activate p53 by blocking protein–protein interactions with MDM2 are in early clinical development. Remarkable progress has also been made in the development of p53-binding molecules that can rescue the function of certain p53 mutants. Finally, cell-based assays are being used to discover compounds that exploit the p53 pathway by either seeking targets and compounds that show synthetic lethality with *TP53* mutations or by looking for non-genotoxic activators of the p53 response.

Cytotoxic chemotherapy
Cell-killing drugs used to treat various cancers by targeting rapidly dividing cells.

The development of new drugs to treat cancer has undergone a dramatic renaissance in the past decade, spearheaded by the development of both small-molecule and biological agents that have shown remarkable clinical activity without the toxicity that is associated with conventional cytotoxic chemotherapy. However, the new agents are highly selective and effective only in a minority of cancers that are identified by specific genetic lesions or alterations. Key examples include gefitinib and erlotinib, which target tumours with some mutations in epidermal growth factor receptor (EGFR)[1,2,3]; imatinib (also known as Glivec or Gleevec), which is effective against tumours harbouring translocations of *ABL1* (REF. 4); and lapatinib, which is efficacious in breast cancers with amplification of *ERBB2* (REF. 5). Therefore, the frustration in the field has been the inability to develop drugs that target the most frequent alterations in human cancer, such as the mutation of the *Ras* genes, the overexpression of *MYC* and (as discussed in this Review) the almost universal alteration of the p53 pathway. The reason for these difficulties lies in the nature of the targets presented by these frequent alterations, which fall outside the scope of targets commonly found to be suitable for small-molecule or biological drug development.

This has produced a wonderful challenge. How can we successfully increase the number of targets that can be approached for therapeutic development and, at the same time, develop new cancer drugs that will benefit the majority of patients? Progress is highly promising owing to the use of two broad approaches, which are both well illustrated in the case of p53. In one approach, new methods of screening, chemistry and structure-based design are yielding effective molecules that target protein–protein interactions and protein folding pathways, which increases the number of direct targets for small-molecule drugs. Protein–protein interaction sites are usually large and shallow, and affinity is achieved through the summation of many weak interactions. A small molecule must effectively mimic these numerous and widespread interactions while maintaining good drug-like properties. The interaction surface of these sites also presents another challenge: shape complementarity. As these sites are usually flat and planar, it is difficult to attain high specificity through maximizing shape recognition. Important work by Clackson and Wells[6] demonstrated that some protein–protein interaction surfaces are not featureless and are not reliant on a wide distribution of weak interactions, but are in fact mediated by a set of key interactions that contribute to a large component of the binding affinity. This subregion of key interactions was termed the 'hot spot', and its dimensions were comparable to the size of a small organic molecule. Examples of this approach to block protein–protein interactions include the BCL-2 inhibitor

**p53 Laboratory (A-STAR), 8A Biomedical Grove, Immunos, Singapore 138648.*
‡Karolinska Institute, Stockholm SE-171 77, Sweden.
§Bioinformatics Institute (A-STAR), 30 Biopolis Street, Matrix, Singapore 138671.‖MRC Centre for Protein Engineering, Hills Road, Cambridge CB2 0QH, UK.
Correspondence to D.P.L.
e-mail: dplane@p53lab.a-star.edu.sg
doi:10.1038/nrc2763

At a glance

- p53 functions as the 'guardian of the genome' by inducing cell cycle arrest, senescence and apoptosis in response to oncogene activation, DNA damage and other stress signals. Loss of p53 function occurs in most human tumours by either mutation of *TP53* itself or by inactivation of the p53 signal transduction pathway.
- In many tumours p53 is inactivated by the overexpression of the negative regulators MDM2 and MDM4 or by the loss of activity of the MDM2 inhibitor ARF. The pathway can be reactivated in these tumours by small molecules that inhibit the interaction of MDM2 and/or MDM4 with p53. Such molecules are now in clinical trials.
- Cell-based screens have been used to find several new non-genotoxic activators of the p53 response, which include inhibitors of protein deacetylating enzymes.
- Molecules that bind and stabilize mutant p53 — restoring wild-type function — have been discovered by both structure-based design and cell-based screens.
- Activating a p53-dependent cell cycle arrest in normal cells and tissues can protect them from the toxic effect of anti-mitotic drugs while not reducing their efficacy in killing p53 mutant tumour cells. This drug combination approach represents a new way to exploit the p53 system.
- The intense study of the p53 pathway is helping to develop new paradigms in drug discovery and development that will have widespread application in other areas of drug discovery.

ABT-373 and the MDM2 inhibitor nutlin[7,8]. In another radically different approach alterations in cellular pathways brought about by oncogenic mutations are targeted; the most dramatic example is the efficacy of the poly (ADP-ribose) polymerase (PARP) inhibitors, which inhibit DNA repair through the base excision repair pathway[9,10] and thereby selectively kill cells that have lost the function of the BRCA tumour suppressor proteins that are required for DNA repair through homologous recombination[11]. This Review provides an update on using both these direct and indirect approaches for targeting the p53 system (TABLE 1).

Crucial concepts in drugging the p53 pathway

The tumour suppressor protein p53 is a transcription factor that has an essential role in guarding the cell in response to various stress signals through the induction of cell cycle arrest, apoptosis or senescence. Recently, other functions of p53 in tumour suppression have been discovered that are independent of its ability to transactivate gene expression. These include direct effects on survival proteins in the mitochondria[12,13], regulation of microRNA processing[14] and reports of possible direct p53 involvement in DNA repair pathways[15–19] (FIG. 1). Other new targets of p53-induced transcription have been identified that are involved in protein translation[20,21]. Impairment of p53 function has a crucial role in tumour evolution by allowing evasion from p53-dependent responses. p53 inactivation in tumours occurs through two general mechanisms. First, the inactivation of p53 function by point mutations in p53 itself or second, through the partial abrogation of signalling pathways or effector molecules that regulate p53 activity. A series of genetic models has, however, established that the restoration of p53 activity in established tumour cells is an exceptionally effective intervention[22–24].

p53 is under precise control by MDM2, which is an E3 ubiquitin ligase that targets p53 for ubiquitin-dependent degradation, functioning as a crucial negative regulator[25] (FIG. 2). MDM2 also inhibits p53 function by modulating its transcriptional activity and by preventing its interaction with the general transcription machinery[26]. In an important feedback loop, p53 activates transcription of *MDM2* (REF. 27). The increased expression of MDM2 leads to a decrease in p53 levels and the inactivation of p53, which in turn leads to a decrease in the rate of *MDM2* transactivation by p53. Besides MDM2, MDM4 (also known as HDM4, MDMX or HDMX) and ARF (also known as $p14^{ARF}$ in humans and $p19^{ARF}$ in mice) also have an important role in controlling p53 stability. MDM4 is a structural homologue of MDM2 that can form a heterocomplex with MDM2 and potentiate the ubiquitylation of p53, and ARF is a tumour suppressor that interacts with MDM2 and inhibits p53 degradation, thereby stabilizing it (FIG. 2). In more than half the tumours with a fault in the p53 pathway, *TP53* itself is not mutated but the p53 pathway is abrogated. Mechanisms that result in this abrogation include increased expression of the p53-negative regulators MDM2 (REF. 28) and MDM4 (REFS 29–31) and deletion or epigenetic inactivation of the p53-positive regulator and MDM2 inhibitor ARF[32,33].

In the other p53-defective tumours *TP53* is mutated, and approximately 95% of these mutations[34] lie in the core DNA-binding domain, which reflects the fact that a key function of p53 is as a transcriptional activator (FIG. 3). Furthermore, 75% of these mutations occur as missense mutations, which result in the tumour-associated form of p53 being predominantly full length, with a single amino acid change in the core domain[34]. These point mutations fall into two broad classes: structural and DNA contact. DNA contact mutations have little or no effect on p53 folding and they directly interfere with residues involved in DNA binding; structural mutations can disrupt the local structure only or they destabilize the whole protein[35]. These mutations usually confer the mutant protein with a dominant-negative activity over the remaining wild-type allele, a mechanism that involves hetero-oligomerization of the mutant protein with the wild-type protein[36–38]. It is also becoming increasingly clear that in addition to losing their tumour-suppressive function many p53 mutants also gain dominant-negative activities and new oncogenic properties (the gain-of-function theory)[39]. These mutations promote an inhibitory interaction between mutant p53 and the homologous p53 family members p73 and p63, thereby reducing their transcriptional activity[40,41]. p53 mutants may also have gained other transcriptional regulatory functions[42,43].

Consequently, the challenge has been — as highlighted by Levine and Oren[44] — to develop molecules that can reactivate p53 function in human tumours. Progress has been made along several distinct lines. In the first of these, small molecules have been sought that will bind to either full-length p53 (REFS 45,46) or the core DNA-binding domain of mutant p53 and restore its normal activity[47]. In the second approach, inhibitors of the protein–protein interaction of p53 with the negative regulator MDM2 have been developed that show clear antitumour activity in preclinical animal models[8,48]. Using a third approach, molecules and drug combinations

Missense mutation
A single nucleotide is changed, resulting in a codon that encodes a different amino acid (non-synonymous).

Table 1 | **Strategies and mechanisms for small molecules that target the p53 pathway**

Molecule	Mechanism of action	Stage in clinical testing
Reactivate mutant p53		
PRIMA-1	Protein folding[49]	Phase I (APR-246)
CP-31398	Protein folding[46]	Preclinical
PhiKan083	Protein thermal stability[69]	Preclinical
Activate wild-type p53		
Nutlin	MDM2 binding[8]	Phase I
MI-219	MDM2 binding[48]	Phase I
Tenovin-6	SIRT1 and SIRT2 inhibition[50]	Preclinical
RITA	p53 binding[51]	Preclinical
Leptomycin B	CRM1 binding[72,135]	Phase I (Elactocin; withdrawn[147])
Actinomycin D	RPL11 and RPL5 release[118]	Approved (Dactinomycin)
Cyclotherapy (temporal combination of p53 activator and mitotic inhibitor)		
Nutlin*	BI-2536 (PLK1 inhibitor[52])‡	Phase I/Phase I§
Nutlin*	VX680 (Aurora inhibitor‖)‡	Phase I/Phase I§
Tenovin-6*	Taxol (Tubulin binding[52])‡	Preclinical/approved§
Actinomycin D*	Taxol‡	Approved/approved§

*p53 activator. ‡Mitotic inhibitor. §Combinations are not in trial together or have been approved together. ‖D.L.P., unpublished observations. CRM1, exportin 1; PLK1, polo-like kinase 1; RITA, reactivation of p53 and induction of tumour cell apoptosis; RPL, ribosomal protein L; SIRT, sirtuin.

have been sought using forward chemical genetics (FCG) cell-based methods to identify molecules that selectively kill tumour cells by activating mutant or wild-type p53 (REFS 49–53). These approaches have identified large numbers of putatively active compounds, but in only a few cases has their precise mode of action been determined. In addition to the research outlined above, p53 recombinant adenovirus-based gene therapy has been approved in China (BOX 1), but remains in extended pre-registration trials in the United States. This approach has provided considerable discussion about the level of efficacy required for regulatory approval. Adenovirus-based gene therapy works by introducing a functional copy of *TP53* into tumours following local injection[54]. As not all cells will be injected, clinical efficacy depends on the bystander effect in the tumour[55]. In a second approach, the ONYX-015 virus has been in extended clinical trials on the basis of the idea that a certain virus may only replicate in tumour cells that lack p53 function[56].

Strategies for reactivating mutant p53

The core domain of wild-type p53 is rather unstable, with a melting temperature of 44 °C and a short half-life of 9 minutes at body temperature[57]. The tumour-associated mutations thermally destabilize the protein further at body temperature, leading to the abrogation of DNA binding and the impairment of the p53 response[58]. Small molecules that stabilize p53 in its active biological conformation — restoring its binding function — could potentially rescue wild-type p53 function. Early results with antibodies that bind the p53 carboxy-terminus (such as pAb421)[59] and a synthetic peptide (p53C)[60] derived from the C-terminal domain showed a stimulatory effect on the DNA binding ability of p53. Importantly, the C-terminal-derived peptide also restores binding to some DNA contact as well as several structurally destabilized mutants (H175, A143 and S249) *in vitro*[61] and it induces apoptosis in cancer cells expressing p53 contact mutants[45,62]. However, the mechanism of action of this interesting peptide is still unclear. Snyder *et al.* synthesized a p53C retro-inverso peptide fused to the protein transduction domain TAT that induced p53 expression in cancer cell lines. This peptide also proved active in animal models of peritoneal carcinomatosis and peritoneal lymphoma, significantly increasing lifespan and generating disease-free animals[63]. However, the challenges that are associated with peptide stability and transport into tumour cells in human clinical trials have yet to be completely addressed[64].

Several non-peptide molecules, identified from small-molecule screens, have also been proposed to restore wild-type p53 activity, including CP31398 (identified by Pfizer on the basis of an *in vitro* assay of mutant p53 unfolding)[46]. Despite the positive initial data for CP31398, which showed that it could induce the expression of both reporter and endogenous p53 target genes following transfection of *TP53*-null cells with two different mutant *TP53* genes[46], more recent work has revealed that the molecule does not directly bind to p53 but instead interacts directly with DNA[65]. The challenge of reactivating mutant p53 with peptides, antibodies or small molecules is whether or not they will be able to target all the mutation classes observed in p53 or a highly specific subset only. If these potentially therapeutic molecules are interacting with p53 itself then this implies that there is a common mechanism, which could be used to rescue the many diverse mutants of p53. The existence of such a mechanism is supported by the fact that many different p53 mutant proteins exhibit

Forward chemical genetics
FCG. Libraries of small molecules are screened for their ability to induce a particular phenotype in cells or cellular extracts. FCG requires three components: a collection of compounds, a biological assay with a quantifiable phenotypic output and a method to identify the target(s) of the active compounds.

Differential scanning calorimetry
Measures the heat changes that occur in biomolecules during controlled increases or decreases in temperature. It measures the enthalpy of unfolding and the change in heat capacity owing to heat denaturation: the higher the thermal transition (melting point) the more stable the molecule.

two common properties: the PAb240 (REF. 66) continuous epitope, which is normally inaccessible in correctly folded p53, is exposed, and the PAb1620 epitope[67], which is sensitive to the loss of the correct p53 tertiary structure, is lost (reviewed in REF. 68).

Another approach using structural data and taking advantage of computational techniques to isolate a refined library of probable p53 stabilizers has yielded exciting data[69]. The determination of the crystal structure of a series of tumour-associated mutant p53 proteins showed that the Y220C mutation creates a binding pocket in the core domain of the mutant protein (FIG. 3) on the opposite face to the DNA-binding domain[47]. This in turn allowed *in silico* screening and the identification of small molecules that could bind to this pocket and, owing to their interaction, stabilize the mutant Y220C core domain in the wild-type conformation. Differential scanning calorimetry confirmed that binding of the compound PhiKan083 raised the melting temperature by 2 °C. An increase in the melting temperature reflects the higher enthalpy required to denature the protein and the extra stabilizing interactions made by the ligand. A high resolution X-ray structure of the p53-Y220C–PhiKan083 complex demonstrated the occupancy of the drug in the predicted binding pocket. This work presents the possibility of developing mutant-specific reactivating drugs. Although it can be argued that such drugs would be difficult to develop as not all mutations in the p53 DNA-binding domain generate a cleft that is ideal for small-molecule binding, precedent suggests that highly active and specific drugs for cancer can find substantial markets. Indeed, tumours with this p53 mutation occur at a similar frequency worldwide to tumours with the BCR-ABL1 translocation, which is the target of imatinib[4].

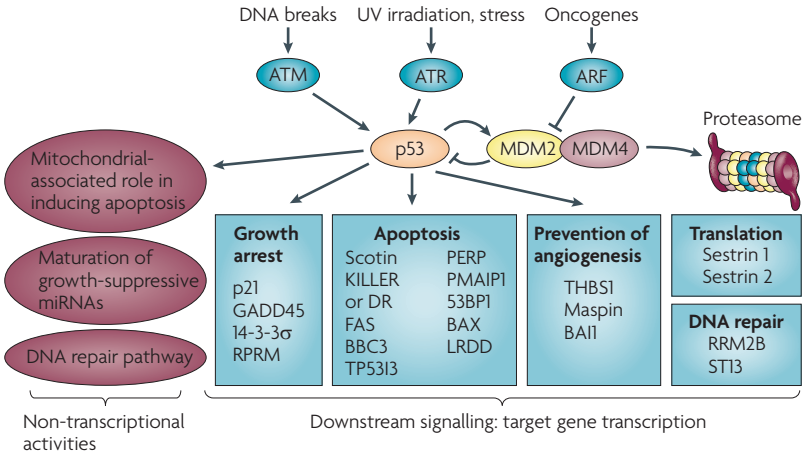


Figure 1 | **The p53 pathway.** p53 is at the centre of a complex web of biological interactions that translates stress signals into cell cycle arrest or apoptosis[26]. Upstream signalling to p53 increases its level and activates its function as a transcription factor in response to a wide variety of stresses, whereas downstream components execute the appropriate cellular response. The principal sensors seem to be MDM2 and MDM4 and their interaction with p53. In non-stressed conditions these proteins bind p53, ubiquitylate it and target it for degradation by the proteasome. In stressed conditions the function of the MDM2–MDM4 complex is blocked by phosphorylation, protein-binding events and/or enhanced degradation[141]. Hence, phosphorylation of MDM4 is essential for the p53 response to ionizing radiation, and the response to oncogene activation depends on the binding of ARF to MDM2. Many p53-activating small molecules function by causing the release of ribosomal proteins from the nucleolus to the nucleoplasm, where they bind to MDM2 and MDM4 and inhibit their function. Molecules that activate wild-type p53 in tumours by disrupting MDM2 activity can compensate for any missing upstream components of the p53 pathway, for example the loss of ARF expression that is frequent in cancer cells[142]. However, defective downstream p53 signalling might substantially decrease their effectiveness. Therefore, the ability to identify tumours in which downstream p53 signalling is unaffected is important. The development of strategies to ensure that the desired p53 response is initiated when it is reactivated might be necessary and could require the judicious use of drug combinations. 53BP1, p53 binding protein 1; ATM, ataxia telangiectasia mutated; ATR, ataxia telangiectasia and Rad3 related; BAI1, brain-specific angiogenesis inhibitor 1; BAX, BCL2-associated X protein; BBC3, BCL2 binding component 3 (also known as PUMA); DR, death receptor; GADD45, growth arrest and DNA-damage-inducible 45; KILLER, p53-regulated DNA damage-inducible cell death receptor (also known as TNFRSF10B); LRDD, leucine-rich repeats and death domain containing; miRNA, microRNA; PMAIP1, phorbol-12-myristate-13-acetate-induced protein 1 (also known as NOXA); RPRM, reprimo; RRM2B, ribonucleotide reductase M2 B; ST13, suppression of tumorigenicity 13 (also known as p48); TP53I3, tumour protein p53 inducible protein 3; THBS1, thrombospondin 1; UV, ultraviolet.

The therapeutic approaches discussed above have focused strongly on traditional high-throughput and computational design strategies (BOX 2). Another attractive approach is to use cell-based screens for identifying p53 pathway-selective drugs. This FCG approach has several advantages and challenges over more traditional screens[70]. 'Hit' compounds from a cell-based screen are generally not cytotoxic, as they are selected for their ability to increase a synthetic event — that is, the accumulation of a reporter gene product. Searching for compounds that activate the transcriptional activity of mutant p53 in cells should lead to the discovery of compounds that directly interact with p53 or compounds that affect the folding and processing of proteins. The most advanced of these molecules, which is now in clinical trials (APREA, Sweden), is the compound PRIMA-1. It was discovered following a cell-based screen in which Bykov and colleagues[49] established a Saos-2 (*TP53*-null) osteosarcoma cell line with a tetracycline-inducible mutant *TP53* gene. They screened a small library of diverse compounds obtained from the US National Cancer Institute (NCI) in a simple 48 hour cell growth assay (using WST-1, a tetrazolium salt that is cleaved to formazan by cellular enzymes) and looked for molecules that reduced growth in a p53 mutant-selective manner. PRIMA-1 was identified and shown to protect several p53 mutants from unfolding *in vitro* and to restore p53-dependent transcription in various cell-based systems expressing the p53 mutants H273 and H175, such as the SKOV, H1299 and SW80 cell lines. However, the exact determination of the mechanism of action of this fascinating molecule has proved elusive. Recent papers have demonstrated that although it has activity in p53-independent cell-based phenotypic assays it can nevertheless form adducts with p53 through a mechanism that involves the covalent modification of cysteine residues[71]. This is a provocative result as several natural products that target particular cellular proteins with high specificity have been shown to use this Michael acceptor-based mechanism. For example, leptomycin B — a known activator of wild-type p53 — binds to the nuclear export protein CRM1 (also known as XPO1) by forming a covalent bond at Cys528 (REF. 72).

The genetic concept of synthetic lethality also provides an alternative framework for identifying genotype-selective anticancer agents. In this approach, changes

Michael acceptor
The Michael reaction occurs between a Michael donor (such as cysteine residues in proteins) and a Michael acceptor molecule (such as leptomycin B) in the presence of a base. The reaction itself is the nucleophillic addition of a carbanion to an α-, β-unsaturated carbonyl compound.

Synthetic lethality
Two genes are in a synthetic lethal relationship if a mutation in both genes leads to cell death but a mutation in one gene alone does not.

Aptamers
Molecules that interact with a specific target molecule usually generated from a large random artificial library, which can be RNA-, DNA-, peptide- or protein-based.

Cis-imidazoline compounds
A class of compounds synthesized around a core imidazole structure, such as the nutlins.

Benzodiazepenes
Chemical compounds with a core chemical structure that is the fusion of a benzene ring and a diazepine ring.

Spiro-oxindole
A molecule with a core scaffold that contains a tryptophan-like structure.

IC_{50}
The concentration of a drug that causes a 50% inhibition of the activity of a target enzyme.

in cellular physiology that arise as a consequence of oncogene activation or tumour suppressor gene loss, rather than oncoproteins themselves, are targeted to achieve tumour selectivity. Therefore, in the case of p53 therapeutics this could lead to compounds that kill p53 mutant cells only. All the approaches described so far rely on the restoration of transcriptional activity to mutant p53. However, the cell-based screening approach might also yield compounds that kill p53 mutant tumour cells because these cells lack selective cell cycle checkpoint or repair mechanisms. This would be directly analogous to the discovery of the sensitivity of BRCA1- or BRCA2-negative cells to PARP inhibitors[73]. PARP1 activity is required for base-excision repair, a DNA repair mechanism that eliminates DNA bases that have been damaged by oxidation during the normal cell cycle. If PARP1 is chemically inhibited, oxidized bases accumulate and DNA replication forks stall at sites of DNA damage, which leads to double-stranded DNA breaks. Homologous recombination normally repairs these breaks, but cell death occurs if this mechanism is absent (as is the case with cancer cells deficient in BRCA1 or BRCA2). These approaches are at an early stage, but the lack of a DNA damage-induced G1 checkpoint in p53 mutant cells has been reported to make them more susceptible to anti-mitotic agents such as polo-like kinase 1 (PLK1) inhibitors[52].

Reactivating wild-type p53

The search for drugs that activate wild-type p53 has used both target-based approaches and FCG methods (BOX 2). Inhibition of p53 activity in tumours by the increased expression of MDM2 has been the target of development for many small-molecule-, peptide- and aptamer-based therapies. MDM2 is overexpressed in many human tumours, often owing to an amplification of a chromosome segment that includes *MDM2* (REF. 74), although overexpression of the protein is possible without gene amplification[75]. There has been extensive validation of MDM2 as a target, ranging from studies with aptamers and peptides through to antisense approaches and, perhaps most tellingly, a set of remarkable experiments using a hypomorphic allele of *Mdm2* in the mouse[76]. In these systems, small reductions in MDM2 levels are sufficient to trigger a mild p53 response (as shown by increased levels of lymphopenia and apoptosis in intestinal crypts) in response to increased p53 activity. The volume of the thymus is also reduced and there is a small effect on weight gain during development. Gene dosage studies have found levels of MDM2 that selectively inhibit the development of colon carcinoma induced by the absence of adenomatous polyposis coli (*APC*) without adverse affects on normal tissues[76]. These powerful studies provided proof of a therapeutic index for MDM2 inhibition that has now been confirmed by the first small molecule candidates, including nutlin[8], MI-219 (REF. 48) and reactivation of p53 and induction of tumour cell apoptosis (RITA; also known as NSC 652287 and discussed below)[51], which produce tumour regression *in vivo* in human tumour xenografts in nude mice.

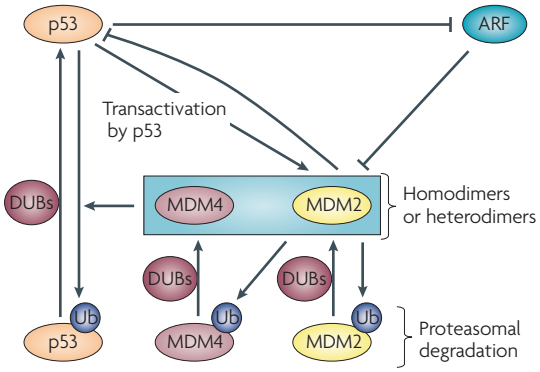


Figure 2 | **A negative feedback loop controls cellular levels of p53.** In normal cells, p53 increases transcription of MDM2 over basal levels. MDM2 also inhibits p53 function by modulating its transcriptional activity by preventing its interaction with the general transcription machinery[28]. MDM2 also forms a heterodimeric complex with MDM4 that promotes the degradation of p53 (REF. 92). ARF adds another level of control to the system by inhibiting MDM2 function[143], the expression of which is in turn also repressed by p53 (REF. 144). MDM2, as well as promoting p53 degradation, ubiquitylates (Ub) MDM4 (REFS 94,96) and promotes the degradation of the MDM2–MDM4 complex in response to ionizing radiation. This is mediated at least in part through ATM- and CHK2- dependent phosphorylation of MDM4, which inhibits binding to 14-3-3 proteins and the deubiquitylating enzyme USP7 (also known as herpes virus-associated ubiquitin-specific protease (HAUSP)), thus permitting its proteasomal destruction[141,145,146]. These cellular mechanisms result in subtle control of p53 levels. ATM, ataxia telangiectasia mutated; DUB, deubiquitylase.

Nutlin-type inhibitors. The first reported small-molecule MDM2 antagonists with *in vivo* activity, the nutlins, were identified from a class of *cis*-imidazoline compounds[8] (FIG. 3). The nutlins could displace p53 from MDM2 *in vitro* with nanomolar potency. Crystal structure studies demonstrated that the nutlins bind to the p53 pocket of MDM2 in a way that mimics the molecular interactions between MDM2 and the crucial amino acid residues from p53 (REF. 8). Nutlins enter many types of cultured cells and inhibit the p53–MDM2 interaction with a high degree of specificity, leading to the stabilization of p53 and the activation of the p53 pathway[8]. Proliferating cancer cells that express wild-type p53 are effectively arrested in the G1 and G2 phases of the cell cycle or can undergo apoptosis when treated with micromolar concentrations of nutlins[77]. This indicates that some cells are more susceptible to nutlin-induced apoptosis than other cells in which a reversible cell cycle arrest is observed. The key cellular characteristics that underlie this difference in response are the subject of intense investigation. This differential response may occur owing to abnormalities further downstream in the p53 pathway.

Other small molecules that have been developed to target the p53–MDM2 interaction include benzodiazepenes[78] and spiro-oxindole[79,80]. The benzodiazepene-based derivatives disrupt the MDM2–p53 interaction *in vitro* with IC_{50} values of 0.5–2 μM and have also been shown to suppress the growth of cell lines containing

wild-type p53. Administration of the benzodiazepene derivative TDP665759 to normal mice led to an increase in p21 (also known as WAF1 and CIP1) levels in liver samples[81]. Finally, TDP665759 synergizes with doxorubicin both in culture and in xenografts of A375 melanoma cells to decrease tumour growth[81–83]. Ding *et al.*[79,80] identified several compounds with a spiro-oxindole core structure that could inhibit the MDM2–p53 interaction *in vitro* in numerous cell lines with IC_{50} values of 30–2000 nM[80]. This work has resulted in a spiro-oxindole derivative, MI-219, which shows good pharmacodynamics and bioavailability in mice compared with the initial spiro-oxindole compounds[84]. This compound induces tumour regression in the absence of tissue-specific toxicity, although it induces low levels of p53 in normal tissues, which activates p21. However, these low levels of p53 are not sufficient to induce apoptosis even in sensitive tissues such as the thymus[48].

There is little doubt that given the range of pharmacophores already identified against the MDM2–p53 interaction, in conjunction with the excellent preclinical profiles reported for nutlin and MI-219, effective inhibitors can be developed. However, several potential drawbacks to targeting the MDM2–p53 interaction can be envisioned. First, MDM2 is induced by p53 activation as part of an inducible feedback loop that negatively regulates the p53 response. Therefore, the drugs would induce their target, limiting their potential efficacy. Second, the current molecules fail to effectively target MDM4.

The interplay of MDM2 and MDM4 in regulating wild-type p53. Genetic studies indicate that MDM2 and MDM4 have non-redundant functions to keep p53 inactive during embryogenesis and throughout development[85,86]. Deficiency in either protein results in embryonic lethality, which is dependent on p53 status. Therefore, *Trp53*$^{-/-}$*Mdm2*$^{-/-}$ mice are viable, whereas *Trp53*$^{+/-}$*Mdm2*$^{-/-}$ results in embryonic lethality; this is also the case for *Mdm4*$^{-/-}$ mice[85–87]. Therefore, the expression of both MDM2 and MDM4 is necessary for the regulation of p53 during development.

MDM4, unlike MDM2, does not have significant intrinsic E3 ubiquitin ligase activity[88]. However, MDM4 forms heterodimers with MDM2 through C-terminal RING domain interactions[89], which stimulates MDM2 to ubiquitylate p53 leading to its degradation[90–93]. Another consequence of MDM2–MDM4 heterodimerization is that MDM4 can be ubiquitylated by MDM2 and is consequently degraded[94–96]. This is an important mechanism for controlling MDM4 levels and thereby fine-tuning the levels of p53 during the stress response. In addition, MDM4 might exhibit greater stability in unstressed cells owing to its preferential deubiquitylation by herpes virus-associated ubiquitin-specific protease (HAUSP; also known as USP7)[97] (FIG. 2).

MDM4 overexpression has been found in 40% (13 of 31) of the tumour cell lines studied[98], and in 9% of breast (41 of 218), 19% of colon (5 of 27) and 18% of lung (16 of 88)[29] human primary tumours studied. More recently, 32 of 49 (65%) of human retinoblastoma samples investigated had extra copies of *MDM4* (REF. 30). In general, increased *MDM4* mRNA occurs in tumours with wild-type p53, and MDM4 knockdown in breast carcinoma and retinoblastoma cell lines leads to p53-dependent growth arrest or apoptosis[29,30,99]. This implies that in the absence of any other regulatory changes MDM4-dependent inhibition of p53 is crucial for tumorigenesis.

The amino-terminal of MDM4 shares 50% sequence similarity with that of MDM2 and binds to the same region of p53 (REF. 100); this region is therefore an obvious target for drug development as it could be specific for both MDM2 and MDM4. Such dual-specific peptides have been described[101]. Although peptides themselves are not often effective drugs, owing to problems of cell penetration and stability, great progress has been reported using chemical methods to 'staple' peptides into a conformation that substantially improves their pharmacodynamics[102]. These peptides have been shown to interact with MDM2 at nanomolar affinities and enter SJSA-1 cells, as well as to induce p53-dependent transactivation of *CDKN1A* (which encodes p21)[102]. It is of great interest whether these peptides retain the activity of the unstapled peptide against MDM4, and the recent solution of the p53 binding domain structure of MDM4 will greatly assist these approaches[101,103].

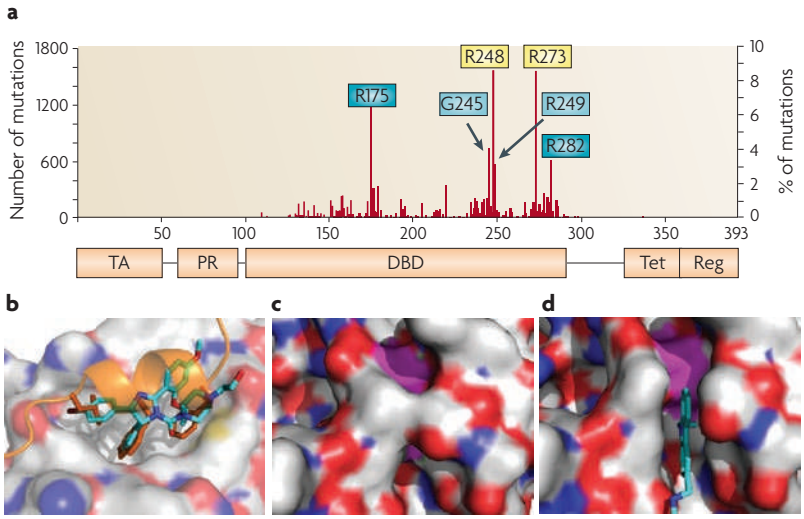


Figure 3 | **Structural applications to p53-based drug therapies. a** | Most inactivating mutations of p53 occur in the DNA-binding domain (DBD), whereas the amino-terminal transactivation domain (TA) is relatively free of point mutations. Mutations in yellow affect DNA contacts, those in light blue cause local distortions and those in dark blue cause global denaturation. **b** | Therapeutics that disrupt MDM2 binding to the TA, for example nutlin[8], are designed to reactivate wild-type p53 by displacing MDM2, which is overexpressed in many p53 wild-type tumours. The crystal structure of nutlin[8] (cyan) in complex with MDM2 shows how it mimics the three key residues from p53 (orange) that are involved in the interaction. The small molecule PhiKan083 has also been designed to specifically interact with the p53-Y220C mutant. This demonstrates the possibility of therapeutically targeting tumours with different inactivating mutations of p53. Parts **c** and **d** of the figure show the cleft generated on the surface of p53 by the missense mutation (**c**), where PhiKan083 (REF. 69) has also been shown to bind using crystallography (**d**). These two therapeutic strategies are designed to reactivate p53 but differ in that the first method reactivates wild-type p53 and the second reactivates mutant p53. PR, proline-rich domain; Reg, carboxy-terminal regulatory domain; Tet, tetramerization domain.

Box 1 | *TP53* gene therapy

Jack Roth was the first scientist to carry out clinical trials of gene therapy for tumours using p53 and he subsequently established Introgen, which has an adenovirus vector-based *TP53* gene therapy (Advexin) in numerous clinical trials in the United States. Although some of these trials have reached Phase III the treatment has not yet crossed that final approval hurdle. Meanwhile, in China, Shenzhen SiBiono GenTech gained approval for its similar product, Gendicine, in October 2004 and has been marketing it ever since. Side effects are mild but the treatment requires local injection into the tumour site and may not be effective in all patients compared with the best standard of care. Attempts to improve efficacy by modifying p53 and enhancing delivery remain an active area of research. The publication of extensive post-registration efficacy data from Shenzhen SiBiono GenTech is eagerly awaited.

Other specific targets in the MDM2–MDM4 regulatory pathway are also being identified. Sites in MDM2 and MDM4 that are involved in the E3 ubiquitin ligase activity, binding ARF and interactions with other regulatory proteins have been shown to be potential targets[104]. Molecules have also been reported by Yang *et al.*[105] that inhibit the E3 ubiquitin ligase activity of MDM2 and as a result cause activation of p53 and the accumulation of MDM2. In addition, a screen for nutlin resistance yielded the DNA repair protein, p53-binding protein 1 (53BP1), as a potential target[106]. RNA interference-based studies of the p53 pathway have identified several deubiquitylating enzymes and proteasome subunits, the inactivation of which induces a p53 response[107].

The binding pocket of the N terminus of MDM2 has shown itself to be eminently druggable, and a future challenge is whether or not these drugs can proceed to the clinic and whether they can also be refined to target MDM4. Apart from this avenue of research, other target sites have been identified in this p53 regulatory pathway that show the potential for drug development, and it remains to be seen if they generate therapeutic leads that have low toxicity in normal tissues.

Cell-based screens for p53-activating molecules

Phenotypic screens offer some advantages over screens using purified components as they select compounds that are cell permeable and active in cells at concentrations that are reasonable for further tests in animals. In the p53 research field, screens typically use the induction of a p53 reporter in a cell line as a readout, and this has led to the identification of a wide variety of compounds, of which some have been proved to have anti-tumour activity *in vivo*. Once a hit compound with interesting properties *in vitro* and *in vivo* is selected, the biggest challenge is to elucidate how the compound achieves p53 activation. This is important from a basic research point of view as it could reveal new p53 regulatory factors. Additionally, it enables further optimization of a primary hit compound based on the structure of the binding site of the compound. So far, p53-based phenotypic screens have led to the discovery of several categories of compounds (discussed below).

Compounds that interact with DNA or affect topoisomerase function. Given the role of p53 as "the guardian of the genome" (REF. 108), DNA-interacting compounds and topoisomerase inhibitors are (not unexpectedly) frequent hits in p53 cell-based phenotypic screens[109,110]. These can easily be identified through reporter assays such as DNA binding experiments, topoisomerase activity assays, the induction of DNA damage-induced p53 phosphorylation at Ser15 or Ser20, the increased phosphorylation of ataxia telangiectasia mutated (ATM) and histone H2AX (which are indicative of a DNA damage response), or comet assays, which detect broken DNA. However, there are interesting examples of DNA intercalators that have been selected from p53 phenotypic screens that do not cause extensive DNA damage, such as the anti-malaria drug quinacrine[111]. Chloroquine, another DNA intercalating anti-malaria drug, activates ATM and induces p53 without causing DNA breaks and prevents lymphoma development in a p53-dependent manner *in vivo*[112]. This work also suggests that the chloroquine-mediated inhibition of lysosomal protein degradation may be related to the induction of p53-dependent cell death.

Inhibitors of nucleic acid synthesis. Deoxyribonucleotide triphosphate (dNTP) biosynthesis inhibitors induce the accumulation of cells in S phase of the cell cycle, leading to extensive genomic damage. When cells are treated with these compounds, or with aphidicolin (which inhibits DNA polymerase-α), p53 is stabilized, but only certain downstream effectors such as PIG3 (also known as TP53I3), but not p21 or MDM2, are expressed[113]. Inhibitors of the ribonucleotide synthesis pathway such as PALA (n-phosphonacetyl-L-aspartate) and mycophenolic acid cause an increase in p53 levels and the accumulation of cells in G1 or G0 of the cell cycle in a p53-dependent manner without inducing chromosomal aberrations[114,115]. An obvious consequence of ribonucleotide depletion is the inhibition of transcription. One possible explanation for the activation of p53 without the induction of DNA damage is that p53 senses ribonucleotide depletion partly through the inhibition of RNA elongation. In fact the mRNA synthesis inhibitor and adenosine analogue DRB (5,6-dichloro-l-β-D-ribofuranosylbenzimidazole) can increase p53 levels and transcriptional activity[116,117].

Screening for activators of wild-type p53-dependent transcription in cells has led to the identification of two ribonucleoside analogues, NCI188491 and NCI154829, that are similar in structure to the purine ribonucleoside analogue sangivamycin[110]. All three compounds increase p53 levels and transcriptional activity in cells at submicromolar concentrations. In the case of the two NCI compounds, p53 activation was not accompanied by substantial increases in genome-damage markers, although the exact mechanism by which these compounds and sangivamycin activate p53 and the effect of these molecules in preclinical models are still unknown

A large screen of a natural product library identified actinomycin D as a potent p53-activating compound[118]. One of the clearest effects of actinomycin D is to inhibit ribosomal RNA synthesis, which disrupts the nucleoli and prevents ribosome biogenesis, thus causing the release of the ribosomal protein L11 (RPL11) and RPL5, which bind and inactivate MDM2 (REF. 119). Mutations in the zinc finger region of MDM2 that abolish RPL11

Topoisomerse inhibitors
Chemotherapy agents that interfere with the actions of topoisomerase 1 and topoisomerase 2, which are involved in DNA replication during the cell cycle.

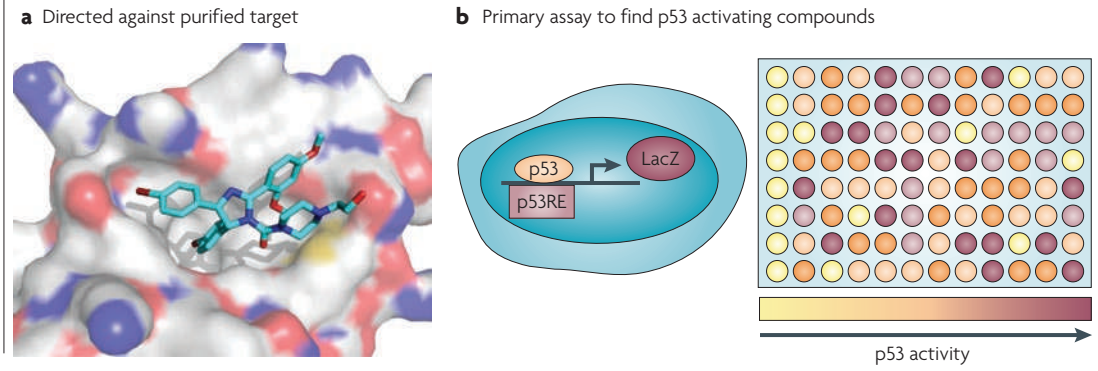
Box 2 | **Routes to drug discovery**

Direct against a purified target

This is the route by which most drugs have recently been discovered and is highly favoured by the pharmaceutical industry[139]. It typically involves the development of a small-molecule inhibitor of an enzyme or receptor that has already been identified as crucial to the disease process, such as the BCR-ABL1 kinase in chronic myelogenous leukaemia (CML)[4]. Large chemical libraries (sometimes as many as 3 million compounds) are then screened in high-throughput assays for the inhibition of the target protein function. Various assay formats are used, with recent developments including reduced assay volumes and extensive automation. The approach is supplemented by structure-based design in which the X-ray or nuclear magnetic resonance (NMR)-determined structure (as shown in part **a** of the figure for the p53–MDM2 interaction) of the active site is used in computational approaches for virtual screening to validate and improve the binding of small molecules to the target protein. Recently, small molecules (less than 350 Da) have also been used to help in lead finding in a process known as fragment-based screening[140]. Once 'hits' have been developed, teams of medicinal chemists work to improve potency, bioavailability and reduce the toxicity of the hit to convert it into a drug lead. This process has been likened to solving a Rubik's cube as all three aspects must be optimized in the final drug. The big advantage of this approach is that the target is known and so biophysical and structural methods can readily be applied. The challenges are that not all hits can be converted to leads (owing to problems with chemistry), not many proteins are suitable targets for this approach (owing to difficulties including low expression and purification) and our incomplete understanding of biology means that target validation may fail such that unexpected side effects are seen in humans.

Indirect against a pathway

This is the route by which most 'old' drugs were discovered. For example, some antibiotics (such as penicillin) and analgesics (such as aspirin) were developed and widely used without knowing how they worked or what their precise molecular targets were. In cases in which no targets are well defined (such as for the development of new malaria treatments) or in which the purified target approach has been unsuccessful (for example, the mutant Ras protein) then these indirect routes are attractive. In this method large numbers of compounds are screened for activity in phenotypic assays searching for selective cell killing or the activation of a transcription reporter, as illustrated in part **b** of the figure for p53. The main advantages of this method are that any phenotype is in principal druggable: the compounds by definition show biological activity and therefore have some drug-like properties. The major difficulty is that the medicinal chemists have few clues as to how to improve potency as the key features of the molecule that interacts with the target are unknown. Therefore, a key area of activity in the field is improving methods by which the targets of unknown active compounds can rapidly be determined so that targets validated by such forward chemical genetic or phenotypic screens can then be developed using direct target-based approaches. An example is the tenovins, which were shown to be active inducers of p53, and their protein target was then identified to be the sirtuins[50].



binding render cells containing wild-type p53 resistant to RPL11 inhibition[120]. Because DRB treatment, nucleotide depletion and DNA damage also lead to nucleolar disruption[121], it is tempting to speculate that increased interaction of MDM2 with free ribosomal proteins is one of the main mechanisms leading to p53 activation.

Although actinomycin D, which causes DNA damage, is a rather toxic drug at high doses, initial studies using low doses showed that it was very specific for inducing p53 activity[118]. Inactivation of p53 had previously been found to render cells completely resistant to the growth inhibitory effects of the drug[122]. Remarkably, using gene expression arrays to compare the effects of actinomycin D with the effects of nutlin showed that low-dose actinomycin D induced an identical pattern of gene expression in p53 wild-type and p53 mutant cells as the pattern induced by nutlin[118]. Actinomycin D is clinically approved and is effective in the treatment of Wilm's tumour[123].

Compounds that disrupt mitosis. The treatment of cells with classic chemotherapeutics such as taxol or the vinca alkaloids markedly increases p53 levels, an event that is essential to avoid polyploidy in response to these tubulin poisons. Exactly how p53 is stabilized and activated in response to mitotic poisons is still under investigation, but p53-based phenotypic screens led to the identification of numerous mitotic poisons. In a recent study, Staples and co-workers[124] identified 17 inhibitors of mitosis among the top 33 hits from a phenotypic screen

Taxol
A compound that stabilizes microtubules by irreversibly binding to the β-subunit of tubulin.

Vinca alkaloids
Anti-mitotic and anti-microtubule agents that prevent tubulin polymerization and so interfere with chromosomal replication and subsequent separation.

(using the activation of p53-dependent transcription) of 30,000 compounds. All but four of these compounds proved to be inhibitors of tubulin polymerization, and an optimized version of one, JJ78:12, showed significant inhibition of xenograft tumour growth.

Compounds that directly interact with p53. So far, RITA is the only compound derived from a phenotypic screen that can be included in this category. This compound was shown to directly bind to p53 and inhibit the interaction of p53 with MDM2 (REF. 51). Whether this is the only mechanism by which RITA increases p53 activity in cells is still controversial[125,126], as there is evidence that RITA can bind to multiple proteins[127] and activate the DNA damage response pathways[126,128]. However, it is intriguing that the induction of markers of DNA damage (namely phosphorylation of CHK1 and histone H2AX) were reported to occur only in cells harbouring wild-type p53 (REF. 129). Although, like the nutlins, RITA can block the MDM2–p53 interaction, RITA is a more effective inducer of apoptosis than the nutlins. It has been shown that this might be due to the ability of MDM2, released from p53 by RITA, to degrade p21 and thus promote apoptosis rather than cell cycle arrest[130]. Another possible mechanism explaining the pro-apototic effect of RITA is its ability to inhibit the expression of survival factors in a p53-dependent manner[131]. These interesting observations and the *in vivo* anti-tumour activity of RITA[51,126] justifies further studies on its mechanism of action.

Compounds that target p53 regulatory factors. Examples of this type of compound that were identified through a p53-based phenotypic screen are tenovin-1 and its more water-soluble derivative tenovin-6 (REF. 50). Tenovins rapidly increase p53 levels in cells treated with low micromolar concentrations, and daily intraperitoneal injection of tenovin-6 at 50 mg per kg delays xenograft tumour growth in mouse models[50]. Through a yeast genetic screen and subsequent enzymatic assays tenovins were shown to inhibit the NAD^+-dependent deacetylase activity of SIRT1 and SIRT2 (REF. 50), two members of the sirtuin family of class III histone deacetylases. p53 deacetylation by SIRT1 impairs p53 stability and transcriptional activity[132–134]. Therefore, inhibiting the sirtuins should lead to increased p53 stability. Indeed, treatment of MCF-7 cells with tenovins led to the accumulation of acetylated p53 and acetylated tubulin, which are established substrates of SIRT1 and SIRT2, respectively. Further chemical optimization of the potency of the tenovins is now possible owing to the elucidation of SIRT1 and SIRT2 as the cellular targets. The discovery and characterization of the tenovins is an example of how current technological advances in target identification and p53 basic research contribute to the understanding of the mechanism of action of bioactive small molecules.

Aside from screening approaches, potent p53 activators have been found by simply testing candidate small molecules that are known to modulate a phenomenon that is likely to impinge on p53 activity. The clearest examples of this are the nuclear export inhibitor leptomycin B and its derivatives. The natural compound leptomycin B inhibits CRM1 and new variants of it — with improved therapeutic windows — are producing promising results in pre-clinical xenograft models[135].

So far, there have been no reports of compounds derived from phenotypic screens that directly interact with MDM2 or MDM4 even though analysis of nutlin activity and MDM2 binding peptides (which were identified with direct screening approaches) shows that such compounds would be highly active in cell-based assays.

Drug combinations and cyclotherapy

The multiple signalling pathways that converge on p53 and the role that p53 has in cell cycle checkpoints has stimulated the search for drug combinations that might exploit the p53 system (TABLE 1). Indeed, low doses of p53-activating drugs such as nutlin, DRB and roscovitine have shown clear synergy in activating p53-induced apoptosis in human wild-type p53 tumour cells[53]. However, the approach that is currently attracting the most excitement is the idea of treating patients with p53 mutant tumours using cylcotherapy[136]. According to this concept, normal cells in the body are placed into a transient cell cycle arrest by activation of p53 with a non-genotoxic drug such as nutlin or low-dose actinomycin D[118,137]. However, the p53-mutant tumour cells would be unaffected and continue to proliferate. The patient would then be treated with a mitotic inhibitor, such as a PLK1 inhibitor or a taxane, to selectively target the cycling tumour cells[52]. The p53-induced arrest in non-tumour cells functions as a chemoprotectant of tissues that would otherwise be cycling and thereby damaged by the anti-mitotic drug. Therefore, side effects such as hair loss, damage to the alimentary canal and loss of white blood cells following chemotherapy would be prevented without reducing the anticancer activity of the anti-mitotic drug. The lack of toxicity of a temporary cell cycle arrest in the normal tissues of an adult mouse has been demonstrated by Gerard Evan's laboratory with an inducible dominant-negative MYC inhibitor[138]. In various tissue culture models cyclotherapy has proved to be remarkably effective, but the real excitement has been generated by the recent study in which nutlin treatment inhibited the neutropenia that was induced by the PLK1 inhibitor BI-2536 without blocking its anti-tumour effect[52] (TABLE 1).

Conclusions and summary

There is little doubt that drugs that exploit the loss of p53 pathway function could have wide applications in the treatment of cancer. As p53 is not a conventional target for the translational industry, the desire to exploit the p53 system has led to many new approaches. These include the search for molecules that affect protein folding and stability, as well as molecules that block protein–protein interactions. It has also been suggested that various phenotypic screening approaches and the combinatorial use of drugs could improve the selectivity and therapeutic index. In the sense that many other pathways in biology offer a similar challenge, the intense effort to target the p53 pathway is encouraging and supportive of the development of new approaches to drug discovery and therapy.

Roscovitine
An olomoucine-related purine flavopiridol, which is a highly potent inhibitor of the kinase activity of cyclin-dependent kinases CDK1, CDK2, CDK5 and CDK7. It induces the activation, stabilization and accumulation of p53 in the nucleus through the suppression of MDM2 expression and partial inhibition of its transcription.

Neutropenia
A haematological disorder characterized by an abnormally low number of neurophils.

1. Paez, J. G. *et al.* EGFR mutations in lung cancer: correlation with clinical response to gefitinib therapy. *Science* **304**, 1497–1500 (2004).
2. Pao, W. *et al.* EGF receptor gene mutations are common in lung cancers from "never smokers" and are associated with sensitivity of tumors to gefitinib and erlotinib. *Proc. Natl Acad. Sci. USA* **101**, 13306–13311 (2004).
3. Lynch, T. J. *et al.* Activating mutations in the epidermal growth factor receptor underlying responsiveness of non-small-cell lung cancer to gefitinib. *N. Engl. J. Med* **350**, 2129–2139 (2004).
4. Capdeville, R., Buchdunger, E., Zimmermann, J. & Matter, A. Glivec (STI571, imatinib), a rationally developed, targeted anticancer drug. *Nature Rev. Drug Discov.* **1**, 493–502 (2002).
5. Baselga, J. & Swain, S. M. Novel anticancer targets: revisiting ERBB2 and discovering ERBB3. *Nature Rev. Cancer* **9**, 463–475 (2009).
6. Clackson, T. & Wells, J. A. A hot spot of binding energy in a hormone-receptor interface. *Science* **267**, 383–386 (1995).
7. Oltersdorf, T. *et al.* An inhibitor of Bcl-2 family proteins induces regression of solid tumours. *Nature* **435**, 677–681 (2005).
8. Vassilev, L. T. *et al. In vivo* activation of the p53 pathway by small-molecule antagonists of MDM2. *Science* **303**, 844–848 (2004).
This paper describes the successful isolation and characterization of the nutlin compounds that activate p53 by binding to MDM2 and blocking its interaction with p53. The authors show that the molecules are highly specific and active in xenograft models.
9. Dantzer, F. *et al.* Base excision repair is impaired in mammalian cells lacking Poly(ADP-ribose) polymerase-1. *Biochemistry* **39**, 7559–7569 (2000).
10. Ame, J. C., Spenlehauer, C. & de Murcia, G. The PARP superfamily. *Bioessays* **26**, 882–893 (2004).
11. Edwards, S. L. *et al.* Resistance to therapy caused by intragenic deletion in BRCA2. *Nature* **451**, 1111–1115 (2008).
12. Yu, J. & Zhang, L. No PUMA, no death: implications for p53-dependent apoptosis. *Cancer Cell* **4**, 248–249 (2003).
13. Moll, U. M., Wolff, S., Speidel, D. & Deppert, W. Transcription-independent pro-apoptotic functions of p53. *Curr. Opin. Cell Biol.* **17**, 631–636 (2005).
14. Suzuki, H. I. *et al.* Modulation of microRNA processing by p53. *Nature* **460**, 529–533 (2009).
15. Ahn, J. *et al.* Dissection of the sequence-specific DNA binding and exonuclease activities reveals a superactive yet apoptotically impaired mutant p53 protein. *Cell Cycle* **8**, 1603–1615 (2009).
16. Mummenbrauer, T. *et al.* p53 protein exhibits 3'-to-5' exonuclease activity. *Cell* **85**, 1089–1099 (1996).
17. Sengupta, S. & Harris, C. C. p53: traffic cop at the crossroads of DNA repair and recombination. *Nature Rev. Mol. Cell Biol.* **6**, 44–55 (2005).
18. de Souza-Pinto, N. C., Harris, C. C. & Bohr, V. A. p53 functions in the incorporation step in DNA base excision repair in mouse liver mitochondria. *Oncogene* **23**, 6559–6568 (2004).
19. Sommers, J. A. *et al.* p53 modulates RPA-dependent and RPA-independent WRN helicase activity. *Cancer Res.* **65**, 1223–1233 (2005).
20. Budanov, A. V. & Karin, M. p53 target genes sestrin1 and sestrin2 connect genotoxic stress and mTOR signaling. *Cell* **134**, 451–460 (2008).
21. Feng, Z. *et al.* The regulation of AMPK β1, TSC2, and PTEN expression by p53: stress, cell and tissue specificity, and the role of these gene products in modulating the IGF-1-AKT-mTOR pathways. *Cancer Res.* **67**, 3043–3053 (2007).
22. Martins, C. P., Brown-Swigart, L. & Evan, G. I. Modeling the therapeutic efficacy of p53 restoration in tumors. *Cell* **127**, 1323–1334 (2006).
This paper shows that restoration of p53 function in tumours using an inducible system is highly effective in inhibiting the growth of even advanced tumours.
23. Ventura, A. *et al.* Restoration of p53 function leads to tumour regression *in vivo*. *Nature* **445**, 661–665 (2007).
This paper demonstrates that p53 restoration induced by a genetic switch in a model system leads to tumour regression by apoptosis.
24. Xue, W. *et al.* Senescence and tumour clearance is triggered by p53 restoration in murine liver carcinomas. *Nature* **445**, 656–660 (2007).
This paper shows that in a mouse liver carcinoma model restoration of p53 activity in tumour cells induces senescence rather than cell death. Strikingly, the senescent cells are cleared by an innate immune response.
25. Bond, G. L., Hu, W. & Levine, A. A single nucleotide polymorphism in the *MDM2* gene: from a molecular and cellular explanation to clinical effect. *Cancer Res.* **65**, 5481–5484 (2005).
In this paper a single nucleotide polymorphism that regulates the expression of MDM2 is shown to affect the probability of developing cancer.
26. Vousden, K. H. & Lane, D. P. p53 in health and disease. *Nature Rev. Mol. Cell Biol.* **8**, 275–283 (2007).
27. Picksley, S. M. & Lane, D. P. The p53-mdm2 autoregulatory feedback loop: a paradigm for the regulation of growth control by p53? *Bioessays* **15**, 689–690 (1993).
28. 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* **69**, 1237–1245 (1992).
The original discovery of the p53–MDM2 interaction is described in this paper.
29. Danovi, D. *et al.* Amplification of *Mdmx* (or *Mdm4*) directly contributes to tumor formation by inhibiting p53 tumor suppressor activity. *Mol. Cell Biol.* **24**, 5835–5843 (2004).
30. Laurie, N. A. *et al.* Inactivation of the p53 pathway in retinoblastoma. *Nature* **444**, 61–66 (2006).
31. Riemenschneider, M. J. *et al.* Amplification and overexpression of the *MDM4* (*MDMX*) gene from 1q32 in a subset of malignant gliomas without *TP53* mutation or *MDM2* amplification. *Cancer Res.* **59**, 6091–6096 (1999).
32. Esteller, M. *et al.* $p14^{ARF}$ silencing by promoter hypermethylation mediates abnormal intracellular localization of MDM2. *Cancer Res.* **61**, 2816–2821 (2001).
33. Sherr, C. J. & Weber, J. D. The ARF/p53 pathway. *Curr. Opin. Genet. Dev.* **10**, 94–99 (2000).
34. Hainaut, P. & Hollstein, M. p53 and human cancer: the first ten thousand mutations. *Adv. Cancer Res.* **77**, 81–137 (2000).
35. Bullock, A. N., Henckel, J. & Fersht, A. R. Quantitative analysis of residual folding and DNA binding in mutant p53 core domain: definition of mutant states for rescue in cancer therapy. *Oncogene* **19**, 1245–1256 (2000).
36. Milner, J. & Medcalf, E. A. Cotranslation of activated mutant p53 with wild type drives the wild-type p53 protein into the mutant conformation. *Cell* **65**, 765–774 (1991).
37. Milner, J., Medcalf, E. A. & Cook, A. C. Tumor suppressor p53: analysis of wild-type and mutant p53 complexes. *Mol. Cell Biol.* **11**, 12–19 (1991).
38. Sigal, A. & Rotter, V. Oncogenic mutations of the p53 tumor suppressor: the demons of the guardian of the genome. *Cancer Res.* **60**, 6788–6793 (2000).
39. Levine, A. J. *et al.* The spectrum of mutations at the p53 locus. Evidence for tissue-specific mutagenesis, selection of mutant alleles, and a "gain of function" phenotype. *Ann. NY Acad. Sci.* **768**, 111–128 (1995).
40. Irwin, M. S. Family feud in chemosensitvity: p73 and mutant p53. *Cell Cycle* **3**, 319–323 (2004).
41. Li, Y. & Prives, C. Are interactions with p63 and p73 involved in mutant p53 gain of oncogenic function? *Oncogene* **26**, 2220–2225 (2007).
42. Strano, S. *et al.* Mutant p53: an oncogenic transcription factor. *Oncogene* **26**, 2212–2219 (2007).
43. Kim, E. & Deppert, W. Transcriptional activities of mutant p53: when mutations are more than a loss. *J. Cell Biochem.* **93**, 878–886 (2004).
44. Levine, A. J. & Oren, M. The first 30 years of p53: growing ever more complex. *Nature Rev. Cancer* **9**, 749–758 (2009).
45. Selivanova, G. *et al.* Restoration of the growth suppression function of mutant p53 by a synthetic peptide derived from the p53 C-terminal domain. *Nature Med.* **3**, 632–638 (1997).
46. Foster, B. A., Coffey, H. A., Morin, M. J. & Rastinejad, F. Pharmacological rescue of mutant p53 conformation and function. *Science* **286**, 2507–2510 (1999).
47. Joerger, A. C., Ang, H. C. & Fersht, A. R. Structural basis for understanding oncogenic p53 mutations and designing rescue drugs. *Proc. Natl Acad. Sci. USA* **103**, 15056–15061 (2006).
This paper describes the first key step in the rational design of specific drugs that can re-activate p53 by binding to it and protecting it from unfolding.
48. Shangary, S. *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. USA* **105**, 3933–3938 (2008).
49. Bykov, V. J. *et al.* Restoration of the tumor suppressor function to mutant p53 by a low-molecular-weight compound. *Nature Med.* **8**, 282–288 (2002).
In this paper the authors use a cell-based screen to search for molecules that only kill cells expressing mutant p53. They identify PRIMA-1 as a compound that has this activity and can restore wild-type p53 function to mutant p53. A modified version of PRIMA-1 (APR-246) is now in clinical trials.
50. Lain, S. *et al.* Discovery, *in vivo* activity, and mechanism of action of a small-molecule p53 activator. *Cancer Cell* **13**, 454–463 (2008).
This paper describes a cell-based screen that leads to the identification of new p53-activating molecules. The target of these new molecules is then defined using a genetic screen in yeast that shows that they function by blocking the deacetylation of p53 by the sirtuins.
51. Issaeva, N. *et al.* Small molecule RITA binds to p53, blocks p53-HDM-2 interaction and activates p53 function in tumors. *Nature Med.* **10**, 1321–1328 (2004).
52. Sur, S. *et al.* A panel of isogenic human cancer cells suggests a therapeutic approach for cancers with inactivated p53. *Proc. Natl Acad. Sci. USA* **106**, 3964–3969 (2009).
An exciting study that shows that prior activation of the p53 pathway with the MDM2 inhibitor nutlin protects against the neutrophil depletion that is induced by mitotic inhibitors that block the activity of PLK1. Using this drug combination can alleviate the side effects of chemotherapy without reducing its ability to kill p53 mutant tumour cells.
53. Cheok, C. F., Dey, A. & Lane, D. P. Cyclin-dependent kinase inhibitors sensitize tumor cells to nutlin-induced apoptosis: a potent drug combination. *Mol. Cancer Res.* **5**, 1133–1145 (2007).
54. Fang, B. & Roth, J. A. Tumor-suppressing gene therapy. *Cancer Biol. Ther.* **2**, S115–121 (2003).
55. Nishizaki, M. *et al.* Recombinant adenovirus expressing wild-type p53 is antiangiogenic: a proposed mechanism for bystander effect. *Clin. Cancer Res.* **5**, 1015–1023 (1999).
56. McCormick, F. Cancer-specific viruses and the development of ONYX-015. *Cancer Biol. Ther.* **2**, S157–160 (2003).
57. Joerger, A. C. & Fersht, A. R. Structural biology of the tumor suppressor p53. *Annu. Rev. Biochem.* **77**, 557–582 (2008).
58. Terzian, T. *et al.* The inherent instability of mutant p53 is alleviated by *Mdm2* or $p16^{INK4a}$ loss. *Genes Dev.* **22**, 1337–1344 (2008).
59. Hupp, T. R., Meek, D. W., Midgley, C. A. & Lane, D. P. Regulation of the specific DNA binding function of p53. *Cell* **71**, 875–886 (1992).
60. Hupp, T. R., Sparks, A. & Lane, D. P. Small peptides activate the latent sequence-specific DNA binding function of p53. *Cell* **83**, 237–245 (1995).
61. Selivanova, G., Ryabchenko, L., Jansson, E., Iotsova, V. & Wiman, K. G. Reactivation of mutant p53 through interaction of a C-terminal peptide with the core domain. *Mol. Cell Biol.* **19**, 3395–3402 (1999).
62. Kim, A. L. *et al.* Conformational and molecular basis for induction of apoptosis by a p53 C-terminal peptide in human cancer cells. *J. Biol. Chem.* **274**, 34924–34931 (1999).
63. Snyder, E. L., Meade, B. R., Saenz, C. C. & Dowdy, S. F. Treatment of terminal peritoneal carcinomatosis by a transducible p53-activating peptide. *PLoS Biol.* **2**, E36 (2004).
64. Lane, D. Curing cancer with p53. *N. Engl. J. Med.* **350**, 2711–2712 (2004).
65. Rippin, T. M. *et al.* Characterization of the p53-rescue drug CP-31398 *in vitro* and in living cells. *Oncogene* **21**, 2119–2129 (2002).
66. Stephen, C. W. & Lane, D. P. Mutant conformation of p53. Precise epitope mapping using a filamentous phage epitope library. *J. Mol. Biol.* **225**, 577–583 (1992).

67. Milner, J., Cook, A. & Sheldon, M. A new anti-p53 monoclonal antibody, previously reported to be directed against the large T antigen of simian virus 40. *Oncogene* **1**, 453–455 (1987).
68. Milner, J. Flexibility: the key to p53 function? *Trends Biochem. Sci.* **20**, 49–51 (1995).
69. Boeckler, F. M. *et al.* Targeted rescue of a destabilized mutant of p53 by an *in silico* screened drug. *Proc. Natl Acad. Sci. USA* **105**, 10360–10365 (2008).
70. Haggarty, S. J. *et al.* Dissecting cellular processes using small molecules: identification of colchicine-like, taxol-like and other small molecules that perturb mitosis. *Chem. Biol.* **7**, 275–286 (2000).
71. Lambert, J. M. *et al.* PRIMA-1 reactivates mutant p53 by covalent binding to the core domain. *Cancer Cell* **15**, 376–388 (2009).
72. Kudo, N. *et al.* Leptomycin B inactivates CRM1/exportin 1 by covalent modification at a cysteine residue in the central conserved region. *Proc. Natl Acad. Sci. USA* **96**, 9112–9117 (1999).
73. Farmer, H. *et al.* Targeting the DNA repair defect in BRCA mutant cells as a therapeutic strategy. *Nature* **434**, 917–921 (2005).
74. 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* **358**, 80–83 (1992).
75. Momand, J., Jung, D., Wilczynski, S. & Niland, J. The *MDM2* gene amplification database. *Nucleic Acids Res.* **26**, 3453–3459 (1998).
76. Mendrysa, S. M. *et al.* Tumor suppression and normal aging in mice with constitutively high p53 activity. *Genes Dev.* **20**, 16–21 (2006).
An elegant paper that uses hypomorphic alleles of *Mdm2* to show that slightly increased levels of p53 activity can allow normal growth while blocking tumour development.
77. Tovar, C. *et al.* Small-molecule MDM2 antagonists reveal aberrant p53 signaling in cancer: implications for therapy. *Proc. Natl Acad. Sci. USA* **103**, 1888–1893 (2006).
78. Grasberger, B. L. *et al.* Discovery and cocrystal structure of benzodiazepinedione HDM2 antagonists that activate p53 in cells. *J. Med. Chem.* **48**, 909–912 (2005).
79. Ding, K. *et al.* Structure-based design of potent non-peptide MDM2 inhibitors. *J. Am. Chem. Soc.* **127**, 10130–10131 (2005).
80. Ding, K. *et al.* Structure-based design of spiro-oxindoles as potent, specific small-molecule inhibitors of the MDM2-p53 interaction. *J. Med. Chem.* **49**, 3432–3435 (2006).
81. Koblish, H. K. *et al.* Benzodiazepinedione inhibitors of the Hdm2:p53 complex suppress human tumor cell proliferation *in vitro* and sensitize tumors to doxorubicin *in vivo*. *Mol. Cancer Ther.* **5**, 160–169 (2006).
82. Leonard, K. *et al.* Novel 1,4-benzodiazepine-2,5-diones as Hdm2 antagonists with improved cellular activity. *Bioorg. Med. Chem. Lett.* **16**, 3463–3468 (2006).
83. Parks, D. J. *et al.* Enhanced pharmacokinetic properties of 1,4-benzodiazepine-2,5-dione antagonists of the HDM2-p53 protein-protein interaction through structure-based drug design. *Bioorg. Med. Chem. Lett.* **16**, 3310–3314 (2006).
84. Shangary, S. & Wang, S. Small-molecule inhibitors of the MDM2-p53 protein-protein interaction to reactivate p53 function: a novel approach for cancer therapy. *Annu. Rev. Pharmacol. Toxicol.* **49**, 223–241 (2009).
85. Parant, J. *et al.* Rescue of embryonic lethality in *Mdm4*-null mice by loss of *Trp53* suggests a nonoverlapping pathway with MDM2 to regulate p53. *Nature Genet.* **29**, 92–95 (2001).
86. Montes de Oca Luna, R., Wagner, D. S. & Lozano, G. Rescue of early embryonic lethality in *mdm2*-deficient mice by deletion of p53. *Nature* **378**, 203–206 (1995).
This paper and reference 75 establish that p53 can induce embryonic lethality if it is not regulated by MDM2.
87. Jones, S. N., Roe, A. E., Donehower, L. A. & Bradley, A. Rescue of embryonic lethality in Mdm2-deficient mice by absence of p53. *Nature* **378**, 206–208 (1995).
88. Stad, R. *et al.* Mdmx stabilizes p53 and Mdm2 via two distinct mechanisms. *EMBO Rep.* **2**, 1029–1034 (2001).
89. Linke, K. *et al.* Structure of the MDM2/MDMX RING domain heterodimer reveals dimerization is required for their ubiquitylation in trans. *Cell Death Differ.* **15**, 841–848 (2008).
90. Tanimura, S. *et al.* MDM2 interacts with MDMX through their RING finger domains. *FEBS Lett.* **9447**, 5–9 (1999).
91. Sharp, D. A., Kratowicz, S. A., Sank, M. J. & George, D. L. Stabilization of the MDM2 oncoprotein by interaction with the structurally related MDMX protein. *J. Biol. Chem.* **274**, 38189–38196 (1999).
92. Linares, L. K., Hengstermann, A., Ciechanover, A., Muller, S. & Scheffner, M. HdmX stimulates Hdm2-mediated ubiquitination and degradation of p53. *Proc. Natl Acad. Sci. USA* **100**, 12009–12014 (2003).
93. Gu, J. *et al.* Mutual dependence of MDM2 and MDMX in their functional inactivation of p53. *J. Biol. Chem.* **277**, 19251–19254 (2002).
94. Pan, Y. & Chen, J. MDM2 promotes ubiquitination and degradation of MDMX. *Mol. Cell Biol.* **23**, 5113–5121 (2003).
95. Kawai, H. *et al.* DNA damage-induced MDMX degradation is mediated by MDM2. *J. Biol. Chem.* **278**, 45946–45953 (2003).
96. de Graaf, P. *et al.* Hdmx protein stability is regulated by the ubiquitin ligase activity of Mdm2. *J. Biol. Chem.* **278**, 38315–38324 (2003).
97. Wade, M. & Wahl, G. M. Targeting Mdm2 and Mdmx in cancer therapy: better living through medicinal chemistry? *Mol. Cancer Res.* **7**, 1–11 (2009).
98. Ramos, Y. F. *et al.* Aberrant expression of HDMX proteins in tumor cells correlates with wild-type p53. *Cancer Res.* **61**, 1839–1842 (2001).
99. Bartel, F. *et al.* Significance of *HDMX*-S (or *MDM4*) mRNA splice variant overexpression and *HDMX* gene amplification on primary soft tissue sarcoma prognosis. *Int. J. Cancer* **117**, 469–475 (2005).
100. Bottger, V. *et al.* Comparative study of the p53-mdm2 and p53-MDMX interfaces. *Oncogene* **18**, 189–199 (1999).
101. Pazgier, M. *et al.* Structural basis for high-affinity peptide inhibition of p53 interactions with MDM2 and MDMX. *Proc. Natl Acad. Sci. USA* **106**, 4665–4670 (2009).
102. Bernal, F., Tyler, A. F., Korsmeyer, S. J., Walensky, L. D. & Verdine, G. L. Reactivation of the p53 tumor suppressor pathway by a stapled p53 peptide. *J. Am. Chem. Soc.* **129**, 2456–2457 (2007).
103. Czarna, A. *et al.* High affinity interaction of the p53 peptide-analogue with human Mdm2 and Mdmx. *Cell Cycle* **8**, 1176–1184 (2009).
104. Karlsson, G. B. *et al.* Activation of p53 by scaffold-stabilised expression of Mdm2-binding peptides: visualisation of reporter gene induction at the single-cell level. *Br. J. Cancer* **91**, 1488–1494 (2004).
105. Yang, Y. *et al.* Small molecule inhibitors of HDM2 ubiquitin ligase activity stabilize and activate p53 in cells. *Cancer Cell* **7**, 547–559 (2005).
106. Brummelkamp, T. R. *et al.* An shRNA barcode screen provides insight into cancer cell vulnerability to MDM2 inhibitors. *Nature Chem. Biol.* **2**, 202–206 (2006).
107. Dayal, S. *et al.* Suppression of the deubiquitinating enzyme USP5 causes the accumulation of unanchored polyubiquitin and the activation of p53. *J. Biol. Chem.* **284**, 5030–5041 (2009).
108. Lane, D. P. Cancer. p53, guardian of the genome. *Nature* **358**, 15–16 (1992).
The news and views commentary that named p53 as "guardian of the genome", emphasizing its role in the DNA damage response.
109. Sohn, T. A., Bansal, R., Su, G. H., Murphy, K. M. & Kern, S. E. High-throughput measurement of the Tp53 response to anticancer drugs and random compounds using a stably integrated Tp53-responsive luciferase reporter. *Carcinogenesis* **23**, 949–957 (2002).
110. Berkson, R. G. *et al.* Pilot screening programme for small molecule activators of p53. *Int. J. Cancer* **115**, 701–710 (2005).
111. Gurova, K. V. *et al.* Small molecules that reactivate p53 in renal cell carcinoma reveal a NF-kappaB-dependent mechanism of p53 suppression in tumors. *Proc. Natl Acad. Sci. USA* **102**, 17448–17453 (2005).
112. Maclean, K. H., Dorsey, F. C., Cleveland, J. L. & Kastan, M. B. Targeting lysosomal degradation induces p53-dependent cell death and prevents cancer in mouse models of lymphomagenesis. *J. Clin. Invest.* **118**, 79–88 (2008).
113. Gottifredi, V., Shieh, S., Taya, Y. & Prives, C. p53 accumulates but is functionally impaired when DNA synthesis is blocked. *Proc. Natl Acad. Sci. USA* **98**, 1036–1041 (2001).
114. Sun, X. X., Dai, M. S. & Lu, H. Mycophenolic acid activation of p53 requires ribosomal proteins L5 and L11. *J. Biol. Chem.* **283**, 12387–12392 (2008).
115. Linke, S. P., Clarkin, K. C., Di Leonardo, A., Tsou, A. & Wahl, G. M. A reversible, p53-dependent G0/G1 cell cycle arrest induced by ribonucleotide depletion in the absence of detectable DNA damage. *Genes Dev.* **10**, 934–947 (1996).
116. te Poele, R. H., Okorokov, A. L. & Joel, S. P. RNA synthesis block by 5,6-dichloro-1-β-D-ribofuranosylbenzimidazole (DRB) triggers p53-dependent apoptosis in human colon carcinoma cells. *Oncogene* **18**, 5765–5772 (1999).
117. Ljungman, M., Zhang, F., Chen, F., Rainbow, A. J. & McKay, B. C. Inhibition of RNA polymerase II as a trigger for the p53 response. *Oncogene* **18**, 583–592 (1999).
118. Choong, M. L., Yang, H., Lee, M. A. & Lane, D. P. Specific activation of the p53 pathway by low dose actinomycin D: A new route to p53 based cyclotherapy. *Cell Cycle* **8**, 2810–2818 (2009).
119. Lohrum, M. A., Ludwig, R. L., Kubbutat, M. H., Hanlon, M. & Vousden, K. H. Regulation of HDM2 activity by the ribosomal protein L11. *Cancer Cell* **3**, 577–587 (2003).
120. Lindstrom, M. S., Jin, A., Deisenroth, C., White Wolf, G. & Zhang, Y. Cancer-associated mutations in the MDM2 zinc finger domain disrupt ribosomal protein interaction and attenuate MDM2-induced p53 degradation. *Mol. Cell Biol.* **27**, 1056–1068 (2007).
121. Rubbi, C. P. & Milner, J. Disruption of the nucleolus mediates stabilization of p53 in response to DNA damage and other stresses. *EMBO J.* **22**, 6068–6077 (2003).
122. Foster, S. A., Demers, G. W., Etscheid, B. G. & Galloway, D. A. The ability of human papillomavirus E6 proteins to target p53 for degradation *in vivo* correlates with their ability to abrogate actinomycin D-induced growth arrest. *J. Virol.* **68**, 5698–5705 (1994).
123. Green, D. M. *et al.* Comparison between single-dose and divided-dose administration of dactinomycin and doxorubicin for patients with Wilms' tumor: a report from the National Wilms' Tumor Study Group. *J. Clin. Oncol.* **16**, 237–245 (1998).
124. Staples, O. D. *et al.* Characterization, chemical optimization and anti-tumour activity of a tubulin poison identified by a p53-based phenotypic screen. *Cell Cycle* **7**, 3417–3427 (2008).
125. Krajewski, M., Ozdowy, P., D'Silva, L., Rothweiler, U. & Holak, T. A. NMR indicates that the small molecule RITA does not block p53-MDM2 binding *in vitro*. *Nature Med.* **11**, 1135–1136 (2005).
126. Yang, J. *et al.* Small-molecule activation of p53 blocks hypoxia-inducible factor 1α and vascular endothelial growth factor expression *in vivo* and leads to tumor cell apoptosis in normoxia and hypoxia. *Mol. Cell Biol.* **29**, 2243–2253 (2009).
127. Rivera, M. I. *et al.* Selective toxicity of the tricyclic thiophene NSC 652287 in renal carcinoma cell lines: differential accumulation and metabolism. *Biochem. Pharmacol.* **57**, 1283–1295 (1999).
128. Nieves-Neira, W. *et al.* DNA protein cross-links produced by NSC 652287, a novel thiophene derivative active against human renal cancer cells. *Mol. Pharmacol.* **56**, 478–484 (1999).
129. Yang, J., Ahmed, A. & Ashcroft, M. Activation of a unique p53-dependent DNA damage response. *Cell Cycle* **8**, 1630–1632 (2009).
130. Enge, M. *et al.* MDM2-dependent downregulation of p21 and hnRNP K provides a switch between apoptosis and growth arrest induced by pharmacologically activated p53. *Cancer Cell* **15**, 171–183 (2009).
131. Grinkevich, V. V. *et al.* Ablation of key oncogenic pathways by RITA-reactivated p53 is required for efficient apoptosis. *Cancer Cell* **15**, 441–453 (2009).
132. Luo, J. *et al.* Negative control of p53 by Sir2α promotes cell survival under stress. *Cell* **107**, 137–148 (2001).
133. Vaziri, H. *et al.* *hSIR2*^{SIRT1} functions as an NAD-dependent p53 deacetylase. *Cell* **107**, 149–159 (2001).
134. Langley, E. *et al.* Human SIR2 deacetylates p53 and antagonizes PML/p53-induced cellular senescence. *EMBO J.* **21**, 2383–2396 (2002).
135. Mutka, S. C. *et al.* Identification of nuclear export inhibitors with potent anticancer activity *in vivo*. *Cancer Res.* **69**, 510–517 (2009).

136. Blagosklonny, M. V. Basic cell cycle and cancer research: is harmony impossible? *Cell Cycle* **1**, 3–5 (2002).
137. Carvajal, D. *et al.* Activation of p53 by MDM2 antagonists can protect proliferating cells from mitotic inhibitors. *Cancer Res.* **65**, 1918–1924 (2005).
138. Soucek, L. *et al.* Modelling Myc inhibition as a cancer therapy. *Nature* **455**, 679–683 (2008).
139. van Montfort, R. L. & Workman, P. Structure-based design of molecular cancer therapeutics. *Trends Biotechnol.* **27**, 315–328 (2009).
140. Nienaber, V. *et al.* Discovering novel ligands for macromolecules using X-ray crystallographic screening. *Nature Biotechnol.* **18**, 1105–1108 (2000).
141. Wang, Y. V., Leblanc, M., Wade, M., Jochemsen, A. G., Wahl, G. M. Increased radioresistance and accelerated B Cell lymphomas in mice with *Mdmx* mutations that prevent modifications by DNA-damage-activated kinases. *Cancer Cell* **16**, 33–43 (2009).
142. Vassilev, L. T. MDM2 inhibitors for cancer therapy. *Trends Mol. Med.* **13**, 23–31 (2007).
143. Zhang, Y. & Xiong, Y. Control of p53 ubiquitination and nuclear export by MDM2 and ARF. *Cell Growth Differ.* **12**, 175–186 (2001).
144. Robertson, K. D. & Jones, P. A. The human ARF cell cycle regulatory gene promoter is a CpG island which can be silenced by DNA methylation and down-regulated by wild-type p53. *Mol. Cell Biol.* **18**, 6457–6473 (1998).
145. Honda, R. & Yasuda, H. Activity of MDM2, a ubiquitin ligase, toward p53 or itself is dependent on the RING finger domain of the ligase. *Oncogene* **19**, 1473–1476 (2000).
146. Fang, S., Jensen, J. P., Ludwig, R. L., Vousden, K. H. & Weissman, A. M. Mdm2 is a RING finger-dependent ubiquitin protein ligase for itself and p53. *J. Biol. Chem.* **275**, 8945–8951 (2000).
147. Newlands, E. S., Rustin, G. J., Brampton, M. H. Phase I trial elactocin. *Br. J. Cancer* **74**, 648–649 (1996).

DATABASES

Entrez Gene: http://www.ncbi.nlm.nih.gov/gene
ABL1 | *ERBB2* | *MYC*
National Cancer Institute Drug Dictionary: http://www.cancer.gov/drugdictionary/
actinomycin D | Advexin | BI-2536 | chloroquine | doxorubicin | erlotinib | gefitinib | imatinib | lapatinib | ONYX-015 | PALA | quinacrine
Pathway Interaction Database: http://pid.nci.nih.gov/p53_pathway
UniProtKB: http://www.uniprot.org
53BP1 | ARF | ATM | BCL-2 | BRCA1 | BRCA2 | EGFR | H2AX | MDM2 | MDM4 | p21 | p53 | PARP1 | PLK1 | RPL11 | RPL5 | SIRT1 | SIRT2

FURTHER INFORMATION

Laboratory homepage: http://www.p53lab.a-star.edu.sg/

ALL LINKS ARE ACTIVE IN THE ONLINE PDF