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REVIEW ARTICLE

Should mutant TP53 be targeted for cancer therapy?

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Mutations in the *TP53* tumour suppressor gene are found in ~50% of human cancers [1–6]. TP53 functions as a transcription factor that directly regulates the expression of ~500 genes, some of them involved in cell cycle arrest/cell senescence, apoptotic cell death or DNA damage repair, i.e. the cellular responses that together prevent tumorigenesis [1–6]. Defects in TP53 function not only cause tumour development but also impair the response of malignant cells to anti-cancer drugs, particularly those that induce DNA damage [1–6]. Most mutations in *TP53* in human cancers cause a single amino acid substitution, usually within the DNA binding domain of the TP53 protein. These mutant TP53 proteins are often expressed at high levels in the malignant cells. Three cancer causing attributes have been postulated for mutant TP53 proteins: the inability to activate target genes controlled by wt TP53 (loss-of-function, LOF) that are critical for tumour suppression, dominant negative effects (DNE), i.e. blocking the function of wt TP53 in cells during early stages of transformation when mutant and wt TP53 proteins are co-expressed, and gain-of-function (GOF) effects whereby mutant TP53 impacts diverse cellular pathways by interacting with proteins that are not normally engaged by wt TP53 [1–6]. The GOF effects of mutant TP53 were reported to be essential for the sustained proliferation and survival of malignant cells and it was therefore proposed that agents that can remove mutant TP53 protein would have substantial therapeutic impact [7–9]. In this review article we discuss evidence for and against the value of targeting mutant TP53 protein for cancer therapy.

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FACTS

- Mutations in the tumour suppressor TP53 are common in diverse human cancers (~50%) and are frequently associated with poor responses to anti-cancer therapy.
- Mutations in TP53 block its tumour suppressive functions by preventing it from binding to target DNA sequences and upregulating genes that mediate several cellular processes, including apoptosis, cell cycle arrest and senescence.
- Mutant TP53 proteins are also reported to have gain-of-function properties that can contribute to tumour growth.

OPEN QUESTIONS

- Are the reported gain-of-function effects of mutant TP53 proteins really critical for the sustained growth and therapy resistance of malignant cells?
- Do the compounds that were reported to specifically target mutant TP53 proteins indeed kill malignant cells by acting on mutant TP53?
- What are the best approaches for treating cancers expressing mutant TP53 protein?

WILD-TYPE (WT) TP53 FUNCTIONS AS A TUMOUR SUPPRESSOR

TP53 is a master transcription factor which directly regulates the expression of ~500 genes involved in diverse cellular responses [1, 2]. The TP53 protein contains several functional domains. Two acidic transactivation domains are located at the N-terminus, and they are critical for TP53 to interact with transcriptional co-activators and co-repressors. An unstructured basic regulatory domain is found at the C-terminus, which assists in the binding of TP53 to DNA and the stabilisation of TP53-DNA complexes. A sequence-specific DNA-binding domain is located in the centre of the TP53 protein. Finally, TP53 functions as a tetramer and the tetramerisation domain is located near the C-terminus [10].

In unstressed cells the TP53 protein is present at only low levels, mostly owing to a negative feedback loop: TP53 can transcriptionally induce MDM2 (called HDM2 in humans), an E3 ubiquitin ligase that ubiquitinates TP53, thereby priming it for proteasomal degradation [11]. When cells are subjected to stress, such as DNA damage, deprivation of metabolites or oncogene activation, the MDM2-TP53 interaction is inhibited as a consequence of several upstream signalling events that are not discussed here (for reviews, see [2–4]), and this results in the stabilisation of the TP53 protein. Stabilised TP53 accumulates in the nucleus where it binds as a homo-tetramer in a sequence-specific manner to target genes to regulate their expression, most likely in conjunction with

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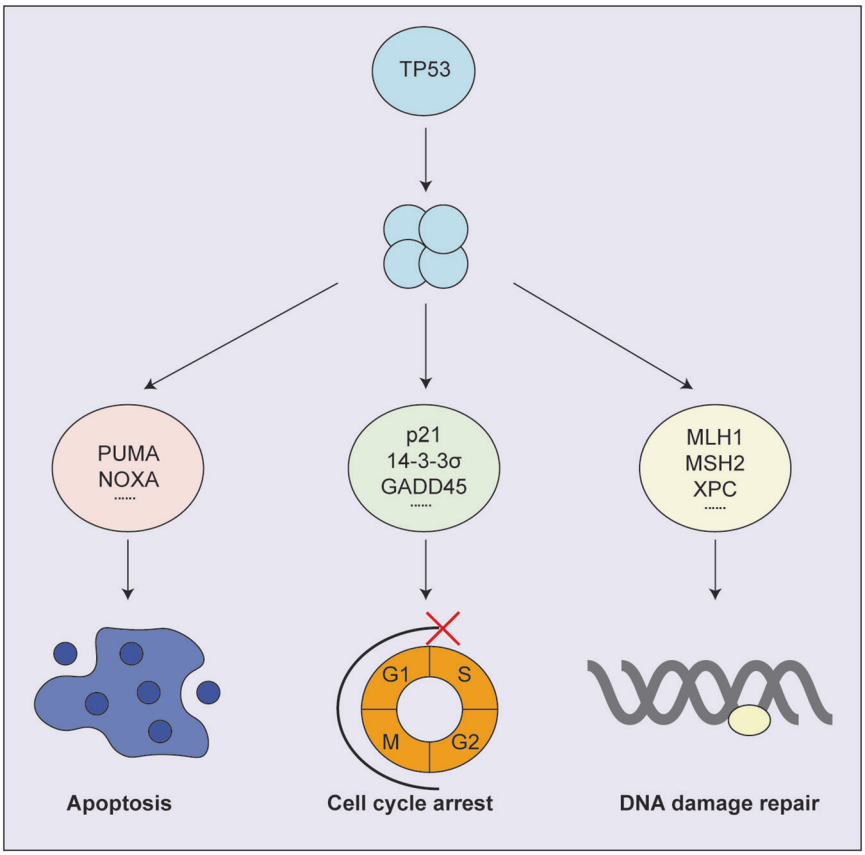

Fig. 1 The functions of wt TP53. Model depicting the target genes activated by wt TP53 and the cellular responses in which their protein products function in wt TP53 mediated tumour suppression.

additional transcription factors [12, 13]. In this way TP53 regulates the cellular responses that are critical for tumour suppression.

TP53 induces cell cycle arrest at the G1/S boundary and cell senescence mainly by direct transcriptional activation of the gene for the cyclin dependent kinase (CDK) inhibitor, p21 [14, 15] (Fig. 1). In addition, 14-3-3σ and GADD45 were reported to be critical for TP53-induced cell proliferation arrest by blocking the G2/M transition [16, 17].

TP53 induces cell death by activating the intrinsic apoptotic pathway that is regulated by the BCL-2 protein family which contains three types of proteins with different functions: the pro-survival family members (e.g. BCL-2, MCL-1) to prevent apoptosis, the pro-apoptotic BH3-only proteins (e.g. BIM, PUMA, NOXA) to initiate apoptosis, and the effectors of apoptosis (BAX, BAK) to kill cells [18, 19]. TP53 can directly transcriptionally activate the genes encoding the pro-apoptotic BH3-only proteins PUMA and NOXA [20–22] that are required for TP53-induced apoptosis, for example after exposure to DNA damage inducing agents [23–25] (Fig. 1). Notably, lymphoid cells from *Puma/Noxa* double knockout mice are as resistant to these agents as those from *Trp53* knockout mice [26, 27], demonstrating that direct transcriptional activation of *Puma* and *Noxa* accounts for all the apoptosis inducing action of TP53 (at least in these cell types).

TP53 was also shown to activate genes that function in the coordination of DNA damage repair [28]. TP53 can orchestrate the nucleotide excision repair (NER) of UV-induced DNA damage through direct transcriptional activation of the *DDB2* (encoding p48) and *XPC* genes [29, 30]. TP53 was also reported to transcriptionally activate several genes involved in DNA mismatch repair, including *MLH1*, *MSH2* and *PMS2* [31, 32]. This DNA repair process is thought to be important in tumour suppression since *Mlh1*, *Msh2* as well as *Pms2* gene deficient mice spontaneously develop tumours (Fig. 1) [33].

Several other cellular responses have also been reported to be activated by wt TP53, including the coordination of metabolism (for an expert review see [34]) and the silencing of large parts of the genome, thereby controlling retrotransposons [35].

Which cellular processes activated by TP53 and which TP53 target genes are critical for the prevention of tumour development?

It remains unclear which of the many processes activated by TP53 are critical for tumour suppression and it is possible that the relative contributions of these processes to tumour suppression may vary depending on cell type and on which oncogenes are active in a given cell. While the absence of TP53 leads to lymphoma or certain other cancers with 100% penetrance in mice within ~250–300 days [36, 37], the loss of individual TP53 target genes that are critical for a given cellular response does not usually cause spontaneous tumour development in mice. For example, mice lacking p21, which is essential for TP53-induced G1/S boundary cell cycle arrest [14] and also plays a major role in TP53-induced cell senescence [38], are not tumour prone on a C57BL/6 background [15]. However, compared to wt controls an increased incidence of several types of cancers was observed on a mixed C57BL/6x129SV background [39] (a genetic background with higher tumour predisposition compared to C57BL/6). Moreover, mice lacking PUMA, NOXA or both of these BH3-only proteins that are essential for TP53-induced apoptosis [27, 40], are not tumour prone [40], although the absence of PUMA did accelerate c-MYC-driven lymphomagenesis [26] and increased the severity of carcinogen induced colon cancer development [41].

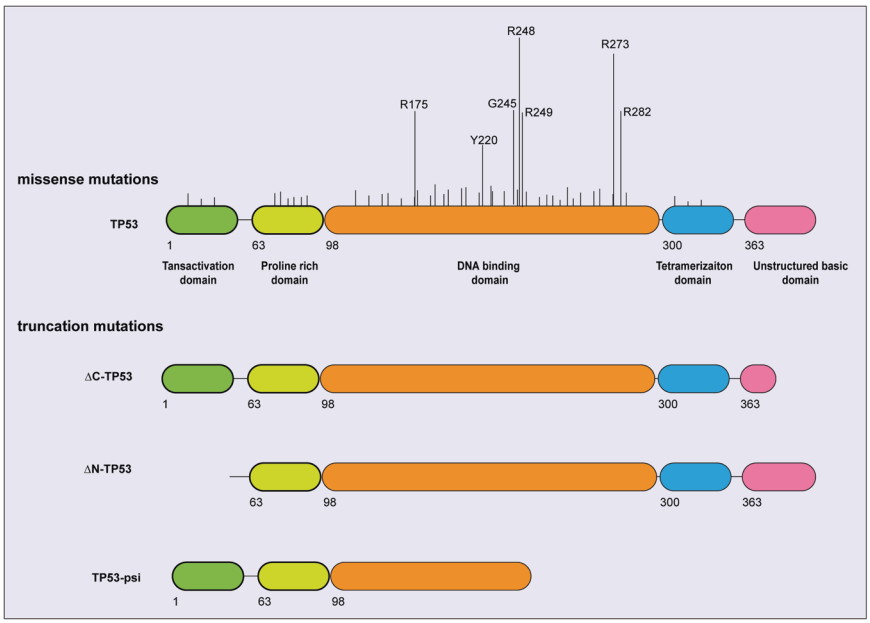

Fig. 2 Localisation of point mutations in TP53. Model depicting the location and relative frequencies of point mutations found in human cancers. Data are derived from [44, 132].

Remarkably, even *Puma/Noxa/p21* triple knockout mice (on a C57BL/6 background) do not spontaneously develop cancer over at least 18 months even though their cells are defective in TP53-induced apoptosis, G1/S boundary cell cycle arrest and cell senescence [42]. In striking contrast, loss of certain genes that function in DNA damage repair and are reported to be directly regulated by TRP53 (e.g. *Mlh1*, *Msh2*) causes a marked predisposition to spontaneous tumour development in mice and also accelerated c-MYC-driven lymphomagenesis [32]. These findings and others [43] indicate that coordination of DNA repair may be the most important TP53 regulated process for tumour suppression.

The role of mutant TP53 in tumorigenesis

TP53 is the most frequently mutated gene (~50%) in human cancers [44]. Although some *TP53* mutations lead to the complete loss of TP53 protein (mimicked experimentally by *Trp53* knockout mice), most are missense mutations that cause single amino acid substitutions, usually within the TP53 DNA-binding domain (Fig. 2). The levels of such mutant TP53 proteins are generally very high in malignant cells although not in mutant TP53 expressing pre-malignant cells in mice in vivo [44, 45]. Mutant TP53 proteins have been proposed to drive malignant transformation and sustain tumour growth through several processes. Of note, the Li-Fraumeni cancer predisposition syndrome in humans, characterised by diverse cancers (e.g. osteosarcoma, acute leukaemia, breast cancer and brain cancer) often arising at a young age, is in ~70% of cases caused by inherited mutations in one allele of *TP53* with loss of the wt *TP53* allele (loss of heterozygosity, i.e. LOH) apparent in the malignancies that arise in these patients (for an expert review see [46]).

Loss of function (LOF)

The TP53 mutations either change the conformation of TP53 proteins (structural mutants) or affect amino acids involved in DNA binding (contact mutants) [47]. Both types of TP53 mutants are unable to transcriptionally activate wt TP53 target genes and thereby cannot induce the essential mediators of apoptosis, cell cycle arrest, cell senescence and DNA damage repair, the processes thought to be critical for wt TP53 mediated tumour suppression.

Elegant experimental systems were developed in which tumour development could be initiated in mice by the absence of wt TP53, but expression of this tumour suppressor could be restored in the malignant cells. This revealed that restoration of wt TP53 in TP53-deficient cancers resulted in tumour regression owing to the activation of apoptosis or cell senescence in lymphomas or solid cancers, respectively [48–50]. Why restoration of wt TP53 causes apoptosis in lymphomas but cell senescence in solid cancers remains an intriguing question. This could be due to differences between these cell types and/or differences in the other oncogenic lesions that drive these malignancies. These factors could impact post-translational modification of TP53 and thereby influence its potency in activating different subsets of its direct target genes (i.e. cell cycle regulating genes *vs* cell death inducing genes), for example by attracting different co-activators or influencing its binding to target sequences in DNA (reviewed in [51]). Regardless, these findings reveal that sustained LOF of TP53 is required for continued tumour expansion.

Dominant negative effects (DNE) of mutant TP53

Whilst advanced tumours that have mutations in *TP53* have often selected for loss of the WT *TP53* allele (loss of heterozygosity (LOH)), at the early stages of malignant transformation, mutant TP53 often co-exists with wt TP53 in the nascent neoplastic cells. The mutant TP53 prevents wt TP53 from exerting its tumour suppressive functions due to the formation of mixed wt/mutant TP53 hetero-tetramers [44]. These mixed tetramers have significantly reduced ability to induce gene expression and cellular responses that are normally driven by tetramers containing only wt TP53 [52, 53]. Initial evidence for a dominant negative effect (DNE) of mutant TP53 came from co-transfection experiments in vitro, showing that processes known to be activated by wt TP53 could be inhibited by concomitant expression of mutant TP53 [54, 55]. A DNE of mutant TP53 was also observed in cells from $Trp53^{R172H/+}$, $Trp53^{R246S/+}$ and $Trp53^{R270H/+}$ mutant knock-in mice. After treatment with stimuli that activate wt TP53 (e.g. γ-radiation), cells from these mice underwent considerably less apoptosis or cell cycle arrest compared to cells from wt ($Trp53^{+/+}$) mice [56, 57]. The DNE of various TP53 mutants could also be seen when they were expressed in haematopoietic stem and progenitor cells

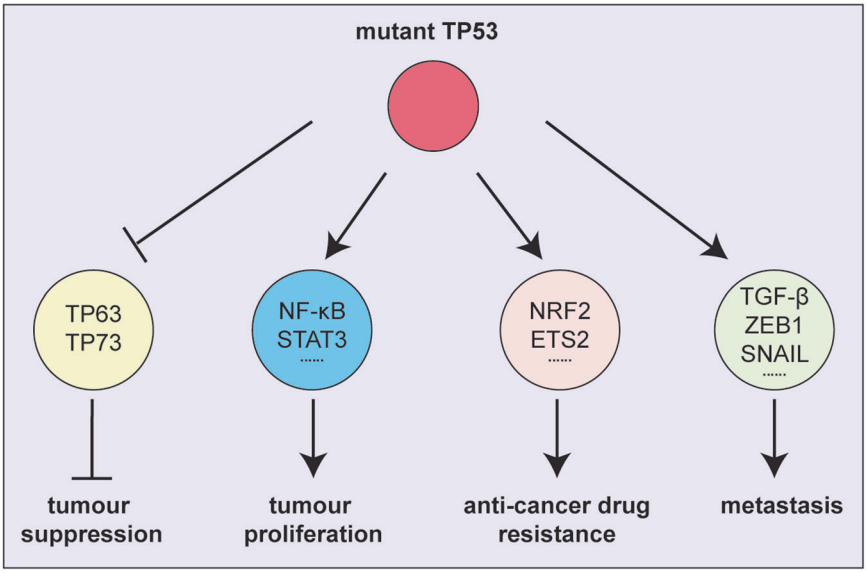

Fig. 3 The mechanisms proposed for mutant TP53 to exert its alleged GOF effects. Model depicting the proposed interactions with other transcription factors that mutant TP53 proteins engage in to drive the development and expansion of tumours.

(HSPCs) from *Trp53*$^{+/-}$ or *Eμ-Myc*;*Trp53*$^{+/+}$ mice but, as expected, not when expressed in HSPCs from *Trp53*$^{-/-}$ mice since the latter do not express wt TP53 on which mutant TP53 could exert its DNE [43, 58]. The loss of the wt *TP53* allele during advanced stages of tumorigenesis (LOH) [59] indicates that the complete loss of wt TP53 function provides further advantages for tumour expansion, even when mutant TP53 is expressed. Interestingly, the levels of mutant TP53 protein are much higher in tumour cells with LOH compared to cells co-expressing both wt TP53 and mutant TP53 [60]. This demonstrates that wt TP53 can somehow repress the levels of mutant TP53 protein.

Gain-of-function (GOF) effects of mutant TP53

Mutant TP53 has also been reported to be able to exert neomorphic gain-of-function (GOF) properties [44], i.e. functions that wt TP53 cannot exert. Removal of mutant TP53, and hence its GOF effects, by using siRNA was reported to inhibit the growth of certain tumour cells in culture and in vivo and to increase their sensitivity to cytotoxic drugs [9, 61]. The GOF effects of mutant TP53 were also reported to enhance tumour metastasis by impacting transcription factors that control the epithelial-mesenchymal transition (EMT) [62–64]. The GOF effects of mutant TP53 were shown to assist malignant cells in metabolic reprogramming to adapt to changes in the availability of growth factors and nutrients by activating glycolysis [65], promoting lipid synthesis [66] and nucleotide synthesis [67].

It has been postulated that mutant TP53 exerts its alleged GOF effects mainly through interactions with other transcription factors to upregulate or downregulate the expression of genes that are not controlled by wt TP53. Some TP53 mutants were reported to inhibit the functions of TP63 and TP73, the two family members of TP53 that can also regulate many of the known TP53 target genes [68–71]. Mutant TP53 was also reported to increase the transcriptional transactivation activity of NF-kB, E2F1, ETS1/ETS2 and YAP1, which are all implicated in promoting tumour growth [72–74] (Fig. 3). Many mechanisms have been proposed to be responsible for these reported GOF effects of mutant TP53 proteins, mostly including protein-protein interactions in which wt TP53 does not engage. It is also important to bear in mind that different mutant TP53 proteins may engage in different protein-protein interactions and thereby exert different GOF effects (for a review see [75]).

Not all TP53 mutants are equivalent

Hundreds of different TP53 mutants have been identified in human cancers, and it appears likely that they do not all function in the same way in driving tumour development. Most TP53 mutations lead to LOF [44]. This may, however, not be universal, since some TP53 mutant proteins were reported to still retain part of the functions of wt TP53. For example, some mutations located in the acidic transactivation domains result in the production of a truncated form of TP53 that retains the ability to induce apoptosis [76]. Moreover, the DNA-binding domain mutant, TP53^{K120R}, was reported to be only defective in the induction of apoptosis but was still able to induce cell cycle arrest and cell senescence [77].

Interestingly, different single-amino acid substitutions that affect the same residue were reported to have different impacts on TP53 function. R175C behaved like wt TP53 and could induce both cell cycle arrest and apoptosis. Conversely, the R175P mutant TP53 was defective in inducing apoptosis but retained the ability to induce cell cycle arrest, whereas R175D mutant TP53 showed loss of both functions [78].

Moreover, not all the TP53 mutants appear to be able to exert a DNE over endogenous wt TP53. Experiments using enforced expression of different TP53 mutants in colon cancer cells expressing endogenous wt TP53 first provided evidence for this notion. Only one hot-spot mutant that was tested, R273H, displayed a DNE, whereas the other TP53 mutants examined, V143A, R175H and R248W, were not able to repress all activities exerted by the endogenous wt TP53 [79]. In vivo experiments with mutant TP53 knockin mice confirmed and extended these findings. For example, *Trp53*$^{R172H/+}$ and *Trp53*$^{R270H/+}$ mutant knockin mice (codons R173H and R273H in human, respectively) displayed comparable tumour-free survival times when compared to *Trp53*$^{+/-}$ mice [56, 80]. However, these findings refer to spontaneous tumour development and it remains possible that in the context of the expression of certain oncogenes, the *Trp53*$^{R172H/+}$ and *Trp53*$^{R270H/+}$ knockin mice would exhibit significantly shorter tumour-free survival compared to the *Trp53*$^{+/-}$ mice.

Although LOF and DNE are widely accepted as critical outcomes of TP53 mutations, only some mutations, especially hot-spot mutations, are thought to give rise to GOF effects [81, 82]. Of note, even for different hot-spot mutations, the reported GOF effects are not equivalent. *Trp53*$^{R172H/-}$ and *Trp53*$^{R270H/-}$ (codons 175 and 273 in human, respectively) mutant knockin mice showed a

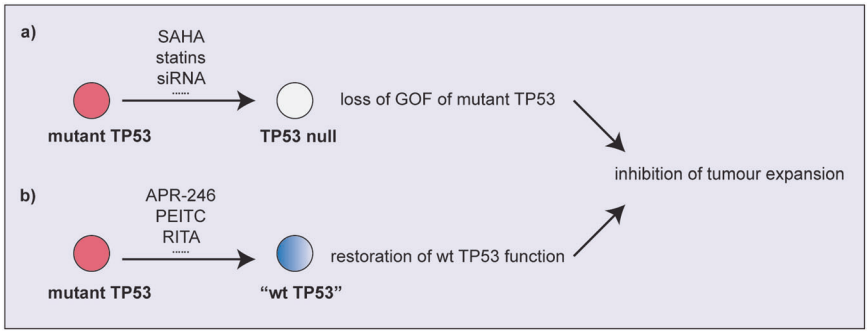

Fig. 4 Therapeutic approaches for targeting mutant TP53 protein for cancer therapy. a Removal of mutant TP53 protein by using drugs or siRNA technology is expected to remove the GOF effects of mutant TP53 and to thereby prevent tumour expansion. **b** Restoring wt TP53 protein conformation in mutant TP53 proteins is expected to restore wt TP53 activated processes (e.g. cell cycle arrest, cell senescence, apoptotic cell death) and to thereby prevent tumour expansion.

Table 1. List of the compounds discussed indicating their reported effects and specificities for different mutant TP53 proteins.

Proposed mode of action	Drugs	Tumour type and TP53 state in which the drug was shown to be active
Depletion of mutant TP53 expression	SAHA	T-cell lymphoma [7] (mutant); breast cancer [86] (mutant);prostate cancer [87] (mutant)
	FK228	Neuroblastoma [88] (wt, mutant); non-small-cell lung cancer [89] (wt, mutant)
	Statins	Breast cancer, sarcoma, lung cancer, pancreatic cancer [96] (structural mutant); ovarian cancer [97] (wt, mutant)
	Gambogic acid	breast cancer [99] (mutant); prostate cancer [100] (null); lung cancer [101] (wt)
	siRNA/shRNA	breast cancer [102] (mutant); prostate cancer [103] (mutant); breast and colon cancer [8] (mutant)
Restoration of wt TP53 functions	PRIMA-1/APR-246	osteosarcoma, lung, ovarian and colon cancer [105] (mutant); multiple myeloma [106] (wt, mutant, null); espohageal cancer [107] (mutant); cholangiocancinoma [108] (mutant); sarcomas [113] (mutant, null); breast, colon cancer [116] (wt, mutant)
	PEITC	breast and lung cancer [117] (R175H); prostate cancer [118] (mutant); breast cancer [119] (mutant)
	RITA	colon cancer [120] (wt); colon, lung, breast, skin cancer, Burkitt lymphoma [121] (mutant); neuroblastoma [122] (wt, mutant)
	CP-31398	lung cancer, melanoma [123] (mutant); colon, breast cancer [124]: mutant; colon, lung, ovarian cancer [125] (wt, mutant); hepatocellular cancer [126] (mutant); colon cancer [127] (mutant); multiple myeloma [128] (wt, mutant, null)
	PK7088	hepatocellular, gastric cancer [129] (Y220C)
	Arsenic Trioxide	acute lymphoblastic leukemia, lung, ovarian, pancreatic cancer, melanoma [130] (structural mutants)

different tumour spectrum compared to that seen in *Trp53*$^{-/-}$ mice but no shortening of tumour-free survival [56]. Humanised *TP53*$^{R248Q/-}$ mutant knockin mice not only developed different types of tumours but also showed shortened tumour-free survival times compared to *Trp53*$^{-/-}$ mice [83]. In contrast, enforced expression of five different TRP53 mutants (two of them hot-spot mutations) in HSPCs from *Trp53*$^{-/-}$ mice did not accelerate tumorigenesis or alter the tumour spectrum compared to control mice reconstituted with HSPCs from *Trp53*$^{-/-}$ mice that had been transduced with an empty vector [43]. Thus, in this experimental system no GOF effects of any of the TRP53 mutants tested could be detected.

Therapeutic targeting of mutant TP53 for cancer therapy

Depleting mutant TP53 in tumour cells. Since the GOF properties of mutant TP53 have been postulated to contribute to tumour development and metastasis and to impair the response of malignant cells to diverse anti-cancer therapeutics, approaches that could specifically deplete mutant TP53 protein levels are postulated to have promise for the treatment of diverse cancers (Fig. 4). The effects and specificities of the compounds described below are summarised in Table 1.

HSP90 and HDAC inhibitors. Heat shock protein 90 (HSP90) is a molecular chaperone that is involved in modulating the folding, stabilisation and degradation of many proteins including some oncogenic proteins, such as mutant TP53. HSP90 has been reported to impact tumour progression, metastasis and high levels of HSP90 are associated with poor prognosis in multiple cancers [84, 85]. The histone deacetylase (HDAC) inhibitor SAHA was reported to synergise with the HSP90 inhibitor 17AAG in degrading mutant TP53, thereby inducing apoptosis and decreasing tumour growth in xenografts [7]. FK228, another HDAC inhibitor, was also shown to inhibit growth and induce apoptosis in tumour cells. However, unlike SAHA, which was reported to kill tumour cells in a manner dependent on the removal of mutant TP53 [86, 87], FK228 induced cell death not only in mutant TP53 expressing tumour cells but also in those expressing wt TP53 [88, 89]. This raises the question of whether HDAC and HSP90 inhibitors, either on their own or in combination, really kill tumour cells by targeting mutant TP53 or through some other process.

Statins. Metabolic reprogramming is a hallmark of cancer [90]. The mevalonate pathway, which regulates the production of cholesterol and isoprenoids, was reported to be involved in the

development and progression of cancer [91]. Mutant TP53 expressing tumours often present with over-activation of the mevalonate pathway [92]. Statins target the rate-limiting enzyme in cholesterol biosynthesis and are used as lipid-lowering agents [93]. Recently, the potential of statins in cancer treatment has been proposed based on the observation that these drugs can trigger apoptosis in certain tumour cells [94] and thereby increase their sensitivity to chemotherapeutic drugs [95]. Interestingly, statins were shown to cause the degradation of mutant TP53 by suppressing the interaction of mutant TP53 with the HSP40 family member, DNAJA1, and it was proposed that this process was responsible for their ability to reduce tumour growth [96]. It was reported that statins inhibit the growth of tumour cells expressing mutant TP53, while having only minimal impact on the growth of tumour cells expressing wt TP53. However, there are also studies showing that statins can kill certain tumour cells independent of their TP53 status [97]. Hence, the mechanisms by which statins kill tumour cells need further investigation.

Gambogic acid. Gambogic acid (GA) has been reported to have potential for the treatment of both solid as well as haematological cancers. GA was first reported to reduce the expression of MDM2, thereby activating wt TP53 and inhibiting tumour cell growth in a wt TP53-dependent manner [98]. Subsequent studies reported that GA also has the ability to reduce the levels of mutant TP53 by preventing the formation of HSP90/mutant TP53 complexes, thereby leading to ubiquitin/proteasome mediated degradation of mutant TP53 [99]. However, other studies found that different mechanisms, rather than effects on mutant TP53 protein, are critical for GA-induced killing of malignant cells. GA was reported to activate the intrinsic apoptotic pathway in TP53-deficient prostate cancer cells by inhibiting the MAPK pathway and the transcription factor c-FOS [100]. GA was also shown to activate ROS-induced endoplasmic reticulum (ER) stress, thereby inducing apoptosis in lung cancer cells [101]. Thus, GA appears to be able to inhibit tumour expansion by activating several cell growth inhibitory pathways, and the degradation of mutant TP53 is only one of the possible mechanisms that may contribute.

siRNA and shRNA. RNA interference (RNAi) can be a potential cancer therapeutic by silencing critical genes that are required for the proliferation and/or survival of tumour cells. Based on some of the considerations outlined above, it has been postulated that siRNA or shRNA mediated knockdown of mutant TP53 could be a promising strategy for inhibiting tumour expansion. Reducing the levels of mutant TP53 by using shRNA or siRNA was reported to elicit substantial apoptosis in mutant TP53 expressing breast cancer cells, but not in wt TP53 expressing cancer cells [102]. Knocking down mutant TP53 was shown to reduce the proliferation of prostate cancer cells by inducing cell cycle arrest at the G1/S or G2/M boundaries [103]. Furthermore, depletion of mutant TP53 by siRNA or shRNA was found to increase the sensitivity of certain tumour cells to anti-cancer drugs [8]. Collectively, these studies established the potential of the removal of mutant TP53 as a strategy for cancer therapy (Fig. 4). It must, however, be noted that a recent study pointed out significant off-target effects of RNAi technology: many putative cancer dependencies, and hence potential anti-cancer drug targets, identified from studies based on RNAi were found to be false leads as they could not be validated when using CRISPR technology to remove these proteins [104].

Restoring wt TP53 function in tumour cells expressing mutant TP53

Mutant TP53 proteins lack the ability to transactivate wt TP53 target genes, resulting in the loss of tumour suppressive functions. Restoring wt TP53 transcriptional activities to mutant TP53 in tumour cells is expected to lead to proliferation arrest, cellular senescence and apoptotic death with a consequent therapeutic benefit (Fig. 4), although this has not yet been proven using genetically engineered mice in which cells can be switched first from expressing wt TRP53 to mutant TRP53 and then, at will, back to wt TRP53.

Mutant TP53 reactivating agent (PRIMA-1) and APR-246. PRIMA-1 was identified in 2002 in a screen of a library of low-molecular-weight compounds [105]. PRIMA-1 was reported to restore wt TP53 conformation to several mutant TP53 proteins that were studied and to thereby reactive wt TP53 transcriptional activities in tumour cells expressing mutant TP53. This was shown to delay the growth and increase apoptosis of tumour cells with mutant TP53, but not of tumour cells containing wt TP53. APR-246, the methylated analogue of PRIMA-1, showed more efficient killing effects in tumour cells containing mutant TP53 and shared many characteristics with PRIMA-1. PRIMA-1 and APR-246 were able to reactivate the expression of wt TP53 target genes in tumour cells expressing mutant TP53 and these compounds were reported to kill tumour cells by inducing the expression of the pro-apoptotic BH3-only protein, NOXA [106, 107]. Moreover, PRIMA-1 and APR-246 were shown to activate the expression of p21 in mutant TP53 expressing tumour cells, thereby inducing cell cycle arrest and cell senescence [105, 108]. Importantly, these two compounds not only killed tumour cells in vitro, but they were also shown to delay tumour growth in vivo, thereby prolonging the survival of mice transplanted with cancer cells [105, 106]. Finally, both PRIMA-1 and APR-246 were shown to cooperate with certain chemotherapeutic drugs to kill tumour cells [109, 110]. Collectively, these findings established PRIMA-1 and APR-246 as promising anti-cancer drugs that target mutant TP53. APR-246 is currently being tested in several phase II clinical trials, including in TP53-mutant myeloid malignancies, high-grade serous ovarian cancer, oesophageal cancer and melanoma. However, PRIMA-1 was also shown to be able to kill tumour cells with wt TP53 [111], and even tumour cells that lack TP53 [112]. Similar observations were also reported for APR-246, which was shown to kill tumour cells irrespective of their TP53 status [113]. A critical underlying mechanism of APR-246 induced killing of malignant cells appears to involve reactive oxygen species (ROS). APR-246 was shown to cause an accumulation of intracellular ROS in a dose-dependent manner and this was reported to induce apoptosis in tumour cells irrespective of their TP53 status. This killing of tumour cells could be inhibited by scavenging intracellular ROS [114]. The ER stress/UPR-pathway was also reported to be involved in PRIMA-1 induced killing of malignant cells [115]. The unfolded and misfolded protein response pathways were significantly up-regulated in multiple myeloma (MM) cells in response to treatment with PRIMA-1, as demonstrated by the increased levels of HSP70, GADD34 and CHOP, all of which are markers of ER stress. Moreover, PRIMA-1 was found to cooperate with the UPR-inducing agent, bortezomib, in the killing of MM cells, and it could even re-sensitise bortezomib-resistant MM cells to this proteasome inhibitor [115]. Finally, PRIMA-1 was reported to induce autophagy in breast cancer cells as well as soft-tissue sarcoma cells, and this was independent of their TP53 status [113, 116]. Collectively, these observations indicate that the mechanisms of APR-246 induced killing of malignant cells still remain unclear. Recently it was reported that expression of SLC7A11 is a more reliable predictor of response to APR-246 that TP53 status in cancer cells [117]. The identification of the mechanisms that are responsible for APR-246 induced killing of tumour cells is predicted to inform the currently ongoing clinical trials of this drug.

Phenethyl Isothiocyanate (PEITC). Phenethyl isothiocyanate (PEITC) is present at high levels in watercress and cruciferous vegetables, and this compound was reported to exert remarkable chemotherapeutic activity. Mutant TP53 was reported to be a

target of PEITC. Unlike APR-246, which was reported to be able to restore wt TP53 function in all contact mutant TP53 proteins tested, PEITC was shown to delay proliferation and induce apoptosis only in tumour cells expressing one specific TP53 mutant protein, R175 [118]. PEITC was reported to restore wt TP53 conformation and transactivation functions to R175 mutant TP53. However, another study revealed that PEITC could exert significant anti-cancer activity not only in malignant cells expressing R175 mutant TP53, but also in cancer cells expressing any of the recognised structural TP53 mutants, including P223L, but it had no impact on tumour cells expressing contact TP53 mutants [119]. Mechanistically, it was reported that PEITC caused a reduction in the levels of mutant TP53 protein in tumour cells through a post-transcriptional process, whereas it had only minimal impact on the levels of wt TP53 in malignant cells [118, 120]. This suggests that PEITC may be able to target mutant TP53 through two different processes, reactivation of wt TP53 functions and a reduction of the levels of mutant TP53 protein. However, considerably more work is needed to validate PEITC as a promising compound for anti-cancer therapy and to identify which of its proposed mechanisms of action are critical for the killing of malignant cells.

Reactivation of TP53 and Induction of Tumour Cell Apoptosis (RITA). RITA (reactivation of TP53 and induction of tumour cell apoptosis) is a small molecule that was identified in a screen of the National Cancer Institute library of compounds. It was initially reported to inhibit the TP53-MDM2 interaction and thereby activate wt TP53-driven anti-tumour effects [121]. However, subsequent studies reached the conclusion that RITA could also suppress proliferation and induce apoptosis in tumour cells expressing mutant TP53, since the compound was found to restore wt TP53 transcriptional activities in several hot-spot TP53 mutants. This was based on the observation that treatment with RITA led to the induction of wt TP53 target genes, including *GADD45*, *BBC3*, *BAX* and *CKDN1A*, in tumour cells expressing mutant TP53 [122]. However, similar to APR-246, other studies showed that RITA was also able to induce apoptosis in tumour cells expressing wt TP53 and even in TP53-deficient cancer cells [123]. Collectively, these studies indicate that the anti-cancer effects of RITA may not be specifically dependent on mutant TP53, and the mechanisms of RITA induced killing of tumour cells still need to be clarified.

CP-31398. CP-31398 is a small molecule that was identified in a screen of a synthetic compound library. It showed the ability to restore a functionally active conformation in mutant TP53 proteins, allowing it to exert wt TP53 transcriptional activity [124]. Subsequent studies revealed that the ability of CP-31398 to induce cell death was TP53-dependent, as this compound could only induce apoptosis in tumour cells expressing wt TP53 or mutant TP53, but not in those deficient for TP53 [125]. CP-31398 was found to increase the levels of wt TP53 by blocking its ubiquitination and proteasomal degradation and this allowed TP53 to activate its canonical cellular responses, including cell cycle arrest and apoptosis in tumour cells [126]. Conversely, CP-31398 was also reported to restore wt TP53 tumour suppressive function in TP53 mutant proteins independent of the nature of the TP53 mutation. CP-31398 was shown to delay the growth of hepatocellular cancer cells expressing R249S or Y220C mutant TP53 and colorectal cancer cells expressing R248Q or P309S mutant TP53, both in vitro and in vivo [127, 128]. The growth inhibitory effects were comparable between cancer cells expressing different TP53 mutants and cancer cells of different cellular origin. CP-31398 was also reported to cause an increase in ROS production and thereby trigger the intrinsic apoptotic pathway in MM cells, regardless of their TP53 status [129]. Collectively, these findings indicate that CP-31398 induced killing of malignant cells may not depend on the expression of mutant TP53 and the mechanisms responsible still require further investigation.

PK7088. The Y220C TP53 mutant protein is a paradigm for studying the restoration of wt TP53 function in a mutant TP53 protein, because it contains a unique surface crevice that is amenable to targeting by small molecules. The small molecule compound PK7088 was reported to bind to this surface crevice on Y220C mutant TP53 and thereby convert its structure from a mutant into the wt conformation with restoration of wt TP53 transcriptional activity [130]. PK7088 was shown to induce TP53-dependent cell cycle arrest and apoptosis by activating the expression of *p21* and *NOXA*, respectively. These effects could be enhanced by addition of the MDM2 inhibitor nutlin-3a, which further indicated the successful restoration of wt TP53 structure and function in Y220C mutant TP53. This work raises the possibility that one specific small molecule might be needed to target each specific TP53 mutant protein, heralding a new paradigm for treating mutant TP53 expressing cancers.

Arsenic trioxide (ATO). Arsenic trioxide (ATO) is a small molecule reported to be able to restore wt TP53 function in tumour cells expressing structural TP53 mutants [131]. ATO can bind to the DNA binding domain only in structural but not in contact TP53 mutants. It thereby induces the transcriptional activities that are characteristic of wt TP53, leading to the suppression of tumour growth both in vitro and in vivo. Thus, ATO may provide a promising therapy against mutant TP53 expressing cancers, but more studies are needed to validate its specificity and efficiency in treating mutant TP53.

Outlook

Many approaches have been tried in pre-clinical tests and even clinical trials to treat cancers by targeting mutant TP53, including reducing the levels of mutant TP53 protein or restoring wt TP53 functions in mutant TP53 proteins. However, for all of these approaches there is evidence in the published literature that the compounds tested can also kill malignant cells through processes that are independent of mutant TP53, or there are other significant limitations. Thus, targeting of mutant TP53 for anti-cancer therapy still remains a challenge that requires further investigation, and importantly, it should first be validated by generating mice in which cells can be sequentially switched from wt TRP53 to mutant TRP53 and then to a TRP53-deficient state that removal (and hence targeting) of mutant TRP53 (TP53) will actually have therapeutic impact.

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ZW, AS and GLK studied the literature and wrote the review article. ZW prepared the figures and the table, which were edited by AS and GLK.

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COMPETING INTERESTS

The authors declare no competing interests.

ADDITIONAL INFORMATION

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