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Review

Mutant p53 reactivation by small molecules makes its way to the clinic



Vladimir J.N. Bykov, Klas G. Wiman*

Karolinska Institutet, Cancer Center Karolinska (CCK), Stockholm, Sweden

ARTICLE INFO

Article history:

Received 1 April 2014

Revised 14 April 2014

Accepted 14 April 2014

Available online 24 April 2014

Edited by Shairaz Baksh, Giovanni Blandino and Wilhelm Just

Keywords:

p53

Mutation

Cancer

Cell death

Drug discovery

Clinical trial

ABSTRACT

The TP53 tumor suppressor gene is mutated in many human tumors, including common types of cancer such as colon and ovarian cancer. This illustrates the key role of p53 as trigger of cell cycle arrest or cell death upon oncogenic stress. Most TP53 mutations are missense mutations that result in single amino acid substitutions in p53 and expression of high levels of dysfunctional p53 protein. Restoration of wild type p53 function in such tumor cells will induce robust cell death and allow efficient eradication of the tumor. Therapeutic targeting of mutant p53 in tumors is a rapidly developing field at the forefront of translational cancer research. Various approaches have led to the identification of small molecules that can rescue mutant p53. These include compounds that target specific p53 mutations, including PK083 and PK5174 (Y220C mutant p53) and NSC319726 (R175H mutant p53), as well as PRIMA-1 and its analog APR-246 that affect a wider range of mutant p53 proteins. APR-246 has been tested in a Phase I/II clinical trial with promising results.

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1. Introduction

The TP53 tumor suppressor gene encodes a DNA-binding transcription factor that induces cell growth arrest, senescence and cell death by apoptosis upon cellular stress [1]. This is achieved by transcriptional regulation of multiple target genes, including p21 that blocks cell cycle progression and Bax and Puma that promote apoptosis. Stress as a result of oncogene activation or disruption of cell cycle control characterizes early stages of tumor evolution. Oncogenic stress leads to aberrant DNA replication which triggers a DNA damage response involving activation of ATM, Chk1 and Chk2 kinases, and p53, and induction of senescence or apoptosis [2,3]. This p53-dependent response serves to eliminate incipient tumor cells or otherwise damaged cells. It is now increasingly clear that p53 is also involved in regulation of various other cellular processes, for instance metabolism, angiogenesis, and aging. Importantly, a large fraction of human tumors carry point mutations in TP53 that give rise to single amino acid substitutions in the p53 protein and disrupt p53 DNA binding (reviewed in [4,5]). As a consequence, mutant p53-carrying tumor cells are able to evade apoptosis and senescence, and progress to more malignant

variants. Moreover, since many conventional anticancer agents, for example cisplatin and doxorubicin, induce DNA damage that triggers a p53 response, mutation of TP53 is often associated with enhanced resistance to conventional chemotherapy.

Accumulating evidence demonstrate that TP53 mutations not only inactivate wild type p53 function but may also confer so called gain-of-function (GOF) activities to mutant p53 that promote tumorigenesis (reviewed in [5,6]). This is manifested as a more aggressive tumor phenotype with increased metastasis in mice carrying mutant p53 compared to p53 null or wild type mice [7–9]. At the molecular level, at least one mechanism responsible for mutant p53 GOF appears to be binding of mutant p53 to other proteins, including p53 family members p63 and p73 [10]. In the case of p63, this interaction has been shown to promote integrin recycling and invasion [11].

The key role of p53 in the cellular response to oncogenic stress, the fact that p53 is the most frequently mutated gene in cancer, the high levels of missense mutant p53 expression in many tumors, and the existence of tumor-promoting GOF activities, all make p53 a relevant and promising target for therapeutic intervention in cancer. This has stimulated intense efforts to design drugs for mutant p53 reactivation in cancer patients. Over the last 15 years, a number of mutant p53-targeting compounds have been identified by various strategies. In this review, we shall focus on the most promising compounds and the clinical development of mutant p53-reactivating drugs.

* Corresponding author.

E-mail address: klas.wiman@ki.se (K.G. Wiman).

2. Is restoration of wild type p53 function sufficient for tumor eradication?

Tumors carry multiple genetic alterations which provide critical functions that characterize cancer cells, the so called Hallmarks of Cancer [12,13]. From a therapeutic standpoint, this raises the question as to how many genetic alterations in a given tumor that must be corrected in order to achieve tumor eradication. Specifically, what is the effect of restoration of wild type p53 function in a tumor that has mutant p53? Will p53 reactivation lead to tumor elimination even if the tumor carries multiple other alterations in critical cancer genes such as PI-3 kinase, PTEN, Ras or Myc? This question has been elegantly addressed in vivo. Several studies have shown that reconstitution of wild type p53 expression by various means in mice induces rapid tumor regression [14–16]. These results demonstrate that restoration of wild type p53 expression is indeed sufficient for elimination of tumors even in the presence of other tumor-associated genetic alterations. This is presumably due to the critical role of p53 in the cellular pro-apoptotic or pro-senescence response to oncogenic stress. Thus, tumors are addicted to loss of p53 function, and genetic alterations that drive tumor growth, e.g. activation of Myc or loss of PTEN, may in fact further enhance p53-dependent cell death upon p53 reconstitution in a tumor cell context.

3. Small molecules that target mutant p53

One can imagine a number of strategies to target mutant p53 in tumors. Two approaches for mutant reactivation by small molecules are shown in Fig. 1. In the first approach, a mutant p53-binding molecule restores wild type folding, DNA binding and p53-dependent transcription, which leads to a biological response and tumor elimination. In the second approach, disruption of mutant p53 binding to p53 family proteins p63 and p73 allows p63/p73-dependent transcription and tumor suppression by several mechanisms. There are also other strategies for targeting mutant p53, including induction of mutant p53 degradation and inhibition of downstream signaling pathways (reviewed in [5]).

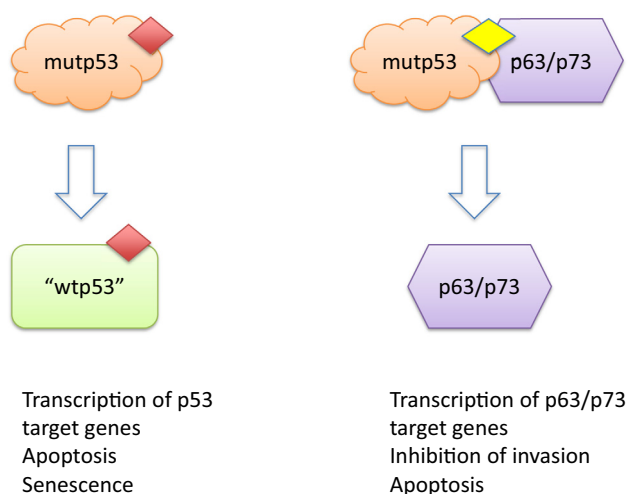


Fig. 1. Two strategies for mutant p53 reactivation. Direct binding of a small molecule may stabilize the p53 core domain and promote wild type folding, leading to restoration of specific DNA binding and transcription of p53 target genes. This will induce tumor cell apoptosis or senescence. Alternatively, disruption of complexes between mutant p53 and p53 family members p63 or p73 by a small molecule may restore p63 and/or p73 function, allowing transcription of p63/p73 target genes and tumor suppression by induction of apoptosis and inhibition of invasion.

In principle, novel targeted drugs can be identified by rational design, based on detailed structural knowledge of the drug target, or random screening of chemical libraries using a suitable assay with a readout such as induction of cell death. Both strategies have advantages and disadvantages, and both have been applied in the case of mutant p53. Further drug development towards the clinic faces numerous challenges, and should address chemical properties of hit compounds, including stability, reactivity, solubility and other aspects related to “druggability”, as well as antitumor efficacy in mouse tumor models.

A rational design approach by Fersht and colleagues has focused on the Y220C hot spot mutation in p53 which occurs in some 75,000 cases of cancer per year. The Y220C substitution creates a cavity that destabilizes the p53 protein. Fersht and colleagues designed Y220C-targeting compounds based on the crystal structure of the p53 core domain, including PK083 and PK7088. The carbazole derivative PK083 binds to the cavity on the p53 surface and raises the melting temperature of Y220C mutant p53 [17]. Similarly, PK7088 raises the melting temperature of the Y220C mutant, and was also shown to induce Y220C-dependent cell cycle arrest and apoptosis in tumor cells [18]. PK7088 promoted correct folding of Y220 mutant p53 and induced expression of p53 targets p21 and the Noxa. In addition, PK7088 was shown to trigger nuclear export of the pro-apoptotic Bax protein to mitochondria, possibly indicating effects on transcription-independent functions of p53. The studies on reactivation of Y220C mutant p53 by PK083 and PK7088 and other compounds raise hopes for efficient targeting of this mutant in cancer patients. It will be of great interest to investigate the efficacy of these compounds in animal models and ultimately in clinical trials.

The compounds PRIMA-1 and APR-246 (PRIMA-1^{MET}) were identified using a different approach. Screening of the Diversity set of 2000 compounds from the National Cancer Institute using a cell-based assay led to the identification of two compounds, PRIMA-1 [19] and MIRA-1 [20]. PRIMA-1 and the PRIMA-1 analog PRIMA-1^{MET}, now named APR-246, can restore wild type conformation to mutant p53, induce apoptosis in tumor cells, and inhibit tumor growth in mice. PRIMA-1 and APR-246 are both converted to methylene quinuclidinone (MQ), Michael acceptor that can bind covalently to cysteines in mutant p53 [21]. Importantly, transfer of MQ-modified mutant p53 protein into p53 null tumor cells induced massive apoptosis, indicating that covalent binding of MQ *per se* is sufficient to reactivate mutant p53 and induce a p53 biological response.

PRIMA-1 and APR-246 have been shown to inhibit tumor growth in vitro and in vivo upon systemic administration in various tumor types and models. PRIMA-1 was first shown to inhibit growth of osteosarcoma xenografts expressing mutant p53 in SCID mice [19]. APR-246 inhibited growth of mutant p53-carrying small cell lung carcinoma (SCLC) cells in vitro and suppressed SCLC xenograft growth in mice [22]. Other studies have demonstrated that APR-246 is active against multiple myeloma cells in vitro and inhibits multiple myeloma tumor growth in mice [23].

MQ can also bind to cysteine residues in wild type p53, and the binding is increased in unfolded p53 [21]. Consistent with this finding, APR-246 was shown to restore the function of inactive wild type p53 in a three-dimensional collagen gel model of melanoma [24]. APR-246 treatment induced expression of pro-apoptotic p53 targets Apaf1 and Puma and triggered cell death by apoptosis. APR-246 also inhibited melanoma xenograft tumors in vivo in a p53-dependent manner. Similarly, APR-246 showed dose-dependent cytotoxic and apoptotic effects in AML cell lines as well as in primary AML patient cells irrespective of p53 status [25], and induced apoptosis and upregulation of p53 target genes p21, Puma and Noxa in Ewing sarcoma cells carrying either wild type or mutant p53 [26]. PRIMA-1 was also shown to significantly

delay formation of adenocarcinomas and induce accumulation of nuclear wild type p53 in a nitrosamine-induced lung cancer model in mice, suggesting a possible use for this type of compounds in chemoprevention [27].

As an electrophilic molecule, MQ could potentially bind to cysteines in many proteins in cells. However, not all such modifications will necessarily have cytotoxic effects [21]. Moreover, cysteine modification will be affected by various factors that limit the number of protein targets, including accessibility of cysteines to the potential ligand, nucleophilicity of the sulfur atom, and flexibility of the targeted structural domain. Nevertheless, it is likely that modification of specific proteins by MQ will lead to cytotoxicity. Modification of TrxR1 (Thioredoxin reductase 1) by APR-246/MQ, which converts this enzyme from a reductase to an oxidase that can induce ROS (reactive oxygen species), may contribute to APR-246-induced cell death and probably accounts, at least in part, for the observed effects of APR-246 on p53 null cancer cells [28].

Rieber and Strasberg-Rieber assessed the efficacy of PRIMA-1 under hypoxic conditions and showed that hypoxia enhanced the sensitivity of SKBR3 breast carcinoma cells carrying mutant p53 to PRIMA-1. Also, combination of PRIMA-1 with peroxidase increased apoptosis and induction of Puma and Mn-SOD in MCF-7 breast carcinoma cells overexpressing mutant p53 [29]. Hypoxia led to increased reactivity with the mutant p53-specific antibody PAb240.

These results indicate that the antitumor activity of PRIMA-1 is influenced by hypoxia and suggest that PRIMA-1 may be useful for addressing chemoresistance in hypoxic tumors.

The p53 family proteins p63 and p73 share high sequence homology with p53 in their DNA binding domains [30]. APR-246 can restore the pro-apoptotic function to mutant TAp63 γ and TAp73 β in tumor cells and induce expression of the p53/p63/p73 downstream targets p21 and Noxa [31]. Moreover, APR-246 can rescue mutant p63 in primary adult skin keratinocytes from ectrodactyly, ectodermal dysplasia, and cleft lip/palate (EEC) syndrome patients with p63 mutations. Keratinocytes from these patients show impaired epidermal differentiation. Treatment with APR-246 partially rescued morphology and gene expression during epidermal stratification, and restored expression of p63-regulated genes [32]. Also, APR-246 was shown to revert corneal epithelial lineage commitment and restore a normal p63-related signaling pathway in induced pluripotent stem cells (iPSC) from EEC patients carrying two different point mutations in the DNA binding domain of p63 [33]. The reactivation of mutant forms of p53 family proteins by APR-246 indicates a common mechanism that presumably involves homologous structural elements in the targeted proteins. Thus, as shown in Fig. 2, targeting two structurally related proteins by APR-246 through a similar molecular mechanism leads to entirely different biological outcomes.

The maleimide MIRA-1 was identified along with PRIMA-1 in our screen of the NCI Diversity set [20]. MIRA-1 and its structural analogs that contain a reactive carbon–carbon double bond are capable of mutant reactivation [20]. MIRA-1 analogs that lack the reactive double bond show no biological mutant p53-dependent activity, consistent with the notion that Michael acceptor activity is critical for mutant p53 reactivation. Indeed, other cysteine-targeting compounds have been shown to affect stability of mutant p53. Kaar et al. [34] identified a series of compounds containing an α,β -unsaturated double bond, characteristic of Michael acceptors, that can bind covalently to p53 cysteines. Binding was shown to raise the melting temperature of wild type and hotspot mutant R175H, Y220C, G245S, R249S, and R282 core domains. Mass spectrometry revealed binding to C124 and C141, followed by C135, C182, C277, C176 and C275. These modifications reduced p53 DNA binding activity. Modification of C277, which is located on the DNA-binding surface of p53, is probably most critical for this effect. Other studies have identified C182 and C277 as preferential sites for modification by *N*-ethylmaleimide [35].

The compound CP-31398 was identified as a substance that protects p53 from thermal denaturation [36]. CP-31398 has anti-tumor activity in vivo in a transgenic UPII-SV40T mouse model of bladder transitional cell carcinoma [37]. Also, the compound has shown strong cancer chemopreventive ability in a nitrosamine-induced lung cancer model [27]. CP-31398 seems to be able not only reactivate mutant p53 but also induce stabilization of wild type p53 [38], probably at least in part due to its ability to bind DNA [39]. CP-31398 has a reactive carbon–carbon double bond but its activity as Michael acceptor is compromised due to steric effects of neighboring functional groups [40]. STIMA-1, a low molecular weight compound and Michael acceptor with some structural similarity to CP-31398, can enhance mutant p53 DNA binding and expression of p53 target proteins and induce mutant p53-dependent apoptosis in human tumor cells [40].

Several other mutant p53-targeting small molecules have been identified by various approaches. The small molecule SCH529074 was shown to bind to the core domain of p53 and act as a chaperone that restores wild type function [41]. In addition, it inhibits p53 ubiquitination by Mdm2. Ellipticine [42] and the compound P53R3 [43] have also been shown to reactivate mutant p53. However, the molecular mechanisms of action of these compounds with respect to mutant p53 reactivation are not fully understood and data on their efficacy in animal models have so far not been reported.

Molecular modeling may potentially be a fruitful strategy for identification of novel compounds that stabilize and/or refold p53, but one problem in such studies is the highly flexible structure of the p53 core domain that prevents reliable predictions of protein structure behavior in solution and upon ligand binding.

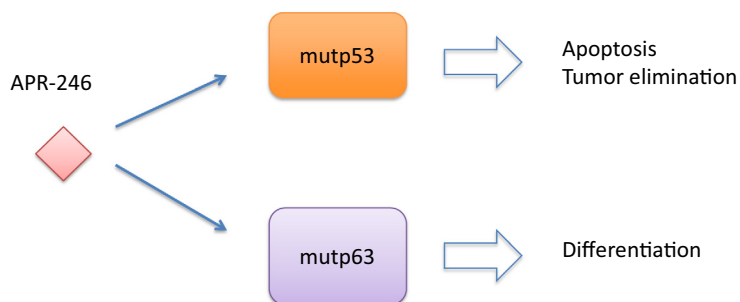


Fig. 2. APR-246 targets both mutant p53 and mutant p63. In the case of mutant p53, reactivation by APR-246 in tumor cells triggers cell death by apoptosis which eliminates the tumor. For mutant p63, reactivation by APR-246 in keratinocytes derived from EEC syndrome patients restores p63-dependent differentiation. This illustrates how conformational rescue by APR-246 may lead to entirely different biological outcomes in different cell types, even if the molecular mechanism is similar.

Nonetheless, Wassman and colleagues used computational methods to identify a transiently open binding pocket between the L1 loop and sheet S3 in the core domain of p53 [44]. The L1/S3 pocket contains C124, C141 and C135. This particular region is known to harbor several targets for secondary mutations that reactivate certain tumor-derived p53 mutants [45,46], thus suggesting its importance for regulation of p53 folding. Docking simulations showed that p53-targeting compounds MQ, MIRA-1, 2, and 3, and STIMA-1, can bind preferentially to the region corresponding to the L1/S3 pocket in human p53. Introduction of a C124A substitution in R175H mutant p53 abolished the effect of PRIMA-1 in Saos-2 osteosarcoma cells. Although these results need to be confirmed, they provide interesting clues as to which cysteine residues in mutant p53 that might be most relevant for conformational rescue by PRIMA-1 and APR-246.

The compound NSC319726 specifically targets R175H mutant p53 [47]. Analysis of information in NCI's database indicated that two compounds from the thiosemicarbazone family increased growth inhibitory activity in mutant p53-carrying tumor cells, and in particular for those that carry R175H mutant p53. NSC319726 was shown to restore wild type structure and function to R175H mutant p53, induce apoptosis in R175H mutant p53-carrying tumor cells, and inhibit growth of R175H mutant p53-carrying tumor xenografts. The mechanism of action of NSC319726 is not fully understood but seems to depend on the zinc ion-chelating properties of the compound. These results are consistent with previous studies showing the importance of zinc for p53 function (reviewed in [5,48]).

4. Targeting complexes between mutant p53 and other proteins

An alternative approach to targeting mutant p53 itself is disruption of complex formation between mutant p53 and p63 or p73 (Fig. 1). As mentioned above, mutant p53 may exert gain-of-function activity by binding and blocking the function of other p53 family proteins. The small molecule RETRA activates p53 target genes and inhibits mutant p53-carrying tumor cells in vitro and tumor xenografts in vivo in mice [49]. RETRA induces expression of p73 and releases p73 from mutant p53 complexes, resulting in tumor suppression similar to functional reactivation of mutant p53. RETRA has activity against various p53 mutants and little effect on normal cells. These results demonstrate that complexes between mutant p53 and p53 family member p73 are potential targets for novel anticancer drugs.

5. Exploring combination treatment

Tumor heterogeneity and dynamics pose major difficulties for efficient treatment of cancer. Treatment with targeted anticancer drugs will inevitably lead to selection for variants that show increased resistance, for example by loss or mutation of the drug target. One possible strategy to prevent the emergence of resistant clones is combination therapy. In the case of the mutant p53-targeting compound APR-246, synergy with chemotherapeutic drugs including adriamycin and danorubicin has been observed in vitro and in vivo [23,25,50]. This effect is presumably due to restoration of wild type p53 activity, which is known to be important for the response to DNA-damaging anticancer drugs. It is also plausible that enhanced levels of mutant p53 in tumor cells exposed to DNA-damaging drugs may increase sensitivity to APR-246. Moreover, combination treatment with mutant p53-targeting molecules and wild type p53-activating compounds such as Nutlin that enhance wild type p53 activity by blocking its binding to the Mdm2 protein (for a review of Nutlin and other compounds that disrupt p53–Mdm2 binding, see [48]) is an attractive strategy, as

it should prevent rapid degradation of p53 upon activation of Mdm2. Indeed, synergy between PK7088 and Nutlin was observed [18].

There may also be more unexpected synergies. PRIMA-1 was shown to restore sensitivity of mutant p53-carrying thyroid tumor cells to 3-Deazaneplanocin A (DZNep), an inhibitor of histone methylation [51]. DZNep depletes EZH2, a core component of the polycomb repressive complex 2 (PRC2), which is overexpressed in many human tumors. While DZNep alone only inhibited growth of wild type p53 thyroid tumor cells, the combination of PRIMA-1 and DZNep had potent antitumor effect on mutant p53-expressing thyroid tumor cells in vitro and in xenograft tumors in vivo.

6. Targeting non-sense mutant p53 by induction of translation read-through

Almost 8% of all TP53 mutations in human tumors are non-sense mutations. The most common non-sense TP53 mutation, R213X, is in fact one of the 10 most common TP53 mutations overall (<http://p53.free.fr>). Tumors that carry non-sense p53 mutations lack full length p53 expression but may express low levels of truncated p53. Non-sense mutant mRNA is degraded by non-sense-mediated RNA decay (NMD). Thus, these tumors are not amenable to mutant p53 reactivation by the refolding strategies described above. Previous studies have indicated that aminoglycoside antibiotics such as G418 and gentamicin can induce read-through and restore expression of non-sense mutant p53 and other genes [52]. However, aminoglycosides are not suitable for long term therapy at the required doses due to severe side effects. Thus, identification of novel efficient inducers of read-through of non-sense mutant p53 is a promising approach for improved treatment of a significant fraction of human tumors. The combination of a read-through drug and a novel inhibitor of NMD was recently shown to restore full length p53 expression in tumor cells carrying non-sense mutant p53 [53].

7. Clinical trials with mutant p53-targeting compounds

Many of the mutant p53-targeting compounds are still at an early stage of preclinical development. The most advanced is APR-246 which has been tested in a Phase I/II clinical trial in 22 patients with hematological malignancies or hormone-refractory prostate cancer [54]. The aims of this first-in-man clinical study were to determine maximum-tolerated dose (MTD), safety, dose-limiting toxicities, and pharmacokinetics of APR-246. Patients received 2 h intravenous infusion once per day for 4 days. APR-246 was well tolerated. The most common adverse effects were dizziness, fatigue, headache, and confusion. Pharmacokinetics showed minor interindividual variation and a half-life of 4–5 h. MTD was defined as 60 mg/kg. Analysis of patient tumor cells revealed induction of cell cycle arrest, apoptosis, and p53 target gene expression in several patients upon treatment. Microarray analysis demonstrated changes in genes that regulate cell growth and cell death. Clinical effects were observed in two patients. One AML patient with a p53 core domain mutation (V173M) showed a marked reduction of bone marrow blasts (46–26%), and one non-Hodgkin's lymphoma patient with a p53 splice site mutation showed a minor clinical response by CT scan. This study demonstrates that APR-246 is safe at predicted therapeutic plasma levels, has a favorable pharmacokinetic profile, and can induce biological effects in tumor cells in vivo that are consistent with reactivation of p53 as the proposed mechanism of action. The clinical efficacy of APR-246 should be studied further in Phase II trials.

8. Conclusions

Ambitious efforts to develop pharmacological strategies for reactivation of p53 in human tumors are underway. Activation of wild type p53 by disrupting the interaction between p53 and Mdm2 has been successfully achieved by Nutlin and a range of other small molecules. Several of these compounds are currently in clinical trials. Pharmacological rescue of mutant p53 is an even greater challenge, due to the heterogeneity of mutant p53 proteins and the need to restore proper folding of a mutant protein, as opposed to blocking protein–protein interactions or inhibiting oncogenic kinases. Rational design and random screening approaches have led to the discovery of a number of small molecules that can reactivate specific p53 mutant proteins or act more broadly on various p53 mutants. Other strategies include disruption of complexes between mutant p53 and p53 family proteins p63 and p73, and induction of read-through of non-sense mutations in p53. Combination treatment with mutant p53-targeting compounds and conventional chemotherapeutic drugs, p53–Mdm2 inhibitors or other compounds has shown promising synergies. Thus, progress has been impressive and even if many problems and challenges undoubtedly remain, we are hopefully getting closer to clinical application of mutant p53-targeting drugs for treatment of cancer.

Conflict of interest

V.J.N.B. and K.G.W. are co-founder and shareholders of Aprea AB, a company that develops p53-based anticancer therapy including APR-246. K.G.W. is a member of its board.

Acknowledgements

We thank the Swedish Cancer Society, the Swedish Research Council (VR), the EU FP6 Framework Program, Radiumhemmets Forskningsfonder, StratCan, Stockholms Läns Landsting (ALF), and Karolinska Institutet for generous support. K.G.W. is the recipient of a Distinguished Professor Award from Karolinska Institutet.

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