OPINION

Assessing *TP53* status in human tumours to evaluate clinical outcome

Thierry Soussi and Christophe Béroud‡*

TP53 is probably the most extensively studied tumour-suppressor gene, and patients with *TP53* mutations are known to have a poor outcome. However, inconsistencies in the analysis of *TP53* status, and failure to realize that different mutations behave in different ways, prevent us from effectively applying our vast knowledge of this protein in clinical practice. What simple steps can be taken to ensure that patients benefit from our understanding of *TP53*?

During their lifespan, normal cells are constantly exposed to various forms of endogenous and exogenous stress that alter their normal behaviour. Genetic insults that can lead to mutations are particularly harmful, as their transmission to daughter cells can lead to cancer. To ensure rigorous homeostasis, mammalian cells have selected for key regulators that control normal cell growth. The *TP53* tumour-suppressor gene (which encodes p53 in humans) was initially found to be essential for the DNA-damage checkpoint, but we now know that it responds to a broad range of cellular stresses, including oncogene activation and hypoxia (BOX 1).

The p53 protein functions as a tetrameric transcription factor and is found at very low levels in normal, unstressed cells[1]. Different forms of stress activate signal-transduction pathways that culminate in post-translational modification and stabilization of p53. This accumulation of p53 activates the transcription of genes that are involved in various activities, including cell-cycle inhibition and apoptosis (depending on the cellular context, the extent of damage and other unknown parameters)[2].

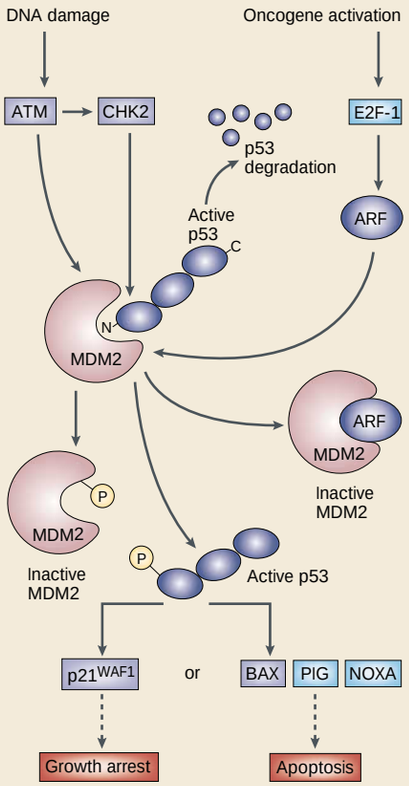
Inactivating *TP53* mutations are the most common genetic alteration found in human cancers[3], and there is growing evidence that inactivation of the p53 pathway occurs in most tumours. Even in cancer types in which *TP53* mutations are rare, p53 function is indirectly abolished either by nuclear exclusion (neuroblastoma), interaction with a viral protein (cervical cancer), interaction with overexpressed MDM2 protein (sarcoma) or inactivation of $p19^{ARF}$ (BOX 1)[4–6]. There are a few tumours in which *TP53* mutations have never been detected, such as testicular cancer and melanoma; but in melanoma the apoptotic pathway that is induced by p53 in response to chemotherapeutic agents is affected by alterations in the *APAF* gene, which acts downstream of p53 (REF. 7). The Li–Fraumeni syndrome is a hereditary predisposition to cancer that is often caused by germ-line mutations of one *TP53* allele. In individuals who have Li–Fraumeni syndrome but lack *TP53* mutations, Bell *et al.* have described germ-line alterations of the CHK2 kinase, which activates p53 after DNA damage[8]. Cells from patients with the radiosensitive and cancer-prone disease ataxia telangiectasia show radioresistant DNA synthesis and a reduced or delayed γ-radiation-induced increase in p53 protein levels[9]. This is due to an inactivating germ-line mutation in ATM, a kinase that activates p53 in response to irradiation. All these data emphasize that most cancer types select cells for loss of p53 function, as it is a central coordinator of cellular responses to stress. Given this important function, inactivation of the p53 pathway would be expected to lead to the selection of more aggressive tumours with a high degree of genetic instability that can be associated with prognosis (disease recurrence and overall survival). Furthermore, the essential function of p53 in apoptosis after DNA damage indicates that its dysfunction could be a predictive factor for the selection of patients who fail to respond to specific therapies (BOX 2).

Since 1989, more than 6,000 papers have described *TP53* alterations in human tumours. However, this vast body of literature contains many conflicting results, making it difficult to obtain a clear picture of whether a particular mutation has a real effect, either as a prognostic or as a predictive marker. How can we best use our state-of-the-art knowledge about the most extensively studied tumour-suppressor protein to develop a strategy that will ensure unbiased analysis of *TP53* alterations in human tumours, so that we can use this information to maximum benefit in clinical practice?

Analysis of p53 status

The first *TP53* mutations were described in 1989 in colon tumours and lung cancer cell lines[10,11]. In the same year, Nigro *et al.* surveyed the *TP53* status of several tumour types and showed that *TP53* mutations are a frequent event in human tumorigenesis[12]. The initial observation that these mutations were localized predominantly in exons 5–8 led to the common belief that most *TP53* mutations are localized in these exons (*TP53* contains 11 exons). We now know that many *TP53* mutations occur outside this region. Fortunately, this focus on exons 5–8 did not lead to an underestimation of the frequency of *TP53* mutations in various cancer types, as many mutation screens have been conducted and some have examined the gene beyond these exons. However, as discussed below, this false assumption can be a source of significant bias in clinical studies.

Box 1 | The p53 pathway in cellular homeostasis and cancer



In normal cells, the transcription factor p53 is inactivated by MDM2, a ubiquitin ligase that targets p53 for degradation in the proteasome and also conceals the transactivation domain of p53. Several types of stress can activate p53, including DNA damage and oncogene activation (see figure), hypoxia, depletion of the cell's nucleotide pool or defects in DNA methylation. Each type of stress is communicated to p53 by distinct mechanisms: p53 is the master switch that integrates signals from these pathways and transforms them into a second series of signals that trigger a cellular response. This switch seems to be flipped by many post-translational modifications. For example, DNA damage triggers inactivation of MDM2 through phosphorylation of p53 and MDM2, leading to dissociation of the p53–MDM2 complex. This phosphorylation is catalysed by several kinases, including ATM and CHK2; the germ-line inactivation of these two kinases has been associated with cancer predisposition (ataxia telangiectasia and Li–Fraumeni syndrome, respectively).

Oncogene activation activates p53 in a different way: in this case, activation of the transcription factor E2F-1 leads to production of ARF, which is thought to sequester MDM2 in the nucleolus.

The number of genes transactivated by p53 might be as many as several hundred — at least when p53 is artificially overexpressed — but only a few of them have been fully validated in normal cells or tissues[65–69]. One well defined p53 target gene encodes the cyclin-dependent kinase inhibitor $p21^{WAF1}$, which blocks cell division. One of the main uncertainties in the p53 pathway concerns whether growth arrest or apoptosis occurs. The apoptosis pathway is more easily triggered in transformed cells than in normal cells, indicating that only studies of normal cells or normal tissues will be able to define the mechanism that decides cell fate after p53 induction. Genes known to be inactivated in human tumours are coloured blue, whereas those that are activated are coloured pink (see REFS 2, 70 for a more complete picture of the complexity of the p53 network).

Two different methodologies have been used to assess *TP53* alterations: DNA sequencing and immunohistochemical staining. Most *TP53* alterations are point missense mutations that lead to the synthesis of a stable, but inactive, protein that accumulates in the nucleus of tumour cells[13]. The correlation between p53 accumulation and *TP53* mutation is about 80%, as frameshift mutations do not lead to p53 accumulation. In a recent update of our p53 database, we analysed the strategy used to search for *TP53* mutations in more than 1,200 publications[3]. As shown in FIG. 1, 40% (500) of the studies examined exons 5–8, whereas only 14% (158) focused on the entire *TP53* gene (except for exon 1, which is noncoding). Similar results are observed when examining each cancer type individually.

Another possible source of bias concerns splice-site mutations. These types of mutation are thought to be relatively infrequent (about 2%) and their effects have not been well characterized. However, in a recent study, Varley *et al.* reported germ-line splice-site mutations in 7 of 40 families (17.5%) with Li–Fraumeni syndrome[14], and splicing was altered in 6 cases. Perhaps the real incidence of splice-site mutations is closer to this figure, as it has been underestimated in the past because splice junctions are rarely analysed.

Analysis of the 158 studies that screened the entire *TP53* gene shows that focusing on exons 5–8 leads to an unacceptable bias. A total of 13.6% of mutations are located outside exons 5–8, with a significant number of mutations in exons 4, 10 and, to a lesser extent, 9 (FIG. 2 and ONLINE TABLE 1). This bias can be observed in all types of cancer, but also for each specific cancer, indicating that the differences are not due to the particular distribution of mutations for a given type of cancer. Furthermore, analysis of *TP53* mutations found in exons 4, 9 and 10 shows that they contain a significantly greater number of frameshift or nonsense mutations than mutations in exons 4–8 (FIG. 3). Such null mutations are usually not detected by immunohistochemical analysis because no protein is produced.

Frameshift mutations can lead to a different phenotype than that observed with missense mutations. Mutations outside the DNA-binding domain can show unusual behaviour, as recently described in a Li–Fraumeni syndrome family with a mutation in exon 4 (REF. 15). By missing 13.6% of all *TP53* mutations, studies designed to determine the clinical value of *TP53* alterations can come to erroneous conclusions that would be highly detrimental to our assessment of the value of this marker. This would also explain the vast heterogeneity of the results in the various published studies, as exemplified by studies of non-small-cell lung carcinoma (NSCLC). Three recent studies focusing on either the entire gene or on exons 4–10 found a good correlation between *TP53* mutations and poor outcome[16–18], whereas no prognostic significance was found when the analysis was restricted to exons 5–8 (REF. 19). In colon or lung cancer, the various studies did not detect any noticeable geographical variation in the pattern of *TP53* mutations. In breast cancer, the situation is very different, with a marked geographical heterogeneity. The frequency of frameshift mutations was high in the United States Mid-West, whereas a GC-to-AT transition at non CpG dinucleotide was high is New Orleans[20].

Behaviour of different mutant proteins

The importance of correlating prognosis or treatment outcome with individual mutations is becoming more apparent as we learn more about their functional differences. These can be understood by mapping them onto the three-dimensional structure of p53. To bind DNA, p53 must first form a homotetramer (FIG. 4). This is mediated by an oligomerization domain in the carboxyl terminus of the protein. Most of the mutations that occur in human tumours produce an altered p53 protein that cannot bind DNA, resulting in impaired transactivation[21–23]. As human carcinomas clearly select for p53 missense mutations rather than deletion of *TP53*, additional oncogenic mechanisms can occur. In some cases,

Box 2 | Prognostic and predictive markers

It is essential to avoid confusion about the terms prognostic and predictive. A prognostic marker can be defined as any factor that, at the time of diagnosis, can provide information on the clinical outcome of the patient, such as survival or disease-free survival. The most powerful prognostic factors are tumour size, clinical spread (stage) and histological grade. Among the molecular markers that have been tested during the past decade, *N-MYC* amplification in neuroblastoma remains the best prognostic marker. A predictive factor is defined as any marker that gives information regarding the response to a specific treatment. Prototype predictive markers are the oestrogen and progesterone receptors that mediate the response to the hormone therapy tamoxifen.

With a few exceptions, none of the potentially useful prognostic or predictive markers have led to any consistent results among independent clinical studies. Factors that influence these studies include inadequate patient recruitment (sample size, diagnostic entry criteria, heterogeneous treatment) and methodological problems (quality of starting tissue, assay variability). This unsatisfactory situation has led several authors to propose a hierarchy of prognostic and predictive studies, analogous to the hierarchical study design in drug trials. Such an approach allows logical exploration and step-by-step validation of potential markers. Phase I studies are early exploratory studies of the association between a prognostic marker and important disease characteristics. They should also lead to the definition of a standardized assay. Phase II studies should define the clinical utility of the marker by identifying the optimal cut-off value between high-risk and low-risk patients. Both of these retrospective phases should be performed in carefully controlled (preferably case-controlled) cohorts of well-defined patients. Phase III studies are large, prospective, confirmatory studies in which the marker is evaluated and compared with other well-defined factors.

The *TP53* status in human cancer could be considered at the end of Phase I. Several meta-analyses have indicated that, despite disagreement in the literature, *TP53* status could have prognostic significance in non-small-cell lung cancer, non-Hodgkin's lymphomas and breast cancer, so the time is ripe to begin Phase II studies to unravel the true potential of using *TP53* status for clinical decision-making.

mutant p53 can have a dominant-negative activity when expressed with wild-type p53. Mixed p53 tetramers with both wild-type and mutant p53 have an altered activity that varies for different mutants[24]. There is also evidence that some mutant p53 proteins might present an increased oncogenic function both *in vitro* and in animal models[25–27]. For example, the H175 mutant is associated with increased resistance to etoposide[28], a DNA-damaging chemotherapeutic agent. Most mutant p53 proteins have lost their DNA-binding activity, leading to loss of their growth inhibition and apoptotic properties. However, some mutants have an impaired apoptotic capacity despite wild-type growth-arrest activity[29]. Mutant p53 behaviour also depends on cell type[30].

Two classes of mutations have been distinguished on the basis of various *in vitro* assays and the three-dimensional structure of the protein[31]: class I mutations, exemplified by mutants at codon 248 (7.6% in the p53 database), affect amino acids that are directly involved in the protein–DNA interaction. They have a wild-type conformation, as probed by conformational monoclonal antibodies, and they do not bind to the heat-shock protein HSP70 (REFS 32,33). Class II mutations, exemplified by the mutant at codon 175 (4.9% in the database), have an altered conformation with intense binding to HSP70. The amino acids that are altered in this class of mutants are involved in stabilizing the tertiary structure of the protein. Class II mutations are associated with a more severe phenotype *in vitro* than class I mutations[32]. Due to an irreversible change of conformation, class II mutants cannot be restored to the wild-type conformation by activating antibodies or peptides[34]. Such heterogeneity can also lie in the nature of the resulting residue. The H273 mutant has a wild-type conformation, whereas the P273 mutant is denatured[32]. This biochemical and biological heterogeneity has been confirmed and refined by structural studies. For example, nuclear magnetic resonance spectroscopy indicates that mutations in the L3 domain can induce either limited or extensive conformational changes, depending on their position or the type of substitution[35,36].

Do these differences in structure and function of the various p53 mutants have clinical implications? Several studies have revealed that specific p53 mutations are associated with either a poorer prognosis or a poor response to treatment (TABLE 1). In breast[37–39] and colon cancer[40,41], there is a strong association between mutations in the L2/L3 loop and shorter survival or poor response to treatment. These data are also emphasized by the observation that the distribution of tumours in *Trp53*$^{-/-}$ mice (*Trp53* encodes p53 in mice) differs from that of mice harbouring a point mutation[42].

It is also essential to consider the genetic background of the patient. Although no p53 modifier genes have been described so far, we cannot rule out the possibility that the efficiency of several DNA-repair pathways could influence p53 behaviour. This has been highlighted by the recent finding that patients with a germ-line mutation in the DNA-repair gene *BRCA1* have a different pattern of *TP53* mutations, associated with unusual biochemical properties[43,44]. This particular observation can be linked to the high frequency of *TP53* mutations in medullary breast cancer (more than 90%), a tumour that is linked to a very good prognosis and is more frequent in families with *BRCA1* mutations than in the general population[45].

The p53 family members, p63 and p73

Two additional p53 family members, p63 and p73, have recently been identified and characterized[46]. p63 and p73 both contain regions that correspond to the amino-terminal transactivation, central DNA-binding and carboxy-terminal oligomerization domains of p53 (REF. 46). Owing to their structural similarities, p63 and p73 can bind to p53 consensus sequences, activate transcription of several p53 target genes, and induce apoptosis when overexpressed in cells. However, unlike *TP53*, which encodes a single polypeptide, *TP63* and *TP73* (the genes that encode p63 and p73 in humans) are more complex and possess at least two main transcriptional promoters, which direct more than six unique

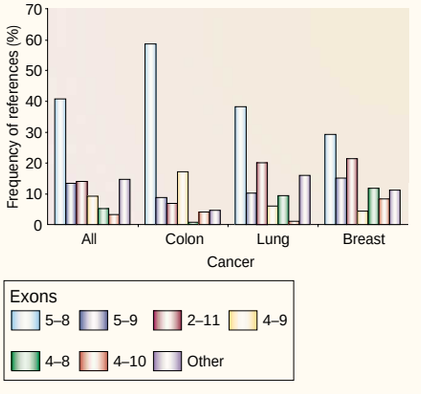


Figure 1 | **Literature survey of strategies used for mutation analysis of *TP53*.** We analysed the sequence region screened in papers published between 1989 and February 2001 for 1,281 references. 'Other' refers to studies in which only partial analysis of the p53 gene was performed, such as single-exon screening.

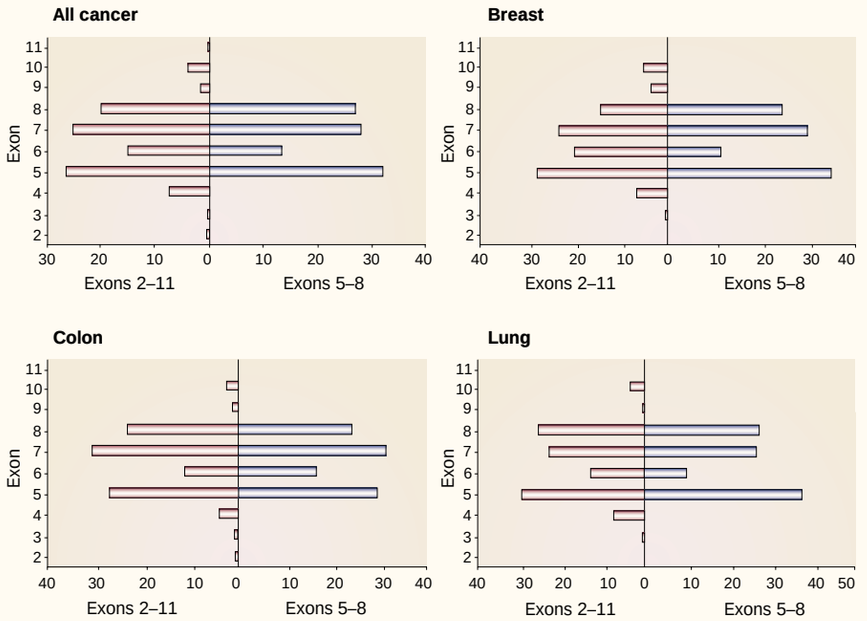


Figure 2 | **Distribution (%) of *TP53* mutations along its exons.** For each cancer type, studies that analysed exons 2–11 (red) are compared with studies that analysed only the central region (exons 5–8, blue).

products that have different activities as transcription factors. All isotypes possess a fully functional DNA-binding domain and the C-terminal oligomerization domains. The two alternate promoters generate isoforms that lack the N-terminal transactivation domain. These isoforms, known as ΔNp63 and ΔNp73, are likely to act as dominant-negative regulators of their full-length counterparts. Several splicing variants generate different C termini, some of which contain a sterile α motif (SAM) domain, known to be involved in protein–protein interactions[47,48]. Biologically, the function of p63 and p73 does not seem to be linked to the protection of genomic integrity, as these genes do not rescue p53 knockout mice from cancer susceptibility. Although the function of p73 is still unclear, a more accurate picture is available for p63. Its expression is particularly high in progenitor or stem cells of epithelial tissues and is gradually lost during differentiation. This function in differentiation is highlighted by the observation that *Trp63* (which encodes p63 in mice) knockout mice have serious epithelial defects.

Molecular analysis has failed to reveal any mutation in these two genes in human cancer, but recent studies have described the accumulation of p63 and p73 in various human tumours[47–49]. Although wild-type p53 cannot form tetramers with full-length p63 and p73, it has been shown that some p53 mutants can form hetero-tetramers with p63 and p73, leading to functional inactivation of their transactivational activity[50–52]. Such behaviour is associated with specific p53 mutants that undergo a conformational change. This association interferes with the transcriptional activity of p63 and p73, and their ability to induce apoptosis. As p73 is phosphorylated in response to the chemotherapeutic agent cisplatin, it is possible that binding of mutant p53 to p73 affects sensitivity to this drug[53] as a consequence of a gain of function for mutant p53. The formation of these heterotetramers is restricted to p53 mutants that carry the Arg72 polymorphism (see below). All these data indicate that a dominant activity of specific p53 mutants, associated with a defined genotype, could act through inactivation of the p63 and p73 pathways.

Wild-type p53 can also bind to the truncated p63 isoform, ΔNp63, and induce its degradation through a caspase-dependent mechanism. This indicates that p53 could act as a negative regulator of p63, which acts as a positive regulator of epithelial cell growth[54]. As 80% of human tumours are of epithelial origin, it is tempting to suggest that p53 mutants that can no longer bind ΔNp63 might have lost this brake on epithelial cell growth.

The Arg/Pro72 polymorphism

Polymorphism at position 72 of the p53 protein leads to a variation in the protein sequence (Arg/Pro variation). It has been shown that the Arg72 form is more sensitive to degradation induced by human papillomavirus (HPV) E6 protein than the Pro72 variant[55]. This sensitivity could be clinically important, as it has been clearly established that p53 degradation is an important feature of HPV-associated tumours, such as cervical or head and neck cancers. Several reports have described an over-representation of the homozygous Arg72 form in patients with cervical cancer compared with the normal population, but this result is highly controversial[55–60]. Although it is beyond the scope of this article to analyse this controversy, it is nevertheless important to take into account the recent discovery, described above, that conformational p53 mutants with an Arg72 polymorphism have a transdominant negative effect on p73 by forming hetero-oligomers with this protein[51]. This activity could lead to an enhanced pathological role for the Arg72 polymorphism in tissues that normally express high levels of p63 or p73. It has also been shown that *TP53* mutations predominantly occur at the Arg72 allele in non-melanoma skin cancer and squamous-cell cancers of the vulva or head and neck[51]. This preference is independent of the HPV genotype. An interesting observation is the variation of this polymorphism in the normal population[61]: the frequency of the Pro allele is 17% in Sweden and Finland, but 63% in black Africans from Nigeria. It has been speculated that the Pro allele was selected for its protective effect against skin cancer. A high level of *TP63* expression is observed in epithelial tissues such as the

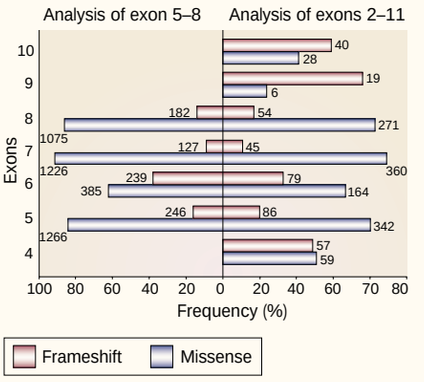


Figure 3 | **Distribution of mutational events in each exon of the *TP53* gene.** Studies focusing on the central region (exons 5–8) are compared with those analysing all coding regions (exons 2–11). Exons 2, 3 and 11 have been omitted owing to the low frequency of *TP53* mutations recorded, which does not allow statistical analysis. Numbers at the end of each column are the numbers of mutations recorded for each category.

skin, and *Trp63* knockout mice lack an epidermis and other squamous epithelia, although whether these two findings are connected remains to be determined. It is, therefore, important to evaluate the role of the Arg/Pro72 polymorphism in various types of cancer. Unfortunately, this polymorphism is located in exon 4 and consequently, as discussed above, has been missed by many studies of p53 status.

Recommendations for analysing p53

We would like to propose some guidelines for analysing *TP53* mutation status in human cancer. We will not address technical recommendations (patient recruitment, starting materials, methods used for prescreening or sequencing methods), as they are beyond the scope of this article. p53 analysis in human tumours is an important challenge, as it can be linked to short survival or poor response to treatment. Either alone or in combination with genotyping of the components of other pathways, p53 analysis can be important for the choice of treatment. This could be highly relevant when comparing the *TP53* mutational status of primary tumours before therapy with that of their therapy-resistant progeny after relapse or in metastases. Such a comparison would highlight specific *TP53* mutations that are more prone to yielding drug-resistant tumours, and the detection of which might affect treatment choices.

As discussed above, the relationship between *TP53* mutation and p53 inactivation is not straightforward and can be influenced by many parameters, including the site of the mutation, the resulting substitution and some natural polymorphisms. In clinical studies that evaluate p53 inactivation as a significant marker, it is therefore important to adopt a clearly standardized strategy. We recommend the following guidelines.

First, only molecular analysis should be performed, as immuno-histochemical analysis cannot distinguish the various types of mutations. It also misses frameshift and nonsense mutation (11.3% and 7.5%, respectively, of mutations found in the p53 mutation database).

Second, *TP53* analysis should not be restricted to exons 5–8, as this leads to an unacceptable bias. Ideally, the entire coding region of *TP53* should be analysed (exons 2–11), including the splice junctions, although analysis of exons 4–10 might be acceptable because it would miss fewer than 1% of all mutations. Richard Iggo and colleagues have developed an assay in yeast that allows the screening of codons 52–364 (68% of exons 4–10) using mRNA as starting material. Ultimately, however, genomic sequencing should be performed, as analysis of RNA can also lead to under-representation of splice-site or nonsense mutations[62]. DNA chip analysis could be one of the favourite methodologies in the future, as it combines good sensitivity with high throughput[63].

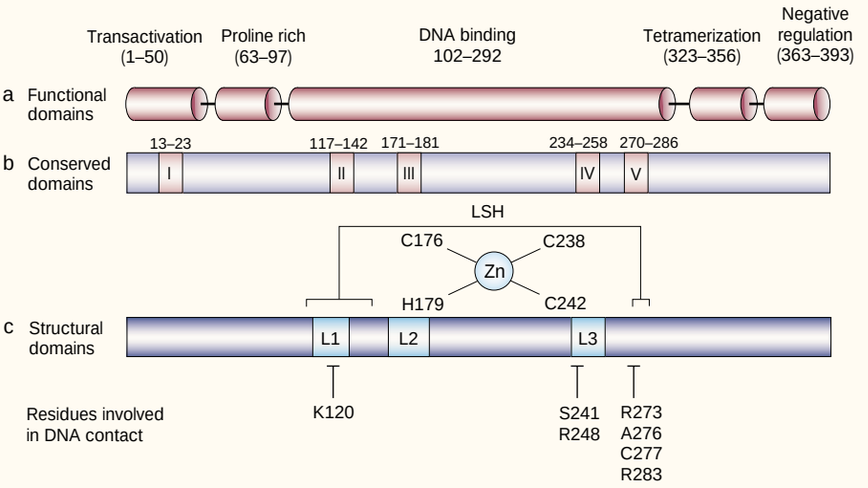


Figure 4 | **Schematic representation of the p53 protein. a** | The functional domains, **b** | regions of sequence conservation, and **c** | structural domains. L1, L2, and L3 indicate loops, and LSH indicates a loop–sheet–helix structure. Tetrahedrally coordinated zinc is necessary for DNA binding. Adapted from REF. 71.

Third, although its association with cancer susceptibility is still uncertain, the polymorphism at codon 72 in exon 4 should be checked and reported with *TP53* mutations. At present, it would probably not be practical to analyse both copies of *TP53* in normal tissue for each patient, and then to work out which allele was preferentially lost in heterozygotes. Nevertheless, large-scale analysis of the distribution of the Arg/Pro72 polymorphism in human tumours should allow the detection of any bias in relation to the normal population. The north–south gradient discussed above should also be taken into account.

Finally, the relationship between specific *TP53* mutations, structural elements of p53 and clinical outcome should be assessed using more rigorous criteria. It cannot be assumed that one mutation in a particular region (the L3 loop, for example) will behave in much the same way as another in the same region. As discussed above, there is a wide heterogeneity in the behaviour of *TP53* mutations, and this can be mutant and/or tissue specific (ONLINE TABLE 1). The observation that particular *TP53* mutations could affect specific treatments should allow the clinician to tailor a therapy to the molecular defect. As discussed by Bullock and Fersht[64], the development of drugs that could rescue some of these mutant p53 proteins emphasizes the need for a thorough molecular analysis when identifying *TP53* mutations.

Studying the relationship between genotype and phenotype is particularly complex for p53. This is not true for all oncogenes and tumour suppressors. In the case of the adenomatous polyposis coli (*APC*) gene, the severity of colorectal carcinoma or the presence of ocular lesions is strictly correlated with the location of the mutation along the *APC* gene. For the *RET* gene, the location of the mutation and other unknown factors determine the type of disease associated with the alteration. But for *TP53*, which is mutated in more than 50% of human cancers, the situation is much more complex, as p53 has a central role in various important pathways that are responsible for maintaining cellular integrity. The observation that some p53 mutants can present a gain of function in relation to other pathways that might be cell specific further encourages a rational strategy for the analysis of p53 alterations, and might allow us to explain the conflicting reports that are published in the literature. Ultimately, understanding the behaviour of each mutation, and analysing it thoroughly for each patient, could allow us to develop sound correlations between *TP53* status and patient outcome.

As mentioned above, p53 is only one element in a network of pathways that link stress to growth control. Several other proteins, such as $p19^{ARF}$, APAF1, ATM, CHK2 and MDM2, can be targets for genetic alterations and contribute to the transformed phenotype. It remains to be determined whether

Table 1 | Prognostic significance of mutations in different structural and functional regions of *TP53*

Number of patients	Screening method*	Exons analysed	Frequency of *TP53* mutations	Clinical findings‡	References
Breast cancer					
63	CDGE	5–8	5 frameshift; 1 nonsense; 12 missense	Patients with mutations in the L2/L3 domain have a poor response to doxorubicin compared with patients who have other types of mutation or wild-type p53 (p=0.01)	72
91§	TTGE	2–11	6 frameshift; 4 nonsense; 16 missense	Patients with mutations in the L2/L3 domain have a poor response to doxorubicin compared with patients who have other types of mutation or wild-type p53 (p=0.014)	73
600‖	NA	5–8	13 frameshift; 14 nonsense; 92 missense	Patients with mutations in the L2/L3 domain have a shorter survival compared with patients who have other types of mutation (p=0.012)	39
76	Yeast assay	–	9 frameshift; 2 nonsense; 21 missense	Patients with DNA contact mutations have a shorter survival compared with patients who have structural mutations (p<0.025)¶	74
1037	SSCP	4–8	178	Patients with mutations in exon 4 have a poor prognosis compared with patients who have wild-type p53 (p<0.0001)	75
222	SSCP	5–8	3 frameshift; 1 nonsense; 43 missense	Patients with mutations in the L2/L3 domain have a shorter survival compared with patients who have wild-type p53 (p=0.02)	37
123	SSCP	5–8	3 frameshift; 1 nonsense; 2 splicing; 18 missense	Patients with mutations in the L2/L3 domain have a shorter survival compared with patients who have other types of mutation or wild-type p53 (p=0.0007)	76
205	TGGE	5–8	10 silent; 9 frameshift; 4 nonsense; 34 missense	Patients with mutations in the L2/L3 domain show no differences in survival compared with patients who have other types of mutation or wild-type p53 (p=0.17)	38
243	Direct sequencing	2–11	17 frameshift; 11 nonsense; 62 missense	Patients with mutations in the L3 domain or in DNA contact residues have a poorer response to tamoxifen than patients with other types of mutation or with wild-type p53 (statistical significance not given)	77
Colon cancer					
273#	CDGE	5–8	15 frameshift; 11 nonsense; 94 missense	Association between p53 mutations and aneuploidy (p<0.00001). Patients with nonsense and frameshift mutations are significantly over-represented in diploid and hyperdiploid tumours compared with aneuploid tumours (p=0.003)	78
222	CDGE	5–8	16 frameshift; 7 nonsense; 77 missense; 5 undefined	Patients with p53 mutations in the L3 domain have a shorter survival compared with patients with other mutations or with wild-type p53 (detected only by CDGE) (p=0.036)	40
192	Direct sequencing	4–9	109 not fully described	Patients with mutations at position 175 (L2 loop) have a shorter survival compared with patients who have other mutations (p=0.0007)	41
Non-small-cell lung cancer					
204	SSCP	5–8	3 frameshift; 2 nonsense; 70 missense	Patients with mutations in exon 8 have a shorter survival compared with patients who have other mutations or no mutation (p<0.001)	79
144	SSCP	4–8,10	10 frameshift; 7 nonsense; 4 splicing; 44 missense	Patients with null mutations have a poor survival compared with patients who have other mutations or missense mutations (p=0.079)	16
103	SSCP	2–11	6 frameshift; 3 nonsense; 40 missense	Patients with missense mutations have a poor prognosis compared with patients who have null mutations in stage I (p<0.001)	18
148	SSCP	4–9	13 frameshift; 8 nonsense; 56 missense; 7 splice	Patients with *TP53* mutations in the L2 + L3 domain or in the zinc-coordinating residues have a shorter survival compared with patients who have other mutations or wild-type *TP53* (HR=2.36;95% CI,1.18-4.74) and (HR=11.7; 95% CI,3,56-38.69), respectively	17
81	SSCP	5–9	17 missense	Patients with mutations in exon 5 have a shorter survival compared with patients who have wild-type p53 (p=0.007)	80
Head and neck cancer					
86	Direct sequencing	5–8	2 frameshift; 2 silent; 35 missense	Patients with contact mutations have a shorter survival compared with patients who have other mutations or wild-type p53 (p=0.0055)	81
Oesophageal cancer					
138	SSCP	5–8	7 nonsense; 12 frameshift; 59 missense	Patients with p53 mutations in the L2 + L3 domain have a shorter survival compared with patients who have other mutations or wild-type p53 (p=0.015)	82

*Prescreening methods used for the localization of *TP53* mutations. For all studies, DNA sequencing was performed to characterize the mutations.
‡The definition of the L2 (residues 163–195) and L3 (residues 236–251) domains of p53 are similar in most studies, whereas the definition of contact and structural mutants varies.
§Includes patients from previous studies published by Aas *et al*. in 1996.
‖Patients from six different countries from a collaborative study of genetic changes in breast cancer.
¶The definition of contact residue used in this study was not described.
#Includes patients from REF. 78.
CDGE, constant denaturant gel electrophoresis; NA, not available; TTGE, temperature gradient gel electrophoresis; SSCP, single-strand conformational polymorphism.

their loss of function (or gain of function, in the case of *MDM2*) is fully equivalent to p53 inactivation, but if mutant p53 gain of function is clearly established, it will obviously remain the most important factor in the development of cell transformation.

Thierry Soussi is at the Institut Curie and Université P. & M. Curie, Laboratoire de Génotoxicologie des Tumeurs, 26 rue d'Ulm, 75248 Paris cedex 05, France. Christophe Béroud is at the Hôpital Necker Enfants Malades, U383 INSERM, 149 rue de Sévres, 75015, Paris, France. Correspondence to T. S. e-mail: thierry.soussi@curie.fr

1. Vousden, K. H. p53. Death star. *Cell* **103**, 691–694 (2000).
2. Vogelstein, B., Lane, D. & Levine, A. J. Surfing the p53 network. *Nature* **408**, 307–310 (2000).
3. Soussi, T., Dehouche, K. & Béroud, C. p53 website and analysis of p53 gene mutations in human cancer: forging a link between epidemiology and carcinogenesis. *Hum. Mutat.* **15**, 105–113 (2000).
4. Moll, U. M., Laquaglia, M., Benard, J. & Riou, G. Wild-type p53 protein undergoes cytoplasmic sequestration in undifferentiated neuroblastomas but not in differentiated tumors. *Proc. Natl Acad. Sci. USA* **92**, 4407–4411 (1995).
5. Oliner, J. D., Kinzler, K. W., Meltzer, P. S., Georges, D. L. & Vogelstein, B. Amplification of a gene encoding a p53 associated protein in human sarcomas. *Nature* **358**, 80–83 (1992).
6. Crook, T. *et al.* Clonal p53 mutation in primary cervical cancer- association with human-papillomavirus-negative tumours. *Lancet* **339**, 1070–1073 (1992).
7. Soengas, M. S. *et al.* Inactivation of the apoptosis effector Apaf-1 in malignant melanoma. *Nature* **409**, 207–211 (2001).
8. Bell, D. W. *et al.* Heterozygous germ line hCHK2 mutations in Li–Fraumeni syndrome. *Science* **286**, 2528–2531 (1999).
9. Rotman, G. & Shiloh, Y. ATM: a mediator of multiple responses to genotoxic stress. *Oncogene* **18**, 6135–6144 (1999).
10. Baker, S. J. *et al.* Chromosome 17 deletions and p53 gene mutations in colorectal carcinomas. *Science* **244**, 217–221 (1989).
11. Takahashi, T. *et al.* p53- a frequent target for genetic abnormalities in lung cancer. *Science* **246**, 491–494 (1989).
12. Nigro, J. M. *et al.* Mutations in the p53 gene occur in diverse human tumour types. *Nature* **342**, 705–708 (1989).
13. Dowell, S. P., Wilson, P. O. G., Derias, N. W., Lane, D. P. & Hall, P. A. Clinical utility of the immunocytochemical detection of p53 protein in cytological specimens. *Cancer Res.* **54**, 2914–2918 (1994).
14. Varley, J. M. *et al.* Characterization of germline *TP53* splicing mutations and their genetic and functional analysis. *Oncogene* **20**, 2647–2654 (1999).
15. Gu, J., Kawai, H., Wiederschain, D. & Yuan, Z. M. Mechanism of functional inactivation of a Li–Fraumeni syndrome p53 that has a mutation outside of the DNA-binding domain. *Cancer Res.* **61**, 1741–1746 (2001).
16. Hashimoto, T. *et al.* p53 null mutations undetected by immunohistochemical staining predict a poor outcome with early-stage non-small cell lung carcinomas. *Cancer Res.* **59**, 5572–5577 (1999).
17. Skaug, V. *et al.* p53 mutations in defined structural and functional domains are related to poor clinical outcome in non-small cell lung cancer patients. *Clin. Cancer Res.* **6**, 1031–1037 (2000).
18. Tomizawa, Y. *et al.* Correlation between the status of the p53 gene and survival in patients with stage I non-small cell lung carcinoma. *Oncogene* **18**, 1007–1014 (1999).
19. Schiller, J. H. *et al.* Lack of prognostic significance of p53 and K-ras mutations in primary resected non-small-cell lung cancer on E4592: a Laboratory Ancillary Study on an Eastern Cooperative Oncology Group Prospective Randomized Trial of Postoperative Adjuvant Therapy. *J. Clin. Oncol.* **19**, 448–457 (2001).
20. Hartmann, A., Blaszyk, H., Kovach, J. S. & Sommer, S. S. The molecular epidemiology of p53 gene mutations in human breast cancer. *Trends Genet.* **13**, 27–33 (1997).
21. Kern, S. E. *et al.* Mutant p53 proteins bind DNA abnormally *in vitro*. *Oncogene* **6**, 131–136 (1991).
22. El-Deiry, W. S., Kern, S. E., Pientenpol, J. A., Kinzler, K. W. & Vogelstein, B. Definition of a consensus binding site for p53. *Nature Genet.* **1**, 45–49 (1992).
23. Kern, S. E. *et al.* Oncogenic forms of p53 inhibit p53-regulated gene expression. *Science* **256**, 827–830 (1992).
24. 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).
25. Dittmer, D. *et al.* Gain of function mutations in p53. *Nature Genet.* **4**, 42–46 (1993).
26. Halevy, O., Michalovitz, D. & Oren, M. Different tumor-derived p53 mutants exhibit distinct biological activities. *Science* **250**, 113–116 (1990).
27. Harvey, M. *et al.* A mutant p53 transgene accelerates tumour development in heterozygous but not nullizygous p53 deficient mice. *Nature Genet.* **9**, 305–311 (1995).
28. Blandino, G., Levine, A. J. & Oren, M. Mutant p53 gain of function: differential effects of different p53 mutants on resistance of cultured cells to chemotherapy. *Oncogene* **18**, 477–485 (1999).
29. Rowan, S. *et al.* Specific loss of apoptotic but not cell-cycle arrest function in a human tumor derived p53 mutant. *EMBO J.* **15**, 827–838 (1996).
30. Forrester, K. *et al.* Effects of p53 mutants on wild-type p53-mediated transactivation are cell type dependent. *Oncogene* **10**, 2103–2111 (1995).
31. Cho, Y. J., Gorina, S., Jeffrey, P. D. & Pavletich, N. P. Crystal structure of a p53 tumor suppressor DNA complex: understanding tumorigenic mutations. *Science* **265**, 346–355 (1994).
32. Ory, K., Legros, Y., Auguin, C. & Soussi, T. Analysis of the most representative tumour-derived p53 mutants reveals that changes in protein conformation are not correlated with loss of transactivation or inhibition of cell proliferation. *EMBO J.* **13**, 3496–3504 (1994).
33. Hinds, P. W. *et al.* Mutant p53 DNA clones from human colon carcinomas cooperate with Ras in transforming primary rat cells: a comparison of the 'Hot Spot' mutant phenotypes. *Cell Growth Differ.* **1**, 571–580 (1990).
34. 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).
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. Wong, K. B. *et al.* Hot-spot mutants of p53 core domain evince characteristic local structural changes. *Proc. Natl Acad. Sci. USA* **96**, 8438–8442 (1999).
37. Berns, E. *et al.* Mutations in residues of *TP53* that directly contact DNA predict poor outcome in human primary breast cancer. *Br. J. Cancer* **77**, 1130–1136 (1998).
38. Kucera, E. *et al.* Prognostic significance of mutations in the p53 gene, particularly in the zinc-binding domains, in lymph node- and steroid receptor positive breast cancer patients. *Eur. J. Cancer* **35**, 398–405 (1999).
39. Borresen, A. L. *et al. TP53* mutations and breast cancer prognosis: particularly poor survival rates for cases with mutations in the zinc-binding domains. *Gene Chromosom. Cancer* **14**, 71–75 (1995).
40. Borresen Dale, A. L. *et al. TP53* and long-term prognosis in colorectal cancer: mutations in the L3 zinc-binding domain predict poor survival. *Clin. Cancer Res.* **4**, 203–210 (1998).
41. Goh, H. S., Yao, J. & Smith, D. R. p53 point mutation and survival in colorectal cancer patients. *Cancer Res.* **55**, 5217–5221 (1995).
42. Liu, G. *et al.* High metastatic potential in mice inheriting a targeted p53 missense mutation. *Proc. Natl Acad. Sci. USA* **97**, 4174–4179 (2000).
43. Chappuis, P. O. *et al.* Prognostic significance of p53 mutation in breast cancer: frequent detection of non-missense mutations by yeast functional assay. *Int. J. Cancer* **84**, 587–593 (1999).
44. Smith, P. D. *et al.* Novel p53 mutants selected in BRCA-associated tumours which dissociate transformation suppression from other wild-type p53 functions. *Oncogene* **18**, 2451–2459 (1999).
45. de Cremoux, P. *et al.* p53 mutation as a genetic trait of typical medullary breast carcinoma. *J. Natl Cancer Inst.* **91**, 641–643 (1999).
46. Yang, A. & McKeon, F. p63 and p73: p53 mimics, menaces and more. *Nature Rev. Moll. Cell Biol.* **1**, 199–207 (2000).
47. Ikawa, S., Nakagawara, A. & Ikawa, Y. p53 family genes: structural comparison, expression and mutation. *Cell Death Differ.* **6**, 1154–1161 (1999).
48. Levrero, M. *et al.* Structure, function and regulation of p63 and p73. *Cell Death Differ.* **6**, 1146–1153 (1999).
49. Hibi, K. *et al.* AIS is an oncogene amplified in squamous cell carcinoma. *Proc. Natl Acad. Sci. USA* **97**, 5462–5467 (2000).
50. Strano, S. *et al.* Physical and functional interaction between p53 mutants and different isoforms of p73. *J. Biol. Chem.* **275**, 29503–29512 (2000).
51. Marin, M. C. *et al.* A common polymorphism acts as an intragenic modifier of mutant p53 behaviour. *Nature Genet.* **25**, 47–54 (2000).
52. Gaiddon, C., Lokshin, M., Ahn, J., Zhang, T. & Prives, C. A subset of tumor-derived mutant forms of p53 down-regulate p63 and p73 through a direct interaction with the p53 core domain. *Mol. Cell Biol.* **21**, 1874–1887 (2001).
53. Agami, R., Blandino, G., Oren, M. & Shaul, Y. The tyrosine kinase c-ABL regulates p73 in apoptotic response to cisplatin-induced DNA damage. *Nature* **399**, 806–809 (1999).
54. Ratovitski, E. A. *et al.* p53 associates with and targets ΔNp63 into a protein degradation pathway. *Proc. Natl Acad. Sci. USA* **98**, 1817–1822 (2001).
55. Storey, A. *et al.* Role of a p53 polymorphism in the development of human papillomavirus-associated cancer. *Nature* **393**, 229–234 (1998).
56. Rosenthal, A. N. *et al.* p53 codon 72 polymorphism and risk of cervical cancer in UK. *Lancet* **352**, 871–872 (1998).
57. Storey, A. *et al.* p53 polymorphism and risk of cervical cancer: reply. *Nature* **396**, 532 (1998).
58. Lanham, S., Campbell, I., Watt, P. & Gornall, R. p53 polymorphism and risk of cervical cancer. *Lancet* **352**, 1631–1631 (1998).
59. Helland, A. *et al.* p53 polymorphism and risk of cervical cancer. *Nature* **396**, 530–531 (1998).
60. Zehbe, I. *et al.* p53 codon 72 polymorphism and various human papillomavirus 16 E6 genotypes are risk factors for cervical cancer development. *Cancer Res.* **61**, 608–611 (2001).
61. Beckman, G. *et al.* Is p53 polymorphism maintained by natural selection? *Hum. Hered.* **44**, 266–270 (1994).
62. Stolzenberg, M. C. *et al.* Germ-line exclusion of a single p53 allele by premature termination of translation in a Li–Fraumeni syndrome family. *Oncogene* **9**, 2799–2804 (1994).
63. Ahrendt, S. A. *et al.* Rapid p53 sequence analysis in primary lung cancer using an oligonucleotide probe array. *Proc. Natl Acad. Sci. USA* **96**, 7382–7387 (1999).
64. Bullock, A. N. & Fersht, A. R. Rescuing the function of mutant p53. *Nature Rev. Cancer* **1**, 68–76 (2001).
65. Zhao, R. *et al.* Analysis of p53-regulated gene expression patterns using oligonucleotide arrays. *Genes Dev.* **14**, 981–993 (2000).
66. Yu, J. *et al.* Identification and classification of p53-regulated genes. *Proc. Natl Acad. Sci. USA* **96**, 14517–14522 (1999).
67. Kostic, C. & Shaw, P. H. Isolation and characterization of sixteen novel p53 response genes. *Oncogene* **19**, 3978–3987 (2000).
68. Kannan, K. *et al.* DNA microarrays identification of primary and secondary target genes regulated by p53. *Oncogene* **20**, 2225–2234 (2001).
69. Tokino, T. & Nakamura, Y. The role of p53-target genes in human cancer. *Crit. Rev. Oncol. Hematol.* **33**, 1–6 (2000).
70. Hansen, R. & Oren, M. p53: from inductive signal to cellular effect. *Curr. Opin. Genet. Dev.* **7**, 46–51 (1997).
71. May, P. & May, E. Twenty years of p53 research: structural and functional aspects of the p53 protein. *Oncogene* **18**, 7621–7636 (1999).
72. Aas, T. *et al.* Specific p53 mutations are associated with *de novo* resistance to doxorubicin in breast cancer patients. *Nature Med.* **2**, 811–814 (1996).
73. Geisler, S. *et al.* Influence of *TP53* gene alterations and c-erbB-2 expression on the response to treatment with doxorubicin in locally advanced breast cancer. *Cancer Res.* **61**, 2505–2512 (2001).
74. Takahashi, M. *et al.* Distinct prognostic values of p53 mutations and loss of estrogen receptor and their cumulative effect in primary breast cancers. *Int. J. Cancer* **89**, 92–99 (2000).
75. Powell, B., Soong, R., Iacopetta, B., Seshadri, R. & Smith, D. R. Prognostic significance of mutations to different structural and functional regions of the p53 gene in breast cancer. *Clin. Cancer Res.* **6**, 443–451 (2000).
76. Gentile, M., Jungestrom, M. B., Olsen, K. E., Soderkvist, P. & Wingren, S. p53 and survival in early onset breast cancer: analysis of gene mutations, loss of heterozygosity and protein accumulation. *Eur. J. Cancer* **35**, 1202–1207 (1999).
77. Berns, E. M. *et al.* Complete sequencing of *TP53* predicts poor response to systemic therapy of advanced breast cancer. *Cancer Res.* **60**, 2155–2162 (2000).
78. Clausen, O. P. F. *et al.* Association of p53 accumulation with *TP53* mutations, loss of heterozygosity at 17p13,

and DNA ploidy status in 273 colorectal carcinomas. *Diagn. Mol. Pathol.* **7**, 215–223 (1998).
79. Huang, C. *et al.* Mutations in exon 7 and 8 of p53 as poor prognostic factors in patients with non-small cell lung cancer. *Oncogene* **16**, 2469–2477 (1998).
80. Vega, F. J. *et al.* p53 exon 5 mutations as a prognostic indicator of shortened survival in non-small-cell lung cancer. *Br. J. Cancer* **76**, 44–51 (1997).
81. Erber, R. *et al. TP53* DNA contact mutations are selectively associated with allelic loss and have a strong clinical impact in head and neck cancer. *Oncogene* **16**, 1671–1679 (1998).
82. Kihara, C. *et al.* Mutations in zinc-binding domains of p53 as a prognostic marker of esophageal–cancer patients. *Jpn J. Cancer Res.* **91**, 190–198 (2000).

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Online links

DATABASES
The following terms in this article are linked online to:
CancerNet: http://cancernet.nci.nih.gov/
breast cancer | cervical cancer | colon tumours | head and neck cancer | melanoma | neuroblastoma | non-Hodgkin's lymphomas | non-small-cell lung carcinoma | sarcoma | testicular cancer
GenBank: http://www.ncbi.nlm.nih.gov/
E6 protein
InterPro: http://www.ebi.ac.uk/interpro/
SAM
LocusLink: http://www.ncbi.nlm.nih.gov/LocusLink/
APAF | *APC* | ATM | *BRCA1* | MDM2 | $p19^{ARF}$ | *RET* | *TP53* | *TP63* | *TP73* | *Trp53* | *Trp63*
Medscape DrugInfo:
http://promini.medscape.com/drugdb/search.asp
cisplatin | etoposide | tamoxifen
OMIM: http://www.ncbi.nlm.nih.gov/Omim/
ataxia telangiectasia | Li–Fraumeni syndrome

FURTHER INFORMATION
The APC database at the Institut Curie:
http://perso.curie.fr/Thierry.Soussi/APC.html
The City of Hope Database of MDM2 Mutations in Human Tumors:
http://www.infosci.coh.org/mdm2asp/default.asp
The IARC *TP53* Mutation Database: http://www.iarc.fr/p53/
The NIH p53 Resources Page:
http://www.nci.nih.gov/intra/lhc/p53ref.htm
The OncoLink p53 Information Center:
http://oncolink.upenn.edu/causeprevent/genetics/p53/
The p53 mutation database:
http://perso.curie.fr/Thierry.Soussi/p53_databaseWh.htm
The TP53 site at the Institut Curie:
http://perso.curie.fr/Thierry.Soussi/
The Universal Mutation Database site:
www.umd.necker.fr/
Access to this interactive links box is free online.

TIMELINE

Comprehensive Cancer Centres and the war on cancer

David Nathan and Edward J. Benz, Jr

Comprehensive Cancer Centres are now recognized as an important weapon in the war on cancer, but they had to fight a very different battle to become accepted by the academic community. Why were these centres developed? How do they contribute to cancer research? Have they achieved the aims for which they were set up? And how should they be improved? It is important to answer these questions because we believe that cancer centres, though in need of improvement, are vital parts of our anticancer strategy.

Dedicated cancer centres now form an important part of the cancer research landscape worldwide, and many of them are recognized as centres of excellence — not only by researchers, but also by those patients seeking state-of-the-art treatment and access to clinical trials. In the United States, the Cancer Centres Programme of the National Cancer Institute (NCI) is now fully accepted as an integral component of the nation's cancer research effort. In the United Kingdom, the newly formed National Cancer Research Network will probably develop a similar programme. Last year, the NCI Cancer Centre Programme used $169 million or 7.7% of the extramural NCI budget. These funds provided partial support for 60 NCI-designated cancer centres in 31 states, of which 40 were deemed 'comprehensive'. Although this commitment is small in comparison to the budget for grants to individual investigators (RO1 grants — $899 million or 41% of the extramural budget), it represents a vital force in cancer research, treatment and prevention, and is firmly based in NCI and National Institutes of Health (NIH) history.

Comprehensive Cancer Centres are designed to join the forces of basic, translational and population cancer research into ever-improving clinical trials in adult and paediatric oncology. Additional aims are to provide effective cancer education and prevention methods to the surrounding community and wider region, and to offer the highest quality surgical, radiotherapeutic, medical and paediatric treatment for cancer. To accomplish these aims, the centres must invest heavily in basic and population science, as well as in clinical science. How did this multidisciplinary approach to cancer research and treatment arise? (see TIMELINE.)

History of the designated cancer centre

The concept of the NCI-designated cancer centre has its roots in the period immediately after the Second World War[1–3] when Vannevar Bush (BOX 1) published *Science, the Endless Frontier*[4] — his tribute to the future of science. Scientific achievements had contributed significantly to victory, convincing Bush and the leadership of the United States that a further investment in basic scientific research by government — in both the public and the private sectors — would greatly enhance the nation's health and welfare, as well as its economic strength. Within the sphere of health research, the then relatively tiny NIH and the Office of Naval Research began to fund basic research grants to a few universities and their medical schools. But from the very beginning of the NIH effort, certain congressmen, particularly Senator Lister Hill of Alabama and Representative John Fogarty of Rhode Island, recognized that the American public would not continue to support basic biomedical research unless it was directly and visibly linked to an expansion of the conquest of disease[5]. Their astute sense of congressional mood was moulded into broad national policy by the efforts of individuals such as Mary Lasker and Sidney Farber, organizations such as the American Cancer Society, and others of a similar stance who supported political and fundraising efforts on behalf of devastating diseases such as cancer, heart disease and stroke.

The list of categorical NIH institutes has grown continuously since then. Within almost all of these institutes — and particularly in the NCI — there was a strong sense that Congress and the tax payers wanted every effort made to close the gap between what could be learned at the bench and the application of that knowledge at the bedside. In the early 1950s, the intramural programme of NIH on its campus in Bethesda, Maryland, responded to that vision and created Building Ten, a 500-bed research hospital that was later named the Warren Magnusen Clinical Centre (FIG. 1). Within the Magnusen Centre, the laboratories of investigators in the various NIH institutes were in close proximity to the beds of what became the largest, best-equipped, and best-funded clinical research centre in the world. NCI rapidly became the largest user of the centre as trials using combinations of