Relevance of TP53 for CLL diagnostics

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ABSTRACT

TP53 disruption in chronic lymphocytic leukaemia (CLL) is a well-established prognostic marker and informs on the appropriate course of treatment for patients. *TP53* status is commonly assessed by fluorescence in situ hybridisation for del(17p) and Sanger sequencing for *TP53* mutations. At present, current screening methods for *TP53* mutations fail to detect diagnostically relevant mutations potentially leading to inappropriate treatment decisions. In addition, low levels of mutations that are proving to be clinically relevant may not be discovered with current less sensitive techniques. This review describes the structure, function and regulation of the *TP53* protein, the mutations found in cancer and CLL, the relevance of *TP53* disruption in CLL and the current screening methods for *TP53* mutations including next-generation sequencing.

INTRODUCTION

Tumour suppressor p53 (*TP53*) gene

Tumour suppressor p53 (*TP53*) was first identified in 1979 when two independent groups published their findings on the interaction of a cellular protein of approximately 53–55 kDa and a large T-antigen of the Simian virus (SV40).^{1,2} P53 was initially thought to act as an oncogene and it was only in the late 1980s that it was correctly assigned as a tumour suppressor gene and then coined as the 'Guardian of the Genome'.³

The protein p53 contains 393 amino acids and is encoded by the *TP53* gene in humans. It is located on the short arm (p) of chromosome 17 (17p13.1) and possesses 11 exons, of which 10 are coding (figure 1).

STRUCTURE AND FUNCTION

P53 is a sequence-specific DNA binding protein that regulates transcription. The protein consists of two N-terminal transactivation domains with a conserved proline-rich domain, a central DNA-binding domain followed by a C-terminus. This encodes its nuclear localisation signals and a domain essential for transcriptional activity.

The p53 protein level is low in normal cells as it is maintained by a series of regulators such as mouse double minute 2 (MDM2) (E3 ubiquitin protein ligase), which facilitates ubiquitin-mediated degradation.⁴ The role of p53 is to control cell proliferation. It is upregulated in response to cellular stress such as DNA damage and activation of oncogenes. When activated, p53 protein levels increase and cell phenotypes change to include apoptosis and elevated DNA repair.⁵

The p53 protein, via two transactivation domains, binds strongly to several specific DNA sequences.^{6,7}

MDM2, a negative regulator of p53, binds to the transactivation domain inducing an alpha-helical conformation thereby inhibiting transcription of p53.⁸ By translocating these genes during periods of cell stress, they become active, producing proteins involved in cell cycle progression and apoptosis.⁹ During genotoxic stress, p53 protein is phosphorylated by ataxia telangiectasia mutated kinase and prevented from undergoing MDM2-mediated degradation (figure 2). Under oncogenic stress, p53 is protected from degradation by p14ARF (alternate reading frame tumour suppressor) protein.

While p53 has a number of functions, the ability to promote cell cycle arrest and apoptosis are the most comprehensively understood. It has been well recognised that p53 is capable of upregulating the cyclin-dependent kinase inhibitor, CDKN1A. It is also well established that the transcriptional activity of p53 is tissue specific and prominent in lymphocytes.¹⁰ In addition, p53 can trigger apoptosis by relying on the induction of proapoptotic *BCL-2* family members facilitating caspase activation and cell death.¹¹

ORIGINS OF P53

TP53 is a member of a broader gene family including *TP63* and *TP73*. Unlike *TP53*, both *TP63* and *TP73* do not have tumour suppressive capabilities. In eukaryotes, the p53 protein sequence is relatively conserved; however, *TP53* has evolved by increasing gene dosage.

At first, this critical anticancer gene was believed to be an oncogene, as it was overexpressed in many cancers including breast and lung cancer.^{12–14} Subsequently, it was discovered that *TP53* was mutated in half of all human cancers, and loss of regulatory function of p53 led to oncogenesis.¹⁵ Germline *TP53* mutations are pathogenic in cases of Li-Fraumeni syndrome, a hereditary cancer predisposition syndrome.¹⁶

The majority of tumour suppressor genes are frequently inactivated by deletions or truncating mutations causing a loss of protein expression. In contrast, the *TP53* gene is unique in comparison to other tumour suppressor genes as inactivation on both alleles is not required to eliminate p53 function. Loss of function of *TP53* is considered a major event in tumorigenesis and is also linked to chemotherapy resistance and a poor prognosis in many cancers.¹⁵

The most common and best characterised mutations in *TP53* are missense mutations within the DNA-binding domain. These account for approximately 75% of mutations and of these, almost 25% occur within six hotspot codons. Interestingly, 25% of *TP53* mutations are non-sense or frameshift mutations, while the remainder are splice site single nucleotide variants and in-frame indels. Inactivation

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Figure 1 Schematic structure of *TP53* gene. The *TP53* gene is located on the short arm (p) of chromosome 17 (17p13.1) and possesses 11 exons, of which 10 are coding.

of the second *TP53* allele occurs most commonly through loss of heterozygosity.¹⁷

A significant body of evidence suggests that certain mutant *TP53* alleles have a gain of function property. This was first suggested over 25 years ago with the introduction of mutant p53 cells that gave rise to a new phenotype distinct from null cells.¹⁸

As previously stated, the *TP53* gene is mutated in half of all human cancers; however, different tumours show a wide spectra of *TP53* mutations. Two broad categories of mutant p53 effects have been described:

1. Those affecting amino acids that contact DNA preventing wild-type transcriptional activity with major disruption to the protein.
2. Those disrupting the three-dimensional structure of the protein.

Although mutations in this gene are most commonly seen in solid tumours, haematological cancers possess these mutations at a lower rate. In contrast, chronic lymphocytic leukaemia (CLL), a common form of adult leukaemia, contains an unusually high number of *TP53* aberrations with well-documented evidence for their role in disease pathogenesis.^{19 20}

Chronic lymphocytic leukaemia

CLL is the most common leukaemia in adults in the Western world and is characterised by clonal expansion of CD5+ B

cells, derived from healthy CD5+ B cells throughout the blood, lymphoid tissues and bone marrow of patients.²¹ CLL has a highly variable presentation and clinical course. The majority of patients at presentation are asymptomatic, have indolent disease and are managed on an active surveillance approach without requiring treatment for many years. A proportion of patients progress and require therapy shortly after diagnosis and can have a rapidly progressive disease leading to early death. This clinical heterogeneity highlights the need for both prognostic and predictive markers in CLL.²²

Considerable advances have been made over the past decade using genome-wide sequencing approaches to decipher the genetic complexity of CLL. However, the key therapy decision-making biomarkers in CLL are *TP53* aberrations: chromosomal defects of 17p13 and *TP53* gene mutations.^{23 24}

TP53 aberrations in patients with CLL

TP53 gene defects in CLL were first described in the early 1990s and the association with inferior clinical survival was established.¹⁹ Initially, the loss of the *TP53* locus was not considered to be an important event in the era of karyotyping.²⁵ However, this was to change with the publication of Dohner's hierarchical CLL classification in 2000.²⁶ This firmly established the inferior survival of patients with deletions of 17p13 (del(17 p)). This was subsequently confirmed in other studies and implemented into routine practice.²⁷ While karyotyping required dividing cells, Dohner's classification used fluorescence in situ hybridisation (FISH). FISH uses fluorophore-labelled DNA probes that hybridise to specific DNA sequences that allows the identification of deletions, additions and translocations. In contrast, *TP53* mutational status was not initially assessed routinely in CLL diagnostics as it was assumed that *TP53* mutations did not occur in the absence of del(17 p).^{28–30} With further studies, it became clear that del(17 p) is usually accompanied by *TP53* mutations in CLL, and studies have shown that both can occur independently with

Figure 2 p53 function and regulation. ATM, ataxia telangiectasia mutated; *MDM2*, mouse double minute 2; UV, ultraviolet.

Figure 3 *TP53* defects: loss of p53 function in chronic lymphocytic leukaemia can occur due to del(17 p) and/or *TP53* variants. The most common cause of *TP53* aberrations results in the combination of del(17 p) and *TP53* mutation.

the frequency dictated by the particular patient cohort studied and the methodology used.

The frequency of *TP53* mutations are low in treatment-naïve patients (approximately 10%) but this increases (up to 25%–50% depending on the study) in patients with disease progression and treatment refractory patients suggesting that these alterations are mostly acquired and provide a mechanism of resistance.³⁰

In general, 90% of patients with del(17 p) carry a mutation and up to 65% of patients with a *TP53* mutation have del(17 p).^{31–33} Patients with a monoallelic *TP53* mutation also have an inferior progression-free and overall survival.^{31,33} Of all patients with *TP53* disruption, mutations and deletions occur together in the majority of patients with monoallelic *TP53* mutations accounting for another 40% of patients, monoallelic deletions and also mutations with copy neutral loss of heterozygosity account for a minority of patients (figure 3).

TP53 AS A TREATMENT RESPONSE PREDICTOR

For more than a decade, chemoimmunotherapy in the form of fludarabine, cyclophosphamide and rituximab was the conventional first-line regimen in fit patients providing a high response rate, improved progression-free survival and an impact on overall survival for the first time in CLL. Not all patients benefit from this treatment, with up to 25% of patients relapsing within 24 months, fulfilling the definition for chemorefractoriness.³⁴ Fludarabine, a purine nucleoside analogue, interferes with DNA synthesis and repair in dividing and non-dividing cells and therefore requires a functional p53 protein to be effective. The presence of *TP53* aberrations reduces progression-free survival due to chemotherapy resistance.^{29,30} The advent of novel treatment options in CLL targeting the B-cell receptor and antiapoptotic molecules targeting BCL-2 have proved beneficial in the treatment of patients with *TP53* aberrations providing alternative methods for cell death.^{35–37} This therefore highlights the need for accurate assessment of *TP53* defects to identify patients who will benefit from novel therapies.

CURRENT TESTING REGIMENS IN ROUTINE PRACTICE

Current guidelines recommend assessment of *TP53* aberrations in patients requiring therapy. Reassessment at each pretreatment time point should be undertaken as clonal evolution can occur.²⁴ As already stated, the assessment of del(17 p) by FISH is routinely performed. The cut-off for a positive result varies within laboratories with the widely accepted threshold that >20% of cells should contain del(17 p) to be a clinically relevant

clone.³⁸ Sanger sequencing is widely used for *TP53* mutational analysis; however, it may misclassify cases of *TP53* mutations as wild type when variants with allelic burdens below the detection limit of Sanger sequencing are present. With the implementation of NGS, large amounts of DNA can be sequenced in parallel at a lower cost than the gold standard Sanger sequencing. Updated guidelines from the *TP53* network of European Research Initiative on CLL (ERIC; www.ericcll.org) suggest a threshold of 10% allelic burden for reporting mutations detected by NGS.³⁹

Targeted NGS panels are also available whereby custom probes are designed to target genes/regions of interest within the genome with much greater sensitivity. This targeted approach facilitates a greater depth of sequencing as a much smaller percentage of the genome is interrogated.^{40,41} This technique has led to improved knowledge on gene mutations and signalling pathways by screening large cohorts.^{42,43} For targeted NGS, if solely one time point is available, intraclonal dynamics can be studied using the variant allele frequency to infer the timing of mutations.⁴³

The general principles of targeted NGS panels are based on sequencing an amplified DNA template. As with other applications, the DNA template is a double-stranded DNA construct consisting of short fragments of DNA flanked by adapters of known sequence. Illumina sequencing is the most broadly used NGS technique and consists of a solid phase amplification of the DNA template on a flow cell with subsequent sequencing by synthesis.⁴⁴ Novel approaches such as capture-based sequencing allows the simultaneous detection of somatic mutations, copy number variations and chromosomal translocations.⁴⁵ As with all technologies, several limitations exist and one of the major limitations of NGS is the lack of standardised bioinformatics pipelines and the requirements to handle large data sets.⁴⁶

As *TP53* mutational status has a major impact on treatment decisions, international collaborations such as ERIC have developed a network of *TP53* reference centres within various countries. ERIC aims to provide standardisation and harmonisation of diagnostic strategies for *TP53* mutational assessment resulting in robust testing strategies and offering quality assurance schemes.

The introduction of NGS also allows the detection of *TP53* mutated subclones. Subclonal *TP53* is the term used to describe variants that are not present in the entire tumour population. This has great clinical relevance in CLL as the disease progresses, different leukaemic cells acquire mutations leading to numerous subclones developing. The subclones are intrinsically linked to their environment, and any changes to this environment such as

chemotherapy, create changes in the cells, allowing outgrowth of resistant clones.²⁰ This is particularly relevant in CLL as *TP53* mutated subclones occur in a significant proportion of cases and have the same unfavourable prognostic impact as that of clonal *TP53* defects.^{47–48}

In conclusion, in CLL, the inactivation of the *TP53* gene by either a deletion or a mutation has a negative impact on the disease course for patients. Detectable *TP53* aberrations are markers of chemorefractoriness, and consequently, treatment decisions are based on this finding. It is a now accepted practice that *TP53* aberrations should be tested before each treatment decision, and NGS represents an ideal technique for mutation assessment and has the additional capability and advantage above historical techniques to detect clinically relevant mutated subclones.

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