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Genomic data in prognostic models—what is lost in translation? The case of deletion 17p and mutant *TP53* in chronic lymphocytic leukaemia

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Summary

Genomic technologies are revolutionizing the practice of haematology-oncology, leading to improved disease detection, more accurate prognostication and targeted treatment decisions. These advances, however, have also introduced new clinical challenges, which include problems of prognostic underdetermination and its attendant risks of over- and undertreatment. Genomic data is generated from different technologies, from cytogenetics to next-generation sequencing, which are often interpreted interchangeably and in a binary fashion—as the presence or absence of a given chromosomal deletion or mutation—an oversimplification which may lead to mistaken prognosis. We discuss the clinical use of one such prognostic marker, represented by sequence and copy number alterations in *TP53*, located on chromosome 17p. Mutations in *TP53* are strongly linked to poor prognosis in a variety of haematological malignancies, including chronic lymphocytic leukaemia (CLL). We review studies in CLL which utilize the 17p deletion or *TP53* mutations for prognostic stratification with specific focus on the technologies used for detection, the thresholds established for clinical significance, and the clinical contexts in which these alterations are identified. The case of CLL illustrates issues arising from simplistic, binary interpretation of genetic testing and highlights the need to apply a critical lens when incorporating genomics into prognostic models.

Keywords: chronic lymphocytic leukaemia, genomics, prognostic models, cytogenetics, next-generation sequencing.

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Genomic technologies are revolutionizing the practice of haematology-oncology. From improved disease detection and prognostication, to mutation-targeted therapies with companion diagnostics, genomic technologies are catalysing rapid changes in how we care for patients with haematological malignancies. These advances, however, have also introduced a new set of challenges in the clinical setting, which include problems of prognostic underdetermination and its attendant risks of over and undertreatment. Prognostic risk scores incorporating a range of clinical and laboratory variables have become ubiquitous in haematology-oncology, and are routinely used to inform treatment decisions. Genomic data increasingly dominate prognostic risk scores and shape treatment algorithms, adding new layers of complexity to clinical management. Although these data have been useful—for example, by allowing for the identification of 'driver' mutations and potential treatment targets—interpretation of genetic testing in the clinic can be complex and context-specific.

For haematological malignancies, genomic data is generated from a variety of technologies from traditional karyotyping and interphase fluorescence *in situ* hybridisation (FISH), to next-generation sequencing (NGS) (Table I). In addition to their variable operating characteristics, information produced by these distinct technologies differs in clinical significance. Despite this, in the clinical setting, genomic data produced by different methodologies are often interpreted interchangeably and in a binary fashion—for example, as the presence or absence of a given chromosomal deletion or mutation—an oversimplification that has the potential to lead to mistaken prognosis and mismanagement.

In this article, we discuss the clinical use of one such prognostic marker, represented by sequence and copy number alterations in *TP53* located at chromosome 17p. Mutations in *TP53* are the most common genomic alterations in cancer and are strongly linked to poor prognosis in many cancer subtypes including haematological malignancies such as chronic lymphocytic leukaemia (CLL) and multiple myeloma. We review the literature on the use of 17p deletion or

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Table I. Comparison of genetic testing methodologies in CLL.

	Advantages	Disadvantages
Karyotyping	• Chromosome banding analysis provides information on chromosome structure and ploidy • Identifies chromosome level abnormalities such as trisomy 12 and subchromosomal, balanced (e.g., gene fusions) and unbalanced structural abnormalities (e.g., del11q22.3-q23.1, del13q14, del17p13) • Provides cell-specific information regarding clonality (e.g., different clonal populations, clonal progression)	• Few dividing cells in CLL • Only 40–50% have chromosomal aberrations • Resolution >10 Mb; therefore, unable to detect gain or loss of single gene • Extreme aneuploidy or multiple structural changes can be difficult to resolve
FISH	• Identifies specific fusions or copy number alterations based on probe specificity • Provides information for single cells, allowing for identification of mutations in different clones and measurement of clone size • Can be used on limited samples	• Limited to specific probes • Limited sensitivity, detects clone size between 5 and 10% depending on the probe set and tissue type
Microarray	• Detection of genome-wide relative copy number changes • Can replace multiple targeted FISH assays for copy number variation analysis • Higher resolution than karyotype	• Requires significant amounts of DNA • Not useful for balanced rearrangements • Not able to detect ploidy changes
NGS	• Simultaneous detection of multiple genomic events • Can identify mutations at single gene level and detect multiple mutations within a specific gene • Can infer copy number variants in relative not absolute terms • Sequential assessment allows identification of newly acquired mutations during course of disease • Ability to detect balanced rearrangements, such as gene fusions (RNA Sequencing) • Higher sensitivity and specificity than cytogenetic methods; detects allelic frequencies between 2 and 5% for DNA, and 1% or less for RNA fusions	• May lack sufficient target density to assess for gross chromosomal structural changes (gain or loss) • Provides average copy number as opposed to single cell resolution • Unable to directly measure clone size or mutations arising in different clones • Variants identified may be of uncertain biological significance • Potential contamination by non-tumour cells, other clonal cell populations, and background somatic or germline mutations • Currently designed to detect mutations primarily within gene coding and not intergenic regions • Unable to assess epigenetic changes

CLL, chronic lymphocytic leukaemia; FISH, fluorescence *in situ* hybridisation; NGS, next-generation sequencing.

TP53 mutations for prognostic stratification in CLL with a focus on the technologies used for detection, the thresholds established for clinical significance, and the clinical contexts in which these genomic alterations are identified. As we move from cytogenetic methodologies, such as FISH, to increasingly sensitive and higher resolution molecular techniques, such as NGS, there is a critical need for understanding the scope and limitations of these technologies to inform appropriate clinical application. In addition to attention to methodology, we argue that appreciating the clinical and molecular context in which genomic alterations are identified remains essential. Using CLL as an example, we caution against a simplistic, binary interpretation of genetic testing in the clinic.

TP53 mutations in human cancers

Much has been written about *TP53* and its role in oncogenesis since its first description as germline mutation in Li-Fraumeni syndrome (Olivier *et al*, 2010). Somatic mutations in *TP53* are the most common genetic alterations in human cancers, and are used as prognostic markers as well as targets of pharmacological intervention. The TP53 protein (also termed p53) has a range of biological effects, which include both inhibitory and activating effects on regulation of the cell cycle and apoptosis through interactions with various pathways (Muller & Vousden, 2014). For its function as a tumour suppressor, TP53 has been described as the "guardian of the genome." Its role, however, has proved more complex than initially described, with a multitude of mutations with a range of functional consequences, including proto-oncogenic gain of function mutations associated with a dominant negative effect on the normally functioning *TP53* allele (Fig 1). The vast mutational spectrum of *TP53* is reflected in the Catalogue of Somatic Mutations in Cancer (COSMIC) database (https://cancer.sanger.ac.uk/cosmic), which currently lists over 5211 different mutations in 40 416 unique samples (approximately 25% of the total 160 297 samples currently in the database), across 46 different tissue types (Tate *et al*, 2018). The pathways and functions of *TP53* are complex and have been extensively reviewed (Olivier *et al*, 2010; Muller & Vousden, 2014). This complexity points

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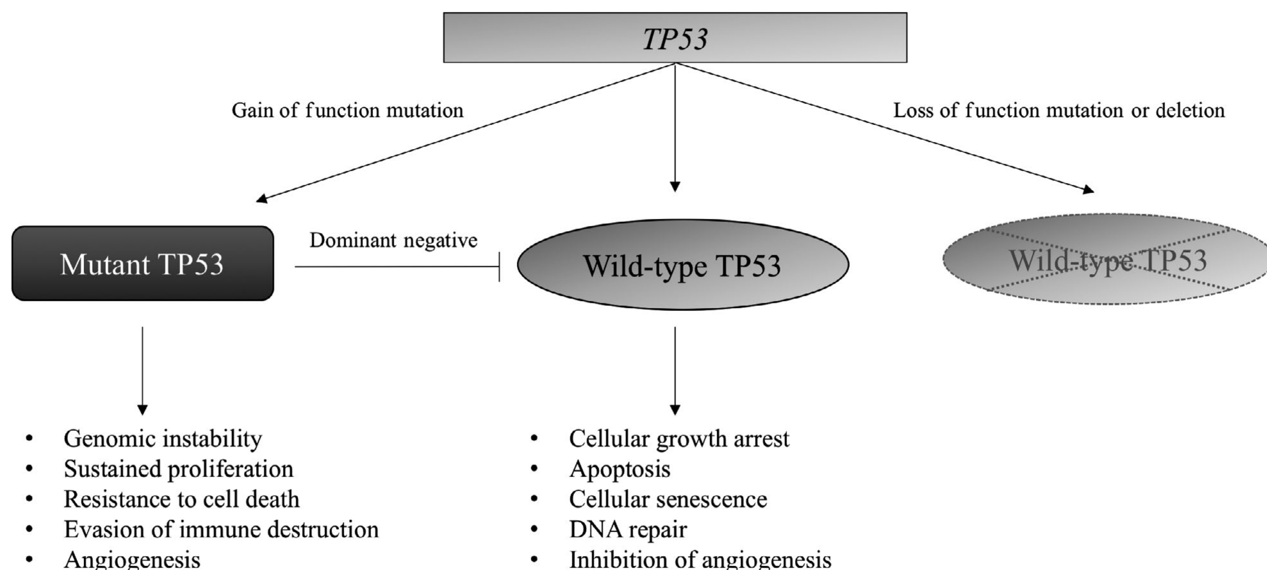

Fig 1. Simplified schematic depicting the role of wild-type and mutant TP53 in cancer. Wild-type *TP53* has wide-ranging roles as a tumour suppressor gene. Gain of function mutations in *TP53* can promote oncogenesis, and some forms exhibit a dominant negative effect, abrogating function of the remaining wild-type allele. Loss of function mutations and/or chromosomal deletions (i.e., deletion 17p) can result in loss of expression of wild-type TP53.

to potential pitfalls that might arise from overly simplistic interpretation of *TP53* mutations clinical prognostic models.

Prognostic impact of *TP53* mutations and 17p deletion in CLL

Prior to 2000, clinical staging, such as the Rai and Binet staging systems (Binet *et al*, 1977), were the cornerstones for determining prognosis and guiding treatment decisions. Döhner *et al* (2000) published the first large study (n = 325) examining the impact of genomic aberrations (identified by interphase FISH) on survival in CLL. These investigators found that 82% of CLL patients had at least one clonal chromosomal abnormality, the most common being the 13q deletion (including the locus of the Retinoblastoma [*RB1*] tumour suppressor gene), which was present in more than half of patients. The most striking finding, however, was the 17p deletion which is associated with loss of *TP53*. The 17p deletion, described in 7% of patients, was associated with a median survival of less than 32 months compared to 133 months for patients with the 13q deletion as their sole abnormality. These observations were reproduced by a number of studies (Catovsky *et al*, 2007; Grever *et al*, 2007; Hallek *et al*, 2010), and reinforced the importance of chromosomal aberrations detected by FISH for predicting response to therapy as well as treatment-free, progression-free and overall survival.

These findings revolutionized prognostic assessment in CLL. Over the last decade new prognostic risk scores have supplanted traditional staging systems (Pflug *et al*, 2014; CLL-IPI Working Group, 2016), incorporating additional clinical factors but largely favouring genomic data (Table II). Combining clinical variables, such as age and clinical stage, with genomic data, such as the 17p deletion and *IGHV* mutation status, could better predict time to first treatment and overall survival in treatment-naïve patients with early stage CLL (Haferlach *et al*, 2010; Wierda *et al*, 2011). More recently, the availability of NGS has reaffirmed the importance of *TP53* mutations as a negative prognostic marker for patients with untreated CLL (Pflug *et al*, 2014; CLL-IPI Working Group, 2016). The latest prognostic risk score, the CLL International Prognostic Index (CLL-IPI), was developed from patient level meta-analysis of 3472 treatment-naïve patients from eight randomized controlled trials (CLL-IPI Working Group, 2016). This study included data on *TP53* mutations (sequence or nucleotide level alterations) or 17p deletions (gene copy number alterations). Data was analysed by comparing patients with no abnormalities *versus* 17p deletion or *TP53* mutation or both. Other variables included in the model were *IGHV* mutational status (mutated *versus* unmutated germline), serum β2-microglobulin concentration (≤3·5 mg/l vs. >3·5 mg/l), clinical stage (Binet A or Rai 0 vs. Binet B–C or Rai I–IV), and age (≤65 years vs. >65 years). Using a weighted grading of the independent factors, a prognostic index was derived that identified four risk groups: low, intermediate, high and very high risk, with 5-year overall survivals of 93%, 79%, 63% and 23%, respectively. Moreover, the 17p deletion and/or *TP53* mutation were of particular prognostic significance in the CLL-IPI, receiving a total of four points meaning that having this genomic alteration alone would automatically place the patient in the high-risk category. This is in contrast to other

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Table II. Prognostic models in CLL.

Reference	*N*	Clinical variables	Laboratory variables	Genomic variables	Prognostic weighting	Prognosis (median OS; 5-year OS)
Wierda *et al* (2007)	1674	Age, sex, Rai stage, involved nodal groups	β-2M, ALC	None	**1 point:** age < 50 years, male, Rai stage III or IV, 3 or more nodal groups, β-2M <2 × ULN, ALC 20–50 × 10^9/l **2 points:** age 50–65 years, β-2M > 2 × ULN, ALC > 50 × 10^9/l **3 points:** age > 65 years	**Low risk (1–3 points):** not reached; 97% **Intermediate risk (4–7 points):** 10·3 years; 80% **High risk (>8 points):** 5·4 years; 55%
Haferlach *et al* (2010)	399	Age	WBC count	del(17p), chromosomal aberrations, *IGHV* mutational status	**1 point:** age > 6 years, WBC count > 2 × 10^9/l, *IGHV* unmutated, 1–2 chromosomal aberrations **2 points:** del(17p), ≥3 chromosomal aberrations	**Favourable risk (0–3 points):** not reached; 92% **Intermediate risk (4–5 points):** not reached; 70% **Unfavourable Risk (>5 points):** 2·1 years; 41%
Rossi *et al* (2013)	637	None	None	*TP53*, *BIRC3*, *NOTCH1*, *SF3B1* mutations, del (11q22-q23), trisomy 12, normal genetics, del(13q14)	N/A	**del(13q) only:** not reached; 87% **Trisomy 12 and normal genetics:** 13·4 years; 78% ***NOTCH1* and/or *SF3B1* mutations and/or del(11q22-q23):** 8·5 years; 66% ***TP53* and/or *BIRC3* disruption:** 5 years; 51%
Pflug *et al* (2014)	1948	Age, sex, ECOG PS	s-TK, β-2M,	del(17p), del(11q), IgVH mutational status	**1 point:** age > 60 years, male, ECOG PS >0, β-2M >1·7 and ≤3·5 mg/l, *IGHV* unmutated **2 points:** β-2M >3·5 mg/l, s-TK > 10 μ/l **6 points:** del(17p)	**Low risk (0–2 points):** not reported; 95% **Intermediate risk (3–5 points):** not reported; 91% **High risk (6–10 points):** not reported; 72% **Very high risk (11–14 points):** not reported; 14%
CLL-IPI Working Group (2016)	3472	Age, clinical stage (Rai or Binet)	β-2M	TP53 deletion/ mutation, IgVH mutational status	**1 point:** age > 65 years, Rai I-IV or Binet B-C **2 points:** β-2M > 3·5 mg/l, *IGHV* unmutated **4 points:** *TP53* deletion/ mutation	**Low risk (0–1 point):** not reached; 91% **Intermediate risk (2–3 points):** 8·7 years; 80% **High risk (4–6 points):** 5·2 years; 53% **Very high risk (7–10 points):** 2·6 years; 19%

ALC, absolute lymphocyte count; β-2M, beta-2 microglobulin; CLL, chronic lymphocytic leukaemia; ECOG PS, Eastern Cooperative Oncology Group performance status; OS, overall survival; PS, performance status; s-TK, serum thymidine kinase; ULN, upper limit of normal; WBC, white blood cell count.

genetic biomarkers, such as the *IGHV* unmutated germline status, which accords only two points. Advanced age or clinical stage only receives one point according to the CLL-IPI.

In CLL, assessment for loss of wild type *TP53* is most commonly measured by detection of 17p deletion by FISH, which is a routine part of clinical diagnostic workup. Its association with a more aggressive disease course and poor response to conventional treatments leading to recommendation to avoid standard chemoimmunotherapy in favour of novel agents (ibrutinib, venetoclax or idelalisib) (Nabhan *et al*, 2015; CLL-IPI Working Group, 2016). Currently, *TP53* status significantly impacts clinical decision making in CLL, highlighted by its incorporation into treatment algorithms at major decision points in patient management. One such

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algorithm suggests that patients with *TP53* mutations or 17p deletion be considered for enrolment in clinical trials of targeted therapies, and even allogeneic haematopoietic stem cell transplantation for eligible patients (Nabhan *et al*, 2015). The impact of early aggressive treatment in these patients is unknown at this time. More recent guidelines acknowledge that, outside of a clinical trial setting, the decision to initiate treatment should not be based on molecular genetics alone but rather consider conventional staging and clinical signs/symptoms of disease progression (Hallek *et al*, 2018).

Clinical heterogeneity of 17p deletion and *TP53* mutation

Although there is a strong association of 17p deletion and *TP53* mutations with poor prognosis in cohort studies, there is also evidence of variability in the clinical course and the types of documented *TP53* mutations in any individual patient. Specifically, not all patients with CLL and 17p deletion have aggressive disease. Indeed, there are reports of patients with early stage CLL and 17p deletions surviving for over 10 years without therapy (Best *et al*, 2008). For patients with 17p deletions detected at diagnosis prior to any treatment, so-called *de novo* 17p deletions which constitute 2–4% of new diagnoses (Shanafelt *et al*, 2006; Stilgenbauer *et al*, 2014), 44% do not require treatment for 2 years, and overall survival reaches 64% at 3 years (Tam *et al*, 2009; Delgado *et al*, 2012). Similarly, in the abovementioned CLL-IPI study of treatment-naïve patients, there was considerable heterogeneity in patients with 17p deletions and/or *TP53* mutations, who could be stratified into subgroups with overall survival ranging from 23% to 63% at 5 years (CLL-IPI Working Group, 2016). In contrast, patients who have received treatment not only have much higher incidence of acquired 17p deletions [ranging from 30% to 50% in multiply treated patients (Gonzalez *et al*, 2011; Pospisilova *et al*, 2012)] but also have significantly worse prognosis, with a 3-year overall survival of 47%. This finding is consistent with the median survival of 3 years in the original study by Döhner *et al* (2000), which included 25% of patients who had received previous therapy. It is clear that clinical context, namely prior treatment, is a crucial determinant of the meaning and impact of this genetic marker.

Context also plays a crucial role in shaping the data upon which prognostic risk scores are built. For example, the CLL-IPI was developed using data from treatment-naïve patients but with progressive symptomatic disease making them eligible for enrolment in clinical trials of first-line therapy. The incidence of the 17p deletion in the clinical trials population (6–8%) (Catovsky *et al*, 2007; Hallek *et al*, 2010; Robak *et al*, 2010) is higher than that found in unselected series of patients at diagnosis (2–4%) (Shanafelt *et al*, 2006; Stilgenbauer *et al*, 2014). The referral bias inherent in the clinical trials population and the models developed from this data has the potential to overestimate the negative prognostic impact of 17p deletion and *TP53* mutation.

More importantly, how we define CLL with 17p deletion appears crucial. The size of the clone harbouring 17p deletions is an independent predictor of outcome, problematizing binary notions of positivity and negativity for this biomarker. While the technical limitations of FISH may produce a lower limit of detection, somewhere in the range 3–10% (Döhner *et al*, 2000), the clone size which predicts poor prognosis varies between studies. Early studies by Döhner *et al* (1995) established 3% as a valid cut-off level, while others showed that only patients with more than 10–20% 17p deleted cells had a significantly inferior outcome (Catovsky *et al*, 2007; Oscier *et al*, 2010). In analysis of 294 patients, Delgado *et al* (2012) found a continuous relationship between the percentage of cells harbouring 17p deletions and overall survival; for patients with clone sizes of <25%, 25–74% and >75%, median overall survival was 64, 39 and 21 months, respectively. Using receiver operating characteristic curves, these investigators suggested the optimal cut-off for designating 17p deletion "positive" was >25% (Tam *et al*, 2009; Delgado *et al*, 2012). Regardless, these results suggest that 17p deletion is not binary variable, as implied in prognostic scoring systems, but rather continuous variable with larger clone sizes associated with worse outcomes.

Conflation of 17p deletion and *TP53* mutations

In the CLL-IPI scoring system, the presence of either a 17p deletion or *TP53* mutation confer the same prognostic significance because these variables were categorically equivalent in their analysis (i.e. there was no significant difference between patients with *TP53* mutation or 17 deletion or both). However, this overlooks the fact that these methodologies measure two different abnormalities. Interphase FISH for 17p deletion quantitates loss of the DNA segment containing the *TP53* locus on the short arm of chromosome 17, along with any adjacent chromatin that is part of that chromosomal deletion. Breakpoints and hence the size of the deleted material may vary between patients. In the context of loss of function mutations, *TP53* is haplosufficient and only biallelic loss of *TP53* abrogates its tumour suppressor function. In practice, homozygous 17p deletion is rarely observed by FISH, and thus monosomy 17p detected by this technique likely represents a surrogate measure for biallelic loss of *TP53* by another mechanism. Indeed, studies have shown that approximately 80% of patients with 17p deletions also have mutations in the remaining *TP53* allele (Yu *et al*, 2017), which probably accounts for the strong association of heterozygous 17p deletion with poor prognosis for this subpopulation as whole. This difference, however, may also partly explain why 17p deletion underdetermines prognosis in CLL, and why some patients with 17p deletions demonstrate long-term survival (Best *et al*, 2008; Tam *et al*, 2009; Delgado *et al*, 2012). Whether patients with 17p deletions

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and an indolent clinical course are those with a remaining wild type *TP53* allele remains to be determined. In support of this hypothesis, Yu *et al* (2017) found that indolent CLL with 17p deletion was characterized by absent or subclonal mutations in the remaining *TP53* gene, as well as fewer chromosomal copy number abnormalities and somatic mutations. Moreover, this study suggested that the prognostic impact of 17p deletion depends upon the associated genomic complexity and additional somatic mutations detected by NGS. Specifically, in the poor prognostic group, 82% had clonal *TP53* mutations in the remaining allele and/or additional driver mutations, such as mutations in *NOTCH1*, *RPS15*, *DDX3X* and *GPS2*. Taken together, these findings highlight the complex nature of genomic data: they are qualitative in their identification of candidate oncogenes/tumour suppressor genes, quantitative in that clone size matters, and context-dependent in their conditioning on the presence of associated mutations and clinical factors.

In multiple myeloma, 17p deletion has also been associated with poor prognosis and a similar relationship between 17p deletions and *TP53* mutations is unfolding. A recent study by Walker *et al* (2018) identified a very high risk group of patients with bi-allelic inactivation of *TP53* who demonstrated a poor prognosis. Notably, once *TP53* mutations were taken into account, 17p deletion was no longer prognostically relevant, suggesting that widespread clinical use of NGS may help subclassify disease and eventually replace the interphase FISH. This may in turn help reduce the underdetermination of prognostic models based on cytogenetic technologies.

Clinical applications of NGS

The disjunction between cytogenetics and molecular testing presents challenges for incorporating these variables into prognostic models to guide clinical practice. Other technologies, such as DNA microarray, have also been used for genetic profiling in CLL (Schwaenen *et al*, 2004); however, these methods have not found widespread clinical application with the growing availability and decreasing costs of NGS. NGS allows for more sensitive detection and provides a resolution not offered by FISH. Studies applying ultra-deep NGS to CLL have enabled the detection of subclonal populations with allelic frequencies as low as 0·3%, which were demonstrated to have prognostic significance in several studies (Rossi *et al*, 2014; Nadeu *et al*, 2016). This technology has yet to find widespread clinical application, and is beyond the sensitivity of molecular testing available in most clinical laboratories.

Despite its higher sensitivity, NGS also has several limitations. The data produced by NGS requires a considerable amount of curation and interpretation, and predicting the functional consequences of mutations detected by these assays is by no means straightforward. In particular, challenges arise in managing so-called "variants of uncertain significance" whose clinical impact are not easily elucidated by population-level analyses, but rather require integrating mechanistic reasoning to guide clinical decision-making (Tonelli & Shirts, 2017). This may be especially relevant for alterations in *TP53*, a gene which has an enormous spectrum of mutations affecting many sites, small proportion of which have been studied in detail (Muller & Vousden, 2014). Particular mutations in *TP53* may be tumour-specific, such as some variants arising in breast, head and neck, and liver cancers (Leroy *et al*, 2017), but may not have the same clinical significance in other cancers. Our evolving understanding for one of the most studied cancer genes only uncovers further complexity, which defies simple incorporation into prognostic models.

Furthermore, current molecular testing in haematological malignancies is performed on samples from the peripheral blood or bone marrow, a heterogeneous mixture of both malignant and normal haematopoietic cells. Current clinical assays do not select tumour cell lineages for mutational analysis. This may be relevant in the presence of concomitant clonal populations, which have been identified in the normal healthy population, so called "clonal haematopoiesis of uncertain significance" (Bejar, 2017). The converse problem is also described by Steensma (2018) wherein NGS performed on patients with myelodysplasia has the potential to be contaminated by populations of monoclonal B lymphocytes, the precursor condition to CLL. New technologies, such as single-cell polymerase chain reaction may help to overcome this problem, and may become important clinical tools in the future. Similar challenges can arise for interpreting testing in patients with pre-existing germline mutations, such as in Li-Fraumeni syndrome for the case of *TP53*, where erroneous interpretation mutations as originating in the tumour could potentially lead to overtreatment. The possibility of background acquired somatic mutations confounding the results of NGS has been raised, supported by data showing high mutation rates in proto-oncogenes and tumour suppressor genes, including *TP53*, in tissue of healthy individuals (Martincorena *et al*, 2015, 2018).

In addition to assessing mutations in DNA sequence, NGS is being adapted to measure larger genomic events, such as exome, gene or chromosomal copy number alterations, as well as RNA for fusion genes, which, in the future, may replace FISH analysis for detection of these type of genomic aberrations. However, it is important to note that current NGS methodologies primarily focus on the coding genetic sequences, as these are currently the most amenable for assessment by clinical laboratories. Potential pathogenic mutations outside of gene coding sequences, such as introns, 5' and 3' untranslated regions, and intergenic regions are largely uninterpretable and are omitted from most NGS approaches.

Another limitation of NGS, or any genomic assessment for that matter, is that it does not address potential changes in epigenetic regulatory mechanisms, such as DNA methylation. Genome-wide DNA methylation analysis in CLL has demonstrated recurring DNA methylation defects across hundreds of

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genomic loci, which correlated with suppressed expression of tumour suppressor genes, and demonstrated potential for prognostic and therapeutic value in CLL (Tong *et al*, 2010). Specifically, Tong *et al* (2010) identified 280 potential targets of DNA methylation in CLL; many are known targets of methylation in other malignancies and some are known to functionally interact with TP53 protein. They demonstrated that methylation silences expression of tumour suppressor genes, and that methylation of specific genes, such as *APP*, was associated with shorter overall survival in multivariate analysis. The specific mechanisms of these methylation patterns in CLL remain to be elucidated. Moreover, they showed that epigenetic modulators (5-azacitidine) were able to reverse gene silencing caused by methylation, raising the possibility of novel therapeutic targets in CLL, which are currently under investigation in clinical trials.

Integrating genomic data in prognostic models

As we untangle the role of *TP53* in CLL, we recognize that a more nuanced understanding is required to more accurately incorporate this biomarker into our evolving prognostic models. While genomics promises to refine our disease categories and prognostic subgroups with the ultimate aim of individualized treatment decisions, several obstacles—both pragmatic and conceptual—lie in the path of this goal.

First, by necessity, prognostic risk scores are developed based on large population-level studies with long-term follow-up. Specifically, the published prognostic scores were based on phase 2 and 3 clinical trials, the most recent being CLL8 and CLL2007FMP, started in 2003 and 2007, respectively (Hallek *et al*, 2010; Lepretre *et al*, 2012). Hence, methods employed are always based on older platforms. This fact alone means that prognostic models are often outdated by the time the model has accumulated sufficient data for validation. Furthermore, such trials used older treatment regimens, and were prior to the advent of newer drugs, such as ibrutinib, venetoclax or idelalisib, which are changing the natural history of CLL. The rapidly evolving genomic and therapeutic landscape of CLL presents a particular challenge for development of prognostic models.

Second, prognostic models commonly use categorical variables. For example, the CLL-IPI prognostic score includes age ≤65 vs. >65 years, advanced *versus* early stage, and, as we have reviewed, *TP53* mutation and/or 17p deletion present *versus* absent. Genomic data, however, resist simplistic, binary interpretation. We have seen how establishing validated cut-offs for clone size or allelic frequency remains a challenge. Converting these data into discrete variables, to be interpreted as "present" or "absent," confers a misplaced concreteness which misrepresents their characteristics as biomarkers and risks misguiding clinical care. *TP53* mutations represent numerous distinct genomic alterations with variable impact on tumour suppressor function as well as a range of other interactions with other pathways.

Third, genomic data is context-dependent. Genetic mutations seldom occur as isolated events within a tumour and the presence of concomitant mutations can alter their clinical impact. As we have seen, a *TP53* mutation or 17p deletion has different meanings when associated with other driver mutations (Yu *et al*, 2017), or in a treatment-naive patient *versus* one who has received prior therapy (Tam *et al*, 2009; Delgado *et al*, 2012). In the latter case, these genomic alterations are often only one factor amongst many biological variables that contribute to more aggressive disease and worse prognosis—a marker of multiply relapsed, treatment-resistant disease within a sicker host. Privileging genetic biomarkers in this setting risks interpreting genes as the only difference makers and ignores the contextual, relational aspects of genetic function.

Genomic data will undoubtedly help improve prognostication in haematology-oncology. But clinicians must apply a critical lens when interpreting the meaning of these biomarkers and incorporating them into prognostic models to inform patient care. Impressive strides in basic science have allowed genomic technologies to enter the clinical laboratory, paralleled by initiatives in clinical research to elucidate impact of genetic biomarkers on prognosis. However, in moving between these domains, from bench to bedside, to guide care for an individual patient, critical knowledge surrounding genetic biomarkers may be lost in translation in our simplified prognostic scores and treatment algorithms.

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Conflicts of interest

None to declare.

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