

Emerging treatment options for patients with p53-pathway-deficient CLL

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Abstract: Over the past 40 years, p53 has been the most widely studied protein in cancer biology. Originally thought to be an oncogene due to its stabilization in many cancers, it is now considered to be one of the most critical tumor suppressors in a cell's ability to combat neoplastic transformation. Due to its critical roles in apoptosis, cell-cycle arrest, and senescence, *TP53* deletions and mutations are commonly observed and are often a portent of treatment failures and poor clinical outcomes. This is particularly true in chronic lymphocytic leukemia (CLL), as patients with p53 alterations have historically had dismal outcomes. As such, the tremendous efforts made to better understand the functions of p53 in CLL have contributed substantially to recent advances in treating patients with p53-pathway-deficient CLL.

Keywords: 17p deletion, CLL, ibrutinib, idelalisib, *TP53* mutation, venetoclax

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Introduction

Chronic lymphocytic leukemia (CLL) is the most common leukemia in developed countries.¹ Incidence in men exists at nearly a 2:1 ratio compared with women, and the median age at diagnosis is 70 years.² The clinical course of CLL is highly variable, as some patients live for decades without complications, while others succumb to the disease within months of diagnosis.

A diagnosis of CLL is made by the presence of $\geq 5 \times 10^9/L$ clonal B cells in the peripheral blood for a minimum of 3 months.³ Commonly, lymphocytosis is the only manifestation of CLL at diagnosis; patients are frequently otherwise asymptomatic. The consensus standard of care for patients in early stages of the disease is to offer close follow up without initiating anti-leukemia therapy.³ However, continued proliferation of CD5+ mature-appearing neoplastic cells can result in further leukocytosis, lymphadenopathy, splenomegaly, and autoimmune cytopenias.⁴ Patients progressing to these symptomatic stages are then eligible for various treatments.

Over the past 2 decades, our understanding of the pathogenesis of CLL has increased dramatically,

leading to the development of new therapeutic strategies for such patients requiring treatment. Treatments have evolved from simple alkylating agents to immune-checkpoint inhibitors, genetically engineered cellular therapies, and targeted molecular agents. From 2010 to 2019, eight new agents have been approved by the US Food and Drug Administration (FDA), marking the most prolific period of drug development for CLL on record. All age groups and risk subtypes of CLL patients have benefited from this therapeutic renaissance. However, patients with CLL harboring *TP53* abnormalities, usually in the form of del(17p) or *TP53* mutations, continue to be a challenging population for durable disease control.

TP53 evaluation

Cytogenetics

Cytogenetic evaluation is a routine and relatively straightforward means to risk-stratify patients with CLL, in which few recurrent cytogenetic abnormalities are seen. Approximately 80% of cases will harbor at least one chromosomal aberration at diagnosis.⁵ Deletion of the long arm of

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chromosome 13 [del(13q)] is the most common alteration (~55% of cases), and is associated with favorable prognosis.⁵ This is followed in frequency by the intermediate-risk category of cytogenetic abnormalities, which encompasses trisomy 12 (10–20% of cases) and deletions in the long arm of chromosome 11 [del(11q); ~15% of cases]. Deletions in the short arm of chromosome 17 [del(17p)] are found in 5–8% of cases at diagnosis, and have been associated with high-risk disease.⁵ Indeed, numerous prospective clinical trials have confirmed a poor prognosis for patients with del(17p).^{6–8} The incidence of del(17p) is even higher in relapsed or refractory CLL (~30% of cases) and confers a similarly dismal prognosis.⁹ However, notable therapeutic advances discussed in this review have mitigated the poor outcomes associated with del(17p).

A critical gene within the deleted portion of chromosome 17p is *TP53*, which encodes for the tumor suppressor p53. p53 is often referred to as the ‘guardian of the genome’ in reference to its critical role in maintaining genomic integrity.¹⁰ Abrogation of wild-type p53 functions allows cells to accumulate mutations, due to loss of p53-dependent apoptotic and cell-cycle arrest functions, and often results in malignancy. Indeed, germline *TP53* mutations are a hallmark of Li-Fraumeni syndrome, wherein patients develop malignancies of various anatomic sites (including hematologic), often before age 30.¹¹

TP53 mutations

Somatic mutations in *TP53* are observed in CLL in ~10% of cases at diagnosis and are often associated with del(17p). Indeed, almost 80% of cases with del(17p) will harbor a *TP53* mutation in the remaining allele, a phenomenon referred to as loss of heterozygosity (LOH).¹² Importantly, mutations in *TP53* have also been observed in the absence of 17p deletions and have been associated with poor prognosis, even in this setting.^{13,14} Such prognoses are evolving in the current era of targeted therapeutics as discussed in this review.

Function

At its simplest, p53 is a transcription factor. In normal cells under basal conditions, the p53 protein exists at levels often below the threshold of antibody-based detection methods. This is largely due to the presence of negative regulators, including

mouse double-minute 2 homolog (MDM2). MDM2 binds p53 and inhibits its transcriptional activity by interfering with its interaction with the transcriptional machinery.¹⁵ However, most importantly, MDM2 also ubiquitinates p53, thereby targeting p53 for proteasomal degradation.^{16–18} In response to cellular stresses, such as deoxyribonucleic acid (DNA) damage or deregulated oncogene activation, this MDM2-mediated negative regulation of p53 is suppressed, allowing for p53 stabilization and transcription of p53 target genes that drive apoptotic and cell-cycle arrest programs.

Classic chemotherapeutic agents exert cytotoxic effects by directly or indirectly inducing DNA damage. Such genomic injury activates a group of protein kinases that include ataxia–telangiectasia mutated (ATM; in the case of double-strand DNA breaks) and ATM and Rad3 related (ATR; in the case of single-strand DNA breaks or replication stress).¹⁹ These kinases begin a cascade of events that include the phosphorylation of both MDM2 and p53. The result is decreased interactions between MDM2 and p53, thus preventing ubiquitination of p53 and thereby facilitating p53 stabilization.^{20–22} As p53 accumulates in the stressed cell and is further phosphorylated, it exerts transcriptional activity, inducing expression of genes that either promote cell-cycle arrest, senescence, or apoptosis. For example, p53-mediated induction of the *CDKN1A* gene, which encodes the p21 protein, arrests the cell cycle and prevents proliferation of cells that have damaged DNA that could promote tumor formation or further tumor evolution.²³

Among p53-responsive genes that induce apoptosis are pro-apoptotic members of the B-cell lymphoma 2 (BCL2) family, such as *BAX*, *PUMA*, and *NOXA*.^{24–26} p53 has also been shown to directly decrease levels of the antiapoptotic protein BCL2.^{27,28} Together, the BCL2 family controls the release of cytochrome c from the outer membrane of mitochondria, resulting in an irreversible apoptotic cascade.²⁹ Thus, upon administration of many chemotherapeutic agents, the desirable result of cell death results in eradication of some or all of the tumor.

In scenarios where p53 cannot bind DNA or cannot be stabilized in order to act as a transcription factor, the desirable cytotoxic effects of chemotherapy are not evident. Importantly, the damage

induced by the chemotherapy may also go unrepaired in the absence of wild-type p53 function. Thus, administration of such drugs may accelerate the accumulation of genotoxic insults while failing to induce cell death. This sets the stage for a challenging therapeutic scenario.

Consequences of TP53 aberrations

In CLL (as well as many other malignancies), these normal wild-type functions of p53 are interrupted. As previously described, in addition to 17p deletions, *TP53* can also be mutated in CLL. These are often missense mutations that predominantly occur in the DNA-binding domain of *TP53* and result in the loss of wild-type p53 functions.³⁰ Indeed, mice that lack *Trp53* (the murine homolog of *TP53*) or express a mutant form (e.g. R172H, R172P, or R270H, corresponding to the R175 and R273 ‘hotspot mutations’ in humans) are tumor prone, often developing tumors of T- or B-cell lineage.^{31–34} Interestingly, some of these mutations have been shown to gain oncogenic activity as they alter the cellular transcriptome. This has been reviewed elsewhere.³⁵ This review will remain focused on the deficiencies of wild-type p53, which confer a fitness advantage to malignant cells.

Even when *TP53* or chromosome 17p are not directly influenced, alterations in p53 regulators may likewise render the p53 pathway deficient. Overexpression of *MDM2* has been observed in B-cell malignancies, including CLL.^{36,37} A single-nucleotide polymorphism in *MDM2* has also been linked to poor outcomes in CLL.^{38,39} Likewise, overexpression of *MDM4*, another negative p53 regulator, has been observed in a subset of p53-wild-type CLL that responds poorly to *MDM2* inhibition.⁴⁰ These observations have been validated in murine models, where increased levels of the p53’s negative regulators *MDM2* or *MDM4* result in hematologic tumors.^{41,42}

Conversely, decreased expression of *MDM2* inhibitors can also abrogate p53 functions. For example, p14^{ARF}, which is encoded from an alternative reading frame in *CDKN2A*, binds to *MDM2* and inhibits *MDM2*-dependent ubiquitination of p53.^{43–45} As such, mutations, deletions, and hypermethylation of the *CDKN2A* locus have been associated with aggressive disease courses in CLL.⁴⁶

Together, these data underscore the importance of p53 deregulation in hematopoietic oncogenesis and response to conventional chemotherapies. These observations also provide substantial rationale for utilizing therapeutic modalities that function without a requirement for wild-type p53.

Treating CLL

Initiation of therapy for CLL is not required for all patients at the time of diagnosis. While cytogenetic [del(17p)] or molecular (*TP53* mutation) aberrations may confer a worse prognosis, the presence of these events alone is not an indication for treatment initiation. Patients may start treatment based on disease-related symptoms or evidence of disease progression, such as physical findings of bulky lymph nodes or splenomegaly, or progressive lymphocytosis or cytopenias. Guidelines for initiation of therapy have been reviewed by the International Workshop on CLL.³ Outside of clinical trials, early therapy is generally not recommended before these criteria are met, as there is currently no evidence that starting chemotherapy earlier in asymptomatic patients improves survival in CLL.⁴⁷ However, early use of targeted molecular agents is an active field of study.^{48,49}

Historic treatments-genotoxic chemotherapies

Alkylating agents

Chlorambucil is an alkylating agent that was approved for medical use in 1957. It exerts its anticancer effects *via* interference with DNA replication, leading to cellular apoptosis in a p53-dependent manner.⁵⁰ The original studies using chlorambucil plus prednisone for treatment of CLL had modest overall response rates (ORRs) and complete response (CR) rates of 47% and 7%, respectively.⁵¹ Subsequently, patients would almost invariably progress with splenomegaly and bulky lymphadenopathy, the majority of whom succumbed to their disease or due to treatment complications.

Chlorambucil is still widely used in Europe and in elderly patients for palliative disease control.⁵² In a meta-analysis of seven clinical trials of the German CLL study group, of 3552 patients, 77% received chlorambucil plus anti-CD20 antibody

therapy.⁵³ Notably, 152 patients were more than 80-years old at treatment initiation.⁵³ Unfortunately, chlorambucil was especially poor in controlling CLL with *TP53* abnormalities, as these patients had significantly inferior ORR (27% versus 87%), shorter progression-free survival (PFS; 5-year PFS: 5% versus 17%), and overall survival (OS; 5-year OS 20% versus 59%) compared with patients without *TP53* abnormalities.^{12,54} Therefore, chlorambucil is currently not recommended for cases of CLL with *TP53* abnormalities, except for palliative purposes. These observations align with our biologic understanding of the consequences of administering such therapies to malignancies deficient in wild-type p53.

Purine analogs

Fludarabine is a purine analog discovered in the early 1970s as having cytotoxic activity *in vitro*.⁵⁵ In a seminal paper by Rai and colleagues, fludarabine showed increased ORR (63% versus 37%), CR (20% versus 4%), and PFS (25 months versus 14 months) compared with chlorambucil.⁵⁶ However, further analysis of patients with *TP53* abnormalities demonstrated continued poor outcomes compared with standard-risk patients.⁵⁶ Approximately 9% of CLL patients had *TP53* abnormalities at diagnosis, but by the time of relapse, 44% of patients had *TP53* abnormalities, suggesting that fludarabine exerts a selection advantage for *TP53* aberrant clones.^{12,57} Nonetheless, fludarabine has been a backbone of frontline CLL chemotherapy since Rai and colleagues' publication in 2000, and its combination with the cytotoxic agent cyclophosphamide, and the monoclonal antibody rituximab have been the genesis of immunochemotherapy (e.g. fludarabine–cyclophosphamide–rituximab, FCR) in hematologic malignancies.

Bendamustine

Bendamustine is a multifunctional alkylating agent containing a purine-like ring that has demonstrated clinical activity in multiple hematologic malignancies. In addition to causing DNA damage responses, this drug has been observed to trigger intrinsic apoptotic pathways, including upregulation of p53 upregulated modulator of apoptosis (PUMA) and phorbol-12-myristate-13-acetate-induced protein 1 (NOXA) and increasing expression of mitochondrial apoptogenic proteins.⁵⁸ In addition, bendamustine can

cause necrotic cell death, and thus shows some efficacy in cells lacking functional p53 or apoptotic pathways.⁵⁹

Bendamustine has been studied in the frontline setting for patients with newly diagnosed CLL. In one study, del(17p) was noted in 7% of patients and the median PFS was less than 12 months, consistent with previous reports using genotoxic chemotherapy approaches.⁶⁰ Bendamustine showed a promising response rate and was better tolerated than FCR, which led to community practices, particularly in the US, to adapt to using bendamustine, particularly for elderly patients who were not eligible for clinical trials. These practices led to a head-to-head study in which it was determined that, in combination with rituximab, bendamustine has a role in the frontline setting for patients lacking del(17p) who are not fit to receive FCR.⁶¹

Antibody therapy

Rituximab

Proteins expressed on the surface of leukemia cells represent a bevy of biologic targets. CD20 is a cell-surface protein expressed on pre-B cells and on differentiated B cells of both normal and malignant origin.⁶² The anti-CD20 antibody rituximab was the first of the targeted antibody therapies to change treatment paradigms for all B-cell malignancies. A combination of signaling-induced cell death, complement-dependent cytotoxicity, and antibody-dependent cellular cytotoxicity is responsible for rituximab-induced cell death.⁶³ These distinct mechanisms of action and manageable side-effect profile allowed for straightforward combinations with already existing chemotherapeutic agents.⁶⁴ FCR was effective in relapsed CLL and set the standard for frontline therapy for newly diagnosed, fit CLL patients requiring treatment.^{65,66} With this combination, ORR improved to 92% with a CR of 70%.⁶⁶ However, even though this treatment modality has proven effective in many patients with CLL, those with *TP53* abnormalities continued to have poorer response rates and shorter remission durations.

Ofatumumab

Ofatumumab is also an anti-CD20 antibody that targets a distinct epitope on CD20 that is not

targeted by rituximab.⁶⁷ In fludarabine-refractory CLL patients, ofatumumab was safe and effective as a single agent, with ORR of 58%, PFS of 5.9 months, and OS of 15.4 months.⁶⁸ While adverse events were minimal, routine use of ofatumumab has not been adapted since it has not demonstrated superiority over rituximab.

Obinutuzumab

Obinutuzumab is another CD20 antibody that was first studied in combination with chlorambucil for elderly patients who were not candidates for standard chemoimmunotherapy.⁶⁹ Patients receiving both drugs had significantly higher CR rates compared with chlorambucil alone (20.7% versus 7%).⁶⁹ However, patients with del(17p) did not see such benefit. Importantly, the addition of obinutuzumab did not elicit substantial toxicity, a crucial consideration in these patient populations.⁶⁹ The results of this study led to trials combining obinutuzumab with fludarabine-cyclophosphamide (FC) or bendamustine, where the combinations demonstrated robust activity, though only one patient harbored del(17p).⁷⁰ For obinutuzumab-FC, the ORR was 62% with 24% CR/CRi with incomplete hematologic recovery (CRi). Obinutuzumab-bendamustine had an ORR of 90% with 45% CR/CRi.⁷⁰ Further studies are ongoing to evaluate which patient populations are best to incorporate this novel CD20 antibody and to investigate its role in p53-pathway-deficient CLL.

Alemtuzumab

Alemtuzumab is an anti-CD52 antibody that was designed for CLL patients refractory to both alkylating and purine analog agents; a population usually harboring *TP53* abnormalities.⁷¹ In the early 2000s, fludarabine-refractory patients had an anticipated survival of only 10 months. This led to the design of a trial treating high-risk CLL patient populations (fludarabine-refractory or harboring *TP53* abnormalities) with alemtuzumab. This trial demonstrated an ORR of 33%, but came with significant infectious complications, including cytomegalovirus reactivation and fungal infections.^{72,73} Alemtuzumab is still available for CLL with abnormal *TP53*; however, its use has been limited due to this significant toxicity.

Targeted agents

To better treat CLL with deficiencies in the p53 pathway, using agents that kill cells independently of p53 is imperative. Understanding the mechanism of action of currently available targeted agents provides rationale for their utilization and combinations.

B-cell expansion in CLL is largely driven by constitutive B-cell receptor (BCR) signaling.⁷⁴ The BCR signals through a series of tyrosine kinases, including phosphatidylinositol 3-kinase delta (PI3K δ) and Bruton's tyrosine kinase (BTK).⁷⁵ Understanding the BCR signaling axis has been pivotal in developing effective drugs for CLL. To this end, PI3K δ inhibitors (idelalisib, duvelisib, and umbralisib) and BTK inhibitors (ibrutinib, acalabrutinib, and zanubrutinib) are in various stages of clinical use. These drugs directly inhibit the pathway that produces and maintains B cells of both normal and malignant origin.

In addition to constitutive BCR signaling, CLL cells often harbor high expression of BCL2, an antiapoptotic protein that sustains cell survival.^{76,77} As such, methods that inhibit or decrease levels of BCL2 in CLL cells have been shown to activate apoptosis.⁷⁸ This understanding led to development of venetoclax, a BCL2 inhibitor, in CLL.

Together, these drugs have been revolutionary, as they inhibit critical mediators of CLL emergence and survival. They are discussed individually in the following section. The major clinical trials utilizing these drugs in patients with p53-pathway-deficient CLL are summarized in Table 1.

Idelalisib

Idelalisib is an orally available small molecule that specifically inhibits the catalytic subunit of PI3K δ .⁷⁹ Mice lacking this isoform have severe B-cell defects, highlighting the importance of this specific protein in the development and function of B cells.^{80–82} In CLL, PI3K δ signals downstream of the BCR, leading to constitutive activation of the PI3K pathway and upregulation of antiapoptotic proteins.^{83,84} Chronic BCR signaling subsequently leads to chronic activation of PI3K δ in B-cell malignancies, including CLL.⁸⁵ Downstream of PI3K δ lies a number of effector

Table 1. Summary of major trials including patients with p53-pathway-deficient CLL.

PMID	Treatment setting	Treatment arm	Median age	del(17p)	TP53 mutation	CR/CRi	Median PFS		Median OS	MRD negativity	MRD assessment
							All patients	p53 deficient			
24401022	Frontline	Obinutuzumab + chlorambucil	74	22/333	ND	20.7%	26.7 months	ND	NR	19.5% (BM); 37.7% (PB)	ASO PCR
24450857	Relapsed/refractory	Rituximab + chlorambucil	73	20/330		7.0%	15.2 months	ND	NR	2.6% (BM); 3.3% (PB)	
		Chlorambucil	72	10/118		0%	11.1 months	ND	NR	ND	
30287523	Relapsed/refractory	Idelalisib + rituximab	71	42/110		0%	NR; 93% at 6 months	NR	NR; 92% at 12 months	ND	
		Rituximab + placebo	71	45/110		0%	5.5 months; 46% at 6 months	4 months	NR; 80% at 12 months	ND	
EHA LB2602	Frontline	Duvelisib	69	21/160	20/160	0.6%	13.3 months	12.7 months	NR	ND	
		Ofatumumab	69	28/159	18/159	0.6%	9.9 months	9 months	NR	ND	
		Ibrutinib	64	6/182	14/182	ND	NR	ND	ND	ND	
23782158	Relapsed/refractory	Placebo	64	7/181	13/181	ND	14.8 months	ND	ND	ND	
		Ibrutinib	66	28/85	ND	2.3%	NR; 75% at 26 months	57% at 26 months	NR; 83% at 26 months	ND	
27637985	Relapsed/refractory	Ibrutinib	64	144/144	107/116	2%	NR; 63% at 24 months		NR; 75% at 24 months	ND	
24881631	Relapsed/refractory	Ibrutinib	67	63/195	ND	2%	NR; 88% at 6 months	NR; 83% at 6 months	NR; 90% at 12 months	ND	
		Ofatumumab	67	64/196		1%	8.1 months; 65% at 6 months	5.8 months	NR; 81% at 12 months	ND	
30501481	Frontline	Rituximab + bendamustine	70	14/481	16/174	26%	43 months; 74% at 2 years	7 months	NR; 95% at 2 years	8%	Flow cytometry
		Ibrutinib	71	9/181	15/168	7%	NR; 87% at 2 years	NR	NR; 90% at 2 years	1%	
		Ibrutinib + rituximab	71	11/180	20/168	12%	NR; 88% at 2 years	NR	NR; 94% at 2 years	4%	

(Continued)

Table 1. (Continued)

PMID	Treatment setting	Treatment arm	Median age	del(17p)	TP53 mutation	CR/CRi	Median PFS		Median OS	MRD negativity	MRD assessment
							All patients	p53 deficient			
30522969	Frontline	Ibrutinib + obinutuzumab	70	14/113	13/112	19%	NR; 79% at 30 months	NR	NR; 86% at 30 months	35%	Flow cytometry
26641137	Relapsed/refractory	Chlorambucil + obinutuzumab	72	18/116	16/110	8%	19 months; 31% at 30 months	11.3 months	NR; 85% at 30 months	25%	
EHA LB2606	Relapsed/refractory	Acalabrutinib	62	18/59	ND	0%	NR	NR	NR	ND	
	Relapsed/refractory	Acalabrutinib	68	28/155	ND	0%	NR; 88% at 1 year	NR; 88% at 1 year*	NR	ND	
	Relapsed/refractory	Rituximab + idelalisib or rituximab + bendamustine	67	21/154	ND	1%	16.5 months; 68% at 1 year	16.2 months*	NR	ND	
31340982	Mixed	Zanubrutinib	69	18/94	ND	2.6%	NR; 100% at 12 months	ND	ND	ND	
26639348	Relapsed/refractory	Venetoclax	66	31/102	ND	20%	25 months; 49% at 2 years	16 months	NR; 84% at 2 years	5%	Flow cytometry
29562156	Relapsed/refractory	Venetoclax + Rituximab	64.5	46/173	48/192	26.8%	NR; 84.9% at 24 months	81.5% at 24 months	91.9% at 24 months	62.4%	Flow cytometry
31166681	Frontline	Rituximab + bendamustine	66	46/169	51/184	8.2%	17 months; 36.3% at 24 months	27.8% at 24 months	86.6% at 24 months	13.3%	
	Frontline	Venetoclax + obinutuzumab	72	17/200	19/171	49.5%	NR; 88.6% at 24 months	73.9% at 24 months	NR; 91.8% at 24 months	75.5% (PB); 56.9% (BM)	ASO PCR
28715249	Relapsed/refractory	Chlorambucil + obinutuzumab	71	14/193	13/157	23.1%	NR; 63.7% at 24 months	32.7% at 24 months	NR; 93.3% at 24 months	35.2% (PB); 17.1% (BM)	
30642819	Relapsed/refractory	CD19 CAR-T cells	61	14/24	ND	17%	8.5 months	ND	NR	88%	Flow cytometry
28424162	Relapsed/refractory	Nivolumab + ibrutinib	65	19/36	ND	0%	NR	NR	NR	ND	
	Relapsed/refractory	Pembrolizumab	72	6/16	1/16	0%	2.4 months	ND	11.2 months	ND	

ASO PCR, allele-specific oligonucleotide polymerase chain reaction; BM, bone marrow; CLL, chronic lymphocytic leukemia; CR, complete response; CRi, CR with incomplete hematologic recovery; MRD, minimal residual disease; ND, no data; NR, not reached; OS, overall survival; PFS, progression-free survival; PB, peripheral blood; PMID, unique identifier number on PubMed.
 Note: * study reported "high risk cytogenetic features", which included p53 abnormalities in addition to other features.

molecules, including AKT (protein kinase B). This is particularly relevant in the context of p53, as AKT phosphorylates MDM2, enhancing its ability to ubiquitinate p53 and target it for proteasomal degradation.⁸⁶ Thus, in addition to blocking the upregulation of antiapoptotic proteins, inhibition of PI3K δ may partly restore p53 activity by allowing p53 stabilization. Indeed, in other cancer models, idelalisib has been shown to induce PUMA.⁸⁷ In the absence of wild-type p53 function, upregulation of PUMA can push cells toward apoptosis. Thus, idelalisib can be useful in treating malignancies with chronic BCR activation, even in the setting of p53 pathway deficiency.

Idelalisib is currently FDA approved for relapsed CLL when used in combination with rituximab. This approval was based on the double-blind, placebo-controlled phase III study using idelalisib plus rituximab *versus* placebo plus rituximab. The median PFS in the idelalisib plus rituximab group was 20.3 months, whereas the placebo plus rituximab group had a median PFS of 5.5 months.^{88,89} OS showed similar trends of 40.6 months *versus* 34.6 months.⁸⁹ This study enrolled a challenging patient population, as more than 40% of patients had del(17p), and the majority had received FCR and had a median of three prior therapies. In long-term follow up, where all patients received idelalisib after initial treatment, patients with del(17p) or *TP53* mutations had an OS of 28.5 months in the idelalisib-rituximab arm compared with only 14.8 months in the placebo-rituximab arm.⁸⁹

While clinically effective, many patients do not tolerate idelalisib, and this agent has a more adverse safety profile than other targeted agents, with relatively high rates of diarrhea and infectious pneumonia.⁹⁰ The FDA issued a black-box warning after frequent immune-mediated occurrences of colitis, pneumonitis, or hepatotoxicity were reported.⁹¹ Cessation of the drug, in addition to use of corticosteroids, appear to alleviate the immune reaction.⁹² Combinations with idelalisib have also been associated with higher rates of infection, including *Pneumocystis jirovecii* pneumonia.^{88,93}

Duvelisib

Duvelisib is an orally available PI3K δ inhibitor that demonstrated superiority to ofatumumab in

a phase III clinical trial of relapsed refractory CLL patients.⁹⁴ In this study, 160 patients were randomized to receive single-agent duvelisib or single-agent ofatumumab. Approximately 30 patients in each group had p53 deficiencies. Duvelisib was able to obtain an overall response rate of 74% *versus* 45% with ofatumumab alone and a median PFS of 13.3 months *versus* 9.9 months. The efficacy of duvelisib was maintained in p53-deficient patients, as their median PFS was 12.7 months compared with 9 months for patients receiving ofatumumab. Based on these data, the FDA approved the use of duvelisib in the treatment of relapsed refractory CLL.⁹⁴

Umbralisib

Umbralisib is a newer inhibitor of PI3K δ that is structurally distinct from idelalisib and duvelisib.⁹⁵ In a phase I study, 17/20 patients with relapsed refractory CLL had an objective response.⁹⁵ Of eight patients with high-risk cytogenetic features, six had a response and none had disease progression. Importantly, umbralisib appeared to have a more favorable safety profile than idelalisib and duvelisib, with fewer incidences of autoimmune-like toxicities.⁹⁵ A study evaluating the use of umbralisib in patients who were intolerant to idelalisib or BTK inhibitors suggests that these patients may benefit from switching to umbralisib.⁹⁶ Median PFS in this cohort, which comprised 24% p53-deficient patients, was 23.5 months. Importantly, patients with prior PI3K δ -targeted therapy did not require umbralisib discontinuation, due to recurrence of adverse events that led to discontinuation of the original drug.⁹⁶

Ibrutinib

Ibrutinib is a tyrosine kinase inhibitor that specifically inhibits Bruton's tyrosine kinase (BTK), a critical molecule mediating BCR signaling. In a phase I/II study of ibrutinib in relapsed refractory patients with high-risk disease, ORR was 71%, and an additional 20% of patients had a partial response (PR) with lymphocytosis. The latter was subsequently described as an ibrutinib-related phenomenon that does not have negative clinical impact. At 26 months of median follow up, PFS was 75% and OS was 83%.⁹⁷

Ibrutinib was the first novel agent to obtain full FDA approval for frontline treatment (March

2016) and relapsed refractory settings in patients with del(17p) CLL. In the frontline setting, ibrutinib was compared with its combination with rituximab *versus* bendamustine–rituximab in older patients.⁹⁸ Critically, for patients in this study with *TP53* abnormalities, the median PFS for the bendamustine plus rituximab group was 6 months compared with the ibrutinib plus rituximab group, where median PFS was not reached at 3 years of follow up.⁹⁸

These encouraging results with ibrutinib in the p53-deficient patients were further validated in the RESONATE-17 trial. Ibrutinib in del(17p) populations showed 2-year PFS of 63% and OS of 75% in this trial, which enrolled 144 patients, all with centrally confirmed del(17p).⁹⁹

Ibrutinib was also evaluated in the frontline setting in combination with obinutuzumab in a phase III trial.¹⁰⁰ Ibrutinib plus obinutuzumab demonstrated superiority compared with chlorambucil–obinutuzumab, as median PFS was not reached in the ibrutinib group and was 19 months in the chlorambucil–obinutuzumab group. In p53-deficient patients, median PFS was 11.3 months in the chlorambucil–obinutuzumab group but was not reached in the ibrutinib–obinutuzumab group.¹⁰⁰

In the longest follow-up study of single-agent ibrutinib, 5-year follow up still showed an ORR of 89% and CR in 29% in treatment-naïve patients, and CR of 10% in the relapsed refractory population. However, the del(17p) population had a median PFS of 26 months compared with 51 months for the whole relapsed refractory population.¹⁰¹

Ibrutinib has also been shown effective in an *in vivo* model of *de novo* p53-mutated CLL.¹⁰² Importantly, this study also demonstrated that frontline ibrutinib treatment in this model did not place undue pressure on the remaining wild-type *Trp53* alleles to undergo LOH. While these findings must be fully substantiated clinically, they are of great interest, since most single trials do not have substantial numbers of patients harboring only heterozygous p53 mutations.

Ibrutinib has been a clear breakthrough in treating CLL patients with *TP53* abnormalities and has been incorporated into all guidelines for the

frontline and relapse settings. While outcomes with this drug are substantially better than those seen with chemoimmunotherapy in similar populations, ibrutinib alone may not be optimal for overcoming *TP53* abnormalities.

Acalabrutinib

Acalabrutinib is a second-generation, more selective, irreversible inhibitor of BTK. This was tested in a phase I/II study of relapsed refractory CLL patients with a median of three prior lines of therapy in which 31% had *TP53* abnormalities. The acalabrutinib arm had an ORR of 95%, partial response rate of 85%, and partial response with lymphocytosis in 10%. Interestingly, del(17p) patients had a 100% ORR, and no observations of Richter's transformation were noted in that trial.¹⁰³

In a phase III study, single-agent acalabrutinib was compared with rituximab–idelalisib (IR) or rituximab–bendamustine (BR) in a relapsed refractory population.¹⁰⁴ PFS was not reached in the acalabrutinib arm *versus* 16.5 months in the IR or BR groups. This improvement in PFS was maintained in the p53-deficient subgroups.¹⁰⁴

Acalabrutinib has also demonstrated efficacy in a small study of patients deemed intolerant to ibrutinib.¹⁰⁵ Patients with del(17p) had an ORR of 67%, and median PFS was not reached in this subgroup at 13.6 months of follow up.¹⁰⁵ Of 33 patients in this study who were previously deemed intolerant to ibrutinib, only 3 discontinued acalabrutinib due to adverse events.¹⁰⁵ Another phase II study has generated similar results, demonstrating that acalabrutinib is tolerable and effective in ibrutinib-intolerant patients.¹⁰⁶ Indeed, a head-to-head comparison of ibrutinib *versus* acalabrutinib is currently underway for relapsed refractory patients with high-risk CLL [ClinicalTrials.gov identifier: NCT02477696].

Zanubrutinib

Zanubrutinib is an orally available kinase inhibitor with greater selectivity for BTK than ibrutinib.¹⁰⁷ This selectivity is desirable, since the off-target effects of ibrutinib are thought to largely mediate the adverse events observed clinically.¹⁰⁷ In a phase I study, ORR was 100% in patients with p53 abnormalities.¹⁰⁷ This high rate of activity is likely due to the fact that zanubrutinib has a

longer half-life compared with acalabrutinib or ibrutinib, thus prolonging exposure of CLL cells to specific BTK inhibition.¹⁰⁷ Zanubrutinib is now being compared with ibrutinib in an ongoing phase III trial of relapsed refractory CLL [ClinicalTrials.gov identifier: NCT03734016].¹⁰⁸

The evidence for the use of BTK inhibitors in *TP53*-aberrant CLL is thus promising, and highlights how targeted agents not requiring the p53 pathway for their efficacy are critical for patients with *TP53* alterations.

Venetoclax

Venetoclax is an orally available inhibitor of BCL2, an antiapoptotic protein that sustains cell survival and is highly expressed in CLL.⁷⁶ BCL2 binds to pro-apoptotic proteins BIM, BAD, BID, and NOXA, thereby inhibiting initiation of the apoptotic cascade.¹⁰⁹ Inhibition of BCL2 by venetoclax thus allows pro-apoptotic proteins to promote cell death. Intact p53 regulates the expression of several pro-apoptotic proteins such as PUMA and BAX, which promote apoptosis. In the absence of wild-type p53 function, venetoclax still holds promise, as it directly antagonizes BCL2 and promotes cell death in a p53-independent manner.¹¹⁰

This lack of p53-dependency is highlighted by studies showing that in relapsed refractory CLL patients with *TP53* mutations or chromosome 17p deletions, single-agent venetoclax led to a 20% CR rate, with 5% of patients having no minimal residual disease (MRD) *via* flow cytometry.¹¹¹ In this difficult-to-treat population, single-agent venetoclax led to 69% PFS at 16 months.¹¹¹

Venetoclax has been studied in combination with obinutuzumab (VO) *versus* chlorambucil-obinutuzumab (CO) in previously untreated patients.¹¹² The use of venetoclax was associated with a longer PFS, including in the p53-aberrant group, where 24 month PFS estimates were 73.9% in the VO arm *versus* 32.7 in the CO arm.¹¹² Further, 70.8% of patients with aberrant p53 treated with VO achieved MRD negativity in peripheral blood compared with only 9.1% in CO.¹¹²

In relapsed refractory CLL, venetoclax plus rituximab (VR) was compared with bendamustine plus rituximab (BR).¹¹³ In this study, venetoclax was

stopped after 2 years of treatment. The 2-year rate of PFS in del(17p) patients was 81.5% in the VR group *versus* 27.8% in the BR group.¹¹³ Post-treatment follow up of this study confirmed these results, showing that median PFS in the VR group for patients with *TP53* mutations was 36 months, *versus* 12.9 months in the BR group.¹¹⁴ Likewise, del(17p) patients did not reach median PFS, *versus* 15.4 months in the BR group.¹¹⁴ Notably, aberrant p53 was associated with an increased risk of disease progression after venetoclax cessation.¹¹⁴

Ibrutinib plus venetoclax

Since chronic BCR signaling is exacerbated by overexpression of BCL2, dual inhibition of these molecules is biologically justified. In the frontline setting, the combination of ibrutinib and venetoclax has been studied to combine mechanistically distinct drugs to minimize the risk of disease resistance developing. In patients with high-risk CLL with *TP53* aberrations, 80% achieved CR with 61% having no MRD.¹¹⁵ While longer follow up is needed to determine the durability of the disease response, this treatment combination represents a completely chemotherapy-free regimen that is highly effective in treating patients with *TP53* alterations.

Currently, a phase III study is investigating the role of venetoclax in combination with ibrutinib and obinutuzumab *versus* ibrutinib plus obinutuzumab in treatment-naïve young patients with CLL. This phase III trial builds from the efficacy of an earlier trial utilizing this combination,¹¹⁶ and hopes to answer the question of whether this time-limited three-drug combination is effective *versus* continuous ibrutinib dosing in CLL patients aged younger than 70 years [ClinicalTrials.gov identifier: NCT03701282]. Yet another study evaluating this combination is also underway, wherein each drug is given as a sequential therapy, beginning with ibrutinib as induction therapy, obinutuzumab as consolidation, and venetoclax as a 1-year maintenance [ClinicalTrials.gov identifier: NCT03755947].

Immunological therapies

Checkpoint inhibitors

Nivolumab is an antibody against programmed-cell death protein 1 (PD-1), the blockade of which

releases the ‘checkpoint inhibition’ of antitumor T-cell responses, allowing for T-cell-induced tumor killing.¹¹⁷ Nivolumab in combination with ibrutinib in a high-risk [del(17p) and del(11q)] relapsed refractory CLL population led to an ORR of 61%.¹¹⁸ Grade 3–4 immune-related toxicities included rash in 8% of patients and elevated alanine transaminase in 2%.¹¹⁸ While this combination was well tolerated, the response rate was similar to single-agent ibrutinib in CLL. Therefore, it is unclear if the addition of nivolumab potentiates the clinical efficacy of ibrutinib. However, it has been noted that 65% of cases with Richter’s transformation responded to the combination of ibrutinib and nivolumab, demonstrating that this combination may be useful in this setting.^{118,119}

Likewise, pembrolizumab, another anti-PD-1-antibody has shown efficacy as a single agent in patients with Richter’s transformation.¹²⁰ However, no patients with CLL responded to pembrolizumab in this study.¹²⁰ A study investigating the combination of pembrolizumab with either idelalisib or ibrutinib is currently underway [ClinicalTrials.gov identifier: NCT02332980].

Chimeric antigen receptor T cells

Modifying one’s own T cells to attack cancer is no longer science fiction. June and colleagues were the first to report the use of autologous chimeric antigen receptor (CAR)-modified T cells in a multirefractory CLL patient, who then achieved a CR.¹²¹ This technology programs T cells to recognize CD19 on the surface of normal and malignant B cells and then selectively kill CD19-expressing cells.¹²¹

Currently, similar CAR-T cells are being evaluated in multiple prospective trials. A phase I CD19 CAR-T trial enrolled very high risk and ibrutinib-refractory CLL patients. Most patients had either complex karyotype or del(17p) (23/24).¹²² ORR was 71% (17/24), and for patients who were alive at the restaging time point, CR was 21% (4/19) and partial response was 53% (10/19).¹²² Toxicities were similar to previous CAR-T studies, with cytokine release syndrome and neurotoxicity observed in 83% and 33%, respectively. In those who had a clinical response and underwent bone marrow biopsy, 88% (15/17) had no MRD by flow cytometry, and 58% (7/12) had no detectable disease by deep *IGH* sequencing.¹²² This demonstrates that

patients who respond to CAR-T therapy may be able to achieve a deep remission without MRD.

Other CAR therapies are also in development, such as ‘off the shelf’ allogeneic CAR-T-cell therapy,¹²³ and natural killer (NK) CAR-cell therapy, the latter of which is being actively developed for refractory CLL [ClinicalTrials.gov identifier: NCT02727803].

Allogeneic transplantation

At this time, the only truly ‘curative’ treatment option for hematologic malignancy is allogeneic stem-cell transplantation, which potentially offers a graft *versus* leukemia effect, leading to a cure. This possible benefit is substantial, but the risk is also high. Given the option, many patients with *TP53* abnormalities seek allogeneic transplantation. At 6 years post-allogeneic transplant, data from four large centers showed a PFS of 40–45% and OS of 50–60% in this patient population.^{124–126} These were patients who had matching donors and were selected to be ‘fit’. Treatment-related mortality and severe graft *versus* host disease was reported in 16–25% and 50–55%, respectively. These results make it daunting to recommend allogeneic transplantation in many elderly patients, as risk will undoubtedly be worse for elderly patients who are under-represented in these clinical trials. However, for a young, fit, high-risk CLL patient with a perfectly matched donor, the opportunity of a potentially curative therapy may be worth the risks associated with stem-cell transplantation. Indeed, the evolving reality of targeted, orally available, and well-tolerated therapies is reason to pause prior to recommending transplant. However, this higher-risk option still represents a viable option while awaiting longer-term outcomes for patients treated with such targeted agents.

p53-targeted therapy

Indirect activation of p53 function by inhibition of MDM2 has been achieved preclinically with a small-molecule antagonist, Nutlin-3a.¹²⁷ However, the development of this agent has not been fully deployed in the clinical setting due to other exciting therapeutic options, deflating clinical interest in MDM2 as a pharmacologic target. Given that p53 mutations represent a significant mechanism driving poor outcomes in patients with CLL,

therapies that restore wild-type p53 function may still be useful additions to the armamentarium of antileukemic agents. In fact, a recently opened phase III clinical trial [ClinicalTrials.gov identifier: NCT03745716] is attempting to reactivate mutant p53 functions using APR-246 (formally known as PRIMA-1MET) in patients with refractory myelodysplastic syndrome. Successful results from this trial could provide impetus to explore the activity of APR-246 in patients with CLL harboring p53 mutations.

Resistance

In cases of CLL that develop resistance to BTK inhibitors ibrutinib or acalabrutinib, a substantially higher percentage appear to harbor del(17p).^{128,129} Such an association suggests that being aware of resistance mechanisms in this population may be particularly prudent.

Restoration of BCR signaling and resistance to ibrutinib have been observed *via* mutations in BTK at Cys481, thus preventing ibrutinib from binding.^{128–131} Activating mutations in the downstream substrate PLC γ 2 leading to autonomous BCR signaling are also commonly observed.^{128–131} In such cases, transition of therapy to venetoclax or a PI3K δ inhibitor can suppresses these resistant clones.^{128,129,132} Development of small molecules to specifically address these resistance mutations may also hold promise. LOXO305 is a reversible BTK inhibitor that retains activity against the most common BTK C481S alteration *in vitro*.¹³³ A phase I trial is underway with this agent [ClinicalTrials.gov identifier: NCT03740529]. Likewise, ARQ 531 is a reversible BTK inhibitor with other kinase inhibitory activity that has been shown in preclinical studies to be effective against both BTK and PLC γ 2 mutant clones.¹³⁴

Resistance to venetoclax can arise from a mutation in BCL2 at Gly101 or Asp103. Mutations at these residue can reduce the affinity of venetoclax for BCL2 substantially. These mutations have been associated with venetoclax resistance and impending relapse in patients with CLL.^{135,136} Other antiapoptotic proteins besides BCL2, such as MCL1 or BCL-X_L, may also be upregulated in CLL cells, leading to disease progression despite venetoclax administration. Importantly, inhibitors of BCR signaling (ibrutinib, idelalisib) and blockade of CD20 (obinutuzumab, rituximab) have demonstrated downregulation of MCL1,

which sensitizes leukemic cells to BCL2 inhibition by venetoclax, lending further credence to the notion of combination therapy.¹³⁷

Pre-emptively understanding these mechanisms of resistance and their impact on the p53 pathway will be key to the sustained success of these agents in the clinic. Understanding the known mechanisms of resistance or anticipating the development of resistance may be useful for keeping patients in deep, durable remissions.

Summary

Patients with CLL have benefited greatly from the explosion of new treatment options. We are rapidly moving away from genotoxic drugs in the frontline setting, and the new reality is that patients with CLL are being treated with targeted small-molecule inhibitors, achieving greater responses and deeper remissions in the context of improved quality of life. Likewise, TP53-abnormal populations have also seen vast improvements in their survival with these new combinations. Likewise, novel CAR-T, NK CARs, and allogeneic CAR therapies have the potential to provide the next steps forward in improving the quality and quantity of life for all patients with CLL.

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