Targeting *TP53* Disruption in Chronic Lymphocytic Leukemia: Current Strategies and Future Directions

Stefano Molica[1] , Constantine Tam[2] ,David Allsup[3] and Aaron Polliack[4]

1) Queens Centre for Oncology and Haematology, Castle Hill Hospital, Hull University NHS Trust, Hull, UK
2) Alfred Hospital, Melbourne, VIC 3004, Australia
3) Centre of Biomedicine, Hull York Medical School, University of Hull, Hull, UK
4) Department of Hematology , Hadassah Hebrew University Medical Center , Jerusalem, Israel

Key words: CLL, Targeting *TP53* aberrations, Current strategies, Novel therapies

Running title: Targeting *TP53* aberration in CLL

Page:22

Words: 3043

Figures:1

Tables: 3

Correspondence:
Stefano Molica
Queens Centre for Oncology and Haematology, Castle Hill Hospital,
Hull University NHS Trust, Hull, UK
Email: smolica@libero.it
Stefano.molica@nhs.net

ABSTRACT

In the modern era of Chronic Lymphocytic Leukemia (CLL) targeted therapy, the loss of p53 function due to genetic abnormalities remains a significant challenge. This is because even targeted agents, which are currently the mainstay of treatment for CLL, do not directly target p53 or restore its disrupted pathway. Consequently, resistance to therapy and unfavorable clinical outcomes often accompany these p53-related abnormalities. An essential goal of future clinical research should be to address the ostensibly "undruggable" p53 pathway. Currently, multiple therapeutic approaches are being explored to tackle *TP53* dysfunction and improve outcomes in high-risk CLL. These approaches include the use of oncoprotein murine double minute 2 (MDM2) inhibitors, small-molecule p53 reactivators, exportin 1 (XPO1) inhibitors, and ataxia-telangiectasia mutated and Rad3-related (ATR) inhibitors. Combinations of these p53-targeting strategies, along with established novel therapies such as B-cell receptor (BCR) or B-cell lymphoma-2 (BCL-2) inhibitors, may shape the future of therapeutic trials in this challenging-to-treat disease.

.

1.0 INTRODUCTION

TP53 aberrations are consistently reliable indicators guiding treatment choices in Chronic Lymphocytic Leukemia (CLL), and their presence is associated with significantly lower survival rates and poor responses to chemotherapy [1-11]. It is important to note that *TP53* gene function can be disrupted in two different ways in CLL: (1) via the deletion of the short "p" arm of chromosome 17, where the *TP53* gene resides [deletion (17p)], or (2) through mutation of the *TP53* gene. The former is typically detected by Fluorescence In Situ Hybridization (FISH) studies, while the latter is detected by sequencing studies [12].

From a therapeutic perspective, agents such as Bruton kinase (BTK) inhibitors and anti-B-cell lymphoma-2 (BCL2) inhibitors have shown effectiveness in patients with *TP53* dysfunction, even though they do not directly target p53 [3-11]. These agents have significantly improved outcomes in CLL. However, patients with disrupted *TP53* generally have a worse prognosis than those with wild-type *TP53*, particularly in the context of relapsed/refractory (R/R) disease or limited-duration anti-BCL2 therapy [3-11]. The efficacy of emerging innovative molecules selectively targeting the p53 mutant protein pathway in CLL is currently under investigation [13].

In this review, we provide a detailed overview of various CLL therapies addressing *TP53* disruption and introduce some promising novel compounds that may potentially restore the function of the mutated p53 protein or enhance the activity of wild-type p53.

2.0 THERAPIES TARGETING PATHWAYS OTHER THAN *TP53*

A prime example of effective agents for treating patients with CLL who have *TP53* disruptions is the use of BTK inhibitors (Table 1). These agents target a p53-independent pathway, adversely affecting the survival of leukemic cells. In previously untreated CLL patients with *TP53* alterations, the estimated progression-free survival (PFS) and overall survival (OS) at 6 years were 61% and 79%, respectively [13]. A pooled analysis across four studies (PCYC-1122e, RESONATE-2, iLLUMINATE, and ECOG-ACRIN E1912), enrolling TP53-aberrant patients treated upfront with ibrutinib-based therapies, reported a four-year PFS and OS rates of 79% and 88%, respectively [15]. In a retrospective analysis at the MD Anderson Cancer Center, CLL patients with baseline del(17p) and/or *TP53* mutations treated in the first-line setting with BTK inhibitors (BTKi), with or without the addition of the BCL2 inhibitor venetoclax or an anti-CD20 antibody, achieved 4-year PFS and OS rates of 72.9% and 83.6%, respectively [16]. Randomized studies have shown no improvement of ibrutinib + rituximab (IR) over ibrutinib. Therefore, the favorable results of ibrutinib + anti-CD20 in *TP53* mutant patients are likely largely due to the effect of ibrutinib [5, 9, 17].

In the context of the ELEVATE-RR trial, which compared ibrutinib versus the second-generation BTKi acalabrutinib in relapsed/refractory CLL patients with high-risk prognostic features (del(17p) and/or del(11q)), the forest plot estimate of PFS shows that the equivalency of ibrutinib and acalabrutinib is maintained in the del(17p) subgroup [7]. The ALPINE study, comparing zanubrutinib to ibrutinib in relapsed/refractory CLL, revealed that zanubrutinib offers superior PFS in patients with del(17p) and/or abnormal TP53, as evidenced by a lower hazard ratio for disease progression or death (0.53) [8]. These differences may, in part, be attributed to zanubrutinib's distinctive pharmacokinetic profile, which permits sustained BTK inhibition by maintaining detectable levels in the peripheral blood over the entire treatment period, even when CLL cells continue to produce additional BTK [18]. The PFS and OS outcomes in the SEQUOIA Arm C, the largest frontline del(17p) CLL study, closely mirror those of SEQUOIA Arm A, which excluded del(17p) patients [19-20]. However, a formal comparison between these patient groups has not been conducted.

In preliminary studies, approximately 76% of patients with 17p deletion or *TP53* mutation who had received prior therapy with covalent BTKIs responded to pirtobrutinib, a non-covalent BTKi developed to overcome pharmacologic and on-target covalent BTKi resistance [21]. Phase 3 studies evaluating pirtobrutinib in earlier lines of therapy and in direct comparison to covalent BTK inhibitors are underway in CLL (Table 2). Results of these trials, when available, will provide a more comprehensive assessment of the efficacy of pirtobrutinib, especially in the context of TP53-mutated CLL patients.

Targeting downstream of p53 for *TP53*-aberrant patients includes venetoclax, a BCL2 inhibitor that directly targets mitochondria [22]. Venetoclax, combined with Obinutuzumab (VO) as upfront fixed-duration therapy, improves PFS in CLL compared to chemoimmunotherapy (CIT). However, *TP53*-aberrant patients only achieve a 5-year PFS of 40.6%. This prompts the question of whether extended therapy and/or BTK inhibition could be more effective against *TP53* aberrations [23]. The CLL17 (NCT04608318) phase-III trial compares continuous ibrutinib monotherapy to fixed-duration VO and venetoclax-ibrutinib (IV) in previously untreated CLL. Patients are randomized 1:1:1 and stratified by del(17p)/*TP53* mutation. The trial's results will help determine whether continuous drug exposure to BTK inhibition is more crucial for improving outcomes in *TP53*-dysfunctional CLL [24].

The combination of ibrutinib and venetoclax (IV) exploits the distinct and complementary mechanisms of action of the two drugs [25]. The CAPTIVATE clinical trial is a phase 2 study assessing both minimal residual disease (MRD)-guided treatment discontinuation and fixed-duration therapy in untreated, fit patients with CLL/SLL [33-34]. In the fixed-duration cohort, IV achieved a 24-month PFS rate of 85% in patients with 17p deletions and *TP53* mutations [26-27]. Notably, PFS and OS rates were similar to those obtained in patients without high-risk features [28]. These data, in addition to those of the GLOW trial [29] (enrolling unfit patients with the exclusion of 17p deletion), led to the approval of the all-oral IV combination as upfront treatment by the European Commission [30].

Other studies have examined the combination of second-generation BTKi and venetoclax. Preliminary results obtained in arm D of the SEQUOIA trial show that combining zanubrutinib with venetoclax is a promising treatment option for patients with del(17p) [31]. In the CLL2-GIVe phase 2 trial, a time-limited, response-adapted combination of obinutuzumab, ibrutinib, and venetoclax was evaluated in previously untreated CLL patients with del(17p) and/or *TP53* mutation [32]. Encouraging results were obtained, and the overall response rate (ORR) at cycle 15 was an impressive 100%, with 58.5% achieving complete response (CR) or CR with incomplete blood count recovery (CRi). Importantly, 87.8% of patients had undetectable MRD at cycles 9 and 12, and after a median follow-up of 38.4 months, the 3-year PFS and OS were 79.9% and 92.6%, respectively [33].

Another triplet regimen using the second-generation BTK inhibitor (BTKi), acalabrutinib, in combination with venetoclax and obinutuzumab (AVO), has also been tested in TP53-aberrant CLL recently (34). In this study, treatment-naïve (TN) patients were enrolled in the all-comers group (n = 37), followed by a multicenter expansion cohort restricted to patients with *TP53*-aberrant disease (n = 31). Patients with *TP53*-aberrant CLL achieved a complete response (CR) rate of 52% and a partial response (PR) of 48%. Ultra-minimal residual disease (uMRD) in the bone marrow was 83% for patients with *TP53* aberrations and 89% in those without. In the whole cohort, enriched for high-risk CLL patients, at a median follow-up of 35 months, progression-free survival (PFS) was 92.6% and overall survival (OS) was 98.5% (34). The AVO regimen is currently under investigation in the phase 3 ACE-CL-311/AMPLIFY trial (NCT038362), comparing AVO vs AV vs chemo-immunotherapy in patients with high-risk CLL.

Finally, it appears that *TP53* abnormalities, with their tendency for genomic instability and clonal complexity, are likely to facilitate the development of resistance mechanisms, such as BTK or Phospholipase C gamma 2 (PLCγ2) mutations, in the case of ibrutinib (35). A large study of 388 patients with diverse clinical risk factors receiving ibrutinib, with up to six years of follow-up, showed that the presence of del(17p)/*TP53* increases the risk of BTK and PLCγ2 mutations in relapsed/refractory patients, as well as in the entire CLL cohort (36).

Since mutations in BTK, resulting in treatment resistance and disease progression, develop with both covalent (cBTKi) and non-covalent inhibitors (ncBTKi), it is clear that novel and more effective therapeutic agents targeting BCR signaling are needed, particularly in patients whose high-risk disease has relapsed or become refractory to available BTK-targeting therapies (37). In this respect, NX-2127, a novel small molecule that drives targeted BTK and IKAROS family zinc finger 3 (IKZF3) degradation through ubiquitination and proteasomal degradation, is of interest (38). This BTK degradation and immunomodulatory activity represents a new mechanism of action and may overcome resistance to currently available novel agents, including cBTKi and ncBTKi. Preliminary results of the NX-2127 phase 1 trial

(NCT04830137) presented at the 2022 American Society of Hematology (ASH) meeting indicate an overall response rate (ORR) of 50% after six months of treatment (38)

3.0 IMMUNOTHERAPY IN THE TREATMENT OF *TP53*-DISRUPTED CLL

Recent advancements in the understanding of interactions between a defective p53 pathway and tumor immunity have opened up new possibilities in the treatment of CLL [39]. One promising approach is allogeneic hematopoietic cell transplantation (allo-HCT), which has shown potential to overcome poor prognosis associated with del(17p) and resistance to fludarabine [40]. However, the utilization of transplants has declined in the past decade due to the introduction of novel agents [41].

A recent study involving 65 patients, mostly younger and carrying *TP53* mutations, found that allo-HSCT led to an estimated 24-month PFS of 63% and OS of 81%. Notably, a reduced-intensity pre-transplant conditioning regimen resulted in low non-relapse mortality at 24 months (13%), though 27% of patients relapsed over time. Interestingly, there were no significant differences in PFS and OS between patients who had received ibrutinib or venetoclax as a bridge to transplant [42]

Immunotherapies, such as Chimeric Antigen Receptor (CAR) T/NK cell therapy, have gained attention for their ability to provide prolonged remissions [43]. In five published studies involving 113 extensively treated CLL patients with *TP53* mutations (ranging from 27% to 96%), the Overall Response Rates (ORR) ranged from 44% to 83%, with Complete Response (CR) rates between 13% and 45% (Table 3) [44-48]. However, CLL patients often develop T-cell dysfunction over time, characterized by altered cytokine secretion, exhaustion markers, decreased cytotoxicity of CD8+ T cells, and a shift toward an effector memory phenotype [49]. To address this, combining small molecules with CD19 CART cell immunotherapy has shown promise, with a recent study reporting a 3-month CR rate of 44% and 72% of patients showing undetectable MRD at 12 months [51]. Notably, ibrutinib may play a role in redirecting the immune response, favoring the expansion and maintenance of CAR T-cell populations [52].

Allogeneic CAR T-cell and CAR natural killer (CAR-NK) therapy, as an extension of adaptive therapy with CAR T-cell products, offers high antitumor efficacy and the advantage of being readily available "off-the-shelf," without extended manufacturing time [53]. Though allogeneic CAR T-cells have primarily been used in a limited number of CLL patients who relapsed following allogeneic stem cell transplants, there have been cases of success, with one patient achieving CR [54].

Therapy with bispecific monoclonal antibodies is also potentially of interest for high-risk CLL patients with disrupted *TP53* [55]. This approach is bespoke for an individual patient and could, thus, have broader applicability than CAR-T therapy. No bispecific antibodies have been approved for CLL as yet, but since their therapeutic efficacy in B-cell lymphomas has become increasingly evident [56], more CLL trials are in development [57].

Blinatumomab, a Bi-specific T-cell engagers (BiTEs) monoclonal antibody targeting CD19, has been shown to be effective in a single case of refractory Richter syndrome (RS) as a bridge to HSCT [58], and two ongoing clinical trials are evaluating its use in combination with lenalidomide (NCT02568553) or blinatumomab-expanded T cells (NCT03823365) in patients with different types of Non-Hodgkin lymphoma (NHL) and high-risk CLL.

An ongoing phase 1b open-label study is evaluating the safety, efficacy, and pharmacokinetics of mosunetuzumab, an anti-CD20 bispecific monoclonal antibody, in patients with relapsed or refractory CLL (NCT05091424) [59], while another phase 1/1b study is testing it as monotherapy or in combination with atezolizumab (a monoclonal antibody that binds to the programmed cell death-ligand 1 [PD-L1]) in patients with R/R B-NHL or CLL (NCT02500407).

Epcoritamab (GEN3013; DuoBody®-CD3×CD20) is another bispecific antibody currently in development for CLL [70]. Preliminary results of a phase 1b/2 trial indicate that epcoritamab has clinical activity in patients with high-risk CLL who have already received two or more lines of systemic therapy, including treatment with, or intolerance to a BTKi (EPCORE CLL-1; NCT04623541). Seven patients who received epcoritamab subcutaneously at 2 dose levels (24 mg [n=3] and 48 mg [n=4]) have been reported, and antileukemic activity has been evident at both doses, with partial responses in 3 of 5 patients [60].

In addition, there are novel immunological strategies currently in development for other hematological malignancies, which could also be applicable to high-risk CLL [57]. One of these is magrolimab, an anti-CD47 antibody, that can harness macrophage-based immunity and enhance phagocytosis [61]. In a phase 1b trial that included 91 previously untreated patients with AML who were unfit for intensive chemotherapy, the efficacy and safety of magrolimab was assessed in combination with azacitidine. Of the 25 *TP53*-mutant patients enrolled in this study, 10 (40%) achieved CR with a median OS of 16.3 months [62]. An ongoing trial of patients with B-cell malignancies, including R/R CLL, is at present also evaluating the combination of venetoclax, obinutuzumab, and magrolimab (VENOM) (NCT04599634), and results are keenly awaited.

All in all, the above results are encouraging and indicate that immunotherapy has therapeutic potential for patients with CLL and *TP53* aberrations, especially those refractory to BTKis and/or venetoclax-based regimens [63]. Future studies should aim at clarifying how approaches like adoptive cellular therapy with CAR T-cells or treatment with bispecific antibodies could be combined with conventional targeted agents, which presently constitute the backbone of treatment for TP53 mutated CLL patients.

4.0 AGENTS TARGETING *TP53* IN CLL

In the era of developing agents targeting *TP53*, small molecules capable of functionally restoring mutant p53 proteins are of increasing interest [64]. One of these agents is the oncoprotein murine double minute 2 (MDM2), which is an E2 ubiquitin ligase controlling the half-life of p53 via ubiquitin-dependent proteasome degradation. The MDM2-p53 interaction is an intriguing therapeutic target, and when it is inhibited, p53 levels increase, thereby improving tumor control [65-66]. A phase 1 study of patients with hematologic malignancies tested RG7112, a small-molecule MDM2 antagonist, demonstrating that most patients with mutant p53 failed to show any response, whereas a single CLL patient with a 2-bp p53 deletion was able to maintain stable disease for more than 2 years [67]. Another pre-clinical investigation involving RG7388, a second-generation MDM2 inhibitor, found that it improved p53 function in CLL cells, and leukemic cells were then able to express the p53 pro-apoptotic gene signature [68].

Unlike MDM2 inhibitors, other drugs promise to restore p53 function in patients carrying pathogenic mutations. Eprenetapopt (APR-246) is a drug that reactivates the mutant and inactivated p53 protein by restoring its conformation and function, thereby re-inducing programmed cell death in cancer cells [69]. The combination of eprenetapopt (APR-246) and azacitidine had a promising safety profile and showed encouraging clinical activity in high-risk patients with *TP53*-mutated myelodysplastic syndrome (MDS) and AML [70]. A phase 1 trial (NCT04419389), based upon two previously approved therapies, is currently exploring the efficacy of APR-246 in CLL; the design of this trial was presented in abstract form at the 2020 ASH meeting [71]. Unfortunately, in the meantime, the sponsors have closed the study for unspecified reasons.

Another interesting target is Exportin-1 (XPO1), also known as chromosomal region maintenance 1 (CRM1) [72]. Although the anti-neoplastic mechanism of action of XPO1 inhibitors is not entirely understood, a recent study reported that the XPO1 inhibitor selinexor may function via selective inhibition of p53 export out of the nucleus, thereby increasing p53 nuclear retention [73-74]. A combination of ibrutinib and selinexor was tested in a phase 1 study (NCT02303392) in 16 patients with CLL/NHL, 4 with del(17p), who had relapsed or were refractory to ≥1 prior therapy. Here, the ORR was 32%, while an additional 47% of patients achieved stable disease (SD), some for a prolonged period of time (up to 36 months) [75]. Unfortunately, in this study, results were presented in an aggregate manner, thus preventing the appreciation of the impact of the selinexor-ibrutinib combination on patients with del(17p).

The theory of synthetic lethality is an additional strategy for overcoming *TP53* dysfunction [76]. In p53- or ataxia-telangiectasia mutated (ATM) deficient CLL cells, the inhibition of ATM and Rad3-related (ATR) signaling by AZD6738 (Ceralasertib) has been shown to lead to an accumulation of unrepaired damaged DNA and, according to preliminary findings, causes death by mitotic catastrophe [77]. In preclinical animal

models, treatment with AZD6738 decreased the tumor burden, as well as the proportion of CLL cells with TP53 or ATM mutations [77]. It is of interest that a phase 1/2 trial (NCT03328273) revealed that the combination of AZD6738 (ceralasertib) and acalabrutinib had clinical activity in high-risk, R/R CLL. Of note, all three patients who received the combination of ceralasertib and acalabrutinib (arm B) achieved a partial response. In contrast, none of the eight patients receiving ceralasertib monotherapy (arm A) responded [78].

In summary, targeting *TP53* disruption in CLL and other malignancies requires a broad understanding of the various mechanisms involved [64]. At present, multiple therapeutic approaches, including the use of MDM2 inhibitors, small molecule P53 reactivators (eprenetapopt), XPO1, and ATR inhibitors, are all being explored to address the issue of TP53 dysfunction and improve outcomes in high-risk CLL [79]

5.0 - CONCLUSIONS

Although the introduction of BTK and BCL2 inhibitors represents a turning point in the treatment of CLL patients with *TP53* mutations, many challenges still remain, as therapies targeting *TP53* dysfunction are not as yet available for use in the clinic [5-11]. In addition, a primary obstacle in this regard is that a single clinically effective agent with an acceptable level of safety is still not yet available for routine use [13]. The future of p53 targeting in CLL will probably rest with the development of effective combinations of p53/ DNA damage response (DDR)-targeting therapies and B-cell receptor (BCR) signaling or Bcl-2 inhibitors [79].

Preclinical studies suggesting that concomitant p53 activation and Bcl-2 inhibition can overcome apoptosis resistance in AML are also encouraging and provide support for further planning of future clinical trials testing this combination in other leukemias [80]. These findings could indeed impact future research and serve to encourage the development of additional novel agents capable of selectively targeting p53-mutated cells aiming at eradicating genetically unstable CLL subclones responsible for disease persistence and inevitable relapse (Fig 1) [81].

ACKNOWLEDGMENT

This paper was not funded.

CONFLICTS OF INTEREST

Authors do not declare any conflicts of interest.

REFERENCES

1. Eichhorst B, Robak T, Montserrat E, et al Chronic lymphocytic leukaemia: ESMO Clinical Practice Guidelines for diagnosis, treatment and follow-up Ann Oncol. 2021;32(1):23-33.
2. Campo E, Cymbalista F, Ghia P, et al. TP53 aberrations in chronic lymphocytic leukemia: an overview of the clinical implications of improved diagnostics. Haematologica. 2018;103(12):1956-1968.
3. Byrd JC, Brown JR, O'Brien S, et al. RESONATE Investigators. Ibrutinib versus ofatumumab in previously treated chronic lymphoid leukemia. N Engl J Med. 2014;371(3):213-223
4. Burger JA, Tedeschi A, Barr PM, et al. RESONATE-2 Investigators. Ibrutinib as initial therapy for patients with chronic lymphocytic leukemia. N Engl J Med. 2015;373(25): 2425-2437.
5. Woyach JA, Ruppert AS, Heerema NA, et al Ibrutinib Regimens versus Chemoimmunotherapy in Older Patients with Untreated CLL. N Engl J Med. 201827;379(26):2517-2528.
6. Moreno C, Greil R, Demirkan F, et al. Ibrutinib plus obinutuzumab in first-line treatment of chronic lymphocytic leukaemia (iLLUMINATE): a multicenter, randomized, open-label, phase 3 trial. Lancet Oncol. 2019 ;20(1):43-56.
7. Byrd JC, Hillmen P, Ghia P, Kater AP, et al Acalabrutinib Versus Ibrutinib in Previously Treated Chronic Lymphocytic Leukemia: Results of the First Randomized Phase III Trial. J Clin Oncol. 2021 ;39(31):3441-3452.
8. Brown JR, Eichhorst B, Hillmen P, et al. Zanubrutinib or Ibrutinib in Relapsed or Refractory Chronic Lymphocytic Leukemia. N Engl J Med 2023 ;388(4):319-332.
9. Sharman JP, Egyed M, JurczakW, et al. Efficacy and safety in a 4-year follow-up of the ELEVATE-TN study comparing acalabrutinib with or without obinutuzumab versus obinutuzumab plus chlorambucil in treatment-naïve chronic lymphocytic leukemia. Leukemia. 2022;36(4):1171-1175.

10. Ghia, P, Barnes G, Yang K, et al Patient-reported outcomes from phase 3 randomized study of zanubrutinib versus bendamustine and rituximab (BR) in patients with treatment-naïve (TN) CLL/SLL. HemaSphere 6():p 560-561, June 2022.)
11. Brown, J.R.; Eichhorst, B.; Hillmen, P.; Jurczak, W.; Kaźmierczak, M.; Lamanna, N.; O'Brien, S.M.; Tam, C.S.; Qiu, L.; Zhou, K.; et al. Zanubrutinib or Ibrutinib in Relapsed or Refractory Chronic Lymphocytic Leukemia. N. Engl. J. Med. 2022, 388, 319–332.
12. Knisbacher BA, Lin Z, Hahn CK, et al Molecular map of chronic lymphocytic leukemia and its impact on outcome. Nat Genet 2022 ;54(11):1664-1674.

.

13. Kwok M Agathanggelou A, Davies N, Stankovic T. Targeting the p53 Pathway in CLL: State of the Art and Future Perspectives. Cancers 2021 ;13(18):4681. doi: 10.3390/cancers13184681.
14. Ahn IE, Tian X, Wiestner A. Ibrutinib for Chronic Lymphocytic Leukemia with TP53 Alterations. N Engl J Med 2020 ;383(5):498-500.
15. Allan JN, Shanafelt T, Wiestner A, et al. Long-term efficacy of first-line ibrutinib treatment for chronic lymphocytic leukaemia in patients with TP53 aberrations: a pooled analysis from four clinical trials. Br J Haematol. 2022 ;196(4):947-953.
16. Cherng HJ, Khwaja R, Kanagal-Shamanna R, et al. TP53-altered chronic lymphocytic leukemia treated with firstline Bruton's tyrosine kinase inhibitor-based therapy: A retrospective analysis. Am J Hematol 2022 ;97(8):1005-1012.
17. Burger JA, Sivina M, Jain N, et al. Randomized trial of ibrutinib vs ibrutinib plus rituximab in patients with chronic lymphocytic leukemia. Blood 2019 ;133(10):1011-1019.
18. Tam, C.S.; Ou, Y.C.; Trotman, J.; Opat, S. Clinical pharmacology and PK/PD translation of the second-generation Bruton's tyrosine kinase inhibitor, zanubrutinib. Expert Rev. Clin. Pharmacol. 2021, 14, 1329–1344.
19. Tam, C.S.; Robak, T.; Ghia, P.; Kahl, et al. Zanubrutinib monotherapy for patients with treatment naïve chronic lymphocytic leukemia and 17p deletion. Haematologica 2020, 106, 2354–2363.
20. Tam, C.S.; Brown, J.R.; Kahl, B.S.; et al. Zanubrutinib versus bendamustine and rituximab in untreated chronic lymphocytic leukaemia and small lymphocytic lym-phoma (SEQUOIA): A randomised, controlled, phase 3 trial. Lancet Oncol. 2022, 23, 1031–1043.
21. Mato AR, Woyach JA, Brown JR,et al Pirtobrutinib after a Covalent BTK Inhibitor in Chronic Lymphocytic Leukemia. N Engl J Med. 2023 ;389(1):33-44.
22. Anderson MA, Deng J, Seymour JF, et al The BCL2 selective inhibitor venetoclax induces rapid onset apoptosis of CLL cells in patients via a TP53-independent mechanism. Blood 2016 ;127(25):3215-24.

23. Al-Sawaf O, Zhang C, Jin HY, Robrecht S,et al. Transcriptomic profiles and 5-year results from the randomized CLL14 study of venetoclax plus obinutuzumab versus chlorambucil plus obinutuzumab in chronic lymphocytic leukemia. Nat Commun. 2023 ;14(1):2147. doi: 10.1038/s41467-023-37648-w.
24. Bennett R, Anderson MA, Seymour JF. Unresolved questions in selection of therapies for treatment-naïve chronic lymphocytic leukemia. J Hematol Oncol 2023 ;16(1):72. doi: 10.1186/s13045-023-01469-7.
25. Lu P, Wang S, Franzen CA, et al. Ibrutinib and venetoclax target distinct subpopulations of CLL cells: implication for residual disease eradication. Blood Cancer J 2021 ;11(2):39. doi: 10.1038/s41408-021-00429-z.
26. Wierda WG, Allan JN, Siddiqi T, et al. Ibrutinib Plus Venetoclax for First-Line Treatment of Chronic Lymphocytic Leukemia: Primary Analysis Results From the Minimal Residual Disease Cohort of the Randomized Phase II CAPTIVATE Study. J Clin Oncol 2021 ;39(34):3853-3865
27. Tam CS, Allan JN, Siddiqi T, Kipps TJ, et al Fixed-duration ibrutinib plus venetoclax for first-line treatment of CLL: primary analysis of the CAPTIVATE FD cohort. Blood. 2022 ;139(22):3278-3289.
28. Allan JN, Flinn IW, Siddiqi T, et al. Outcomes in Patients with High-Risk Features after Fixed-Duration Ibrutinib plus Venetoclax: Phase II CAPTIVATE Study in First-Line Chronic Lymphocytic Leukemia. Clin Cancer Res 2023 ;29(14):2593-2601.
29. Munir T, Moreno C, Owen C, et al. Impact of Minimal Residual Disease on Progression-Free Survival outcomes after fixed-duration Ibrutinib-Venetoclax Versus Chlorambucil-Obinutuzumab in the GLOW study. J Clin Oncol 2023, 41 (21);3689-3699.
30. News release. Johnson & Johnson. August 4, 2022. Accessed August 25, 2022. https://bit.ly/3AMP4yh
31. Tedeschi, A.; Ferrant, E.; Flinn, I.W.;et al. Zanubrutinib in Combination with Venetoclax for Patients with Treatment-Naïve (TN) Chronic Lymphocytic Leukemia (CLL) or Small Lymphocytic Lymphoma (SLL) with del(17p): Early Results from Arm D of the SEQUOIA (BGB-3111-304) Trial. In Proceedings of the American Society of Hematology Annual Meeting, Atlanta, GA, USA, 11–14 December 2021.
32. Huber H, Tausch E, Schneider C, et al. Final analysis of the CLL2-GIVe trial (obinutuzumab, ibrutinib, and venetoclax) in untreated CLL with del(17p)/TP53mut. Blood. 2023, Huber H, Tausch E, Schneider C, et al. Final analysis of the CLL2-GIVe trial (obinutuzumab, ibrutinib, and venetoclax) in untreated CLL with del(17p)/TP53mut. Blood. 2023, 142 (11);961-972..
33. Ryan CE, Lampson BL, Tyekucheva S, et al. Updated Results from a Multicenter, Phase 2 Study of Acalabrutinib, Venetoclax, Obinutuzumab (AVO) in a Population of Previously

Untreated Patients with CLL Enriched for High-Risk Disease. Blood (2022) 140 (Supplement 1): 837–838.

34. Woyach, J.A.; Ruppert, A.S.; Guinn, D.; et al. BTKC481S-Mediated Resistance to Ibrutinib in Chronic Lymphocytic Leukemia. J. Clin. Oncol. 2017,35 (13);1437-1443.
35. Wiestner A, Ghia P, Byrd JC, et al Rarity of B-Cell Receptor Pathway Mutations in Progression-Free Patients With Chronic Lymphocytic Leukemia (CLL) During First-Line Versus Relapsed/Refractory (R/R) Treatment With Ibrutinib. Blood (2020) 136 (Supplement 1): Abstr 2219.
36. Lew TE, Lin VS, Cliff ER, et al. Outcomes of patients with CLL sequentially resistant to both BCL2 and BTK inhibition. Blood Adv. 2021;5(20):4054-4058.
37. Mato AR, Wierda WG, Ai WZ, et al NX-2127-001, a First-in-Human Trial of NX-2127, a Bruton's Tyrosine Kinase-Targeted Protein Degrader, in Patients with Relapsed or Refractory Chronic Lymphocytic Leukemia and B-Cell Malignancies. Blood (2022) 140 (Supplement 1): 2329–2332.
38. Kwok M, Agathanggelou A, Davies N, Stankovic T. Targeting the p53 Pathway in CLL: State of the Art and Future Perspectives. Cancers (Basel). 2021;13(18):4681.
39. Dreger P, Schetelig J, Andersen N, et al. Managing high-risk CLL during transition to a new treatment era: stem cell transplantation or novel agents? Blood. 2014;124(26):3841-3849.
40. Dreger P, Ghia P, Schetelig J, et al. High-risk chronic lymphocytic leukemia in the era of pathway inhibitors: integrating molecular and cellular therapies. Blood. 2018;132(9):892-902.
41. Roeker LE, Dreger P, Brown JR, et al. Allogeneic stem cell transplantation for chronic lymphocytic leukemia in the era of novel agents. Blood Adv. 2020;4(16):3977-3989.
42. Porter DL, Levine BL, Kalos M, Bagg A, June CH. Chimeric antigen receptor-modified T cells in chronic lymphoid leukemia. N Engl J Med. 2011;365(8):725-733.
43. Porter DL, Hwang WT, Frey NV, et al. Chimeric antigen receptor T cells persist and induce sustained remissions in relapsed refractory chronic lymphocytic leukemia. Sci Transl Med. 2015;7(303):303ra139.
44. Frey NV, Gill S, Hexner EO, et al. Long-term outcomes from a randomized dose optimization study of chimeric antigen receptor modified T cells in relapsed chronic lymphocytic leukemia. J Clin Oncol. 2020;38(25):2862-2871.
45. Turtle CJ, Hay KA, Hanafi LA, et al. Durable molecular remissions in chronic lymphocytic leukemia treated with CD19-specific chimeric antigen receptor-modified T cells after failure of ibrutinib. J Clin Oncol. 2017;35(26):3010-3020.
46. Siddiqi T, Soumerai JD, Dorritie KA, et al. Phase 1 TRANSCEND CLL 004 study of lisocabtagene maraleucel in patients with relapsed/refractory CLL or SLL. Blood. 2022;139(12):1794-1806
47. Fraietta JA, Beckwith KA, Patel PR, et al. Ibrutinib enhances chimeric antigen

receptor T-cell engraftment and efficacy in leukemia. Blood. 2016;127(9):1117-1127.

48. Forconi F, Moss P. Perturbation of the normal immune system in patients with CLL. Blood. 2015;126(5):573-581.
49. Iovino L, Shadman M. CAR T-cell therapy for CLL: a new addition to our treatment toolbox? Clin Adv Hematol Oncol. 2023;21(3):134-141.
50. Gill S, Vides V, Frey NV, Hexner EO, et al. Anti-CD19 CAR T cells in combination with ibrutinib for the treatment of chronic lymphocytic leukemia. Blood Adv. 2022;6(21):5774-5785.
51. Liu Y, Song Y, Yin Q. Effects of ibrutinib on T-cell immunity in patients with chronic lymphocytic leukemia. Front Immunol 2022;13:962552. doi: 10.3389/fimmu.2022.962552. eCollection 2022.
52. Strati P. CAR T-cell therapy: which product for which patient? Blood. 2022;139(26):3673-3674.
53. Brudno J.N., Somerville R.P., Shi V., et al. Allogeneic T cells that express an anti-CD19 chimeric antigen receptor induce remissions of B-cell malignancies that progress after allogeneic hematopoietic stem-cell transplantation without causing graft-versus-host disease. J. Clin. Oncol. 2016;34:1112–1121.
54. Iyer P, Wang L. Emerging Therapies in CLL in the Era of Precision Medicine. Cancers (Basel). 2023;15(5):1583. doi: 10.3390/cancers15051583.
55. Hutchings M. The evolving therapy of DLBCL: Bispecific antibodies. Hematol Oncol. 2023 Jun;41(S1):107-111. doi: 10.1002/hon.3154.
56. Perutelli F, Jones R, Griggio V, Vitale C, et al Immunotherapeutic Strategies in Chronic Lymphocytic Leukemia: Advances and Challenges. Front Oncol. 2022 Feb 21;12:837531. doi: 10.3389/fonc.2022.837531. eCollection 2022.
57. Alderuccio JP, Mackrides N, Chapman JR, Vega F, Lossos IS. Rapid Complete Response to Blinatumomab as a Successful Bridge to Allogeneic Stem Cell Transplantation in a Case of Refractory Richter Syndrome. Leuk Lymphoma 2019, 60(1):230–233.
58. Cao Y, Marcucci EC, Budde LE. Mosunetuzumab and lymphoma: latest updates from 2022 ASH annual meeting. J Hematol Oncol. 2023 Jun 28;16(1):69. doi: 10.1186/s13045-023-01462-0.
59. Kater AP, Christensen JH, Bentzen HH, et al Subcutaneous Epcoritamab in Patients with Relapsed/Refractory Chronic Lymphocytic Leukemia: Preliminary Results from the Epcore CLL-1 Trial. Blood (2021) 138 (Supplement 1): Abstr 2627.
60. Melo Garcia L, Barabé F. Harnessing Macrophages through the Blockage of CD47: Implications for Acute Myeloid Leukemia. Cancers (Basel) 2021 Dec 13;13(24):6258. doi: 10.3390/cancers13246258.

61. Sallman DA, Al Malki MM, Asch AS, et al Magrolimab in Combination With Azacitidine in Patients With Higher-Risk Myelodysplastic Syndromes: Final Results of a Phase Ib Study. J Clin Oncol 2023;41(15):2815-2826.
62. Scarfò L. Novel therapies and combinations in CLL refractory to BTK inhibitors and venetoclax. Hematology Am Soc Hematol Educ Program 2022 Dec 9;2022(1):316-322.
63. Wang H, Guo M, Wei H, Chen Y. Targeting p53 pathways: mechanisms, structures, and advances in therapy. Signal Transduct Target Ther. 2023;8(1):92. doi: 10.1038/s41392-023-01347-1.
64. Hassin O, Oren M. Drugging p53 in cancer: one protein, many targets. Nat Rev Drug Discov. 2023;22(2):127-144.
65. Zhu H, Gao H, Ji Y, Zhou Q, et al Targeting p53-MDM2 interaction by small-molecule inhibitors: learning from MDM2 inhibitors in clinical trials. J Hematol Oncol 2022 ;15(1):91. doi: 10.1186/s13045-022-01314-3.
66. Andreeff M, Kelly KR, Yee K, et al Results of the Phase I Trial of RG7112, a Small-Molecule MDM2 Antagonist in Leukemia. Clin Cancer Res 2016;22(4):868-76.
67. Ciardullo C, Aptullahoglu E, Woodhouse L, et al, Non-genotoxic MDM2 inhibition selectively induces a pro-apoptotic p53 gene signature in chronic lymphocytic leukemia cells. Haematologica 2019;104(12):2429-2442.
68. Wang Z, Hu H, Heitink L, et al The anti-cancer agent APR-246 can activate several programmed cell death processes to kill malignant cells. Cell Death Differ 2023;30(4):1033-1046.
69. Cluzeau T, Sebert M, Rahmé R, et al Eprenetapopt Plus Azacitidine in TP53-Mutated Myelodysplastic Syndromes and Acute Myeloid Leukemia: A Phase II Study by the Groupe Francophone des Myélodysplasies (GFM). J Clin Oncol 202;39(14):1575-1583.
70. Thompson MC, MD1, Davids MS, Jain N, et al. Phase 1 and Dose Expansion Study of APR-246 in Combination with Ibrutinib or Venetoclax-Based Therapy in Subjects with TP53-Mutant Relapsed and/or Refractory Non-Hodgkin Lymphomas (NHL) Including Chronic Lymphocytic Leukemia (CLL) and Mantle Cell Lymphoma (MCL). 62TH ASH ANNUAL MEETING AND EXPOSITION December 5-8, 2020, Abstr 1311.
71. Azizian NG, Li Y. XPO1-dependent nuclear export as a target for cancer therapy. J Hematol Oncol. 2020;13(1):61. doi: 10.1186/s13045-020-00903-4.
72. Subhash V.V., Yeo M.S., Wang L., Tan S.H., Wong F.Y., Thuya W.L., Tan W.L., Peethala P.C., Soe M.Y., Tan D., et al. Anti-tumour efficacy of Selinexor (KPT-330) in gastric cancer is dependent on nuclear accumulation of p53 tumour suppressor. Sci. Rep. 2018;8:12248. doi: 10.1038/s41598-018-30686-1.

73. Allegra A., Innao V., Allegra A.G., Leanza R., Musolino C. Selective Inhibitors of Nuclear Export in the Treatment of Hematologic Malignancies. Clin. Lymphoma Myeloma Leuk. 2019;19:689–698.
74. Stephens DM, Huang Y, Ruppert AS, et al Selinexor Combined with Ibrutinib Demonstrates Tolerability and Safety in Advanced B-Cell Malignancies: A Phase I Study. Clin Cancer Res 2022;28(15):3242-3247.
75. Shaheen M, Allen C, Nickoloff JA, Hromas R. Synthetic lethality: exploiting the addiction of cancer to DNA repair. Blood. 201;117(23):6074-6082.
76. Kwok M, Davies N, Agathanggelou A, et al. ATR inhibition induces synthetic lethality and overcomes chemoresistance in TP53- or ATM-defective chronic lymphocytic leukemia cells. Blood 2016;127(5):582-595.
77. Jurczak W, Elmusharaf N, Fox CP, et al. Phase 1/2 results of ceralasertib as monotherapy or in combination with acalabrutinib in high-risk relapsed/refractory chronic lymphocytic leukemia. Presented at: 2022 AACR Annual Meeting; April 8-13, 2022; New Orleans, LA. Abstract CT532.
78. Moia R, Boggione P, Mahmoud AM, et al Targeting p53 in chronic lymphocytic leukemia. Expert Opin Ther Targets. 2020;24(12):1239-1250.
79. Kojima K, Konopleva M, Samudio IJ, et al Concomitant inhibition of MDM2 and Bcl-2 protein function synergistically induce mitochondrial apoptosis in AML. Cell Cycle 2006 ;5(23):2778-2786.
80. Zapatka M, Tausch E, Öztürk S, et al. Clonal evolution in chronic lymphocytic leukemia is scant in relapsed but accelerated in refractory cases after chemo(immune) therapy. Haematologica 2022 Mar 1;107(3):604-614.

Legend Figure 1

Available strategies and approaches under clinical investigation targeting metabolic and immunological pathways for patients with TP53 mutant CLL.