original report

# Next-Generation Sequencing Reveals Potentially Actionable Alterations in the Majority of Patients With Lymphoid Malignancies

abstract

**Purpose** **Next-generation sequencing (NGS) identifies potentially targetable alterations by US Food and Drug Administration (FDA)–approved drugs and/or by available experimental agents that may not have otherwise been contemplated. Many targeted drugs have been developed for diverse solid cancers; a smaller number of genomically targeted drugs have been approved for lymphoid malignancies.**

**Materials and Methods** **We analyzed NGS results from 60 patients with various lymphoid malignancies and found 224 alterations (median per patient, three alterations).**

**Results** **Forty-nine patients (82%) had potentially actionable alterations with the use of FDA-approved drugs and/or experimental therapies; only 11 patients (18%) had no theoretically actionable alterations. Only three patients (5%) had an alteration for which an approved drug in the disease is available (on label); 45 patients (75%) had an alteration for which an approved drug is available for another disease (off label). The median number of alterations per patient potentially actionable by an FDA-approved drug was one. Of note, 19 (32%) of 60 patients had intermediate to high tumor mutational burden, which may predict response to certain immunotherapy agents.**

**Conclusion** **NGS identifies alterations that may be pharmacologically tractable in most patients with lymphoid malignancies, albeit with drugs that have usually been developed in the context of solid tumors. These observations merit expanded exploration in the clinical trials setting.**

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## INTRODUCTION

The lymphoid malignancies have diverse biologic and clinical behavior and typically are treated with multiagent chemotherapy. Many therapeutic regimens for B-cell non-Hodgkin lymphomas and leukemias also incorporate the anti-CD20 monoclonal antibody rituximab, which has improved patient outcomes.[1] Treatment of metastatic solid tumors, like lymphoid malignancies, has also relied heavily on the use of cytotoxic chemotherapy. However, over the past decade, the treatment paradigm for metastatic solid tumors has shifted away from chemotherapy toward matching oncogenic driver mutations with targeted therapy (precision medicine).[2-4] For example, in patients with *BRAF*-mutated metastatic melanoma, the BRAF inhibitor vemurafenib markedly increases overall survival compared with chemotherapy.[5]

Numerous targeted therapies are now approved by the US Food and Drug Administration (FDA) for patients with metastatic solid tumors across a wide array of histologies (Data Supplement). Occasionally, targetable alterations are found that change the treatment paradigm for a disease, which is notable for solid tumors such as *EGFR*-mutated non–small-cell lung cancer. The first example of targeted therapeutic efficacy is the hematologic disorder chronic myelogenous leukemia, which has been transformed by the use of agents that affect Bcr-Abl kinase activity.

Despite the rapid success in development, approval, and use of small-molecule–targeted agents in patients with solid malignancies, a paucity of such therapies approved for use in lymphoid malignancies remains (Data Supplement). For many patients with lymphoid malignancies, oral

small-molecule–targeted therapeutics are not an available option.

The rapid technological advances in next-generation sequencing (NGS) have allowed oncologists to sequence tumor exomes in a clinically meaningful period of time.[6-8] Some studies have shown that patients with solid malignancies treated with matched therapy have improved outcomes,[9] and meta-analyses in approximately 85,000 patients have supported this finding.[10-12] The targeting of alterations such as *BRAF* can result in responses across a wide variety of cancers, including lymphoid malignancies (eg, hairy cell leukemia).[13,14] Although not all malignancies that harbor *BRAF* mutations will respond equally well to BRAF inhibition, the strategy of cross-cancer basket trials has been established as highly worthwhile.

NGS accurately identifies substitutions, indels, copy number alterations, and gene fusions in hematologic malignancies.[15] In this report, we use this technology to analyze the genomic alterations in a cohort of 60 patients with various lymphoid malignancies to estimate the frequency of theoretically actionable alterations. These results may help to inform further development of clinical trials in this field.

## MATERIALS AND METHODS

### Patients

We retrospectively reviewed the medical charts of 220 patients with hematologic malignancies who had undergone NGS. Only patients with lymphoid malignancies were selected for additional review. Patients were seen at the University of California San Diego Moores Cancer Center from October 2012 until March 2016. This study was performed and consents obtained in accordance with University of California San Diego institutional review board guidelines.

### NGS

Tumor samples from tissue (Table 1) or peripheral blood were collected from 60 patients and submitted for NGS to Foundation Medicine, a clinical laboratory improvement amendments–certified laboratory for NGS. The FoundationOne Heme panel was used, which is a hybrid capture–based NGS test.[16] The methods used in this assay have been described in detail in previous reports.[7,15] The FoundationOne Heme assay simultaneously detects all genomic alterations, including base pair substitutions, indels, copy number alterations, and select gene rearrangements, in 405 cancer-related genes. For tumor mutational burden (TMB), the number of somatic mutations detected on NGS are quantified, and that value was extrapolated to the whole exome by using a validated algorithm described in detail in earlier publications.[17,18] Alterations with known and likely effects on functional status are not counted.

### Definition of Actionable Alteration

An alteration was defined as potentially actionable if its protein product is a component of a molecularly defined pathway for which there is at least one available FDA-approved drug or investigational drug that may affect the function of the protein product of the alteration or the immediate downstream effectors of the protein product or that differentially recognizes the protein in tumor versus normal cells. The protein products of genomic alterations were considered to be functional if the genomic alterations have been previously identified as relevant to cancer in the COSMIC database,[19] which catalogs recurrent somatic alterations in cancer. Novel base substitution, indel, and rearrangement alterations that result in truncations and homozygous copy number deletions that occur in tumor suppressor genes were considered to have likely functional implications. Novel genomic alterations that occur at the same position as known alterations as well as alterations with conflicting evidence with regard to implication for function were subject to review by an internal panel of subject matter experts to determine functional status of the relevant protein product on the basis of all available evidence, including, but not limited to, the ExAC, dbSNP, and ClinVar databases.[20-22]

### Data and Statistical Analyses

Patient characteristics were obtained through electronic medical record review. Descriptive statistics were used, including medians, ranges, and frequencies.

## RESULTS

### Patient Characteristics

Sixty patients (35 men [58%] and 25 women [42%]) with lymphoid malignancies were identified (Table 1). Forty-six patients (77%) were white. The most common malignancy in the cohort was chronic lymphocytic leukemia (CLL; 32%), followed by acute lymphoblastic leukemia (ALL; 20%), multiple myeloma (18%), diffuse large B-cell lymphoma (DLBCL; 10%), follicular lymphoma (8%), and other lymphoid neoplasms (5%). The most common site for obtaining tissue

**Table 1.** Patient Characteristics

| Characteristic | No. (%) |
|---|---|
| No. of patients | 60 |
| Sex | |
| Male | 35 (58) |
| Female | 25 (42) |
| Median age at diagnosis, years (range) | 57.84 (22.21-83.55) |
| Race | |
| White | 46 (77) |
| Asian | 3 (5) |
| African American | 1 (2) |
| Hispanic | 2 (3) |
| Other | 8 (13) |
| Malignancy | |
| CLL | 19 (32) |
| B-ALL | 10 (17) |
| T-ALL | 2 (3) |
| DLBCL | 6 (10) |
| FL | 5 (8) |
| MM | 11 (18) |
| Marginal zone lymphoma | 2 (3) |
| NK/T NHL | 1 (2) |
| LPL | 1 (2) |
| CTCL | 1 (2) |
| Primary CNS lymphoma | 1 (2) |
| Testicular lymphoma | 1 (2) |
| Biopsy site | |
| Blood | 16 (27) |
| Bone marrow | 22 (37) |
| Lymph node | 10 (17) |
| Soft tissue | 3 (5) |
| Brain | 3 (5) |
| Skin | 2 (3) |
| Other | 3 (5) |
| Unknown | 1 (2) |
| Timing of NGS | |
| At diagnosis | 14 (23) |
| At relapse | 46 (77) |
| Median alterations per patient (range) | 3 (0-14) |
| Median potentially FDA-actionable alterations per patient (range) | 1 (0-9) |

Abbreviations: B-ALL, B-cell acute lymphoblastic leukemia; CLL, chronic lymphocytic leukemia; CTCL, cutaneous T-cell lymphoma; DLBCL, diffuse large B-cell lymphoma; FDA, US Food and Drug Administration; FL, follicular lymphoma; LPL, lymphoplasmacytic lymphoma; MM, multiple myeloma; NGS, next-generation sequencing; NHL, non-Hodgkin lymphoma; NK/T, natural killer/T-cell lymphoma; T-ALL, T-cell acute lymphoblastic leukemia.

used for NGS was bone marrow (37%) followed by peripheral blood (27%) and lymph nodes (17%).

## NGS Results

Two hundred twenty-four alterations were identified by NGS in the entire cohort of 60 patients. Types of alterations identified were substitutions, indels, copy number alterations, and gene fusions. Fig 1A shows the 15 most frequent alterations among the cohort, with *TP53* mutations (10 patients), *IGH* translocations (nine patients), loss of *CDKN2A/B* (eight patients), and *BCL2* mutations (eight patients) within the top five. All alterations identified in the cohort are listed in the Data Supplement.

All patients but two had a unique portfolio of alterations. One patient with CLL and one with multiple myeloma each had a solo *NRAS* mutation. However, the actual alteration in *NRAS* differed between the two patients (*NRAS* G13D *v* *NRAS* Q61R).

The median number of alterations detected per patient was three (range, zero to 14). As demonstrated in Figure 1B, seven patients (12%) had no reportable alterations, 10 (17%) had one alteration, and 43 (71%) had two or more alterations. The maximum number of alterations identified was 14, which was observed in two patients (3%), one with CLL, the other with DLBCL. Of note, all patients with DLBCL had five or more alterations.

## Actionable Alterations

Potentially actionable alterations were identified in all disease subtypes (Table 2). All disease subtypes, except marginal zone lymphoma, had alterations targetable by FDA-approved drugs. Forty-nine patients (82%) had potentially actionable alterations with FDA-approved drugs and/or experimental therapies (clinical trials), whereas 11 patients (18%) had no theoretically actionable alterations.

Depicted in Figure 2 is the number of patients with potentially FDA actionable alterations per disease group. For example, 11 (92%) of 12 patients with ALL and 11 (58%) of 19 patients with CLL had at least one theoretically targetable alteration. All patients with DLBCL had targetable alterations. The median number of potential FDA actionable alterations detected per patent was one. Twenty (33%), 15 (25%), and 10 (17%) patients had one, two, and three or more hypothetically targetable alterations, respectively.

Figure 3 demonstrates that of the 49 patients (82%) with potentially targetable mutations,

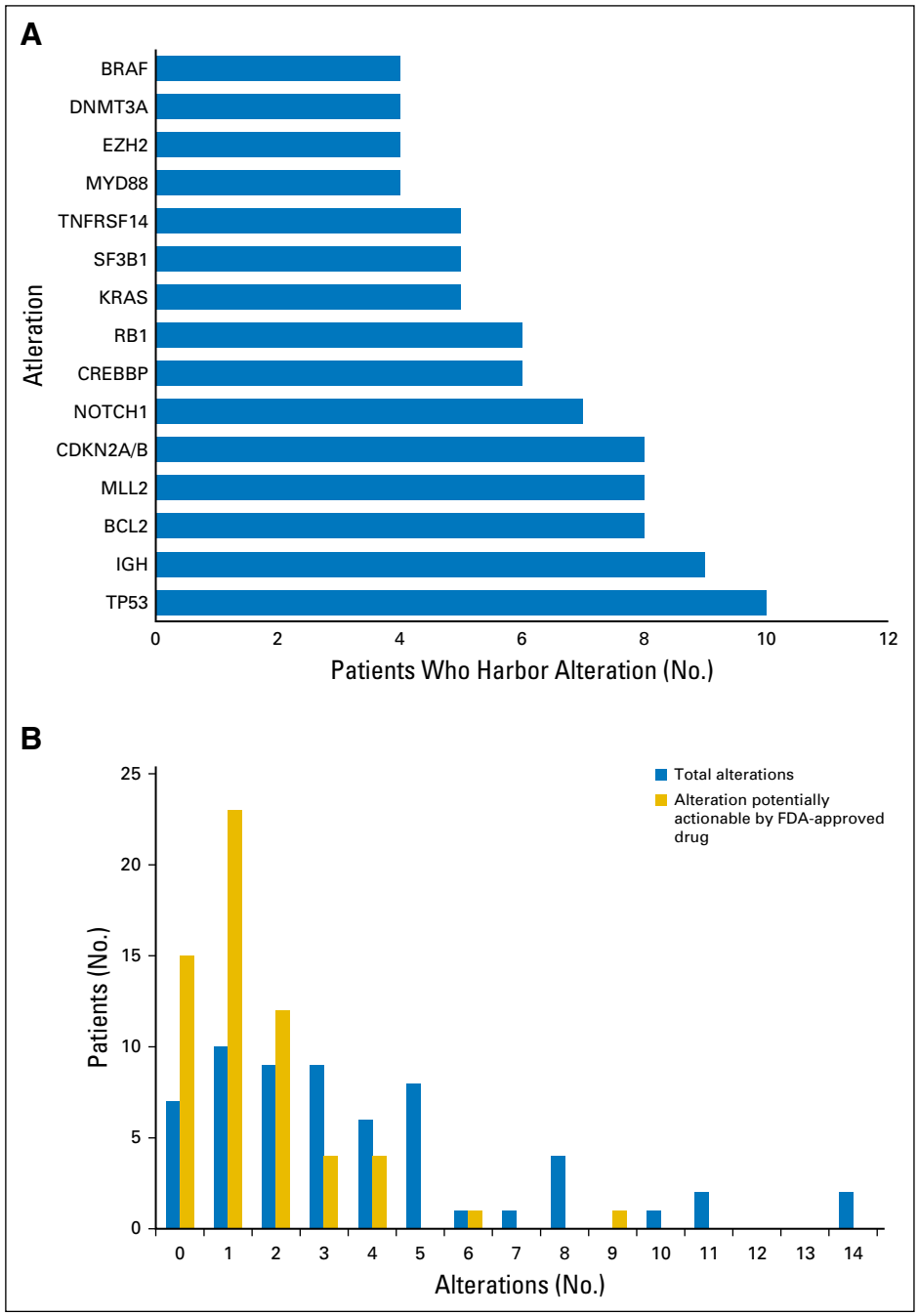


**Fig 1.** (A) Frequency and type of various molecular alterations found among 60 individuals with lymphoid malignancies. Only the 15 most common alterations are shown. The full list of alterations is shown in the Data Supplement. (IGH refers to IGH translocations that involve a partner gene.) (B) The number of patients with the designated number of total alterations and the number of patients with the designated number of potentially actionable alterations by an US Food and Drug Administration (FDA) –approved drug (on or off label).

45 had alterations that were targetable by FDA-approved drugs. Only three patients had an alteration for which an approved drug in the disease is available (on label), whereas 45 patients (75%) had an alteration for which an approved drug is available in another disease (off label). Forty-six patients (77%) had at least one alteration theoretically targetable by an experimental therapy (clinical trial). Eleven patients had no targetable alterations; these patients included seven with no detected genomic alterations.

Two patients with B-cell ALL (B-ALL) and one with Waldenström macroglobulinemia had alterations targetable with on-label–approved drugs (Fig 3). One patient with B-ALL was Philadelphia chromosome positive and already receiving dasatinib before testing, whereas the patient with Waldenström macroglobulinemia was switched to ibrutinib upon finding the *MYD88* L265P mutation.

## TMB

TMB ranges in a large cohort were defined as one or fewer to five (low), six to 19 (intermediate), and

**Table 2.** Number of Potentially Actionable Alterations in 60 Patients With Various Lymphoid Malignancies

| Diagnosis (No.) | No Reportable Alterations, No. (%) | Patients With Alterations but None Potentially Actionable by FDA-Approved Drug and/or Clinical Trial, No. (%) | Patients With One or More Alterations Potentially Actionable by Approved and/or Experimental Drug, No. (%) | Approved Drugs in the Disease Available (on label), No. (%) | Approved Drugs in Another Disease Available (off label), No. (%) | Experimental Treatment (clinical trials), No. (%) |
|---|---|---|---|---|---|---|
| CLL (19) | 4 (21) | 2 (11) | 13 (68) | 0 (0) | 11 (58) | 12 (63) |
| B-ALL (10) | 0 (0) | 0 (0) | 10 (100) | 2 (20) | 9 (90) | 9 (90) |
| T-ALL (2) | 0 (0) | 0 (0) | 2 (100) | 0 (0) | 2 (100) | 2 (100) |
| DLBCL (6) | 0 (0) | 0 (0) | 6 (100) | 0 (0) | 6 (100) | 6 (100) |
| FL (5) | 1 (20) | 1 (20) | 3 (60) | 0 (0) | 3 (60) | 3 (69) |
| MM (11) | 2 (18) | 0 (0) | 9 (82) | 0 (0) | 9 (82) | 9 (82) |
| Marginal zone lymphoma (2) | 0 (0) | 1 (50) | 1 (50) | 0 (0) | 0 (0) | 1 (50) |
| NK/T NHL (1) | 0 (0) | 0 (0) | 1 (100) | 0 (0) | 1 (100) | 0 (0) |
| LPL (1) | 0 (0) | 0 (0) | 1 (100) | 1 (100) | 1 (100) | 1 (100) |
| CTCL (1) | 0 (0) | 0 (0) | 1 (100) | 0 (0) | 1 (100) | 1 (100) |
| Primary CNS lymphoma (1) | 0 (0) | 0 (0) | 1 (100) | 0 (0) | 1 (100) | 1 (100) |
| Testicular lymphoma (1) | 0 (0) | 0 (0) | 1 (100) | 0 (0) | 1 (100) | 1 (100) |
| Overall (60) | 7 (12) | 4 (7) | 49 (82) | 3 (5) | 45 (75) | 46 (77) |

Abbreviations: B-ALL, B-cell acute lymphoblastic leukemia; CLL, chronic lymphocytic leukemia; CTCL, cutaneous T-cell lymphoma; DLBCL, diffuse large B-cell lymphoma; US FDA, Food and Drug Administration; FL, follicular lymphoma; LPL, lymphoplasmacytic lymphoma; MM, multiple myeloma; NHL, non-Hodgkin lymphoma; NK/T, natural killer/T-cell lymphoma; T-ALL, T-cell acute lymphoblastic leukemia.

20 or more (high) mutations per megabase of sequenced DNA.[23] In the current cohort, TMB ranged from one or fewer to 140 (Data Supplement). High TMB was seen in five patients (8%), intermediate in 14 (23%), and low in 40 (67%). TMB was not available for one patient (2%). Intermediate to high TMB was noted across almost all histologies (except for marginal zone lymphoma, natural killer/T-cell lymphoma, and cutaneous T-cell lymphoma). One patient with CLL had a TMB of 140, whereas two patients with DLBCL had a TMB of 20. Two patients with B-ALL had an intermediate level of TMB (both 11). Three patients with multiple myeloma had an intermediate level of TMB.

## DISCUSSION

This study demonstrates that in the majority of patients (82%) with lymphoid malignancies whose disease was interrogated by NGS, alterations were found that might be pharmacologically tractable, a percentage similar to the 77% reported by He et al[15] for diverse hematologic malignancies. Another study reported that 70% of patients with various solid tumors had an alteration that was theoretically actionable with an approved drug.[24] This finding is similar for lymphoid malignancies, with 75% of the current study patients having an alteration potentially actionable by an approved drug. Table 3 lists therapeutics with their corresponding targets that were identified in the patient cohort.

One of the major obstacles to performing comprehensive genomic profiling of clinical specimens is the necessity for an adequate tumor sample. Unlike solid malignancies where invasive biopsy is almost always required to obtain a tissue sample, comprehensive genomic profiling of lymphoid malignancies often can be performed with the use of peripheral blood and/or bone marrow. In this cohort, 54% of patients had specimens obtained from blood and/or bone marrow.

Tumors can acquire new mutations as they progress, which underscores the importance of obtaining new tissue when available for sequencing at the time of therapeutic decision making. For instance, the hallmark of chronic myelogenous leukemia is accumulation of genomic alterations with disease progression.[44] Similarly, patients with lung cancer and *EGFR* mutations that are sensitive to first-generation EGFR inhibitors will acquire secondary genomic alterations in *EGFR* (eg, *EGFR*

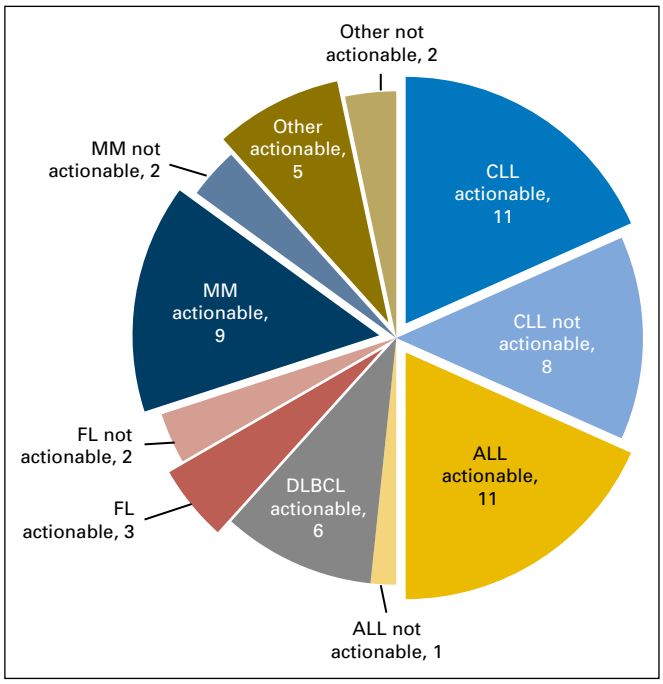


**Fig 2.** Number of patients with and without potentially US Food and Drug Administration–actionable alterations in each malignancy type. ALL, acute lymphoblastic leukemia; CLL, chronic lymphocytic leukemia; DLBCL, diffuse large B-cell lymphoma; FL, follicular lymphoma; MM, multiple myeloma.

T790M) that are resistant to these agents but sensitive to third-generation inhibitors.[45] In solid malignancies, NGS often is performed on tissue available from diagnosis to avoid repeat biopsy. In one study, 42% of patients did not have metastatic disease at the time of their biopsy.[24] Lymphoid malignancies are more amenable to repeat biopsy, with more than one half of the current cohort having tissue available from the blood and/or bone marrow. In addition, 77% of patients had NGS performed on tissue from relapse as opposed to diagnosis. This accessibility facilitates repeat NGS to guide therapy.

Over the past decade, 26 orally administered targeted therapies have been FDA approved for the treatment of 13 different solid malignancies (Data Supplement). Over the same period, only five orally administered targeted therapies have been approved for the treatment of lymphoid malignancies (Philadelphia chromosome–positive ALL, CLL, follicular lymphoma, mantle cell lymphoma, and Waldenström macroglobulinemia). The current data demonstrate that 45 patients (75%) had an alteration that theoretically could have been targeted with an approved, albeit off-label, drug, whereas only three patients (5%) had an alteration for which an approved on-label drug was available. In a similar study in patients with solid malignancies, 20% had an alteration targeted by on-label agents, whereas 67% had an alteration targetable by off-label use.[24] (Of note, the study used the same definitions for actionability as were used in this analysis.) Currently, a paucity of orally administered targeted therapy is available for on-label use in lymphoid malignancies. However, as our findings suggest, the majority of cases likely will have potentially targetable alterations.

The mutational landscape for numerous lymphomas has now been well characterized by several whole-exome sequencing studies.[46,47] For example, *MLL2*, *CREBBP*, and *TP53* alterations in DLBCL, as described by Pasqualucci et al,[46] were some of the most common recurrent mutations in the current cohort of patients with DLBCL. In the 19 patients with CLL, the most prevalent aberrations were alterations in *NOTCH1* found in five (26%). This alteration was also the most common (12%) in a cohort of patients with CLL sequenced by Puente et al.[47] Furthermore, 82% of our patients had alterations that were pharmacologically tractable, similar to the 77% reported by He et al[15] in diverse hematologic malignancies. These similar findings in other cohorts provide further support of the generalizability of our findings to other large cohorts of lymphoid malignancies.

MYD88, an adapter protein used by toll-like receptors, was shown to be mutated (*MYD88* L265P) in > 90% of patients with Waldenström macroglobulinemia (lymphoplasmacytic lymphoma).[48] This mutation not only is relatively specific for the disease but also predicts clinical presentation and survival.[49] MYD88 signaling is important in lymphomagenesis and signals through BTK.[50] In a phase II trial, ibrutinib produced an overall response rate of 91% in a group of previously treated patients with Waldenström macroglobulinemia, which led to its FDA approval.[37] Furthermore, response rates to ibrutinib were significantly higher in patients with *MYD88* mutations versus wild-type MYD88.[51] In the current cohort, *MYD88* L265P mutation was found in the one patient with Waldenström macroglobulinemia. However, *MYD88* alterations were also identified in one patient with DLBCL (*MYD88* L265P), one with follicular lymphoma (*MYD88* S219C), and one with primary CNS lymphoma (*MYD88* L265P). *MYD88* mutations have been discerned in marginal zone B-cell lymphoma,[52] CLL,[53] DLBCL,[54] and primary CNS lymphoma.[55] Preliminary data from a phase I trial of single-agent ibrutnib in four patients with CNS lymphoma demonstrated responses in two of the three patients evaluated. The current data further confirm that mutations in *MYD88* are readily identified by NGS and that trials in patients with lymphoid malignancies and *MYD88* mutations with BTK inhibitors are warranted.

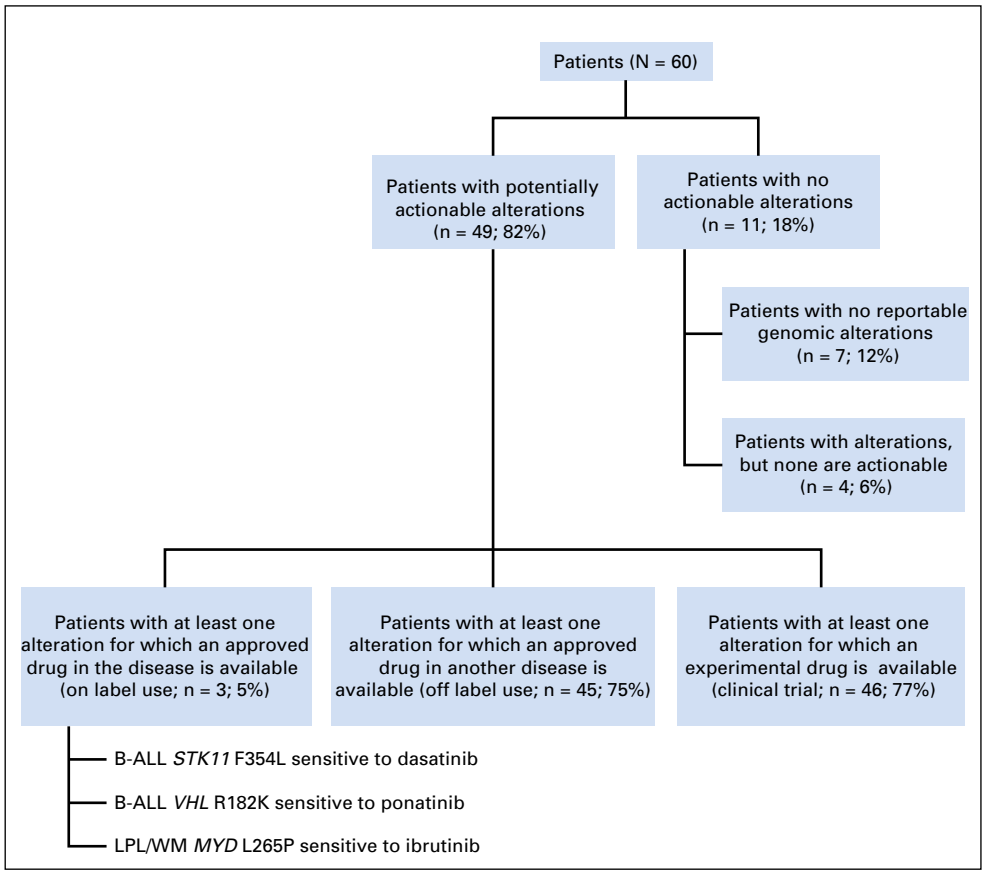


**Fig 3.** Breakdown of patients with potentially actionable alterations. B-ALL, B-cell acute lymphoblastic leukemia; LPL, lymphoplasmacytic lymphoma; WM, Waldenström macroglobulinemia.

One of the most common recurrent alterations in the current cohort was loss of *CDKN2A/B*. CDKN2A/B encodes for the $p16^{INK4A}$ protein, which is a negative regulator of the cyclin D–dependent protein kinases CDK4 and CDK6 important in cell cycle progression from the G1 to S phase.[28] Theoretically, this alteration can be targeted by palbociclib, a CDK4/6 inhibitor FDA approved for use in hormone receptor–positive/human epidermal growth factor 2–negative metastatic breast cancer in combination with letrozole[56] and fulvestrant.[57] Mantle cell lymphoma is a disease characterized by t(11;14)(q13;q32) translocation, which places the CCND1 gene under control of the IGH locus. High levels of CCND1 should upregulate CDK4/6. In a phase I trial of 17 patients with relapsed mantle cell lymphoma treated with single-agent palbociclib, five (18%) achieved progression-free survival of $>$ 1 year, with one complete and two partial responses.[58] The high percentage of *CDKN2A/B* alterations in the current study population gives further rationale for designing trials with CDK inhibitors in lymphoid malignancies.

Activating mutations in *BRAF* were found in three patients with CLL (two with *BRAF* G469A and one with *BRAF* V600E) and one patient with multiple myeloma (*BRAF* V600E). These mutations are potentially targetable by the BRAF inhibitors vemurafenib and dabrafenib and the MEK inhibitors trametinib and cobimetinib.[5,27] *BRAF* alterations have been identified as a driver mutation and as a biomarker for sensitivity to BRAF inhibition in both hairy cell leukemia and Erdheim-Chester disease.[59-61] BRAF alterations have been found in approximately 3% of patients with CLL.[62] In the current study, two patients had mutations that led to an alanine substitution for a glycine at position 469. This mutation is activating in melanoma and confers sensitivity to BRAF inhibition.[63] To our knowledge, this mutation has not been described in CLL before this report. Further studies that assess the role of BRAF inhibitors in patients with CLL who harbor *BRAF* mutations are warranted.

In addition to numerous theoretically targetable alterations, NGS identified mutations of prognostic significance in many of the tumor types studied. For example, alterations with known prognostic significance in CLL were identified, including *TP53*, *SF3B1*, *BIRC3*, *NOTCH1*, and *ATM*.[64] Furthermore, NGS identified mutations in *BTK*

**Table 3.** Potentially Actionable Targets and Examples of Their Corresponding FDA-Approved Drugs

| Actionable Gene | Examples of FDA Approved Drugs | Comment | First Author |
|---|---|---|---|
| *APC* | Sulindac | Sulindac is an NSAID. | Samadder[25] |
| *ATM* | Olaparib | Olaparib is a PARP inhibitor. | |
| *AXL* | Cabozantinib | Cabozantinib is a multitargeted kinase inhibitor. | |
| *BCL2* | Venetoclax | Venetoclax is approved for the treatment of relapsed CLL. | Roberts[26] |
| *BRAF* | Vemurafenib Trametinib Cobimetinib Dabrafenib | Trametinib and cobimetinib are MEK inhibitors. | Robert[27] |
| *CCND2* | Palbociclib | Palbociclib is a CDK4 and CDK6 inhibitor. | Sherr[28] |
| *CD79B* | Ibrutinib | Ibrutinib is approved for the treatment of CLL, relapsed mantle cell lymphoma, and Waldenström macroglobulinemia. | Davis[29] |
| *CDK4* | Palbociclib | Palbociclib is a CDK4 and CDK6 inhibitor. | Sherr[28] |
| *CDKN2A/B* | Palbociclib | Palbociclib is a CDK4 and CDK6 inhibitor. | Sherr[28] |
| *CSF1R* | Sunitinib Imatinib Nilotinib | | |
| *CXCR4* | Plerixafor | Plerixafor is approved for mobilization of peripheral blood stem cells. | Scala[30] |
| *DNMT3A* | Azacitidine Decitabine | Azacitidine and decitabine are hypomethylating agents. | Metzeler[31] |
| *ERRB3* | Pertuzumab Afatinib | | |
| *FGF23* | Lenvatinib Pazopanib Ponatinib Regorafenib | FGF23 acts through FGFR1, FGFR2, FGFR3, and FGFR4. | |
| *FGF3* | Lenvatinib Pazopanib Ponatinib Regorafenib | FGF3 acts through FGFR1, FGFR2, and possibly FGFR3. | |
| *FGF6* | Lenvatinib Pazopanib Ponatinib Regorafenib | FGF6 acts through FGFR1, FGFR2, and FGFR4. | |
| *FLT3* | Sorafenib Sunitinib | Both sorafenib and sunitinib are multitargeted kinase inhibitors. | |
| *FLT4* | Sorafenib Pazopanib Sunitinib | Sunitinib is a multitargeted kinase inhibitor. | |
| *GNAS* | Trametinib | Trametinib is an MEK inhibitor. | |
| *IDH1* | Azacitidine Decitabine | Azacitidine and decitabine are hypomethylating agents. | Emadi[32] |
| *IDH2* | Azacitidine Decitabine | Azacitidine and decitabine are hypomethylating agents. | Emadi[32] |
| *JAK1* | Tofacitinib | Tofacitinib is FDA approved for the treatment of rheumatoid arthritis. | Boyle[33] |
| *JAK2* | Ruxolitinib Tofacitinib | Ruxolitinib is approved for use in polycythemia vera and primary myelofibrosis. | Harry[34] |
| *JAK3* | Tofacitinib | Tofacitinib is FDA approved for the treatment of rheumatoid arthritis. | Furumoto[35] |
| *KRAS* | Trametinib | Trametanib is FDA approved for the treatment of melanoma. | Infante[36] |
| *MAP2K1* | Trametinib | Trametinib is an MEK inhibitor. | |
| *MSH2* | Pembrolizumab Nivolumab Atezolizumab | These drugs target PD-1 or PD-L1. | |
| *MSH6* | Pembrolizumab Nivolumab Atezolizumab | Pembrolizumab, nivolumab, and atezolizumab target PD-1 or PD-L1. | |
| *MYD88* | Ibrutinib | Ibrutinib is approved for use in CLL, relapsed mantle cell lymphoma, and Waldenström macroglobulinemia. | Treon[37] |

(Continued on following page)

**Table 3.** Potentially Actionable Targets and Examples of Their Corresponding FDA-Approved Drugs (Continued)

| Actionable Gene | Examples of FDA Approved Drugs | Comment | First Author |
|---|---|---|---|
| *NF1* | Trametinib Temsirolimus Everolimus | Temsirolimus and everolimus are mTOR inhibitors. | |
| *NF2* | Temsirolimus Everolimus Trametinib Lapatinib | Temsirolimus and everolimus are mTOR inhibitors. | |
| *NRAS* | Trametinib | Trametinib is an MEK inhibitor. | |
| *PALB2* | Olaparib | Olaparib is a PARP inhibitor. | |
| *PIK3CA* | Temsirolimus Everolimus | Temsirolimus and everolimus are mTOR inhibitors. | |
| *PTCH1* | Vismodegib Sonidegib | Both vismodegib and sonidegib are approved for the treatment of metastatic basal cell carcinoma. | Midgen[38] Sekulic[39] |
| *STK11* | Dasatinib Everolimus Temsirolimus Bosutinib | | |
| *TET2* | Azacitidine Decitabine | Azacitidine and decitabine are hypomethylating agents. | Bejar[40] |
| *TP53* | Bevacizumab Pazopanib | Bevacizumab (anti-VEGF-A antibody) has been associated with longer median PFS in patients with tp53 than tp53 wild type (11 *v* 5 months; retrospective study), and *TP53* mutation is associated with increased VEGF-A. In patients with sarcoma, pazopanib (a VEGFR inhibitor) response is associated with the presence of *TP53* mutations. | Said[41] Schwaederlé[42] Koehler[43] |
| *VHL* | Axitinib Bevacizumab Everolimus Pazopanib Sorafenib Sunitinib Temsirolimus Vandetanib | | |

Abbreviations: CLL, chronic lymphocytic leukemia; US FDA, Food and Drug Administration; FGF, fibroblast growth factor; FGFR, fibroblast growth factor receptor; mTOR, mammalian target of rapamycin; NSAID, nonsteroidal anti-inflammatory drug; PARP, poly (ADP-ribose) polymerase; PFS, progression-free survival; VEGF, vascular endothelial growth factor; VEGFR, vascular endothelial growth factor receptor.

and *PLCγ2*, which confer resistance to ibrutinib and would require a change in therapy.[65] Recently, studies identified a group of BCR-ABL–negative B-ALL with a Philadelphia chromosome–like gene expression signature that carries an inferior prognosis.[66] Mutations associated with this gene signature include *IKZF1* deletions or mutations, kinase fusions, *JAK2* mutations, and *CRLF2* mutations. In the 10 patients with B-cell ALL in the current study, *IKZF1* deletions and *JAK2* were identified in three and two, respectively. NGS readily identified Philadelphia chromosome–like B-ALL and can assist in selecting patients for clinical trials of targeted agents aimed at improving the poor outcome of these patients.[15]

Higher neoantigen burden, which may be largely predicted by TMB, has been associated with higher objective response rates and progression-free survival in patients treated with PD-1 blockade.[67,68] Melanoma, lung cancer, and renal cell carcinoma, all of which are highly responsive to PD-1 blockade, have been shown to have a high degree of mutational burden from whole-genome and -exome sequencing studies.[69] In the current cohort, TMB ranged from one or fewer to 140 (Data Supplement). Intermediate to high TMB was noted across almost all histologies (except for marginal zone lymphoma, natural killer/T-cell lymphoma, and cutaneous T-cell lymphoma). For lymphoid malignancies, high TMB was observed in 8% of patients. In comparison, high TMB has been reported in approximately 10% of adenocarcinomas of the lung[23] and approximately 42% of melanomas.[70] Dramatic responses to PD-1 blockade have already been reported in Hodgkin lymphoma and have led to FDA approval of nivolumab for relapsed/refractory Hodgkin lymphoma.[71] TMB has begun to be validated as a marker of response to immunotherapy in several different solid tumors.[16,17,67] On the basis of the current findings, TMB likely merits investigation as a marker of response to immunotherapy in lymphoid malignancies as well.[72]

This study has several limitations. The cohort was small, and not all lymphoid malignancies were represented. The majority of the patients had CLL, multiple myeloma, and ALL, whereas other non-Hodgkin lymphomas were less well represented. Although the majority of patients had actionable mutations theoretically targeted by FDA-approved drugs, in practice, insurance approval for off-label drug use often is difficult to obtain.[73] In addition, no standard definition exists for a targetable alteration, and the level of evidence needed to support this is a matter of debate. The number of actionable alterations discussed in this article may be overestimated, but even so, these patients should still be directed toward clinical trials that target these alterations so that the responsiveness or lack thereof can be determined. Recent guideline papers, such as Li et al,[74] have begun to address this issue and formulate standardized criteria for the definition of a targetable alteration. Numerous additional clinical trials with a standardized definition of what constitutes a targetable alteration are needed to determine the extent to which patients respond when an alteration is theoretically druggable. Currently, a number of such trials are ongoing (ClinicalTrials.gov identifiers NCT02534675, NCT00851032, and NCT02465060).

In conclusion, we found that most patients with lymphoid malignancies have unique and complex molecular portfolios. In > 80% of patients were one or more genomic alterations that are potentially actionable with existing drugs. Therefore, patients with lymphoid malignancies who have exhausted standard therapy or who are unable to tolerate chemotherapy may be excellent candidates for matched targeted therapies ideally administered in the context of a clinical trial.

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## AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

**Next-Generation Sequencing Reveals Potentially Actionable Alterations in the Majority of Patients With Lymphoid Malignancies**

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