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Hematologic Malignancies

Comprehensive Genomic Profiling of Hodgkin Lymphoma Reveals Recurrently Mutated Genes and Increased Mutation Burden

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Disclosures of potential conflicts of interest may be found at the end of this article.

Key Words. Genomic profiling • Hodgkin lymphoma • *B2M* mutation • *TP53* mutation • *XPO1* mutation

Abstract

Background. The genomic landscape of Hodgkin lymphoma (HL) has been difficult to characterize due to the paucity of neoplastic cells and an abundant microenvironment. Such characterization is needed in order to improve treatment strategies.

Materials and Methods. We performed comprehensive genomic profiling (CGP) using targeted next-generation sequencing on archival formalin-fixed paraffin embedded tumor samples from 63 patients to analyze the landscape of HL.

Results. CGP was successful for 49/63 archival specimens (78%), and revealed aberrations impacting genes including *B2M*, *TP53*, and *XPO1* (E571). Of the 34 patients for whom total mutation burden (TMB; mutations/megabase [Mb]) was assessed, 5 (15%) had high TMB (≥20 mutations/Mb), 18 (53%) had intermediate TMB (6–19 mutations/Mb), and 11 (32%) had low TMB (≤5 mutations/Mb). We next tested 13 patients' plasma cell-free DNA with droplet digital polymerase chain reaction for the presence of *XPO1* E571 mutation, which was confirmed in the plasma of 31% of patients. In three patients with serially collected plasma samples, *XPO1* E571K allelic frequency changes corresponded with changes in tumor size on conventional radiographic imaging.

Conclusion. The study demonstrates that comprehensive genomic profiling of archival Hodgkin lymphoma tumor samples is feasible and leads to the identification of genes that are recurrently mutated and that Hodgkin lymphoma has increased mutation burden in the majority of samples analyzed. Furthermore, tracking of *XPO1* E571 mutant allele frequency in a subset of patients may also represent a potential disease-monitoring strategy and warrants further investigation. ***The Oncologist*** 2019;24:219–228

Implications for Practice: This study provides the first evidence that comprehensive genomic profiling can be performed to map the genomic landscape of Hodgkin lymphoma and that a subpopulation of patients has mutations in *TP53*, *B2M*, *XPO1*, and other genes. It was found that 15% of patients have high mutation burden, which, in cancers such as melanoma, may indicate sensitivity to immune checkpoint inhibitors, and may thus be explored for Hodgkin lymphoma. Lastly, this work demonstrates that changes in the mutant allele frequency of *XPO1* in serially collected plasma cell-free DNA samples correspond with treatment outcomes measured with conventional radiographic imaging.

Introduction

Advances in genomic technologies have led to identification of genes commonly perturbed in cancer. Genomic testing has been quickly adopted and widely implemented in cancer care to leverage treatment decision-making and improve treatment outcomes [1, 2]. Although technologies such as next-generation sequencing (NGS) are increasingly accepted in nonhematologic malignancies, the genomic landscape of Hodgkin lymphoma (HL) remains to be described.

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In 2016, an estimated 8,500 new cases of HL were diagnosed in the U.S. [3]. Although rates of survival have improved over the years, approximately 10% of early-stage HL patients and 20%–30% of advanced-stage patients become refractory after initial therapy [4]. Furthermore, late treatment-related sequelae may lead to significant morbidity and mortality. Therapeutic options for patients with relapsed or refractory disease include salvage chemotherapy, followed by high-dose chemotherapy and autologous stem cell transplantation (ASCT). For patients refractory to primary therapy, even with aggressive approaches such as ASCT, overall survival rates do not exceed 30% [5]. Patients who relapse after ASCT may be candidates for allogeneic stem cell transplantation (alloSCT), which yields a median overall survival of 29 months with an approximate 20% treatment-related mortality [6]. Patients relapsing after ASCT and/or alloSCT have only limited treatment options, which include brentuximab vedotin, lenalidomide, bendamustine, or programmed cell death protein 1 (PD-1) antibodies, with median survival usually not exceeding 24 months [4, 7]. Therefore, a pressing need exists to better understand the underlying disease biology, which may lead to improvement in treatment outcomes. Investigation of the genomic landscape of HL has been difficult because of the low tumor content in these inflammatory cell- and stroma-rich tissue samples. Therefore, we performed a study using comprehensive genomic profiling (CGP) with a targeted NGS panel to test for genomic aberrations in archival tumor samples from patients with HL in order to identify potentially actionable molecular targets.

Materials and Methods

Patients and Tumor Samples

Formalin-fixed paraffin-embedded (FFPE) archival tumor samples, procured during routine clinical procedures for 63 HL patients, were reviewed and evaluated.

Comprehensive Genomic Profiling

A single hematoxylin and eosin-stained slide was examined in order to confirm the presence of tumor. CGP was performed from FFPE tissue using the Clinical Laboratory Improvement Amendments-certified, College of American Pathologists-accredited, New York State-approved FoundationOne (DNA-seq) [8] or FoundationOne Heme (DNA/RNA-seq) [9] assay. A minimum of 50 ng of DNA and 300 ng of RNA were extracted, RNA was converted to complementary DNA, and DNA was sonicated with subsequent adaptor ligation and hybridization capture, as described previously [8, 9]. DNA-based captured libraries (up to 405 cancer-related genes plus select introns from 31 genes) were sequenced to a median unique exon coverage depth of 500× using Illumina sequencing, and, following removal of duplicate reads, were analyzed for base substitutions, insertions, deletions, copy number alterations (focal gene amplifications and homozygous deletions), and select gene fusions. RNA-based captured libraries (265 genes) achieved ≥3M on-target unique pairs and were analyzed only for the presence of rearrangements. Total mutation burden (TMB) was defined as the number of somatic coding base substitutions and indel alterations minus known driver alterations per megabase (Mb) of genome examined, adjusted for the tumor content to allow for cross-study comparisons. A TMB of ≥20 mutations/Mb was considered high; 6–19 mutations/Mb intermediate; and ≤5 mutations/Mb low.

Plasma Collection and Cell-Free DNA Testing for the XPO1^{E571} Mutation

Whole blood was collected in ethylenediaminetetraacetic acid-containing tubes and centrifuged and spun twice within 2 hours to yield plasma. We extracted cell-free DNA (cfDNA) from plasma samples collected from 13 relapsed/refractory Hodgkin lymphoma patients before initiation of systemic therapy using the QIAamp Circulating Nucleic Acid Kit (Qiagen, Valencia, CA) according to the manufacturer's instructions. In order to identify common *XPO1* mutations in plasma cfDNA, we designed droplet digital polymerase chain reaction (ddPCR) probes against the E571K and E571V mutations. Sixteen nanograms of unamplified cfDNA was tested using ddPCR for the presence of the *XPO1* E571K or *XPO1* E571V mutation to distinguish the wild-type allele from the mutant using the QX200 Droplet Digital PCR platform (Bio-Rad, Pleasanton, CA) according to the manufacturer's standard protocol. The lower limit of detection was approximately <0.1% mutant allele frequency (MAF) per single well.

Results

Comprehensive Genomic Profiling of Archival Tumor Tissue

Molecular profiling was successfully performed in 49 (78%) of the 63 classical Hodgkin lymphoma FFPE specimens (Fig. 1; Table 1). Of these 49 specimens, 9 (18%) had no alterations, and in 40 specimens, a total of 44 genes with 95 alterations, including nonsynonymous mutations, splice site changes, rearrangements, or copy number alterations, were identified. Detailed patient characteristics were available for 18 patients treated at MD Anderson Cancer Center (Table 2).

The most commonly mutated gene was *TP53* (tumor protein p53) with 13 mutations in 11 patients (22%; Table 1; Fig. 2). The next most commonly mutated gene was *B2M* (beta-2 microglobulin) with 12 mutations in 11 patients (22%; Table 1; Fig. 2). For 82% (9/11) of patients with *B2M* alterations (with patient 2 demonstrating two *B2M* mutations), mutations occurred at the M1 position (M1I, n = 2; M1K, n = 5; M1R, n = 1; M1T, n = 1; M1V, n = 1; Table 1). The next most frequently mutated gene was *XPO1* (exportin 1; *CRM1*), for which nine (18%) cases harbored an E571 mutation (E571K: n = 8, E571V: n = 1; Table 1; Fig. 2). These mutations were not mutually exclusive of *TP53* or *B2M* aberrations. For two of these nine patients, an *XPO1* mutation (E571K: n = 1, E571V: n = 1)

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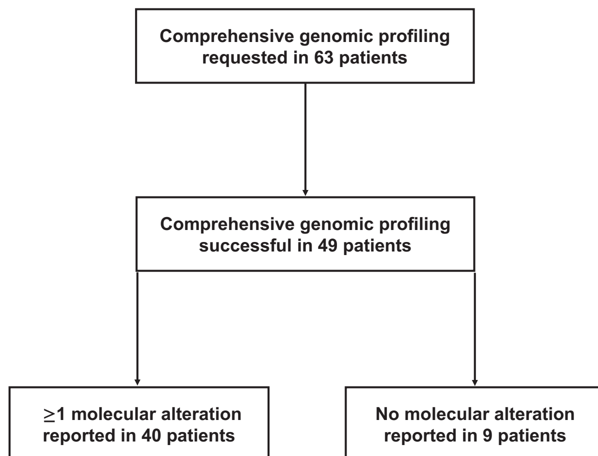

Figure 1. Enrollment of Hodgkin lymphoma patients whose archival tumor tissue was submitted for comprehensive genomic profiling.

was the only panel gene demonstrating an aberration, whereas the remaining seven patients demonstrated 1 through 20 additional mutations. The next most frequently mutated genes include *TNFAIP3* (tumor necrosis factor, alpha-induced protein 3) and *SOCS1* (suppressor of cytokine signaling 1). Eight aberrations in *TNFAIP3* were identified in seven patients (14%; Table 1; Fig. 2). Notably, two events were nonsense mutations and the remaining six were frameshift events, with two of these occurring in patient 23. Five events were additionally identified in *SOCS1* across five patients (10%; Table 1; Fig. 2). Three missense mutations, each occurring in three separate patients, were identified, along with rearrangements in patients 7 and 39. In both patients 7 and 39, an *IGH-SOCS1* rearrangement was identified, with a breakpoint occurring in exon two, the only coding exon, of *SOCS1.* In three of the five patients demonstrating *SOCS1* aberrations, the identified *SOCS1* mutation was the only event identified from CGP (Fig. 2). Programmed death-ligand 1 (PD-L1) amplification was also uncommon with only one case (patient 2) whose tumor harbored amplification of *CD274.*

Of the 34 patients for whom TMB was assessed, 5 (15%) had high TMB, 18 (53%) had intermediate TMB, and 11 (32%) had low TMB (Fig. 3). Only two patients, both with intermediate TMB, received anti-PD-1 antibodies, and both patients responded to treatment.

ddPCR of Plasma cfDNA for *XPO1* E571 Mutations

To confirm if recurrent *XPO1* E571 mutations are measureable in blood plasma and have the potential to act as a biomarker, ddPCR of cfDNA was performed. Of the 13 patients whose tumor samples were subjected to ddPCR analysis, 3 had *XPO1* E571K mutations (MAFs of 6.10%, 2.07%, and 6.10%, respectively) and 1 had the *XPO1* E571V mutation (MAF of 2.70%). Among these four patients' cases, CGP of the tumor tissue had not been performed in one case, *XPO1* mutation was not detected by profiling in one case, no results were obtained by profiling for one case, and the *XPO1* E571V mutation was confirmed in the tumor in one case (Table 3). Among the remaining nine cases in which ddPCR did not detect *XPO1* E571K or E571V mutations in cfDNA, CGP did not produce results in one case, demonstrated no *XPO1* mutation in one case, and was not performed for seven cases (Table 3). All three patients whose cfDNA had *XPO1* E571K mutations had longitudinal collection of plasma during systemic therapy, and changes in *XPO1* E571K MAFs corresponded to changes in tumor size on conventional radiographic imaging with positron emission tomography-computed tomography (Fig. 4).

Discussion

Our study demonstrates that CGP using targeted NGS of FFPE archival tumor samples from Hodgkin lymphoma patients is feasible. We observed a spectrum of alterations distributed across 44 cancer-related genes; the most commonly mutated genes were *TP53* (22% of patients), *B2M* (22% of patients), and *XPO1* (18% of patients). We also detected *XPO1* E571K or E571V mutations in 31% of the assayed plasma cfDNA samples. To our knowledge, our study is the first to report analysis of the genomic landscape of Hodgkin lymphoma using archival tumor samples.

Large-scale genomic profiling has been challenging because of the nature of the disease, as Hodgkin and Reed-Sternberg (HRS) cells are relatively sparse in the stroma-rich environment [10]. To address this problem, researchers have performed flow sorting of these cells to prepare samples for whole exome sequencing. Reichel et al. [10] used this approach for 10 fresh tumor samples and detected *B2M* alterations in 80% of cases. Other alterations including *XPO1* mutations have also been reported; however, the need for fresh tumor tissue limits the clinical utility of this approach. Notably, we did not perform microdissection of HRS cells in this study.

Others have also assessed the genomic profiles of tumor biopsies and plasma-derived cfDNA. Camus et al. [11] reported *XPO1* E571K mutations in 24% of tumor biopsies and in approximately 50% of plasma cfDNA samples from Hodgkin lymphoma patients; however, the investigators used ddPCR, which was not designed to detect alterations in cancer-related genes other than *XPO1.* We improve upon these studies by performing genomic profiling of more readily available archival tumor specimens in order to evaluate the genomic landscape of Hodgkin lymphoma.

In our study, *TP53* mutations were among the most frequent molecular abnormalities, occurring in 22% of patients. Previous studies have reported lower frequency of *TP53* mutations, which occurred in approximately 9%–11% of Hodgkin lymphoma patients [12, 13]. However, previous studies suggested that *TP53* mutation frequency might be associated with enrichment for HRS cells [14, 15]. Arguably, samples meeting quality standards for the CGP could have been plausibly enriched for HRS cells. Unfortunately, a small sample size precluded analysis of prognostic

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Table 1. Comprehensive genomic profiling of archival tumor tissue in Hodgkin lymphoma patients

Patient	Sample type	Test type	DNA test	RNA test	Gene	Alteration	Alteration type	% reads	TMB	TMB
1	unknown	F1	X		*BCL6*	R459H	substitution	43	9.7	intermediate
2	unknown	F1H	X	X	*CD36*	Y325*	substitution	51	166.8	high
					FBXO11	R640fs*5	indel	9		
					ASXL1	Q1517*	substitution	9		
					CDC73	splice	splice	10		
					TSC2	V1711M	substitution	50		
					PTCH1	G288D	substitution	12		
					DNMT3A	P896L	substitution	11		
					BRCA2	R2842C	substitution	42		
					ATR	W2104*	substitution	7		
					MSH6	T1219I	substitution	9		
					B2M	M1K	substitution	26		
					AXIN1	P24S	substitution	9		
					B2M	M1I	substitution	13		
					XPO1	E571K	substitution	32		
					TP53	R248Q	substitution	21		
					TP53	K291N	substitution	19		
					CD274	amplification	copy number			
					JAK2	amplification	copy number			
					KDM4C	amplification	copy number			
					CCND3	amplification	copy number			
					PDCD1LG2	amplification	copy number			
3	unknown	F1	X		*ATM*	R189K	substitution	8	4.4	low
4	unknown	F1H	X	X	*MEF2B*	Y69H	substitution	18	ND	ND
					TNFAIP3	S79fs*22	indel	12		
					BCL7A	splice	splice	14		
					TP53	E286K	substitution	14		
					IGH	fusion	rearrangement	16 read pairs		
5	unknown	F1H	X	X	*B2M*	M1K	substitution	5	8.8	intermediate
6	unknown	F1H	X	X	*MDM4*	amplification	copy number		4.4	low
7	unknown	F1H	X		*SOCS1*	rearrangement	rearrangement	15 read pairs	10.3	intermediate
8	relapsed	F1H	X		*B2M*	A8fs*36	indel	6	11	intermediate
					TNFAIP3	A67fs*29	indel	5		
9	relapsed	F1H	X	X	*B2M*	M1K	substitution	7	49.2	high
					HIST1H1D	R80H	substitution	3		
					TP53	A159V	substitution	2		
10	newly diagnosed	F1H	X	X	*ZRSR2*	R448_R449insSR	indel	48	1.5	low
					ICK	splice site	831+2_831+2delT	41		
11	unknown	F1	X		*B2M*	M1T	substitution	3	4.4	low
12	relapsed	F1H	X	X	*IRF8*	S55A	substitution	5	17.6	intermediate
13	relapsed	F1H	X	X	*LRP1B*	R2772H	substitution	44	8.1	intermediate
14	unknown	F1H	X	X	*XPO1*	E571K	substitution	3	2.2	low
15	relapsed	F1H	X	X	*TP53*	Q165E	substitution	17	28.7	high
					GNA13	R166*	substitution	33		
					XPO1	E571K	substitution	22		
					B2M	M1R	substitution	13		
					STAT3	S614R	substitution	9		

					TNFAIP3	R569fs*123	indel	31		
					CCND3	amplification	copy			
					PDCD1LG2	fusion	rearrangement	18 read pairs		
16	relapsed	F1H	X	X	*TP53*	T284P	substitution	2	8.8	intermediate
17	relapsed	F1H	X	X	*BLM*	P30L	substitution	1	NA	NA
18	newly diagnosed	F1H	X	X	*CD36*	Y325*	substitution	51	8.1	intermediate
					TP53	I255T	substitution	19		
					XPO1	E571K	substitution	5		
19	unknown	F1H	X	X	*B2M*	M1V	substitution	1	1.5	low
20	unknown	F1H	X	X	*B2M*	M1K	substitution	3	18.4	intermediate
					XPO1	E571K	substitution	2		
21	relapsed	F1H	X	X	*SOCS1*	S116N	substitution	5	20.6	high
22	relapsed	F1H	X	X	*XPO1*	E571K	substitution	7	11.8	intermediate
					ARID1A	S2179fs*23	indel	6		
					B2M	M1K	substitution	6		
					TNFAIP3	R271*	substitution	8		
23	newly diagnosed	F1H	X	X	*B2M*	L7*	substitution	35	45.6	high
					TNFAIP3	C627fs*44	indel	16		
					TNFAIP3	R71fs*29	indel	12		
					AXL	R190H	substitution	55		
24	relapsed	F1H	X	X	*ATR*	S1038fs*11	indel	43	1.5	low
25	relapsed	F1H	X	X	*TP53*	S215G	substitution	3	5.9	low
26	relapsed	F1H	X	X	*STAT3*	D661H	substitution	10	8.1	intermediate
					PRDM1	N242fs*28	indel	9		
27	relapsed	F1	X		*BCL6*	P473L	substitution	47	NA	NA
					TP53	Y234H	substitution	2		
28	relapsed	F1	X		*XPO1*	E571K	substitution	3	14.3	intermediate
					TSC2	loss	copy			
29	relapsed	F1	X		*TP53*	L130R	substitution	1	3.3	low
30	relapsed	F1	X		*TP53*	R181C	substitution	3	13.2	intermediate
31	relapsed	F1	X		*SOCS1*	A3T	substitution	3	8.8	intermediate
32	relapsed	F1	X		*BRCA1*	splice	splice	46	13.2	intermediate
33	relapsed	F1	X		*TNFAIP3*	W85*	substitution	4	18.7	intermediate
					XPO1	E571K	substitution	3		
34	relapsed	F1	X		*ATM*	splice	splice	47	1.1	low
35	relapsed	F1	X		*TP53*	K305R	substitution	10	20.3	high
					TP53	G293fs*13	indel	12		
					PIM1	K24N	substitution	12		
					TNFAIP3	C627fs*44	indel	15		
36	relapsed	F1	X		*B2M*	M1I	substitution	10	14.1	intermediate
					PIM1	L2F	substitution	16		
					EPHA5	T856I	substitution	8		
					REL	amplification	copy number			
37	relapsed	F1	X		*PCLO*	R1666Q	substitution	1	7.1	intermediate
					SOCS1	A17T	substitution	2		
38	relapsed	F1	X		*MCL1*	amplification	copy number		1.1	low
39	relapsed	F1H	X	X	*IGH-SOCS1*	fusion	rearrangement		9.6	intermediate
40	relapsed	F1H	X	X	*XPO1*	E571V	substitution	4	4.4	low
41	relapsed	F1H	X	X	none				ND	ND
42	relapsed	F1	X		none				ND	ND

(continued)

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Table 1. (continued)

Patient	Sample type	Test type	DNA test	RNA test	Gene	Alteration	Alteration type	% reads	TMB	TMB
43	relapsed	F1	X		none				ND	ND
44	unknown	F1H	X	X	none				ND	ND
45	unknown	F1H	X	X	none				ND	ND
46	unknown	F1H	X	X	none				ND	ND
47	unknown	F1H	X	X	none				ND	ND
48	unknown	F1H	X	X	none				ND	ND
49	unknown	F1H	X	X	none				ND	ND

Abbreviations: F1, FoundationOne (DNA-seq); F1H, FoundationOne Heme (DNA/RNA); NA, not available; ND, not done; TMB, total mutation burden; X, a test was performed for this patient (DNA test, or RNA test).

significance. Molecular alterations in *B2M* were also among the most frequently detected molecular abnormalities. *B2M* inactivating mutations result in a loss of expression of the major histocompatibility complex class I complex, which is involved in antigen presentation. Decreased expression of *B2M* has also been reported to be associated with worse prognosis in non-Hodgkin lymphoma patients [16]. Notably, inactivating *B2M* mutations have been observed in Hodgkin lymphoma patients, and lack of *B2M* expression has been associated with a favorable prognosis [10]. With respect to therapeutic insights, one recent study suggested that truncating *B2M* mutations may be associated with acquired resistance to PD-1 blockade in metastatic melanoma [10]. These studies, along with our findings, demonstrate the benefits of genomic profiling to improve our understanding of the genomic landscape of Hodgkin lymphoma tumors, as well as to improve therapeutic selection.

Other frequent recurrent alterations in the present study were *XPO1* E571 mutations, which were detected in 18% of archival tumor samples and 31% of plasma cfDNA samples. Small numbers precluded assessment of concordance between plasma cfDNA and tumor tissue testing. XPO1 regulates the nuclear export and localization of proteins involved in cell proliferation and the cell cycle. Increased expression of *XPO1* in cancer patients has been shown to be associated with worse survival and increased metastasis [17], and mutations in this gene have been reported in B-cell lymphoma [18]. A recent study using ddPCR reported recurrent *XPO1* E571K mutations in 24% of tumor biopsies and about 50% of plasma cfDNA samples from Hodgkin lymphoma patients [11]. Currently, XPO1 inhibitors such as selinexor are being evaluated in solid tumors and hematologic malignancies in clinical trials; however, the possible relationship between *XPO1* mutations and sensitivity or resistance to XPO1 inhibition remains unknown because preclinical data on B-cell lymphoma and osteosarcoma cell lines demonstrated efficacy independent of *XPO1* mutation status [18].

Other potentially targetable molecular alterations identified in our study include loss of *TSC2* (tuberous sclerosis 2), for which mammalian target of rapamycin inhibitors may be effective [19]; and a *BRCA1* mutation, which suggests treatment with DNA-damaging platinum agents or poly(ADP-ribose) polymerase inhibitors [20, 21]. However, the one *BRCA1* mutation we identified may be a germline event given the event's allelic frequency of 46% and the patient's family history of breast cancer. Previous studies have reported that germline *BRCA1* mutations are infrequent in Hodgkin lymphoma [22].

We also identified recurrent events in *TNFAIP3* (14% of patients) and *SOCS1* (10% of patients). *TNFAIP3* is involved in the regulation of apoptosis and nuclear factor κB signaling, and the nonsense and frameshift mutations we identified are predicted to be inactivating due to the predicted translation of a truncated protein. Deletions, mutations, and decreased protein expression of *TNFAIP3* have been reported in approximately 24%–44% of Hodgkin lymphoma patients and in 10%–38% of non-Hodgkin lymphoma patients [23, 24]. With respect to *SOCS1*, S116N has been reported in diffuse large B-cell lymphoma [25], and A17T has been reported in B-cell lymphoma [26]. A17T is outside of the gene's functional domains, but S116N is in the gene's conserved Src Homology 2 domain and thus may affect protein interactions if expressed. Mutations in *SOCS1*, which has roles in apoptosis, and in cell survival and growth through JAK/STAT signaling, have also been reported in Reed-Sternberg cells and Hodgkin cell lines as inactivating mutations [27]. The *SOCS1* rearrangement we identified in patients 7 and 39 is also predicted to be inactivating as it disrupts the only coding exon of *SOCS1.* This event is similar to a t(14;16)(q32;p13.1) event that was previously described in a Hodgkin lymphoma patient [28].

High TMB was recently found to be predictive of favorable response to PD-1- and PD-L1-targeted therapies in lung cancer [29]. In our study, 15% patients with TMB data had high TMB. However, only two patients with known TMB received PD-1 antibodies, and both responded despite having intermediate TMB. Thus, the relationship between TMB and response to immune checkpoint inhibitors remains to be clarified.

We further show that ddPCR is a highly sensitive approach that can detect *XPO1* E571 mutations in plasma cfDNA samples from Hodgkin lymphoma patients. Using ddPCR, we detected *XPO1* E571 mutations in 31% of cases, and we were able to confirm the mutation in archival tumor samples in three of the four cases for which tumor tissue was available. In addition, dynamic tracking of *XPO1*

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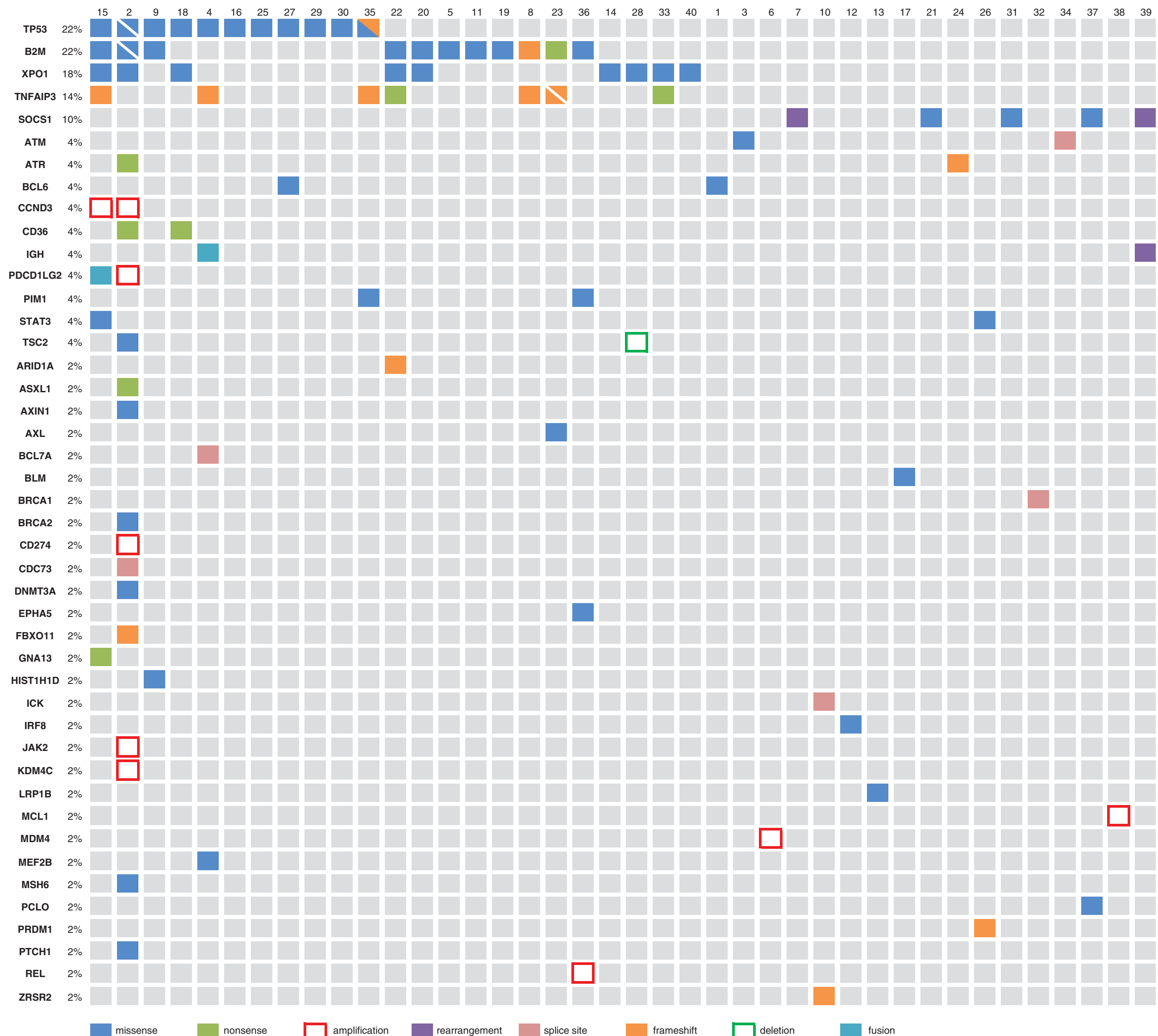

Figure 2. Molecular alterations in archival tumor samples from 40 Hodgkin lymphoma patients. The nine patients for whom comprehensive genomic profiling did not identify alterations are not shown. Split squares are shown if two events were identified in the same gene (e.g., *B2M* and *TP53* for patient 2).

MAF corresponded with changes observed during routine radiographic imaging. Previously, Camus et al. [11] reported that ddPCR detected *XPO1* E571K mutations in about 50% of plasma cfDNA samples; however, in nearly half those cases, the *XPO1* mutation was not confirmed in the tumor tissue. Also, patients with detectable *XPO1* mutation in plasma cfDNA at the end of therapy had a trend toward shorter progression-free survival.

Our study has several potential limitations. First, the sample size was relatively small, and second, CGP encompassed up to 405 selected cancer-related genes such that novel events may be missed. In addition, our method did not include testing of normal DNA to reliably exclude possible germline alterations. Third, our method did not include sorting the HRS cells. Therefore, it is unclear if all reported variants are truly from HRS cells. Fourth, although we were able to obtain TMB data for a considerable number of patients, only a few patients received checkpoint inhibitors; thus, the value of TMB in predicting immunotherapy response remains unknown. In addition, whether any of the recurrent molecular alterations we identified may be used for therapeutic decision-making remains unclear. Furthermore, profiling of degraded FFPE DNA lends itself to the possibility of false negatives. For example, previous studies have reported a recurrence of *PD-L1/CD274* and *PD-L2/PDCD1LG2* amplification in newly diagnosed HL cases [30] and nodular sclerosis HL [31]. However, only one patient profiled here (33 of 49 were tested for *PD-L1/CD274* and *PD-L2/PDCD1LG2* amplification as these genes were not initially included in the panel) demonstrated PD-L1 amplification such that this caveat must be considered during data interpretation. Finally, the roles of *B2M* and *XPO1* mutations as possible biomarkers of response to XPO1 inhibitors or immune checkpoint inhibitors,

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Table 2. Patients' characteristics for 18 patients treated at MD Anderson Cancer Center

Patient	Hodgkin lymphoma type	Gender	Age at diagnosis, years	Stage at diagnosis	Associations with EBV (EBER)
26	Classical HL, NOS	Male	69	IV	Negative
27	Nodular sclerosis	Female	59	III	Negative
28	Nodular sclerosis	Male	28	IV	Positive
29	Nodular sclerosis	Male	36	II	Unknown
30	Nodular sclerosis	Female	32	II	Positive
31	Nodular sclerosis	Male	22	II	Unknown
32	Nodular sclerosis	Male	29	II	Unknown
33	Classical HL, NOS	Female	31	IV	Unknown
34	Nodular sclerosis	Male	33	IV	Positive
35	Nodular sclerosis	Male	38	IV	Negative
36	Nodular sclerosis	Male	34	II	Negative
37	Nodular sclerosis	Female	43	II	Negative
38	Nodular sclerosis	Male	44	IV	Unknown
39	Nodular sclerosis	Male	23	II	Negative
40	Nodular sclerosis	Female	27	II	Negative
41	Classical HL, NOS	Male	21	IV	Positive
42	Nodular sclerosis	Female	39	III	Negative
43	Nodular sclerosis	Male	30	II	Unknown

Abbreviations: EBER, EBV-encoded RNA; EBV, Epstein-Barr virus; HL, Hodgkin lymphoma; NOS, not otherwise specified.

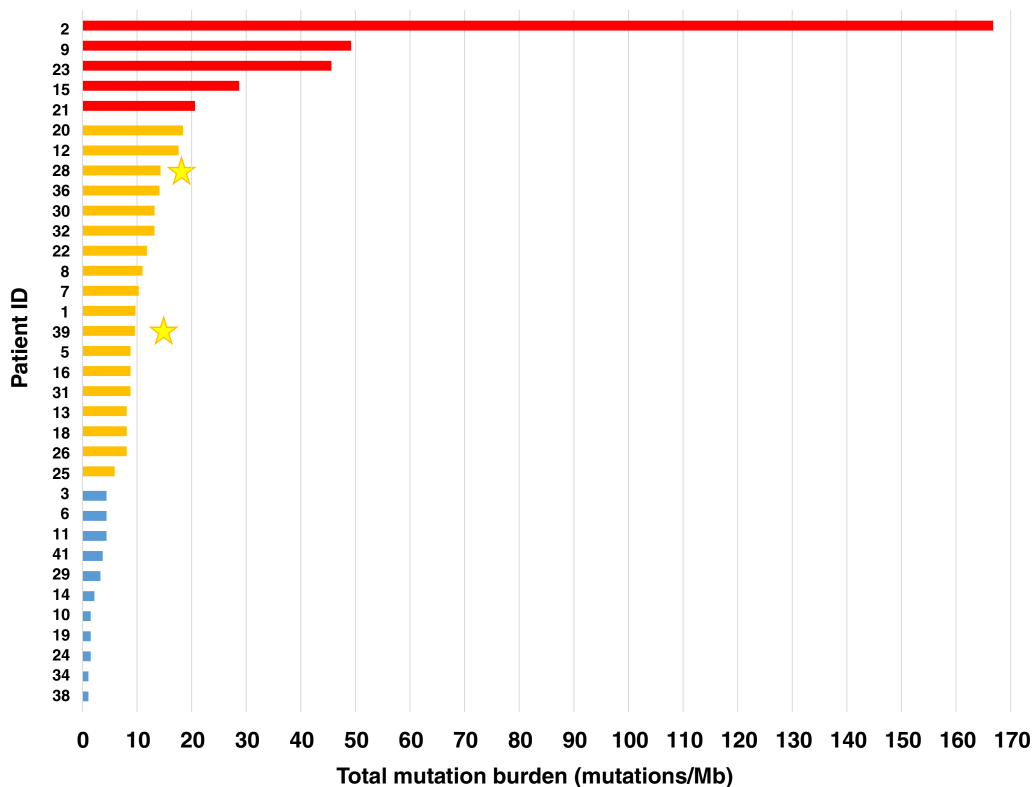

Figure 3. Total mutation burden for 34 Hodgkin lymphoma patients. High mutation burden is depicted in red, intermediate in orange, and low in blue. Two patients (yellow stars) received anti-programmed cell death protein 1 antibodies and responded to treatment.
Abbreviation: Mb, megabase.

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Table 3. *XPO1* mutations in plasma cfDNA

Patient	*XPO1* mutation in plasma cfDNA (MAF)	*XPO1* mutation in tumor detected by ddPCR (MAF)	*XPO1* mutation in tumor detected by CGP
A	Wild-type	Wild-type	Not done
B	E571K (6.10%)	E571K (0.86%)	Not done
7	E571K (2.07%)	E571K (4.30%)	Wild-type
C	Wild-type	Not done	Not done
D	Wild-type	Not done	Not done
E	Wild-type	Not done	Not done
F	Wild-type	Not done	Not done
G	Wild-type	Not done	Failed
H	Wild-type	Not done	Not done
41	Wild-type	Not done	Wild-type
I	E571K (6.10%)	No tissue available	Failed
40	E571V (2.70%)	E571V (2.60%)	E571V
J	Wild-type	Not done	Not done

Three patients that were profiled by CGP also had cfDNA analysis (patients 7, 40, 41). Patients with letter identifiers were not profiled by CGP.
Abbreviations: cfDNA, cell-free DNA; CGP, comprehensive genomic profiling; ddPCR, droplet digital polymerase chain reaction; MAF, mutant allele frequency.

respectively, remain to be evaluated in preclinical and prospective clinical studies.

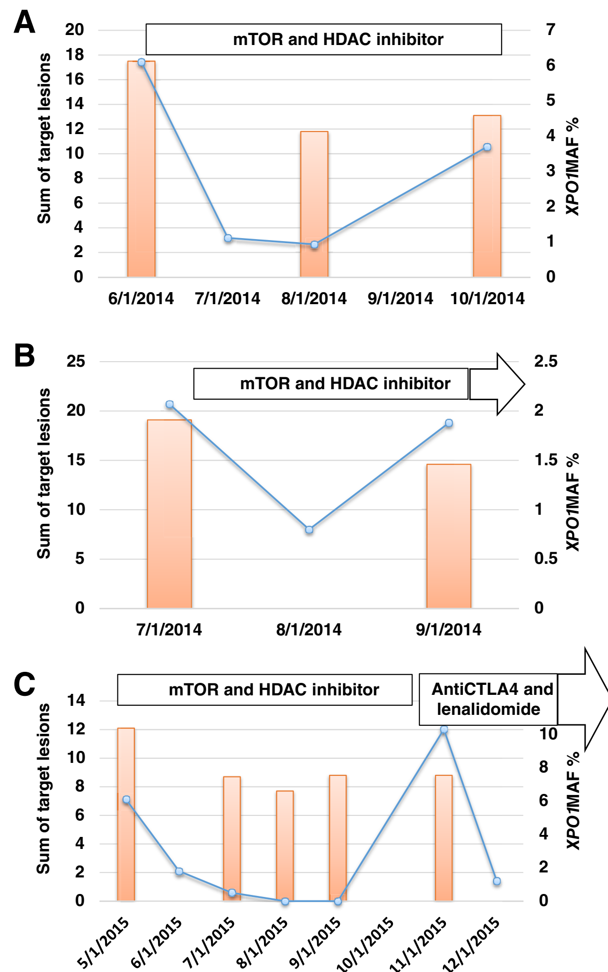

Figure 4. Dynamic changes in *XPO1* E571K MAF. Changes in *XPO1* E571K MAF in plasma cell-free DNA are shown as blue graph lines. Changes in tumor size, represented by the sum of perpendicular diameters of target tumor lesions evaluated by Cheson criteria (orange bars), in two Hodgkin lymphoma patients treated with mTOR and HDAC inhibitors **(A and B)** and one patient treated with mTOR and HDAC inhibitors followed by an anti-CTLA4 antibody plus lenalidomide **(C)**, are also shown.
Abbreviations: CTLA4, cytotoxic T-lymphocyte associated protein 4; HDAC, histone deacetylase; MAF, mutant allele frequency; mTOR, mammalian target of rapamycin.

Conclusion

Overall, we demonstrate the feasibility of performing CGP on archival tumor specimens from Hodgkin lymphoma patients. In addition to improving our understanding of the genomic landscape of Hodgkin lymphoma, we identified recurrent alterations in the *TP53*, *B2M*, *XPO1*, and *TNFAIP3* genes. Fifteen percent of patients demonstrated high TMB, which in other cancers was found to be associated with activity of immune checkpoint inhibitors. We further show that *XPO1* mutations may be detected in patient cfDNA, and that changes in the MAF of cfDNA *XPO1* mutations correlate with changes in tumor size. As a result, these findings provide potential strategies for therapeutic selection and molecular monitoring of patients. Continued genomic analysis of Hodgkin lymphoma patients thus creates a foundation for improving patient care.

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Disclosures

Jo-Anne Vergilio: Foundation Medicine, Inc. (E, OI); **David Fabrizio:** Foundation Medicine, Inc. (E, OI); **Vincent A. Miller:** Foundation Medicine, Inc. (E, OI); **Philip J. Stephens:** Foundation Medicine, Inc. (E, OI); **Jeffrey S. Ross:** Foundation Medicine, Inc. (E, OI); **Filip Janku:** Biocartis, Trovagene, Foundation Medicine, Inc. (RF), Trovagene, Guardant Health (SAB). The other authors indicated no financial relationships.

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