Published in final edited form as:

Am J Hematol. 2016 November ; 91(11): E478–E479. doi:10.1002/ajh.24496.

Clinical and molecular characteristics of *XPO1* mutations in patients with chronic lymphocytic leukemia

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Abstract

The *XPO1* (exportin) gene (also referred to as chromosome region maintenance 1; *CRM1*) is a karyopherin that exports proteins and RNA fragments from the nucleus into the cytoplasm.^{1,2} The human *XPO1* gene, located on chromosome 2 (2p15), is believed to encode an oncogenic protein since many of the molecules exported by *XPO1* into the cytoplasm are associated with either known tumor-suppressor genes, such as *TP53* or *FOXO3A*, or transcription factors that contribute to cell proliferation and survival such as IκB-α³ or *STAT3*⁴ whose accumulation in the nucleus results in cell death. The binding of *XPO1* to various proteins is mediated by recognizing the leucine-rich nuclear export signals (LR-NES) on the N-terminus of snurportin 1 (SNUPN) forming a nuclear pore complex or a cargo, thereby transporting proteins out of the nuclear membrane. Overexpression, deregulation, or dysfunction of *XPO1* has been reported in various types of cancer.⁵ *XPO1* is a therapeutic target in CLL,⁶ and selective inhibitors of nuclear transport (SINE) such as selinexor are now being investigated in clinical trials in CLL.

Keywords

Exportin; *XPO1* gene; CRM1; CLL; chronic lymphocytic leukemia

Next-generation sequencing (NGS)-based mutational studies in patients with chronic lymphocytic leukemia (CLL) have demonstrated that *XPO1* is recurrently mutated in <5% of patients with CLL.⁷ The integration of commonly occurring gene mutations with current prognostic factors including cytogenetic aberrations improves prognostication for CLL patients.^{8–10} In this study, we investigated the clinical significance of *XPO1* mutations in patients with CLL.

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Authorship Contributions

P.J., R.K-S. and Z.E. contributed to the study design, data collection (N.S. and U.R.), wrote the paper and analyzed results. R. K-S., K.P. and R.L. analyzed molecular data, Z.E., M.K., P.T., H.K., J.B. and W.W. contributed patient samples.

Conflicts-of-Interest Disclosure: None from any authors.

Peripheral blood and/or bone marrow cells from 486 CLL patients were analyzed using an amplicon-based NGS panel of 53 cancer-related genes using MiSeq system (Illumina, Inc., San Diego, CA). Details of this panel are listed in *supplemental Table 2*. In 13 patients, we also performed NGS-based mutation analysis with a panel composed of CLL-related genes including *NOTCH1*, *SF3B1*, *POT1*, *BTK*, and *BIRC3*.

The study was approved by Institutional Review Board, and written informed consent was obtained from the study patients. Conventional cytogenetic and fluorescence *in situ* hybridization (FISH) studies were performed. Survival analysis was conducted by using GraphPad Prism version 6.00 for Windows (GraphPad Software, La Jolla, CA).

Patient population

XPO1 mutations were detected in 38 of 486 (7.8%) CLL patients. Of these 38 patients, 16 were treatment-naïve and 22 were previously treated. Clinical characteristics of the patients are summarized in *supplemental table 1*. The median age of patients with *XPO1* mutations was 59 years (range, 37–81 years). Nine patients (24%) were ≥ 65 years of age. Among the 38 patients with *XPO1* mutations, 28 of 38 (74%) had early Rai stage disease, whereas 10 (26%) had advanced disease. Fifteen patients (40%) had sole del(13q), 11 (29%) had del(11q) [10 of whom had coexisting deletion del(13q)], 3 had del(17p), and 3 had trisomy 12. Complex karyotype was detected in 2 patients. Thirty patients (79%) had unmutated immunoglobulin heavy chain variable region (*IGHV*) status and 3 were mutated. There was no predilection to a specific VH gene usage.

The median follow-up duration of patients after the detection of *XPO1* mutation was 13.5 months (range, 0–74 months). At the last follow-up, one patient with relapsed/refractory CLL had died of disease progression and infection, and 37 patients were alive; the median overall survival duration of these patients was not reached (Supplemental Figure 1A).

Among the 16 previously untreated patients, 7 required treatment, and 9 did not. Among the 22 previously treated patients, 18 were pretreated with fludarabine-based therapies, and none were pretreated with ibrutinib. 21 of the 22 patients required subsequent treatment, and 1 one remains under observation. Of these 21 patients, 15 (71%) received ibrutinib-based therapy and 13 (87%) responded. Overall, one patient transformed into Hodgkin's lymphoma.

Molecular characteristics

The clinical and molecular characteristics of the patients with *XPO1* mutations are detailed in Table 1. All *XPO1* mutations were missense mutations in exon 15 leading to protein alteration in codon 571. The most frequent *XPO1* mutation was p.E571K (n=29), followed by p.E571V, p.E571G and p.E571Q in 12 patients (3 of whom had coexisting p.E571K with p.E571G, p.E571K, and p.E571V mutations). The median allelic frequency of *XPO1* mutation was 20% (range, 1.6%–46.2%). Nine patients (23%) had a mutant allelic burden of 10%.

Concurrent CLL specific mutations were detected in various genes in addition to *XPO1* in 10/13 patients (supplemental figure-2), most frequently in *NOTCH1*, *SF3B1* and *TP53*. (Annotated in Table-1).

Clinical relevance of *XPO1* mutations

We compared patients who had *XPO1*-mutated CLL and unmutated *IGHV* gene to CLL patients with unmutated *IGHV* gene (n=136) without *XPO1* or *TP53* mutations (control group). Supplemental figure 1B and C shows that the patients with *XPO1*-mutated CLL and the patients in the control group had a similar TTFT and survival duration (P=NS).

Although the leukemogenic mechanisms of mutations in patients with CLL are unclear, the recurrent nature of *XPO1* mutation in CLL strongly supports its involvement in the pathogenesis of the disease. We found that *XPO1* mutations are commonly found in patients with unmutated *IGHV* (79%), early Rai stage (73%), CD38 overexpression, ZAP-70 positivity, and del(13q). Few patients had coexisting *TP53* mutations. During an approximate 14-month follow-up after testing, we observed that *XPO1* mutations may not be associated with poor prognosis. Overall, our results agree with two previously published reports.^{7,8} Jeromin et al.⁸ found that among 33 (3%) of 969 patients who had *XPO1* mutations, the majority (30 patients [91%]) had unmutated *IGHV* status. We were unable to determine the mutations in *NOTCH1* in our panel in all patients. However, using a different gene panel in 13 patients, we found that *NOTCH1* mutations were seen in 5/10 (50%) patients with *XPO1* mutations. On comparing the survival duration and TTFT in a matched control group to *XPO1*-mutated patients, we found that patients with *XPO1* fared better. The majority of patients who needed treatment responded to ibrutinib-based therapy. This suggested that although patients with *XPO1* mutations had markers of poor prognosis, the overall outcome was not poor in those receiving ibrutinib. Low frequency of *XPO1* mutations and good response to ibrutinib suggest that the *XPO1* mutation may not be a marker of poor prognosis in patients with CLL.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

This study was supported in part by the NIH/NCI under award number P30CA016672 and by the NCI under award number P01CA049639.

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Clinical and genotypic characteristics of CLL patients with *XPO1* mutations (n=38). All *XPO1* mutations were of missense type, noted in codon 571 of exon 15.

Table 1

Gene mutations	HGVs (Human Genome Variation Society) nomenclature	Mutant allelic frequencies (%)	Treatment status	FISH abnormalities	VH gene usage	IGHV mutation status
<i>XPO1</i>	NM_003400.3(<i>XPO1</i>):c.1711G>A p.E571K	33.6	TN	del(13q)	VH4-30	UM
<i>XPO1</i>	NM_003400.3(<i>XPO1</i>):c.1711G>A p.E571K	1.6	TN	del(11q);del(13q)	VH3-33	UM
<i>XPO1</i>	NM_003400.3(<i>XPO1</i>):c.1712A>T p.E571V	42.7	TN	del(13q)	VH3	UM
<i>XPO1</i>	NM_003400.3(<i>XPO1</i>):c.1711G>A p.E571K	18.5	TN	Negative	VH1-69	UM
<i>XPO1</i>	NM_003400.3(<i>XPO1</i>):c.1712A>T p.E571V	44.4	TN	del(13q)	VH1-69	UM
<i>XPO1</i>	NM_003400.3(<i>XPO1</i>):c.1711G>A p.E571K	42.6	TN	tetrasomy 12;13q	VH4-34	UM
<i>XPO1</i>	NM_003400.3(<i>XPO1</i>):c.1712A>G p.E571G, NM_003400.3(<i>XPO1</i>):c.1711G>A p.E571K	37.5, 2.0	TN	del(13q)	ND	UM
<i>XPO1</i>	NM_003400.3(<i>XPO1</i>):c.1712A>T p.E571V	41.5	TN	del(13q)	VH4-30	UM
<i>XPO1</i>	NM_003400.3(<i>XPO1</i>):c.1712A>T p.E571V	35.7	TN	Negative	VH1-69	M
<i>XPO1</i>	NM_003400.3(<i>XPO1</i>):c.1711G>A p.E571K	5.90	Treated	del(11q), del(13q)	VH3-11	UM
<i>XPO1</i>	NM_003400.3(<i>XPO1</i>):c.1711G>A p.E571K	44.10	Treated	del(17q),11q,13q	VH4-31	M
<i>XPO1</i>	NM_003400.3(<i>XPO1</i>):c.1711G>A p.E571K	8.5	TN	trisomy 12	VH1-69	UM
<i>XPO1</i>	NM_003400.3(<i>XPO1</i>):c.1711G>A p.E571K	2.7	Treated	del(13q)	VH3-74	UM
<i>XPO1</i>	NM_003400.3(<i>XPO1</i>):c.1711G>A p.E571K	20.6	Treated	13q	VH1-46	UM
<i>XPO1</i>	NM_003400.3(<i>XPO1</i>):c.1711G>A p.E571K	8.3	Treated	13q	ND	UM
<i>XPO1</i>	NM_003400.3(<i>XPO1</i>):c.1711G>A p.E571K	32.3	Treated	11q;13q	VH4-31	UM
<i>XPO1</i>	NM_003400.3(<i>XPO1</i>):c.1711G>A p.E571K	6.6	Treated	11q;13q	VH3	UM
<i>XPO1</i>	NM_003400.3(<i>XPO1</i>):c.1712A>G p.E571G	29.1	Treated	11q;13q	ND	ND
<i>XPO1</i>	NM_003400.3(<i>XPO1</i>):c.1711G>A p.E571K	46.2	Treated	11q;13q	VH 4-59	UM
<i>XPO1</i>	NM_003400.3(<i>XPO1</i>):c.1711G>A p.E571K	12	Treated	13q	ND	ND
<i>XPO1</i>	NM_003400.3(<i>XPO1</i>):c.1711G>A p.E571K	39	Treated	13q	VH-1	UM
<i>XPO1</i>	NM_003400.3(<i>XPO1</i>):c.1712A>T p.E571V, NM_003400.3(<i>XPO1</i>):c.1711G>A p.E571K, NM_003400.3(<i>XPO1</i>):c.1712A>G p.E571G	11.8,4.7 and 2.8	Treated	11q;	VH4-59	UM

Gene mutations	HGVs (Human Genome Variation Society) nomenclature	Mutant allelic frequencies (%)	Treatment status	FISH abnormalities	VH gene usage	IGHV mutation status
<i>XPO1, K-RAS</i>	NM_003400.3(<i>XPO1</i>):c.1711G>A p.E571K,, NM_004985.3(<i>KRAS</i>):c.38G>A p.G13D	37.9 (<i>XPO1</i>); <i>KRAS</i> (7.1)	Treated	13q	ND	ND
<i>XPO1, TP53</i>	NM_003400.3(<i>XPO1</i>):c.1711G>A p.E571K, NM_003400.3(<i>XPO1</i>):c.1712A>T p.E571V,, NM_000546.5(<i>TP53</i>):c.514G>T p.V172F	9.3 (<i>XPO1</i>), 6.5 (<i>TP53</i>)	TN	17p;13q	VH3	UM
<i>XPO1, TP53</i>	NM_003400.3(<i>XPO1</i>):c.1711G>A p.E571K, NM_000546.5(<i>TP53</i>):c.830G>T p.C277F	<i>XPO1</i> (19.2); <i>TP53</i> (23.9)	Treated	13q	VH1-69	UM
# <i>XPO1, TP53</i> and <i>ATM</i>	NM_003400.3(<i>XPO1</i>):c.1711G>A p.E571K, NM_000051.3(<i>ATM</i>):c.1888G>A p.V630M, NM_000546.5(<i>TP53</i>):c.376-2A>G	10.9 (<i>XPO1</i>), 8.5 (<i>TP53</i>) and 39.7 (<i>ATM</i>)	Treated	11q, 13q	VH3-30.3	UM
# <i>XPO1, ATM, NOTCH1, SF3B1</i>	NM_003400.3(<i>XPO1</i>):c.1711G>A p.E571K, NM_000051.3(<i>ATM</i>):c.7976T>C p.L2659S, NM_017617.3(<i>NOTCH1</i>):c.7540_7542del p.P2514del, NM_012433.2(<i>SF3B1</i>):c.2110A>T p.I704F	22.2 (<i>XPO1</i>), 21.6 (<i>ATM</i>), 33.1 (<i>NOTCH1</i>), 31.5 (<i>SF3B1</i>)	Treated	Negative	ND	UM
# <i>XPO1, ATM, POT1, NOTCH1</i>	NM_003400.3(<i>XPO1</i>):c.1711G>C p.E571Q, NM_000051.3(<i>ATM</i>):c.4564G>C p.G1522R, NM_015450.2(<i>POT1</i>):c.1781_1782del p.G594fs*, NM_017617.3(<i>NOTCH1</i>):c.7541_7542del p.P2514fs*	18 (<i>XPO1</i>), 9.5 (<i>ATM</i>), 8.2 (<i>NOTCH1</i>), 31 (<i>POT1</i>)	Treated	Del13q	ND	ND
# <i>XPO1, NOTCH1</i>	NM_003400.3(<i>XPO1</i>):c.1711G>A p.E571K, NM_017617.3(<i>NOTCH1</i>):c.7541_7543del p.P2514_E2515delinsQ	19.8 (<i>XPO1</i>), 30.5 (<i>NOTCH1</i>)	TN	Negative	VH2	UM
# <i>XPO1, SF3B1</i>	NM_003400.3(<i>XPO1</i>):c.1711G>A p.E571K, NM_012433.2(<i>SF3B1</i>):c.2094_2101del p.Q698fs*	19.7 (<i>XPO1</i>), 34.8 (<i>SF3B1</i>)	TN	TP53, Trisomy 12	ND	UM
# <i>XPO1, SF3B1</i>	NM_003400.3(<i>XPO1</i>):c.1711G>A p.E571K, NM_012433.2(<i>SF3B1</i>):c.2219G>A p.G740E	23.9 (<i>XPO1</i>), 22.8 (<i>SF3B1</i>)	Treated	Negative	ND	ND
# <i>XPO1, NOTCH1 and SF3B1</i>	NM_003400.3(<i>XPO1</i>):c.1711G>A p.E571K, NM_017617.3(<i>NOTCH1</i>):c.7541_7542del p.P2514fs, NM_012433.2(<i>SF3B1</i>):c.1988C>T p.T663I	7.9, 2.9 and 5.5	TN	13q	VH3	UM
# <i>XPO1, ATM</i>	c.1711G>A p.E571K	2.8 and 2.6	TN	T12	VH4-39	UM
# <i>XPO1, CARD11, K-RAS, PTPN11</i>	NM_003400.3(<i>XPO1</i>):c.1712A>G p.E571G and NM_032415.4(<i>CARD11</i>):c.383C>T p.T128M	19.6 and 21.4	Treated	11q;13q	VH3-11	UM

Gene mutations	HGVs (Human Genome Variation Society) nomenclature	Mutant allelic frequencies (%)	Treatment status	FISH abnormalities	VH gene usage	IGHV mutation status
# <i>XPO1</i> , <i>TP53</i> , <i>NOTCH1</i> , <i>POT1</i>	NM_003400.3(<i>XPO1</i>):c.1712A>G p.E571G;NM_000546.5(<i>TP53</i>):c.743G>A p.R248Q;NM_017617.3(<i>NOTCH1</i>):c.7442del p.F2481fs* and NM_015450.2(<i>POT1</i>):c.281A>G p.Q94R	2 (<i>XPO1</i>); 2.2 (<i>TP53</i>);2.2 (<i>NOTCH1</i>); 2.7 (<i>POT1</i>)	Treated	13q	VH4-59	UM
<i>XPO1</i> #	NM_003400.3(<i>XPO1</i>):c.1711G>A p.E571K	26.4	TN	Del (11q);del(13q); Trisomy12	VH2-5	M
<i>XPO1</i> #	NM_003400.3(<i>XPO1</i>):c.1711A>G p.E571G	2	Treated	Del11q;del(13q)	VH1	UM
<i>XPO1</i> #	NM_003400.3(<i>XPO1</i>):c.1711G>A p.E571K	21.1	Treated	Not done	VH3-43	UM

TN: treatment naïve; treated; previously treated; UM: unmutated immunoglobulin heavy chain (IGHV)

underwent additional testing for mutations in *NOTCH1*, *POT1*, *SF3B1* and *BIRC3* genes using a broader panel