Co-shared genomic alterations within tumors from patients with both myeloproliferative neoplasms and lymphoma

Myeloproliferative neoplasms (MPN) and lymphomas are generally considered to be distinct malignant diseases. MPN are clonal hematopoietic stem cell disorders characterized by proliferation of one or more of the myeloid-derived cell lineages. Lymphomas comprise a wide range of B- or T/NK-cell-derived malignancies.[1,2] However, in patients diagnosed with both malignancies, some lymphoid entities are overrepresented.[3,4] In a nationwide cohort of patients presenting with both MPN and lymphoma, we observed that the occurrence of T-follicular helper cell lymphoma, angioimmunoblastic type (AITL), was five to seven times higher than in the general population.[5] This, together with observations of similarities at genomic and proteomic level in patients with concurrent MPN and lymphoma,[6,7] has contributed to the hypothesis that MPN and some lymphoma subtypes may share common pathogenetic steps. To further explore this hypothesis, we investigated the mutational landscape in archival tumor samples from patients with both diagnoses.

We performed whole exome sequencing (WES) of paired bone marrow (BM) and lymphoma tissue samples from patients diagnosed, either simultaneously or metachronously, with both MPN and lymphoma, of either AITL or diffuse large B-cell lymphoma (DLBCL) type. To our knowledge, the present study is the first to report the occurrence of shared genomic alterations within the disease-specific tumor samples from patients diagnosed with MPN and either AITL or DLBCL.

A Danish cohort of patients diagnosed with both MPN and lymphoma between 1990-2015 was previously described.[5] Diagnostic tumor samples from 14 patients with MPN, diagnosed prior or synchronous to either AITL (N=5) or DLBCL (N=9), were identified through the Danish National Pathology Registry (DNPR).[8] We excluded patients with MPN diagnosed >6 months after lymphoma to reduce the probability of therapy-induced MPN. Clinicopathological characteristics are summarized in Table 1.

The study was approved by the Danish National Committee on Health Research Ethics (record no. 1609521) and the Danish Data Protection Agency (record no. 1-16-02-420-15) and was performed in compliance with the principles of the Helsinki Declaration. Before inclusion, written informed consent was obtained. Exception was made in cases where the patient had died at the time of the study, to which separate permission was granted in the ethics approval.

All patients were diagnosed with MPN based on BM samples, while the lymphoma diagnoses were based on either lymph node or extra-nodal tissue biopsies. Non-neoplastic tissue from archived specimens in the DNPR or saliva (in cases where no archived non-neoplastic tissue was available) were used as germline control. All specimens were reviewed by an experienced hematopathologist at a tertiary-care center. Immunohistochemical characterizations were performed to verify the adequacy of study specimens regarding the presence of MPN and lymphoma in relation to tumor content and to assess for any reciprocal infiltration of MPN into lymphoma or *vice versa*. A DNA library triad encompassing the 3-way paired MPN, lymphoma, and non-neoplastic tissue specimens was constructed for WES following standard protocols. Captured targets were paired-end sequenced according to standard protocols. Raw sequencing data was quality checked. We observed a high level of indels and C:G-T:A substitutions, probably induced by formalin-fixation of the specimens. To reduce the false-positive rate, the data was processed on two independent mutation-calling pipelines. Variants with allele frequencies (VAF) <10% were excluded from the analyses, and only somatic variants reported in COSMIC[9] were retained for downstream evaluation. Variants within genes with a well-established relevance for the diseases addressed in the present study, were manually curated regardless of VAF. This involved variants in *DNMT3A, TET2, IDH2, RHOA, and JAK2*.[1,2] Alleles with depth coverage of ≥20 reads were evaluated. Variants present in the gnomAD database (version 2.1.1),[10] European non-Finnish population, at a frequency above 0.01 were removed. Quality and read depth assessment were complemented with the inspection of focus variants in Integrative Genomics Viewer.[11]

In patients diagnosed with MPN and AITL, the fraction of myelopoietic tissue in the MPN BM samples varied between 40-95% (Table 1). Tumor cell content in the AITL samples was estimated ranging between 60-90%. In patients diagnosed with MPN and DLBCL (patients #6-14), the fraction of myelopoietic tissue in the MPN BM samples varied between 80-95%. Tumor cell content in the DLBCL samples was estimated in the range 70-90%, except for patient #7 in which there was a low content of 15% due to adjacent salivary gland tissue.

Figure 1 shows an overview of the identified mutations with VAF ≥10% for both MPN/AITL and MPN/DLBCL patients.

Specific mutations, well characterized in the literature as being associated with MPN and AITL are presented in Table 2 in more detail. Shared mutations were defined by an

Table 1. Demographic and clinicopathological characteristics.

Patient #	Sex	MPN diagnosis	Age in years at MPN diagnosis	MPN treatment	ΔDx	MPN tissue	Lymphoma tissue	Non-neoplastic germline tissue	Tumor content		First line lymphoma treatment	Survival from lymphoma diagnosis in years
									MPN sample %	Lymphoma sample %		
AITL patients												
1	M	PMF	64	None	0	BM	LN	Lung	40	70	CHOP	5.3
2	F	ET	73	ASA	2.6	BM	LN	Skin	90	80	CHOP	8.2
3	M	ET	60	ASA	4.8	BM	LN	Skin	95	85	CHOEP+ASCT	6.0
4	F	PV	72	ASA, P, HC	3.5	BM	LN	Skin	95	60	Palliation	<0.1
5	F	MPN-U	62	None	0	BM	LN	Skin	85	90	CHOEP+ASCT	Alive*
DLBCL patients												
6	M	PV	71	ASA, P, HC	8.3	BM	Skin, bone	Skin	95	90	Palliation	<0.1
7	M	MPN-U	71	ASA, P, HC	3.8	BM	SG	GIT	95	15	R-CHOP	8.4
8	F	PV	68	ASA, P, HC	2.6	BM	LN	Ovary	90	70	R-CHOP	4.5
9	F	ET	68	NR	16.6	BM	SG	Skin	90	90	NR	NR
10	F	PV	65	None	0	BM	LN	GIT	90	75	R-CHOP	0.3
11	F	ET	76	HC	9.5	BM	GIT	Saliva	80	90	R-CHOP	1.5
12	F	MPN-U	66	ASA, P	0	BM	LN	Saliva	90	90	R-CHOP	Alive*
13	M	ET	65	ASA, HC	12.3	BM	GIT	Saliva	80	90	R-CHOP	0.6
14	M	PV	71	I, P	3.1	BM	Pleura	Saliva	80	75	R-CHOP+MTX	Alive*

AITL: angioimmunoblastic T-cell lymphoma; ASA: acetylsalicylic acid; ASCT: autologous stem cell transplantation; BM: bone marrow; CHOEP: cyclophosphamide, hydroxydaunorubicin, oncovin, etoposide, prednisolone; CHOP: cyclophosphamide, hydroxydaunorubicin, oncovin, prednisolone; ΔDx: time between myeloproliferative neoplasms (MPN) and lymphoma diagnosis in years; DLBCL: diffuse large B-cell lymphoma; ET: essential thrombocythemia; F: female; GCB: germinal-center B-cell like; GIT: gastrointestinal tract; HC: hydroxycarbamide; I: interferon; LN: lymph node; M: male; MPN: myeloproliferative neoplasm; MPN-U: MPN unclassifiable; MTX: methotrexate; NR: not reported; P: phlebotomy; PMF: primary myelofibrosis; PV: polycythemia vera; R: rituximab; SG: salivary gland; * alive at time of data analysis but exact survival time not reported.

A

Genes	MPN/AITL (patients #1-5)										Function impact*
	1		2		3		4		5§		
	M	L	M	L	M	L	M	L	M	L	
SF1	11										Medium
DNMT3A			29	31							Medium
TET2	21	38			48	33					High
IDH2		15							2	2	Medium
RHOA		29							9		Medium
JAK2			9#	41#	36#		72				Medium
NOTCH2						25					Medium
NCOR1					33			18	10		Medium
ABL1		13									Medium
NSD1		10									Medium
IRF4		17									Medium
INVS		11									Medium
DICER1		12									Medium
TAF1							63				Medium
BCOR		11									Medium
MTOR		22									Medium
SMC1A		20									Medium
BCL2		21									High
NRAS						12			80		Medium
STAT3										16	Medium
PTEN							35				High
CDC73							29				Medium
TET1							21				Medium
BCOR							16				Medium

Frameshift mutation
Missense mutation
Stop-gain mutation

Figure 1. Overview of shared and private mutations in the study cohort. Shared and private mutations with variant allele frequencies (VAF) ≥10% identified by whole exome sequencing. Canonical myeloproliferative neoplasm (MPN) and lymphoma-entity associated mutations are also reported at lower VAF levels. The numbers in the individual boxes indicate VAF (%). (A) Mutations identified in patients with angioimmunoblastic T-cell lymphoma (AITL) and MPN. §In patient #5, MPN and AITL were diagnosed simultaneously. The bone marrow was morphologically and immunohistochemically found to be infiltrated by both neoplasms. The mutational findings in this patient should therefore be interpreted with caution. (B) Mutations identified in patients with diffuse large B-cell lymphoma (DLBCL) and MPN. #Identical variant found in non-neoplastic tissue (germline mutation). *According to International Cancer Genome Consortium terminology, www.dcc.icgc.org. AITL: angioimmunoblastic T-cell lymphoma; DLBCL: diffuse large B-cell lymphoma; M: myeloid sample; L: lymphoid sample.

B

Genes	MPN/DLBCL (patients #6-14)																		Function impact*
	6		7		8		9		10		11		12		13		14		
	M	L	M	L	M	L	M	L	M	L	M	L	M	L	M	L	M	L	
NRAS		16																	Medium
MYD88		63										58							High
JAK2	92#	15#	12		72#	14#	13										20		Medium
MPL			10												10				High
TP53												26							High
POU2F2				13															Medium
TET2							15												High
PIM1								30				26							Medium
DUSP2								24				32						22	Medium
CD58								20											High
B2M										19									High
EZH2										12									Medium
TBL1XR1												29							Medium
ETV6												34							Medium
SOCS1														15					Medium
HIST1H1E														14					Medium
NFKBIA														11					Medium
BCL2																44			High
CCND3																49			Medium
SPEN																48			High
TNFAIP3																		27	High
NFKBIE																		29	High
PIK3R1																		19	High
IKBKB																		31	Medium
PAX5																		28	Medium

Frameshift mutation
Missense mutation
Stop-gain mutation

Table 2. Detailed representation of selected mutations identified in the study cohort.

Patient #	Somatic mutations					MPN	Lymphoma	Present in germline control (VAF %)
	Gene	Position	Nucleotide change	Mutation type	Amino acid change	VAF %	VAF %	
AITL patients								
1	*DNMT3A*	chr2:25470979	G>A	Missense	p.T261M	12	-	No
	DNMT3A	chr2:25470521	C>T	Missense	p.R318Q	-	7	No
	TET2	chr4:106157053	C>T	Stop gain	p.Q673	21	38	No
	IDH2	chr15:90631839	T>C	Missense	p.R172G	-	15	No
	RHOA	chr3:49412973	C>A	Missense	p.G17V	-	29	No
2	*DNMT3A*	chr2:25462017	T>G	Missense	p.N797T	29	31	No
	JAK2	chr9:5073770	G>T	Missense	p.V617F	9	31	Yes (15)
3	*TET2*	chr4:106164778	C>T	Stop gain	p.R1237	48	33	No
	JAK2	chr9:5073770	G>T	Missense	p.V617F	36	-	Yes (34)
4	*JAK2*	chr9:5072541	G>A	Missense	p.R64Q	72	-	No
5[§]	*IDH2*	chr15:90631838	C>A	Missense	p.R172M	2	2	No
	RHOA	chr3:49412973	C>A	Missense	p.G17V	9	-	No
DLBCL patients								
6	*JAK2*	chr9:5073770	G>T	Missense	p.V617F	92	15	Yes (22)
8	*JAK2*	chr9:5073770	G>T	Missense	p.V617F	72	14	Yes (8)

Identical mutations shared between the myeloproliferative neoplasm (MPN) and lymphoma, within the same patient, are highlighted in grey. Several shared mutations were identified in the MPN/angioimmunoblastic T-cell lymphoma group while shared mutations were identified in only two MPN/diffuse large B-cell lymphoma patients involving exclusively p.V617F *JAK2* mutation canonical for MPN. [§]In patient #5, MPN and lymphoma were diagnosed simultaneously. The bone marrow was infiltrated by both neoplasms. The mutational findings in this patient should therefore be interpreted with caution. AITL: angioimmunoblastic T-cell lymphoma; DLBCL: diffuse large B-cell lymphoma; MPN: myeloproliferative neoplasm; VAF: variant allele frequency.

identical position and nucleotide change within the given gene sequence. In four of five AITL patients, mutations in either of the epigenetic modifier genes *DNMT3A, TET2*, or *IDH2* were identified. Notably, a mutation in *IDH2* leading to amino acid change at position R172 was found in two of five patients. A *RHOA* mutation resulting in the G17V change was found in the AITL sample of patient #1. In patient #5, MPN and AITL were simultaneously diagnosed. This patient had discrete lymphoma infiltration in the BM, where a *RHOA* mutation was identified. The same mutation could not be detected in the lymph node biopsy.

Both AITL patients with *IDH2* mutations had a concurrent *RHOA* mutation. Mutations in the *JAK2* gene were found in three of the MPN samples. Two cases harbored the *JAK2* V617F amino acid change, while the third was *JAK2* exon 12 mutation-positive.

In the nine DLBCL patients, mutations in genes commonly associated with lymphoid neoplasms (*B2M, BCL2, CCND3, EZH2*, TP53, *NKFBIE, PAX5* and *MYD88*), were identified with high allelic burdens (Figure 1). *JAK2* mutations resulting in V617F amino acid change were found in five of these patients' MPN samples. One patient harbored a *MPL* mutation in the MPN sample.

Shared mutations were found in three of five (60%) MPN/AITL patients and involved *DNMT3A, JAK2* and *TET2*. *IDH2* was found in both tissue samples of patient #5, but due to simultaneous diagnosis of MPN and AITL and BM infiltration of lymphoma, this mutation could not confidently be classified as shared. Shared mutations were found in two of nine (22%) MPN/DLBCL patients involving only *JAK2* (Table 2).

DNMT3A and *TET2* play essential roles in hematopoietic stem cell differentiation and abnormal function of these genes may lead to impaired hematopoietic differentiation capacity and to the accumulation of clonal hematopoiesis (CH).[12-14] Shared mutations of *DNMT3A* (patient #2) and *TET2* (patients #1 and #3) were present with high allelic burdens (range 19-49%), supporting the hypothesis that these mutations may be involved in the early common pathogenetic steps of both MPN and AITL. The allelic burden of *DNMT3A* and *TET2* mutations were higher than those of *IDH2* and *RHOA* in the AITL samples, suggesting that the latter mu-

tations could represent more downstream events.[14]
These observations support the possible parallel evolution of two distinct neoplastic proliferations, a myeloid and a lymphoid, from a common hematopoietic progenitor cell population that carry CH features such as *TET2* and *DNMT3A* mutations. These findings extend previous reports of the development of metachronous AITL and myeloid neoplasms from a common *TET2/DNMT3A*-mutated stem cell population in patients with CH.[15]
Eight of the 14 patients harbored *JAK2* mutations: three of five (60%) of the MPN/AITL patients and five of nine (56%) of the MPN/DLBCL patients. In patient #2 (AITL) and patients #6 and #8 (DLBCL), the *JAK2* mutation was shared. The non-neoplastic tissue of these patients also carried the same *JAK2* variant with a low allele frequency (Table 2). While this may represent *in vivo* tumor cell contamination of the samples, another possible interpretation is that the presence of a *JAK2* germline mutation predisposed to the subsequent development of MPN and lymphoma.
The risk of cross-contamination between MPN and lymphoma cannot be excluded with certainty, as demonstrated by the findings in patient #5. However, this risk is mitigated by factors such as: (i) expert specimen review by a tertiary-center hematopathologist; (ii) the diagnosis of MPN dating several years before the lymphoma diagnosis in most patients (10/14), reducing the probability of lymphoma cells being present in the MPN samples. Of the five patients with shared mutations (3 MPN/AITL and 2 MPN/DLBCL), four had an interval between the diagnosis of MPN and lymphoma of ≥2.6 years. In the last of these five patients (patient #1), AITL was diagnosed simultaneously with MPN, but without evidence of lymphoma in the BM; (iii) a high tumor cell content in most specimens, increasing the probability that the DNA extracted and sequenced from MPN and lymphoma tissue is representative of the respective neoplasm.
For future investigations, the application of single-cell and spatial multi-OMICS will likely improve the level of precision of clonal recognition and development.
In conclusion, we identified shared and private mutations in patients with co-occurrent MPN and lymphoma. Some of these mutations, particularly in the setting of MPN/AITL, may reflect ancestral pathogenetic alterations related to CH (e.g., *TET2, DNMT3A*), while others (e.g., *IDH2, RHOA*) may facilitate downstream clonal divergence and expansion. These events seem to be less frequent in DLBCL than AITL. However, additional data from larger, independent studies are required to provide support for these hypotheses.

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Contributions
JMH, MBP, WCC, GI, and FdA conceptualized the study. TLP, JMH, GI, and SJHD validated tissue specimens. JMH, TLP, HF, MBM, SJHD, PN, and BKM collected the samples. JMH, MCH, and NJB performed the biostatistical analyses. JMH, MBP, MBE, MCH, PRN, TLP, HF, MBM, SJHD, PN, BKM, HBO, JS, WT, NJB, ML, WCC, GI, and FdA made substantial contributions to the acquisition and interpretation of data. JMH, MBP, PRN, MBE, NJB, and FdA drafted the original manuscript. All authors critically revised and contributed to the completion of the final manuscript.

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Data-sharing statement

Due to patient confidentiality and Danish data protection regulations, original clinico-pathological and genomic data (including raw sequencing data) cannot be made publicly available. However, data can be shared upon reasonable request and in accordance with GDPR and current national legislation.

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