REGULAR ARTICLE

blood advances

Somatic mutations in FAS pathway increase hemophagocytic lymphohistiocytosis risk in patients with T- and/or NK-cell lymphoma

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Key Point

- Somatic mutations in FAS pathway are associated with HLH in T- and/or natural killer–cell lymphoma.

Although significant progress has been made in understanding the genetic basis of primary hemophagocytic lymphohistiocytosis (HLH), the pathogenesis of secondary HLH, the more prevalent form, remains unclear. Among the various conditions giving rise to secondary HLH, HLH in patients with lymphoma (HLH-L) accounts for a substantial proportion. In this study, we investigated the role of somatic mutations in the pathogenesis of HLH-L in a cohort of patients with T- and/or natural killer–cell lymphoma. We identified a 3-time higher frequency of mutations in FAS pathway in patients with HLH-L. Patients harboring these mutations had a 5-time increased HLH-L risk. These mutations were independently associated with inferior outcome. Hence, our study demonstrates the association between somatic mutations in FAS pathway and HLH-L. Further studies are warranted on the mechanistic role of these mutations in HLH-L.

Introduction

Hemophagocytic lymphohistiocytosis (HLH) is a severe hyperinflammatory syndrome caused by abnormal systemic activation of macrophages and cytotoxic T cells.[1] Primary (inherited/familial) HLH, most common in children, is caused by germline mutations affecting lymphocyte cytotoxicity and immune regulation.[2,3] Secondary HLH (sHLH) usually affects adults and is commonly triggered by infections, malignancies, and/or other conditions, resulting in macrophage hyperactivation.[3] Lymphoma is among the most frequent malignancies associated with HLH, with T- and/or natural killer (NK)-cell (T/NK-cell) lymphoma being more common than B-cell lymphoma.[4,5] HLH-L has a poor prognosis, underscoring the need to improve the prediction and management of this condition.[6,7] However, the pathogenesis of HLH-L remains poorly understood.[6] Besides infections such as the Epstein-Barr virus (EBV), the underlying malignancy itself, and therapeutic interventions as known sHLH triggers, variants in genes involving familial HLH have recently been reported by a few groups in adult patients with sHLH, but the pathogenicity of these variants remains inconclusive,[8-10] suggesting different pathogenesis between familial HLH and adult-onset sHLH.[10] A recent study demonstrated high prevalence of clonal hematopoiesis in sHLH,[11] implicating that somatic mutations in blood cells may lead to hyper-inflammatory responses, a critical feature of HLH. There is lack of studies on the association between

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The full-text version of this article contains a data supplement.

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somatic mutations in neoplastic cells and sHLH. Germline and somatic *FAS* mutations, which lead to defective activation-induced cell death and the accumulation of self-reactive T cells, are the most common cause of autoimmune lymphoproliferative syndrome (ALPS).[12-14] ALPS and HLH have many overlapping clinical and laboratory features,[15] raising the possibility of a shared etiology, for example, *FAS* mutations. In this study, we set out to investigate the frequency of somatic mutations including *FAS* and its association with sHLH in patients with T/NK-cell lymphomas.

Methods and patients

Patients

The pathology archives between January 2016 and August 2023 at Memorial Sloan Kettering (MSK) Cancer Center were searched to identify patients with a diagnosis of a T/NK-cell lymphoma/ lymphoproliferative disorder (LPD). The key words "non-Hodgkin" or "NK cell lymphoma or leukemia" or "T cell lymphoma (TCL)" or "mature T cell lymphoma" or "atypical NK cell" or "atypical T cell" or "T cell LPD" were used to identify patients. Only patients who had next-generation sequencing (NGS) studies with matched germline DNA control for analysis (see below) were further reviewed. A detailed chart review was performed to obtain clinical and laboratory parameters, to confirm the diagnosis and to identify patients with HLH. Diagnosis of HLH was made according to the HLH-2004 criteria.[16] In addition, a positive or negative optimized HLH inflammatory (OHI) index[17] based on elevated soluble CD25 (>3900 U/mL) and ferritin (>1000 ng/mL) was also assigned to each patient at the time of their lymphoma diagnosis or during treatment courses. Informed consent was obtained from patients. This study was approved by the MSK Institutional Review Board.

NGS and analysis

Mutational profiling was performed on formalin-fixed, paraffin–embedded lymphoma tissues (bone marrow or peripheral blood for patients with leukemia) by a targeted NGS panel MSK Integrated Mutational Profiling of Actionable Cancer Targets (MSK-IMPACT).[18] Nail DNA was collected as a germline control. However, germline analysis was not permitted in this study. To evaluate the pathogenicity, somatic variants were classified as oncogenic, likely oncogenic, or variant of unknown significance as assessed according to the Clinical Genome Resource, Cancer Genomics Consortium, and Variant Interpretation for Cancer Consortium guidelines. The impact of missense variants was further evaluated by Polyphen-2 and sorting intolerant from tolerant (SIFT).[19,20]

Immunohistochemistry studies

Staining was performed on the Leica Bond-3 auto staining system (Leica, Deer Park, IL), using enzymatic digestion as pretreatment (AR9551; 10 minutes; Enzyme 1, Bond Enzyme Pre-treatment Kit, Leica), 30-minute primary incubation time, and a polymer detection system (DS9800; Bond Polymer Refine Detection, Leica). FAS antibody used is clone LT95 (NB500-503, Novus) with the dilution of 1:200 and the final concentration of 5 mg/mL.

Statistical analysis

Patient characteristics were summarized by frequency (percentage) or medians with interquartile range. Associations between mutation frequencies and disease characteristics were tested by Fisher exact test, Pearson χ^2 test, and Wilcoxon rank sum test. Cumulative incidence curves for time-to-HLH diagnosis from lymphoma diagnosis were estimated with a death as competing event and left truncation at the date of genomic testing. Associations with genetic and clinical characteristics and risk of HLH were assessed using cause-specific Cox proportional hazards model. Overall survival (OS) from the time of HLH testing, with left truncation at date of genomic testing, was evaluated by Kaplan-Meier method, and the difference between groups were determined by Cox proportional hazards models. OS from the time of lymphoma diagnosis, with left truncation at date of genomic testing, was also evaluated by Kaplan-Meier method, and the differences between groups were determined by Cox proportional hazards models. All statistical analyses were performed using R 4.3.2.

Results

HLH in patients with T- and NK-cell lymphoma

In total, 433 patients were included in this study meeting the following criteria: carrying a diagnosis of T/NK-cell lymphoma or LPD, and having matched MSK-IMPACT analysis on neoplastic cells (supplemental Table 1). HLH was diagnosed in 29 patients (6.7%; 17 men and 12 women; median age, 60 years) based on 2004 criteria, none of whom were after hematopoietic stem cell transplant. These patients included angioimmunoblastic T-cell lymphoma (AITL; 2/89 [2.2%]), peripheral T-cell lymphoma, not otherwise specified (PTCL, NOS; 13/78 [16.7%]), anaplastic large cell lymphoma (ALCL; 2/47 [4.3%]), large granular lymphocytic leukemia (LGLL; 3/46 [6.5%]), adult T-cell leukemia/ lymphoma (ATLL; 2/25 [8.0%]), extranodal T/NK-cell lymphoma (ENKTL; 1/12 [8.3%]), hepatosplenic T-cell lymphoma (2/7 [28.6%]), aggressive NK-cell leukemia (ANKL; 2/3 [66.7%]), mycosis fungoides/Sézary syndrome (1/62 [1.6%]), and other types of cutaneous T-cell lymphoma (CTCL; 1/19 [5.3%]). Notably, HLH was not found in patients with T-prolymphocytic leukemia (T-PLL; 0/23 [0%]) and intestinal T-cell lymphoma (0/11 [0%]). HLH was diagnosed at a median of 9.4 months (interquartile range, 3.5-15.8) after the diagnosis of lymphoma. Of the 29 patients, 27 had markedly increased soluble CD25 (>10 000 pg/mL), and 28 had ferritin levels >1000 ng/mL.[17] EBV reactivation was detected in 17 cases at or before the onset of HLH (Table 1). Of the 29 patients with positive HLH, 28 patients succumbed to disease with a median survival of 22 days (95% confidence interval [CI], 13-60) after HLH diagnosis (supplemental Figure 1). Because the OHI index may be more sensitive for identifying malignancy-associated HLH,[17] we also evaluated OHI index in our cohort. Forty-nine patients were positive for OHI index (OHI-positive), and 28 of them also met the 2004 criteria (supplemental Table 2). Patients meeting either the 2004 criteria or OHI index were both associated with inferior OS (supplemental Figure 1).

Somatic mutations in FAS pathway associated with HLH in patients with T- and NK-cell lymphoma

We evaluated the mutational landscape of T/NK-cell lymphoma in our cohort (Figure 1). The most frequently mutated genes were *TET2*, *TP53*, *STAT3*, *ROHA*, and *DNMT3A*. As expected,

Table 1. Clinicopathologic characteristics of patients with HLH and/or *FAS* mutations

PID	HLH	FAS mutation	Mutation significance*	VAF	IHC	LOH	Age	Sex	Lymphoma subtypes	ALC, K/mL	HLH, mo	Death, mo	Hemophagocytosis	EBV titer, IU/mL	sIL2R, pg/mL	Ferritin, ng/mL
1	Yes	No	NA	NA	ND	NA	19	F	ENKTL	0.8	3	3	No	>800 000	10 997	135 680
2	Yes	No	NA	NA	ND	NA	53	M	ATLL	2.8	1	1	No	7966	>20 000	123 756
3	Yes	No	NA	NA	ND	NA	69	M	AITL	0.2	8	9	Yes	0	>20 000	19 962
4	Yes	No	NA	NA	ND	NA	23	M	HSTCL	1.9	5	5	No	<400	>80 000	30 529
5	Yes	No	NA	NA	ND	NA	48	F	HSTCL	3.3	15	16	No	0	>20 000	54 647
6	Yes	No	NA	NA	ND	NA	62	M	PTCL, NOS	0.1	1	12	Yes	0	14 250	5463
7	Yes	No	NA	NA	ND	NA	69	F	PTCL, NOS	0.5	6	7	No	0	19 525	47 693
8	Yes	No	NA	NA	ND	NA	61	F	PTCL, NOS	0.1	18	18	No	7313	6699	140 740
9	Yes	No	NA	NA	ND	NA	16	M	PTCL, NOS	0.3	1	alive	Yes	0	>20 000	11 782
10	Yes	No	NA	NA	ND	NA	55	F	PTCL, NOS	0.4	12	13	Yes	3105	18 355	13 393
11	Yes	No	NA	NA	ND	NA	65	M	PTCL, NOS	6.7	7	8	No	0	>20 000	19 502
12	Yes	No	NA	NA	ND	NA	59	M	PTCL, NOS	0.3	22	22	No	12 408	>20 000	5547
13	Yes	No	NA	NA	ND	NA	62	M	PTCL, NOS	34.3	9	9	ND	2333	>20 000	17 800
14	Yes	No	NA	NA	ND	NA	54	M	PTCL, NOS	0.6	1	1	Yes	0	38 945	42 659
15	Yes	No	NA	NA	ND	NA	77	M	PTCL, NOS	0.8	5	8	No	6621	>20 000	21 966
16	Yes	No	NA	NA	ND	NA	72	M	PTCL, NOS	0.7	17	18	Yes	0	>20 000	9246
17	Yes	No	NA	NA	ND	NA	61	F	PTCL, NOS	0.2	6	14	No	<400	>20 000	8906
18	Yes	No	NA	NA	ND	NA	64	F	T-LGLL	1.2	13	15	No	3214	8989	4021
19	Yes	No	NA	NA	ND	NA	69	M	T-LGLL	0.5	14	17	Yes	<400	>20 000	27 328
20	Yes	No	NA	NA	ND	NA	71	M	T-LGLL	0.5	12	13	Yes	1683	10 975	82 206
21	Yes	No	NA	NA	ND	NA	56	F	MF	1.0	16	16	No	<400	>20 000	2282
22	Yes	No	NA	NA	ND	NA	68	F	CD8+ PCAETL	0.5	42	42	No	3557	>20 000	972
23	Yes	No	NA	NA	ND	NA	54	F	ALCL	3.1	12	12	Yes	0	>20 000	22 621
24	Yes	No	NA	NA	ND	NA	49	M	ALCL	0.3	79	80	ND	0	>20 000	15 073
25	Yes	L224*	Likely oncogenic	0.42	Pos	No	68	F	AITL	0.4	25	27	Yes	5773	>20 000	12 277
26	Yes	W281*	Likely oncogenic	0.41	Neg	No	57	F	PTCL, NOS	0.3	2	2	No	1356	>20 000	64 718
27	Yes	D260N	predicted damaging	0.26	Neg	ND	47	M	ATLL	0.7	55	58	No	0	>20 000	2286
28	Yes	S230Lfs*4	predicted damaging	0.05	Neg	No	67	M	ANKL	0.6	3	3	Yes	1408	>20 000	20 597
29	Yes	N264K	predicted damaging	0.10	Neg	No	71	M	ANKL	0.1	0	10	Yes	0	38 866	>33 500
30	No	C135Vfs*52	Likely oncogenic	0.03	ND	No	52	M	MEITL	2.2	NA	alive	No	ND	ND	ND
31	No	V220Wfs*3	Likely oncogenic	0.18	Neg	Yes	61	F	PTCL, NOS	0.5	NA	alive	No	0	ND	ND
32	No	X218_splice	Likely oncogenic	0.12	ND	ND	86	F	PTCL, NOS	0.3	NA	70	No	ND	ND	ND
33	No	I233Yfs*14	Likely oncogenic	0.21	ND	ND	68	F	PTCL, NOS	1.6	NA	alive	No	0	ND	ND
34	No	N302Vfs*57	Likely oncogenic	0.66	ND	Yes	75	M	PTCL, NOS	1.4	NA	49	No	0	ND	ND

HLH represents months from TCL diagnosis to HLH diagnosis; and death represents months from TCL diagnosis to decease.

ALC, absolute lymphocyte counts; $CD8^+$ PCAETL, Primary cutaneous $CD8^+$ epidermotropic cytotoxic T-cell lymphoma; ENKTL, extranodal T/NK-cell lymphoma, nasal type; IHC, immunohistochemistry; LOH, loss of heterozygosity; MEITL, monomorphic epitheliotropic intestinal T-cell lymphoma; NA, not applicable; ND, not done/determined; Neg, negative; PCGDTCL, primary cutaneous gamma delta T-cell lymphoma; Pos, positive; T-LGLL, T-large granular lymphocytic leukemia; VAF, variant allelic frequency.

*Mutations were classified as oncogenic, likely oncogenic, or variant of unknown significance (VUS) as assessed according to the Clinical Genome Resource, Cancer Genomics Consortium, and Variant Interpretation for Cancer Consortium guidelines. VUS were further evaluated by Polyphen 2 and SIFT. A score of Polyphen2 >0.9 and/or of SIFT <0.05 was deemed as damaging.

Table 1 (continued)

PID	HLH	FAS mutation	Mutation significance*	VAF	IHC	LOH	Age	Sex	Lymphoma subtypes	ALC, K/mL	HLH, mo	Death, mo	Hemophagocytosis	EBV titer, IU/mL	sIL2R, pg/mL	Ferritin, ng/mL
35	No	S230Efs*2	Likely oncogenic	0.45	Neg	No	56	M	PTCL, NOS	4.5	NA	19	No	0	ND	ND
36	No	X66_splice	Likely oncogenic	0.12	Neg	No	82	F	PTCL, NOS	0.7	NA	9	No	ND	ND	ND
37	No	E272I	predicted damaging	0.07	ND	Yes	48	M	PTCL, NOS	ND	NA	alive	No	ND	ND	ND
38	No	D269G	predicted damaging	0.33	ND	ND	77	F	PTCL, NOS	1.6	NA	44	No	0	ND	1476
39	No	L242Pfs*5	Likely oncogenic	0.44	Pos	No	74	M	ATLL	99.8	NA	3	No	0	>20 000	595
40	No	E261V	predicted damaging	0.37	ND	ND	74	F	ATLL	NA	NA	alive	No	ND	ND	ND
41	No	G286A	predicted damaging	0.25	ND	ND	65	F	ATLL	5.1	NA	alive	No	ND	ND	ND
42	No	L298Yfs*8	Likely oncogenic	0.77	Pos	Yes	86	M	T-LGLL	3.2	NA	8	No	0	ND	ND
43	No	D260G	predicted damaging	0.35	ND	No	68	M	T-LGLL	1	NA	alive	No	ND	ND	ND
44	No	Q273*	Likely oncogenic	0.09	ND	No	71	M	T-LGLL	0.6	NA	alive	No	ND	ND	484
45	No	D265G	predicted damaging	0.39	ND	ND	58	M	ENKTL	2.1	NA	alive	No	0	ND	ND
46	No	N252D	predicted damaging	0.12	ND	ND	80	M	AITL	1.1	NA	alive	No	0	ND	4138
47	No	E150*	Likely oncogenic	0.49	Neg	Yes	76	M	PCGDTCL	2.9	NA	alive	No	ND	ND	34
48	No	C119Afs*68	Likely oncogenic	0.03	Neg	No	41	F	SS	4.1	NA	alive	No	0	ND	239
49	No	H142D	Predicted damaging	0.97	Neg	Yes	81	M	SS	1.2	NA	alive	No	ND	ND	ND
50	No	X66_splice	Likely oncogenic	0.12	ND	Yes	62	M	MF	0.2	NA	19	No	0	ND	1674
51	No	X226_splice	Likely oncogenic	0.45	ND	No	65	M	MF	1.1	NA	alive	No	0	ND	ND
52	No	F134_C135ins*	Likely oncogenic	0.46	Neg	Yes	59	F	T-PLL	1.2	NA	alive	No	ND	ND	ND

HLH represents months from TCL diagnosis to HLH diagnosis; and death represents months from TCL diagnosis to decease.

ALC, absolute lymphocyte counts; CD8$^+$ PCAETL, Primary cutaneous CD8$^+$ epidermotropic cytotoxic T-cell lymphoma; ENKTL, extranodal T/NK-cell lymphoma, nasal type; IHC, immunohistochemistry; LOH, loss of heterozygosity; MEITL, monomorphic epitheliotropic intestinal T-cell lymphoma; NA, not applicable; ND, not done/determined; Neg, negative; PCGDTCL, primary cutaneous gamma delta T-cell lymphoma; Pos, positive; T-LGLL, T-large granular lymphocytic leukemia; VAF, variant allelic frequency.

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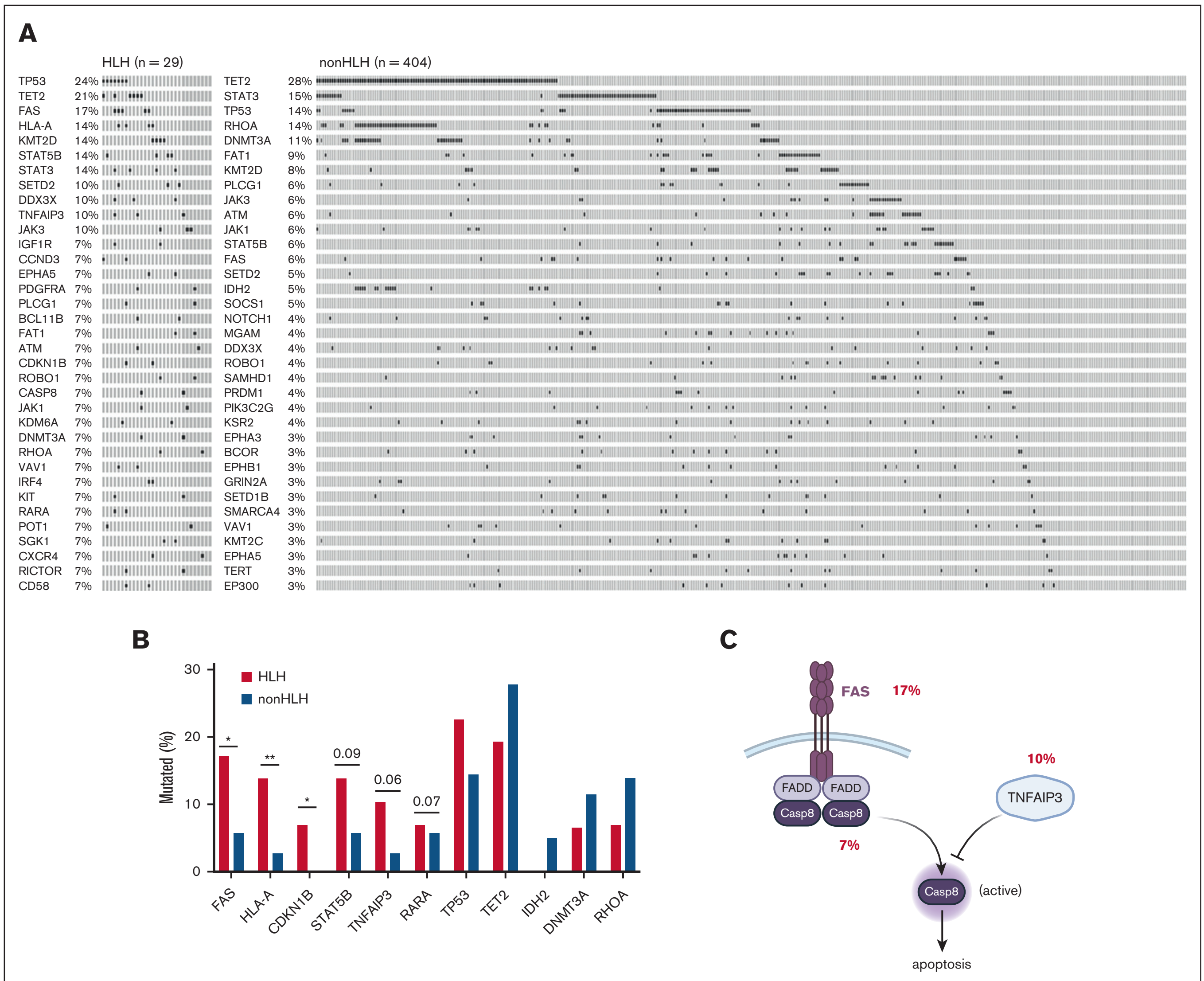

Figure 1. Mutational profiles between patients with and without HLH. (A) Oncoprints (only top 30 mutated genes were shown). (B) Bar plots showing the comparison of mutations in various genes. (C) Illustration of mutations in FAS pathway; figure created with BioRender.com.

mutations in *TET2*, *DNMT3A*, and *RHOA* were highly prevalent in AITL, whereas *STAT3* mutations were enriched in indolent TCL, mostly LGLL. Interestingly, *TP53* mutations were present in nearly a third of PTCL, NOS.

To identify genetic alterations associated with increased HLH risk, mutational profiles were compared between patients with and without HLH (Figure 1A). The frequent mutated genes were *TP53* (24.1%), *TET2* (20.7%), *FAS* (17.2%), and *HLA-A* (13.8%) in patients with HLH and *TET2* (27.7%), *STAT3* (14.6%), *TP53* (14.4%), and *RHOA* (13.9%) in patients without HLH. Importantly, *FAS*, *HLA-A, CDKN1B*, and *CASP8* mutations were more frequent in patients with HLH than those without ($P = .03$; $P = .01$; $P = .01$; and $P = .03$, respectively; Figure 1B). The frequencies of *STAT5B*, *RARA*, and *TNFAIP3* mutations were also borderline increased in patients with HLH ($P = .09$; $P = .07$; and P = 0.06, respectively). Notably, *FAS*, *CASP8*, and *TNFAIP3* encode proteins critical for the FAS pathway (Figure 1C). Among patients with HLH, 27.6% had at least 1 mutation in the FAS pathway compared with 8.9% of patients without HLH ($P = .004$).

We next examined the association of gene mutations with HLH risk using univariable cause-specific Cox regression. Among all the patients undergoing HLH evaluation, we found an increased risk of HLH in patients harboring somatic mutations in *FAS* (hazard ratio [HR], 4.24; 95% CI, 1.46-12.4; $P = .008$), *CASP8* (HR, 13.4; 95% CI, 3.14-57.5; $P < .001$), *HLA-A* (HR, 5.71; 95% CI, 1.96-16.6; $P = .001$), *CDNK1B* (HR, 19.2; 95% CI, 4.40-83.5; $P < .001$), *STAT5B* (HR, 3.45; 95% CI, 1.19-10.0; $P = .022$), *TNFAIP3* (HR, 5.20; 95% CI, 1.56-17.4; $P = .007$), and *RARA* (HR, 6.72; 95% CI, 1.58-28.6; $P = .010$; Figure 2A; supplemental Table 3). Interestingly, *TP53* mutations were not a risk factor for HLH. The presence of at least 1 mutation in FAS pathway genes (*FAS, CASP8*, and *TNFAIP3*) was significantly associated with increased

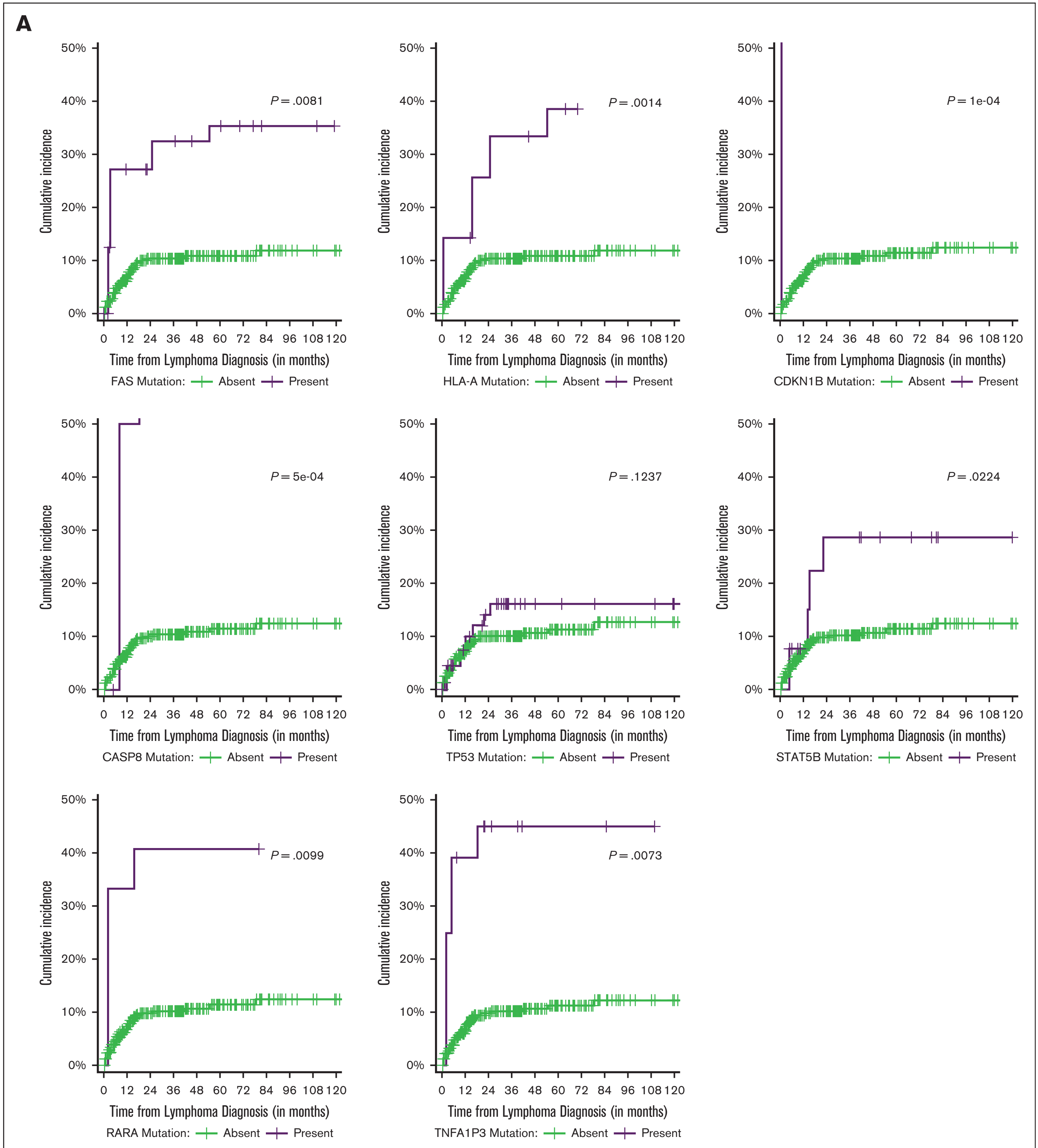

Figure 2. Association of HLH risk by time-to-HLH (cumulative incidence) for patients undergoing complete HLH workup and mutations or specific T-cell malignancy. (A) Association between HLH risk and mutations of *FAS*, *CASP8. HLA-A*, *CDKN1B*, *TP53*, *TNFA1P3*, *RARA*, and *STAT5B.* (B) Association between HLH risk and specific T-cell malignancy.

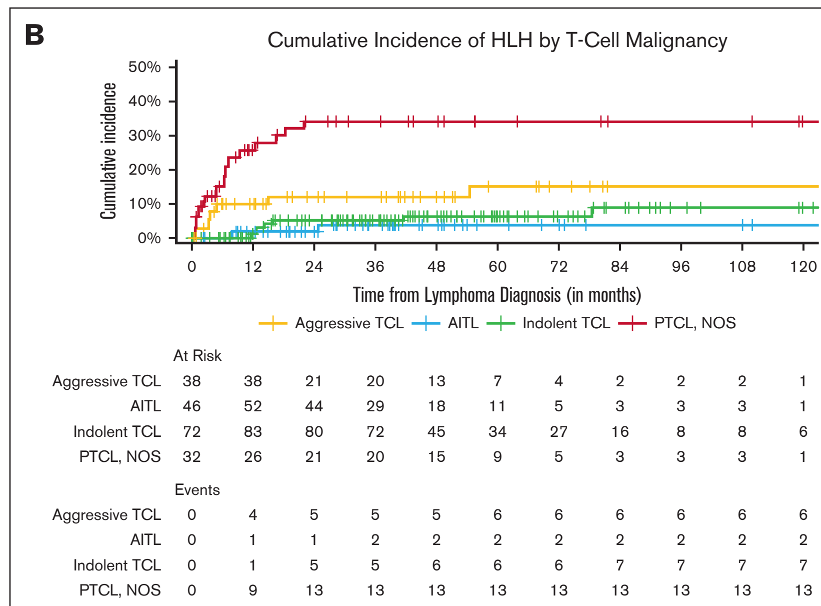

Figure 2 (continued)

HLH risk (HR, 4.98; 95% CI, 2.11-11.8; $P < .001$). HLH risk may be different between T- and NK-cell lymphoma subtypes.[7] Indeed, after grouping the T/NK-cell lymphomas into 4 major categories (indolent TCL [LGLL, SS/MF/other CTCL, ALCL, and LPD], AITL, PTCL, NOS, and aggressive TCL [ANKL, ATLL, T-PLL, HSTCL, ENKTL, and intestinal TCL]), we demonstrated that PTCL, NOS appeared to be significantly associated with an increased risk of HLH (HR, 2.79; 95% CI, 1.06-7.35; $P = .038$; Figure 2B; supplemental Table 3). To disentangle these confounding factors, multivariable cause-specific time-to-HLH analysis was performed for each mutation, stratified by specific T-cell malignancy and adjusting for age and sex. To this end, we identified mutations in *HLA-A*, *CDKN1B*, *CASP8*, and *TNFAIP3* as independent HLH risks (supplemental Table 4). The presence of at least 1 mutation involving the FAS pathway (*FAS*, *CASP8*, and *TNFAIP3*) was independently associated with increased HLH risk (HR, 3.56; 95% CI, 1.44-8.77; $P = .006$; supplemental Table 4). This association remained significant even when OHI criteria were applied (HR, 3.21; 95% CI, 1.48-6.93; $P = .003$).

Association between FAS pathway mutations and survival

We first evaluated clinical risk factors associated with OS. Univariable analysis identified both HLH and lymphoma subtypes as poor risk factors, whereas age, sex, or EBV status were not (supplemental Table 5). Because an increased risk of HLH was associated with multiple mutations, the impacts of individual mutations on OS were also evaluated. Univariable OS analysis showed an association between inferior OS and mutations in *TP53* (HR, 1.99; 95% CI, 1.36-2.93; $P < .001$), *CASP8* (HR, 6.95; 95% CI, 2.54-19.05; $P < .001$), *HLA-A* (HR, 2.19; 95% CI, 1.18-4.07; $P = .01$), *RARA* (HR, 3.24; 95% CI, 1.43-7.36; $P = .005$), *STAT5B* (HR, 1.84; 95% CI, 1.04-3.25; $P = .04$; supplemental Figure 3), and *CDKN1B* (HR, 2.19; 95% CI, 1.18-4.07; $P = .013$) (data not shown). Multivariable analysis, however, after stratification by lymphoma subtypes and adjusting for age and sex, showed that the association with inferior OS remained significant only for *TP53* (HR, 1.7; 95% CI, 1.03-2.80; $P = .037$), *CASP8* (HR, 5.44; 95% CI, 1.62-18.3; $P = .006$), and *CDKN1B* (HR, 16.2; 95% CI, 3.11-84.4; $P < .001$; Table 2). Importantly, the presence of at least 1 mutation in FAS pathway genes (*FAS*, *CASP8*, and *TNFAIP3*) was still significantly associated with inferior OS in this multivariable model (HR, 1.91; 95% CI, 1.10-3.34; $P = .022$). The association between FAS pathway mutations and outcome was lost when stratified by HLH (data not shown), further validating the close interaction between FAS pathway mutations and HLH. However, the significance of *TP53* mutations remained even after stratification by HLH. The findings, together with the significant association seen between other mutations and T-cell subtypes (supplemental Table 6), suggests the survival impact was largely determined by specific lymphoma subtypes, but mutations in FAS pathways and *TP53* also carry independent risks for poor outcome.

FAS mutations in patients with T- and NK-cell lymphoma

Because *FAS* appears frequently mutated in T- and NK-cell lymphoma, we decided to further evaluate the clinicopathologic features of patients with *FAS* mutations. *FAS* mutations were identified in 28 patients (19 men and 9 women; median age, 66 years), with an overall frequency of 6.5% (Figure 1A; Table 1). These 28 patients included AITL (2/89 [2.2%]), PTCL, NOS (9/78 [11.5%]), ATLL (4/25 [16.0%]), ENKTL (1/12 [8.3%]), LGLL (3/46 [6.5%]), ANKL (2/3 [66.7%]), T-PLL (1/23 [4.3%]), intestinal T-cell lymphoma (1/11 [9.1%]), and mycosis fungoides/Sézary syndrome/CTCL (5/81 [6.2%]). Interestingly, the prevalence of *FAS* mutations in PTCL, NOS was significantly higher than that in AITL ($P = .02$). *FAS* mutations were not identified in ALCL and hepatosplenic T-cell lymphoma. Seventeen of these 28 patients had a nodal biopsy, and none showed features suggestive of ALPS. Nineteen patients had documented flow cytometric analysis on peripheral blood, and none showed CD4/CD8 double negative TCR alpha/beta positive T cells >1.5% of lymphocytes or 2% of total T cells. Fifteen mutations were

Table 2. **Multivariable OS after HLH assessment for each mutation stratified by specific T-cell malignancy, adjusting for age and sex**

Characteristics	Mutations	n	Event, n	HR	95% CI	*P* value
FAS mutation	Absent	187	98	Ref.	Ref.	
	Present	19	11	1.66	0.87-3.16	.12
HLA-A mutation	Absent	195	100	Ref.	Ref.	
	Present	11	9	1.82	0.88-3.78	.11
CDKN1B mutation	Absent	204	107	Ref.	Ref.	
	Present	2	2	16.2	3.11-84.4	<.001
CASP8 mutation	Absent	203	106	Ref.	Ref.	
	Present	3	3	5.44	1.62-18.3	.006
TP53 mutation	Absent	173	87	Ref.	Ref.	
	Present	33	22	1.7	1.03-2.80	.037
TET2 mutation	Absent	139	75	Ref.	Ref.	
	Present	67	34	1.10	0.63-1.91	.7
STAT3 mutation	Absent	181	99	Ref.	Ref.	
	Present	25	10	0.61	0.32-1.19	.15
STAT5B mutation	Absent	191	98	Ref.	Ref.	
	Present	15	11	1.29	0.66-2.51	.5
ROHA mutation	Absent	172	92	Ref.	Ref.	
	Present	34	17	1.27	0.64-2.54	.5
RARA mutation	Absent	200	104	Ref.	Ref.	
	Present	6	5	1.69	0.64-4.43	.3
TNFA1P3 mutation	Absent	198	105	Ref.	Ref.	
	Present	8	4	1.78	1.63-5.04	.3
KMT2D mutation	Absent	191	99	Ref.	Ref.	
	Present	15	10	1.37	0.70-2.09	.4
DNMT3A mutation	Absent	177	93	Ref.	Ref.	
	Present	29	16	1.41	0.79-2.52	.2
Any mutations involving FAS, CASP8, and TNFA1P3	Absent	179	93	Ref.	Ref.	
	Present	27	16	1.91	1.10-3.34	.022
No. of mutations involving FAS, CASP8, and TNFA1P3	0	179	93	Ref.	Ref.	
	1	24	14	1.80	1.00-3.24	.048
	2	3	2	3.45	0.79-15.0	.10

nonsense or frameshift, predicted to cause decreased/absent protein expression, thus deemed as likely oncogenic (Figure 3A). Ten patients had missense mutations, mostly involving death domain, and initially deemed as variant of unknown significance. Further evaluation predicted these 10 missense mutations to be damaging by Polyphen 2 (score $>$ 0.9), SIFT (score $<$ 0.05), and 3-dimensional structural modeling (Figure 3B-C; supplemental Table 7). Several amino acids affected by these mutations are highly conserved in evolution (supplemental Figure 4). The variant allelic frequency ranged from 0.03 to 0.97 (median, 0.30 $\pm$ 0.09). Because all patients had nail DNA as germ line control, these mutations were deemed as somatic. By reviewing NGS data, loss of heterozygosity of *FAS* mutations was identified in 8 patients, with variant allelic frequency ranging from 0.07 to 0.97 (Table 1).

In total, 5 of 28 (17.9%) patients carrying *FAS* mutations (all locating at cytoplasmic portion) developed HLH compared with only 24 of 405 patients (5.9%) without *FAS* mutations ($P = .03$; Figure 3D-E; Table 1). *FAS* mutations were not detected in any of the 21 patients who were OHI-positive but did not meet 2004 criteria. Relatedly, there was no significant association between *FAS* mutations and OHI index status (HR, 2.39; 95% CI, 0.85-6.74; $P = .1$).

Because most somatic *FAS* mutations in our cohort were nonsense or frameshift, it would predict decreased or absent FAS protein expression levels. To test this, immunohistochemical stain using anti-FAS antibody was performed on formalin-fixed, paraffin-embedded tissues of 48 patients, obtained from our archives (Figure 3F). FAS protein expression was absent in 11 of 14 patients (78%) with *FAS* mutations. In contrast, only 9 of 34 patients (32%) with no *FAS* mutation had loss of FAS protein expression ($P = .004$; Figure 3G). There appeared no correlation between FAS protein expression and types of mutations.

Figure 3. *FAS* mutations in patients with T- and NK-cell lymphoma. (A) Lollipop illustration of *FAS* mutations in protein coding sequence (modified from cBioPortal). Red circle represents nonsense mutations; black circle represents frameshift mutations; and green circle represents missense mutations. Red arrowed line represents mutations identified in patients with HLH (only 1 patient with D260N or N264K mutations had HLH). (B-C) 3-dimensional modeling showing the amino acid changes of several representative missense mutations (https://www.ncbi.nlm.nih.gov/Structure/pdb/3OQ9). (D) Graphic illustration of the frequency of *FAS* mutations in patients with HLH. (E) The

Discussion

Our study demonstrated a previously unrecognized association between FAS pathway mutations and HLH-L in patients with T/NK-cell lymphoma. The etiology of HLH-L may be multifactorial including EBV infection, chemotherapy-related injury, and genetic susceptibility. Our study identified somatic, pathogenic mutations in FAS pathway overrepresented in patients with HLH-L, suggesting acquired genetic susceptibility as an underlying etiology. Notably, 28 of 29 patients developed HLH after treatment, and many of them had EBV and/or other viral infections, suggesting a possible intricate interplay between genetic alterations, viral infection, and chemotherapy, and more studies are warranted for better understanding the underlying mechanisms. A recent study has shown somatic mutations in *TET2* in blood cells are more prevalent in patients with sHLH and may contribute to sHLH by inducing hyperactivation of macrophages.[11] Our study showed comparably frequent *TET2* mutations in T/NK-cell lymphoma in both patients with HLH and those with non-HLH. Notably, mutations in several genes (*FAS*, *CASP8*, and *TNFAIP3*) identified in our cohort are implicated in FAS pathways. It will be interesting to study whether these somatic mutations in lymphoma cells also lead to dysregulation of immune response, including macrophage hyperactivation.[21]

Both germline and somatic *FAS* mutations are characteristic of ALPS.[13,14,22,23] Patients with ALPS have increased risks of lymphoma.[24] Because ALPS and HLH share some clinical and laboratory features, the presence of somatic *FAS* mutations in T/NK-cell lymphoma in our cohort raises the possibility of an underlying, undiagnosed ALPS in these patients. However, several lines of evidence argue against this. First, patients with ALPS with germline *FAS* mutations are mostly aged <5 years, and those with somatic *FAS* mutations are aged <20 years, with only rare exceptions.[22,23] Our cohort with *FAS* mutations were mostly aged >50 years. Second, CD4/CD8 double negative TCR alpha/beta positive T cells, characteristic of ALPS, were not increased in our patients. Third, reviewing of histology did not show features suggestive of ALPS. Fourth, the patients with *FAS* mutations and HLH had extremely high levels of sCD25 (>20 000 pg/mL) and serum ferritin (>10 000 ng/mL), which is very rare in ALPS.[15,25]

FAS/FASL pathway is essential for the downregulation of immune reactions as well as in T-cell–mediated cytotoxicity via inducing apoptosis.[26] Mutations in *FAS* or *FASL* lead to defective apoptosis and lymphoproliferation in ALPS.[13,14] It is conceivable to speculate that loss-of-function mutations along this pathway in our cohort provides survival advantage in lymphoma cells. However, the mechanistic link between these mutations in lymphoma and HLH is yet to be established. FAS/FASL signaling pathway may regulate the activation of macrophages.[27] The interplay between mutated lymphoma cells and macrophages warrants further studies.

We identified mutations in *TP53* and FAS pathway as independent risk factors for poor outcomes. *TP53* mutations has been previously shown to correlate with inferior progression-free survival in patients with PTCL treated with chemotherapy.[28] Intriguingly, *TP53* mutations were not associated with HLH, and the prognostic significance was not affected by HLH stratification in patients with T- and NK-cell lymphoma. In comparison, the prognostic value of mutations in FAS pathways was lost after HLH stratification. Therefore, we posit that *TP53* mutations lead to inferior outcome by conferring therapeutic resistance to chemotherapy, whereas FAS pathway mutations do this by increasing HLH risk. These pieces of evidence collectively support that genetic information may be incorporated into current risk-stratification models in clinical practice for these patients.[29,30]

One major caveat of our study is the lack of germline analysis to exclude the rare possibility of coexisting germ line *FAS* and other primary HLH-related mutations in these patients. Although these germline mutations may not be sufficient to induce sHLH as suggested by a recent study,[10] they may collaborate with somatic mutations. In addition, other germline mutations such as Hepatitis A virus cellular receptor 2/T-cell immunoglobulin and mucin-domain containing-3 (TIM-3), commonly seen in subcutaneous panniculitis-like TCL, resulting in loss of TIM-3 protein function, may lead to macrophage activation and HLH.[31] Second, due to the retrospective in nature, our study might have underestimated the frequency of HLH, despite extensive manual chart review being performed. Third, the median survival of our patients was only 25 days after the diagnosis of HLH, indicating an extremely poor prognosis. However, the possibility of a delayed diagnosis cannot be ruled out because an early, accurate diagnosis of HLH has been very challenging. Nevertheless, this study sheds new light on the potential clinical relevance of somatic mutations in the FAS pathway in HLH-L and calls for further investigations and eventually the development of methods for early diagnosis and targeted therapeutic approaches for these patients at high risk.

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Authorship

Contribution: Y.L. and R.S. compiled and annotated the patient cohort; Y.L. and M.A. annotated the mutations; D.N. and A. Derkach performed statistical analyses; W.T.J., S.V., and S.M.H. provided clinical information; A. Dogan helped the pathology and provided guidance; W.X. designed and supervised the study; Y.L., R.S., and W.X. wrote the manuscript; and all authors approved the manuscript.

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Figure 3 (continued) distribution of patients with HLH related to *FAS* mutations and the types of T/NK-cell lymphoma. (F) Representative pictures of FAS immunohistochemical staining in patients with T-cell lymphoma with wild-type *FAS* (left) and mutant *FAS* (right). Original magnification, 400×; scale bar, 50 mm. (G) Summary of *FAS* immunohistochemical staining in 48 patients with and without *FAS* mutations. IHC, immunohistochemistry; Mut, mutated; N, no; neg, negative; pos, positive; Y, yes.

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