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American Society of Hematology
2021 L Street NW, Suite 900,
Washington, DC 20036
Phone: 202-776-0544 | Fax 202-776-0545
bloodadvances@hematology.org

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

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Ying Liu (Memorial Sloan Kettering Cancer Institute, United States) Rohan Sardana (Memorial Sloan Kettering Cancer Center, United States) David Nemirovsky (Memorial Sloan-Kettering Cancer Center, United States) Denise Frosina (Memorial Sloan Kettering Cancer Center, United States) Achim Jungbluth (Memorial Sloan-Kettering Cancer Center, United States) William Johnson (Memorial Sloan Kettering Cancer Center, United States) Santosha Vardhana (Memorial Sloan Kettering Cancer Center, United States) Maria Arcila (Memorial Sloan Kettering Cancer Center, United States) Steven Horwitz (Memorial Sloan Kettering Cancer Center, United States) Andriy Derkach (Memorial Sloan-Kettering Cancer Center, United States) Ahmet Dogan (Memorial Sloan-Kettering Cancer Center, United States) Wenbin Xiao (Memorial Sloan Kettering Cancer Center, United States)

Abstract:
While 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 lymphoma patients (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 NK-cell lymphoma. We identified a 3-time higher frequency of mutations in FAS pathway in patients with HLH-L. Patients harbouring 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.

Conflict of interest: COI declared - see note

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

Ying Liu[1,2], Rohan Sardana[1], David Nemirovsky[3], Denise Frosina[1], Achim Jungbluth[1], William T. Johnson[4,5], Santosha Vardhana[4,5,6], Maria Arcila[1,2], Steven M. Horwitz[4,5], Andriy Derkach[3], Ahmet Dogan[1], Wenbin Xiao[1]

Running Title: Somatic mutations in FAS pathway and HLH in T- and/or NK-cell lymphoma

[1]Hematopathology Service, Department of Pathology and Laboratory Medicine, Memorial Sloan Kettering Cancer Center, New York, NY, USA
[2]Diagnostic Molecular Service, Department of Pathology and Laboratory Medicine, Memorial Sloan Kettering Cancer Center, New York, NY, USA
[3]Department of Epidemiology and Biostatistics, Memorial Sloan Kettering Cancer Center, New York, NY, USA
[4]Lymphoma Service, Department of Medicine, Memorial Sloan Kettering Cancer Center, New York, NY, USA
[5]Department of Medicine, Weill Cornell Medical College, Cornell University, New York, NY, USA.
[6]Human Oncology&Pathogenesis Program, Memorial Sloan Kettering Cancer Center, New York, NY, USA

Corresponding author: Wenbin Xiao MD, PhD, Department of Pathology and Laboratory Medicine, Memorial Sloan Kettering Cancer Centre, 1275 York Avenue, New York, NY 10065; Phone: 212-639-6457; Fax: 929-321-1513; Email: xiaow@mskcc.org

Current address for R.S.: Sardana labs, Punjab, India

Additional data and materials are available upon request.

Key points:

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

Abstract

While 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 lymphoma patients (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 NK-cell lymphoma. We identified a 3-time higher frequency of mutations in FAS pathway in patients with HLH-L. Patients harbouring 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.

Keywords: FAS, CASP8, TNFAIP3, mutations, genetic susceptibility, hemophagocytic lymphohistiocytosis, T-cell lymphoma, NK-cell lymphoma, EBV

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 malignancy associated with HLH with T- and/or NK-cell lymphoma (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 hyperinflammatory responses, a critical feature of HLH. There is lack of studies on the association between 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, e.g. *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- and/or 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- or NK-cell lymphoma/lymphoproliferative disorder (LPD). The key words “non-Hodgkin" OR "NK cell lymphoma or leukemia" OR "T cell lymphoma" OR "mature T cell lymphoma" OR "atypical NK cell" OR "atypical T cell" OR "T cell lymphoproliferative disorder" were used to identify patients. Only patients who had next generation sequencing 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.

Next generation sequencing and analysis

Mutational profiling was performed on formalin fixed, paraffin embedded (FFPE) lymphoma tissues (bone marrow or peripheral blood for patients with leukemia) by a targeted next generation sequencing (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 (VUS) 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 SIFT[19,20].

Immunohistochemistry studies

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

Statistical analysis

Patient characteristics were summarized by frequency (percentage) or medians with interquartile range (IQR). Associations between mutation frequencies and disease characteristics were tested by Fisher's exact test, Pearson's Chi-squared 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-hazard model. Overall survival 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-hazard models. Overall survival from 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-hazard models. All statistical analyses were performed using R 4.3.2.

This study was approved by the MSK Institutional Review Board.

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- or NK-cell lymphoma or lymphoproliferative disorder AND having matched MSK-IMPACT analysis on neoplastic cells (**Supplemental Table S1**). HLH was diagnosed in 29 patients (6.7%, 17 men/12 women, median age 60 years old) based on 2004 criteria, none of whom were post 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 NK/T-cell lymphoma (ENKTL, 1/12, 8.3%), hepatosplenic T-cell lymphoma (HSPTCL, 2/7, 28.6%), aggressive NK-cell leukemia (ANKL, 2/3, 66.7%), mycosis fungoides/Sezary syndrome (MF/SS, 1/62, 1.6%) and other types of cutaneous T-cell lymphoma (CTCL, 1/19, 5.3%). Notably, no HLH was found in patients with T-prolymphocytic leukemia (T-PLL, 0/23) and intestinal T-cell lymphoma (0/11). HLH was diagnosed a median of 9.4 (IQR: 3.5-15.8) months after diagnosis of lymphoma. 27 of the 29 patients had markedly increased soluble CD25 (>10000 pg/mL) and 28 of the 29 patients 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 HLH-positive cases, 28 patients succumbed to disease with a median survival of 22 (95% CI, 13-60) days after HLH diagnosis (**Supplemental Figure 1**). As the OHI index may be more sensitive for identifying malignancy associated HLH[17], we also evaluated OHI index in our cohort. 49 patients were positive for OHI index (OHI-positive) and 28 of them also met 2004 criteria (**Supplemental Table S2**). Patients meeting either 2004 criteria or OHI index were both associated with inferior overall survival (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- and/or NK-cell lymphoma in our cohort (**Figure 1**). The most frequently mutated genes were *TET2, TP53, STAT3, ROHA, DNMT3A*. As expected, mutations in *TET2, DNMT3A* and *RHOA* were highly prevalent in AITL, while *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=0.03, p=0.01, p=0.01 and p=0.03, respectively, **Figure 1B**). The frequencies of *STAT5B*, *RARA* and *TNFAIP3* mutations were also borderline increased in patients with HLH (p=0.09, 0.07 and 0.06, respectively). Notably, *FAS*, *CASP8* and *TNFAIP3* encode proteins critical for FAS pathway (**Figure 1C**). 27.6% of HLH patients had at least one mutation in FAS pathway as compared to 8.9% of non-HLH patients (p=0.004).

We next examined the association of gene mutations with HLH risk using univariable cause-specific Cox regression. Among all the patients undergoing HLH evaluated, we found an increased risk of HLH in patients harbouring somatic mutations in *FAS* (HR: 4.24, 95% CI: 1.46-12.4, p=0.008), *CASP8* (HR: 13.4, 95% CI: 3.14-57. 5, p<0.001), *HLA-A* (HR: 5.71, 95% CI: 1.96-16.6, p=0.001), *CDNK1B* (HR: 19.2, 95% CI: 4.40-83.5, p<0.001), *STAT5B* (HR: 3.45, 95% CI: 1.19-10.0, p=0.022), *TNFAIP3* (HR: 5.20, 95% CI: 1.56-17.4, p=0.007), and *RARA* (HR: 6.72, 95% CI: 1.58-28.6, p=0.010) (**Figure 2A**, **Supplemental Table S3**). 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 HLH risk (HR: 4.98, 95% CI: 2.11-11.8, p<0.001). HLH risk may be different between T- and NK-cell lymphoma subtypes[7]. Indeed, after grouping the T- and/or NK-cell lymphomas into four 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=0.038)(**Figure 2B, Supplemental Table S3**). To disentangle these confounding factors, multivariable cause-specific time-to-HLH analysis was performed for each mutation after 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 S4**). The presence of at least 1 mutation involving FAS pathway (*FAS*, *CASP8* and *TNFAIP3*) was independently associated with increased HLH risk (HR: 3.56, 95% CI: 1.44-8.77, p=0.006) (**Supplemental Table S4**). This association remained significant even when OHI criteria were applied (HR: 3.21, 95% CI: 1.48-6.93, p=0.003).

Association between FAS pathway mutations and survival

We first evaluated clinical risk factors associated with overall survival (OS). Univariable analysis identified both HLH and lymphoma subtypes as poor risk factors, while age, sex or EBV status were not (**Supplemental Table S5**). As 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<0.001), *CASP8* (HR: 6.95, 95% CI: 2.54-19.05, p<0.001), *HLA-A* (HR: 2.19, 95% CI: 1.18-4.07, p=0.01), *RARA* (HR: 3.24, 95% CI: 1.43-7.36, p=0.005), *STAT5B* (HR: 1.84, 95% CI: 1.04-3.25, p=0.04) (**Supplemental Figure S3**) and *CDKN1B* (HR: 2.19, 95% CI: 1.18-4.07, p=0.013) (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=0.037), *CASP8* (HR: 5.44, 95% CI: 1.62-18.3, p=0.006) and *CDKN1B* (HR: 16.2, 95% CI: 3.11-84.4, p<0.001) (**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=0.022). 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 stratified by HLH. The findings, together with the significant association seen between other mutations and T-cell subtypes (**Supplemental Table S6**), 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

As FAS appears frequently mutated in T- and NK-cell lymphoma, we decided to further evaluate the clinicopathologic features of *FAS* mutated patients. *FAS* mutations were identified in 28 patients (19 men/9 women, median age of 66 years old) with an overall frequency of 6.5% (**Figure 1A and 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 MF/SS/CTCL (5/81, 6.2%). Interestingly, the prevalence of *FAS* mutations in PTCL, NOS was significantly higher than that in AITL (p=0.02). *FAS* mutations were not identified in ALCL and HSPTCL. 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$\alpha\beta$ T cells greater than 1.5% of lymphocytes or 2% of total T cells. Fifteen mutations were 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 (VUS). Further evaluation predicted these 10 missense mutations to be damaging by Polyphen 2 (score>0.9), SIFT (score<0.05) and 3D structural modelling (**Figure 3B and C**, **Supplemental Table S7**). Several amino acids affected by these mutations are highly conserved in evolution (**Supplemental Figure S4**). The variant allelic frequency ranged from 0.03 to 0.97 (median 0.30$\pm$0.09). As all patients had nail DNA as germline control, these mutations were deemed as somatic. By reviewing NGS sequencing data, loss of heterozygosity of *FAS* mutations was identified in 8 patients with VAF 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 as compared to only 24 of 405 (5.9%) patients without *FAS* mutations (p=0.03, **Figure 3D and E and Table 1**). *FAS* mutations were not detected in any of the 21 patients who were OHI-positive while 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=0.1).

As 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 FFPE tissues of 48 patients all obtained from our archives (**Figure 3F**). FAS protein expression was absent in 11 of 14 (78%) patients with *FAS* mutations. In contrast, only 9 of 34 (32%) patients with no *FAS* mutations had loss of FAS protein expression (p=0.004, **Figure 3G**). There appeared no correlation between FAS protein expression and types of mutations.

Discussion

Our study demonstrated a previously unrecognized association between FAS pathway mutations and HLH-L in patients with T- and/or 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 HLH-L patients, suggesting acquired genetic susceptibility as an underlying etiology. Notably, 28/29 patients developed HLH after treatment, and many of them had EBV and/or other viral infection, 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 sHLH patients and may contribute to sHLH by inducing hyperactivation of macrophages[11]. Our study showed comparably frequent *TET2* mutations in T- and/or NK-cell lymphoma in both HLH and non HLH patients. Notably, mutations in several genes (*FAS, CASP8 and TNFAIP3*) identified in our cohort are implicated in FAS pathways. It will be interesting to study if 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 for ALPS [13,14,22,23]. Patients with ALPS have increased risks of lymphoma[24]. As ALPS and HLH share some clinical and laboratory features, the presence of somatic *FAS* mutations in T- and/or 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, ALPS patients with germline *FAS* mutations are mostly younger than 5 years old and those with somatic *FAS* mutations are younger than 20 years old with only rare exceptions[22,23]. Our cohort with *FAS* mutations were mostly older than 50 years. Second, CD4/CD8 double negative TCRα/β T-cells, characteristic for 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 down-regulation 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 signalling 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 impacted 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 while 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 lack of germline analysis to exclude the rare possibility of co-existing germline *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 HAVCR2/TIM-3, commonly seen in SPTCL resulting in loss of TIM-3 protein function, may lead to macrophage activation and HLH[31]. Second, due to retrospective in nature, our study might have underestimated the frequency of HLH despite an extensive manual chart review was performed. Third, the median survival of our patients was only 25 days after diagnosis of HLH, indicating an extremely poor prognosis. However, the possibility of a delayed diagnosis cannot be ruled out as 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 FAS pathway in HLH-L and calls for further investigations and eventually developing methods for early diagnosis and targeted therapeutic approaches for these high-risk patients.

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Author 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.A.V., and S.M.H. provided clinical information. A.Dogan. helped the pathology and provided guidance. W.X. designed and supervised the entire study. Y.L, R.S., and W.X. wrote and all authors approved the manuscript.

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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/μL)	HLH (m)	Death (m)	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	>800000	10997	135680
2	Yes	No	NA	NA	ND	NA	53	M	ATLL	2.8	1	1	No	7966	>20000	123756
3	Yes	No	NA	NA	ND	NA	69	M	AITL	0.2	8	9	Yes	0	>20000	19962
4	Yes	No	NA	NA	ND	NA	23	M	HSTCL	1.9	5	5	No	<400	>80000	30529
5	Yes	No	NA	NA	ND	NA	48	F	HSTCL	3.3	15	16	No	0	>20000	54647
6	Yes	No	NA	NA	ND	NA	62	M	PTCL, NOS	0.1	1	12	Yes	0	14250	5463
7	Yes	No	NA	NA	ND	NA	69	F	PTCL, NOS	0.5	6	7	No	0	19525	47693
8	Yes	No	NA	NA	ND	NA	61	F	PTCL, NOS	0.1	18	18	No	7313	6699	140740
9	Yes	No	NA	NA	ND	NA	16	M	PTCL, NOS	0.3	1	alive	Yes	0	>20000	11782
10	Yes	No	NA	NA	ND	NA	55	F	PTCL, NOS	0.4	12	13	Yes	3105	18355	13393
11	Yes	No	NA	NA	ND	NA	65	M	PTCL, NOS	6.7	7	8	No	0	>20000	19502
12	Yes	No	NA	NA	ND	NA	59	M	PTCL, NOS	0.3	22	22	No	12408	>20000	5547
13	Yes	No	NA	NA	ND	NA	62	M	PTCL, NOS	34.3	9	9	ND	2333	>20000	17800
14	Yes	No	NA	NA	ND	NA	54	M	PTCL, NOS	0.6	1	1	Yes	0	38945	42659
15	Yes	No	NA	NA	ND	NA	77	M	PTCL, NOS	0.8	5	8	No	6621	>20000	21966
16	Yes	No	NA	NA	ND	NA	72	M	PTCL, NOS	0.7	17	18	Yes	0	>20000	9246
17	Yes	No	NA	NA	ND	NA	61	F	PTCL, NOS	0.2	6	14	No	<400	>20000	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	>20000	27328
20	Yes	No	NA	NA	ND	NA	71	M	T-LGLL	0.5	12	13	Yes	1683	10975	82206
21	Yes	No	NA	NA	ND	NA	56	F	MF	1.0	16	16	No	<400	>20000	2282
22	Yes	No	NA	NA	ND	NA	68	F	CD8+ PCAETL	0.5	42	42	No	3557	>20000	972
23	Yes	No	NA	NA	ND	NA	54	F	ALCL	3.1	12	12	Yes	0	>20000	22621
24	Yes	No	NA	NA	ND	NA	49	M	ALCL	0.3	79	80	ND	0	>20000	15073
25	Yes	L224*	Likely oncogenic	0.42	+	No	68	F	AITL	0.4	25	27	Yes	5773	>20000	12277
26	Yes	W281*	Likely oncogenic	0.41	-	No	57	F	PTCL, NOS	0.3	2	2	No	1356	>20000	64718

27	Yes	D260N	predicted damaging	0.26	-	ND	47	M	ATLL	0.7	55	58	No	0	>20000	2286
28	Yes	S230Lfs*4	predicted damaging	0.05	-	No	67	M	ANKL	0.6	3	3	Yes	1408	>20000	20597
29	Yes	N264K	predicted damaging	0.10	-	No	71	M	ANKL	0.1	0	10	Yes	0	38866	>33500
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	-	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
35	No	S230Efs*2	Likely oncogenic	0.45	-	No	56	M	PTCL, NOS	4.5	NA	19	No	0	ND	ND
36	No	X66_splice	Likely oncogenic	0.12	-	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	+	No	74	M	ATLL	99.8	NA	3	No	0	>20000	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	+	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	-	Yes	76	M	PCGDTCL	2.9	NA	alive	No	ND	ND	34
48	No	C119Afs*68	Likely oncogenic	0.03	-	No	41	F	SS	4.1	NA	alive	No	0	ND	239
49	No	H142D	Predicted damaging	0.97	-	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	-	Yes	59	F	T-PLL	1.2	NA	alive	No	ND	ND	ND

*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 greater than 0.9 and/or of SIFT lower than 0.05 was deemed as damaging.
Abbreviations: VAF, variant allelic frequency; IHC, immunohistochemistry; LOH, loss of heterozygosity; ALC, absolute lymphocyte counts; NA, not applicable; ND, not done/determined; AITL, angioimmunoblastic T-cell lymphoma; CD8+ PCAETL, Primary cutaneous CD8-positive epidermotropic cytotoxic T-cell lymphoma; PTCL, NOS, peripheral T-cell lymphoma, not otherwise specified; ATLL, adult T-cell leukemia/lymphoma; T-LGLL, T-large granular lymphocytic leukemia; ENKTL, extranodal NK/T-cell lymphoma, nasal type; ANKL, aggressive NK-cell leukemia; MEITL, monomorphic epitheliotropic intestinal T-cell lymphoma; PCGDTCL, primary cutaneous gamma delta T-cell lymphoma; T-PLL, T-prolymphocytic leukemia; MF, mycosis fungoides; SS, Sezary syndrome. HLH (m): Months from TCL diagnosis to HLH diagnosis. Death (m): Months from TCL diagnosis to decease.

Table 2. Multivariable OS Post-HLH Assessment for Each Mutation Stratified by Specific T-Cell Malignancy, Adjusting for Age and Sex

Characteristics		N	Event N	HR	95% CI	p-value
FAS mutation	Absent	187	98	Ref.	Ref.	
	Present	19	11	1.66	0.87, 3.16	0.12
HLA-A mutation	Absent	195	100	Ref.	Ref.	
	Present	11	9	1.82	0.88, 3.78	0.11
CDKN1B mutation	Absent	204	107	Ref.	Ref.	
	Present	2	2	16.2	3.11, 84.4	**<0.001**
CASP8 mutation	Absent	203	106	Ref.	Ref.	
	Present	3	3	5.44	1.62, 18.3	**0.006**
TP53 mutation	Absent	173	87	Ref.	Ref.	
	Present	33	22	1.7	1.03, 2.80	**0.037**
TET2 mutation	Absent	139	75	Ref.	Ref.	
	Present	67	34	1.10	0.63, 1.91	0.7
STAT3 mutation	Absent	181	99	Ref.	Ref.	
	Present	25	10	0.61	0.32, 1.19	0.15
STAT5B mutation	Absent	191	98	Ref.	Ref.	
	Present	15	11	1.29	0.66, 2.51	0.5
ROHA mutation	Absent	172	92	Ref.	Ref.	
	Present	34	17	1.27	0.64, 2.54	0.5
RARA mutation	Absent	200	104	Ref.	Ref.	
	Present	6	5	1.69	0.64, 4.43	0.3
TNFA1P3 mutation	Absent	198	105	Ref.	Ref.	
	Present	8	4	1.78	1.63, 5.04	0.3
KMT2D mutation	Absent	191	99	Ref.	Ref.	
	Present	15	10	1.37	0.70, 2.09	0.4
DNMT3A mutation	Absent	177	93	Ref.	Ref.	
	Present	29	16	1.41	0.79, 2.52	0.2

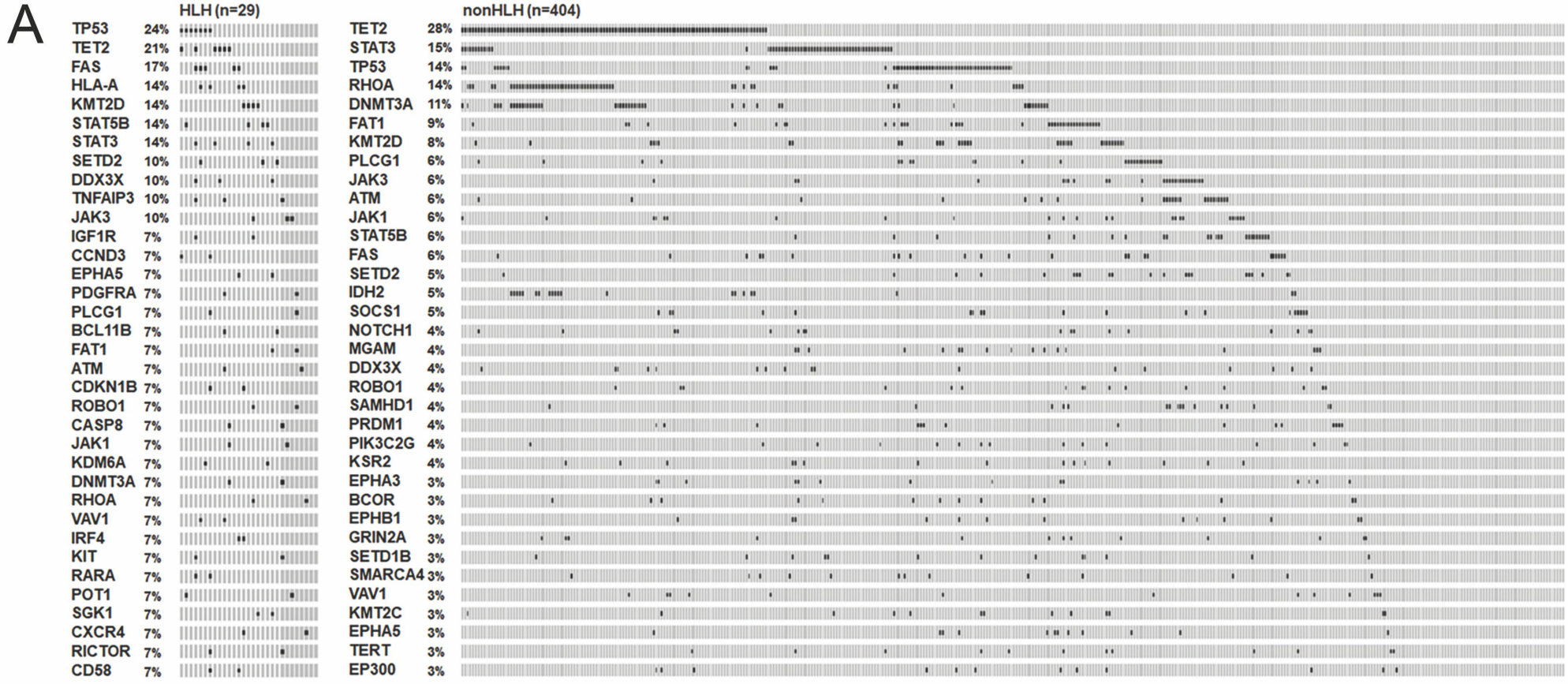
Any mutations involving FAS, CASP8, TNFA1P3	Absent	179	93	Ref.	Ref.	
	Present	27	16	1.91	1.10, 3.34	**0.022**
Number of mutations involving FAS, CASP8, TNFA1P3	0	179	93	Ref.	Ref.	
	1	24	14	1.80	1.00, 3.24	0.048
	2	3	2	3.45	0.79, 15.0	0.10

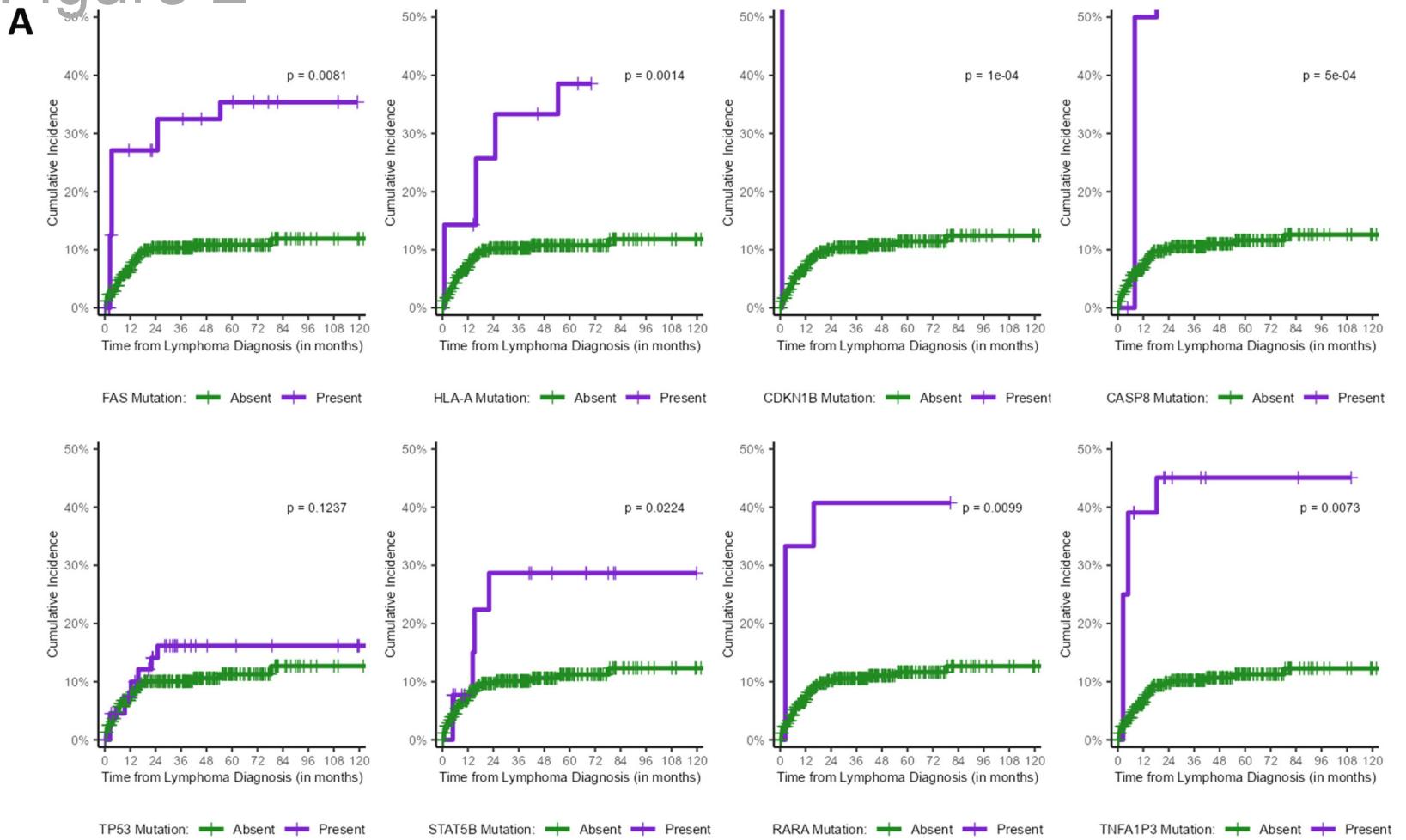
Figure 1. Mutational profiles between patients with and without HLH. **A.** Oncoprints (only top 30 mutated genes were shown). **B.** Bar plots showing the comparison of mutations in various genes. **C**. Illustration of mutations in FAS pathway (created by BioRender).

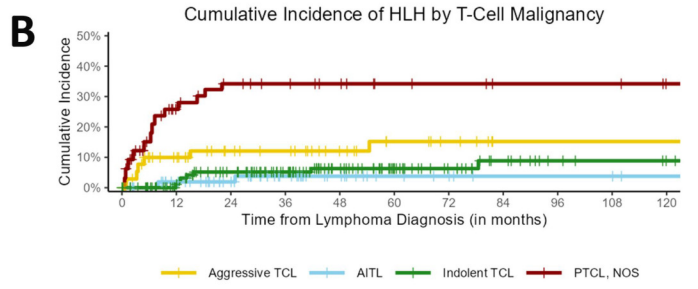
Figure 2. Association of HLH risk by time-to-HLH (cumulative incidence) for patients undergoing complete HLH work-up and mutations or specific T-cell malignancy. **A**. Association between HLH risk and mutations of *FAS*, *CASP8*. *HLA-A*, *CDKN1B*, *TP53*, *TNFA1P3*, *RARA* and *STAT5B*. **B**. Association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

Figure 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 one patient with D260N or N264K mutations had HLH, respectively). **B-C**. 3-D modelling 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 distribution of HLH patients related to *FAS* mutations and the types of T/NK-cell lymphoma. **F**. Representative pictures of FAS immunohistochemical staining in T-cell lymphoma patients with wild-type *FAS* (left) and mutant *FAS* (right), respectively. Original magnification x400. Scale bar, 50 μm. **G**. Summary of *FAS* immunohistochemical staining in 48 patients with and without *FAS* mutations.

Fig 2







	At Risk										
Aggressive TCL	38	38	21	20	13	7	4	2	2	2	1
AITL	46	52	44	29	18	11	5	3	3	3	1
Indolent TCL	72	83	80	72	45	34	27	16	8	8	6
PTCL, NOS	32	26	21	20	15	9	5	3	3	3	1
	Events										
Aggressive TCL	0	4	5	5	5	6	6	6	6	6	6
AITL	0	1	1	2	2	2	2	2	2	2	2
Indolent TCL	0	1	5	5	6	6	6	7	7	7	7
PTCL, NOS	0	9	13	13	13	13	13	13	13	13	13

Fig 3