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TO THE EDITOR:

Recurrent *STAT3-JAK2* fusions in indolent T-cell lymphoproliferative disorder of the gastrointestinal tract

Ayush Sharma,[1] Naoki Oishi,[2,3] Rebecca L. Boddicker,[2] Guangzhen Hu,[2] Hailey K. Benson,[2] Rhett P. Ketterling,[2] Patricia T. Greipp,[2] Darlene L. Knutson,[2] Sara M. Kloft-Nelson,[2] Rong He,[2] Bruce W. Eckloff,[4] Jin Jen,[2] Asha A. Nair,[5] Jaime I. Davila,[5] Surendra Dasari,[5] Konstantinos N. Lazaridis,[1,6] N. Nora Bennani,[7] Tsung-Teh Wu,[2] Grzegorz S. Nowakowski,[6,7] Joseph A. Murray,[1,*] and Andrew L. Feldman[2,*]

[1]Division of Gastroenterology and Hepatology and [2]Department of Laboratory Medicine and Pathology, Mayo Clinic, Rochester, MN; [3]Department of Pathology, University of Yamanashi, Chuo, Yamanashi, Japan; and [4]Medical Genome Facility, [5]Department of Health Sciences Research, [6]Center for Individualized Medicine, and [7]Division of Hematology, Mayo Clinic, Rochester, MN

Indolent T-cell lymphoproliferative disorder of the gastrointestinal tract (GI TLPD) is a newly recognized entity in the World Health Organization (WHO) classification of lymphoid neoplasms.[1] GI TLPD is defined as a clonal T-cell proliferation occurring in the GI tract, most commonly in the colon and small bowel, with bland cytologic appearance, a generally noninvasive architectural pattern, and indolent clinical behavior. Patients typically are adults (with a predominance of males) who present with GI symptoms, including abdominal pain, vomiting, diarrhea, and weight loss. Unlike enteropathy-associated T-cell lymphoma (EATL), from which it must be distinguished, GI TLPD is not associated with underlying celiac disease, and its etiology and molecular pathogenesis remain unknown. GI TLPD tends not to respond to chemotherapy and, despite its indolent nature, symptoms are often debilitating and challenging to treat.[2]

We examined the pathological and genetic features of GI TLPD to better understand this new entity (see supplemental Data, available on the *Blood* Web site). By reviewing clinical records and pathological material, we identified and confirmed 10 patients with GI TLPD and 1 patient with natural killer cell enteropathy, which is considered a separate entity by WHO criteria.[1-3] There were 6 males and 5 females (mean age, 56 years; range, 39-74 years; Table 1). Histologic features of GI TLPD were similar to those previously reported (Figure 1A-B).[1,2] Of the patients with GI TLPD, 5 had a $CD4^+$ T-cell phenotype, 4 had a $CD8^+$ phenotype, and 1 had a double-positive phenotype (Table 1). Clonal T-cell receptor gene rearrangements were identified in all 10 patients with GI TLPD but not in the patient with natural killer cell enteropathy. Samples from four patients underwent testing by FoundationOne Heme (Foundation Medicine, Cambridge, MA; supplemental Table 1). Notably, 1 $CD4^+$ GI TLPD sample was found to have a *STAT3-JAK2* fusion. *JAK2* fusions have been identified in a variety of myeloid and precursor lymphoid neoplasms[4,5] but are rare in mature T-cell lymphomas[6-9]; among 200 Mayo Clinic patients with a variety of T-cell lymphoma subtypes previously studied by fluorescence in situ hybridization (FISH) using a *JAK2* break-apart probe, all were negative for rearrangement.[7]

To confirm the *JAK2* rearrangement and determine whether it was recurrent in GI TLPD, we performed FISH on formalin-fixed, paraffin-embedded tissue sections using a previously published break-apart probe.[7,10] The initial sample identified by FoundationOne Heme and samples from 3 additional patients showed rearrangements of the *JAK2* gene region on 9p24.1; they represented 4 of 5 patients with a $CD4^+$ phenotype, whereas patients with other phenotypes lacked rearrangements (Table 1; Figure 1C-F). We tested 23 EATLs (in addition to 4 EATLs previously studied[7]) using a *JAK2* break-apart FISH probe, including 9 type II EATLs, now designated as monomorphic epitheliotropic intestinal T-cell lymphomas in the WHO classification[11]; all 27 samples were negative. We then designed a *STAT3-JAK2* dual-fusion FISH probe and demonstrated that all 4 GI TLPDs demonstrated fusion signals (Figure 1G), confirming a recurrent t(9;17)(p24.1;q21.2) rearrangement.

To evaluate the precise breakpoints of the fusion transcripts, we performed RNA sequencing from formalin-fixed, paraffin-embedded tissue and obtained interpretable results in 3 of the 4 patients with t(9;17)(p24.1;q21.2) rearrangements. All 3 showed *STAT3-JAK2* fusion with the identical messenger RNA breakpoint (Figure 1J), confirmed by Sanger sequencing (Figure 1I). The predicted fusion protein retained the C-terminal JH1 kinase domain of JAK2 and a portion of the JH2 pseudokinase domain but lacked the upstream SH2 and FERM domains (Figure 1J), similar to other *JAK2* fusions.[4,5,7] Reciprocal *JAK2-STAT3* fusion transcripts were also detected in all 3 sequenced samples; however, the breakpoint was different in each sample and likely nonfunctional (1 sample out of frame and 2 with predicted proteins lacking the JAK2 tyrosine kinase domain and virtually all of the STAT3 coding region). These data demonstrate recurrent *STAT3-JAK2* fusions in GI TLPDs. The exclusivity for $CD4^+$ samples and identical breakpoints strongly suggest that *STAT3-JAK2* fusions contribute to the pathogenesis of this disorder.

STAT3 activation is critical in many T-cell neoplasms, and activating mutations of the *STAT3* gene are relatively common.[12-16] However, Perry et al[2] found minimal phosphorylated STAT3 (pSTAT3) in GI TLPDs and did not observe any *STAT3* SH2 domain hotspot mutations. To determine whether pSTAT3 might be present in GI TLPDs with *STAT3-JAK2* fusions, we performed immunohistochemistry for $pSTAT3^{Y705}$ and samples from all 4 patients were negative (Figure 1K), as were samples from 5 of 7 patients who did not have *STAT3-JAK2* fusion (Table 1). Although

Table 1. Characteristics of 11 patients with indolent GI TLPD or NK-cell enteropathy

Patient	Age	Sex	GI site*	CD3	CD4	CD5	CD7	CD8	CD56	TIA1	GrzB	TCR βF1	TCR γδ	*STAT3-JAK2* fusion	pSTAT1^{Y701}	pSTAT3^{Y705}	pSTAT5^{Y694}
CD4$^+$ GI TLPD																	
1	54	F	D,J	+	+	+	−	−	−	−	−	+	−	Yes	−	−	+
2	48	F	S,D,J,I	+	+	+	+	−	−	−	−	+	−	Yes	−	−	+
3	51	M	D,J	+	+	+	−	−	−	−	−	+	−	Yes	+	−	+
4	70	M	S,D,J	+	+	−	+	−	−	−	−	+	−	Yes	−	−	−
5	51	M	D,J,I,C	+	+	+	+	−	−	−	−	+	−	No	−	+	−
CD8$^+$ GI TLPD																	
6	47	F	S,D,J,I	+	−	+	−	+	−	+	−	+	−	No	−	−	+
7	39	M	S,D,J,I,C	+	−	+	+	+	−	+	−	+	−	No	−	−	−
8	74	F	D,J	+	−	+	+	+	−	+	−	+	−	No	+	−	−
9	57	M	I	+	−	+	+	+	−	+	−	+	−	No	−	−	−
CD4$^+$/CD8$^+$ GI TLPD																	
10	60	M	D,J	+	+	+	−	+	−	−	−	+	−	No	†	+	†
NK-cell enteropathy																	
11	68	F	S,D,J	+	−	−	−	−	+	+	+	−	−	No‡	†	−	−

F, female; M, male.

*GI sites of involvement: C, colon; D, duodenum; I, ileum ; J, jejunum; S, stomach.

†Insufficient material or technically uninterpretable.

‡No fusion reported by FoundationOne Heme testing; FISH studies technically uninterpretable.

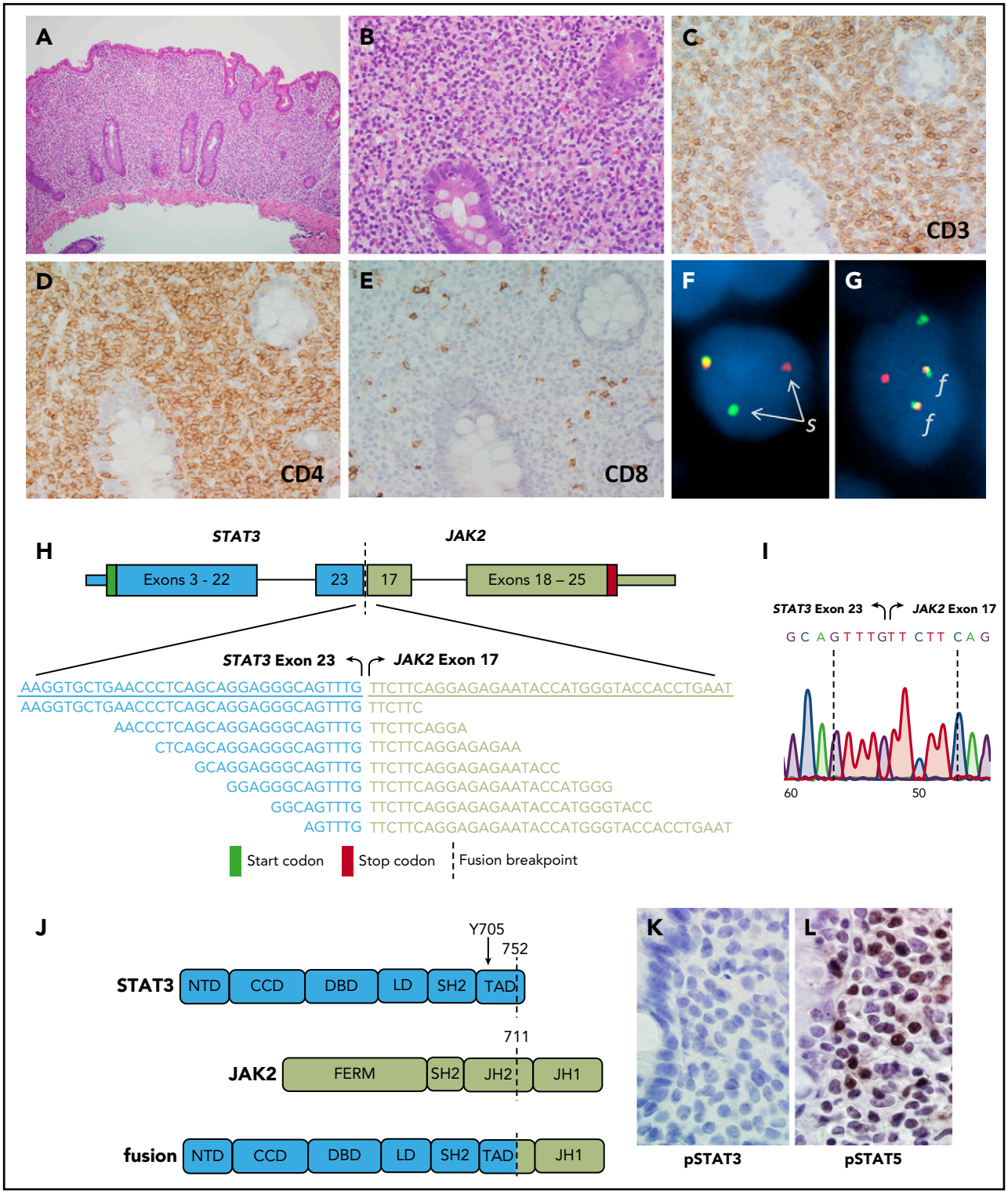

Figure 1. Indolent T-cell lymphoproliferative disorder of the gastrointestinal tract with *STAT3-JAK2* fusion. (A-B) Duodenal biopsy shows a monotonous population of small lymphocytes in the lamina propria with preservation of the glands (hematoxylin and eosin stain; patient 2; original magnification ×40 [A] and ×400 [B]). By immunohistochemistry, the cells are positive for (C) CD3 and (D) CD4 and negative for (E) CD8 (original magnification ×400 [C-E]). (F) FISH using a break-apart probe to the *JAK2* gene region shows separations of red and green signals, indicating the presence of a rearrangement (original magnification ×600). (G) FISH using a dual-fusion probe for the *STAT3* (red) and *JAK2* (green) gene regions shows red-green fusion signals (*f*), indicating the presence of fusion of these 2 regions (original magnification ×600). (H) RNA sequencing identifies chimeric reads supporting the presence of *STAT3-JAK2* fusion joining the end of exon 23 of *STAT3* with the beginning of exon 17 of *JAK2*. The identical breakpoint was found in all 3 patients who had GI TLPD with *STAT3-JAK2* fusion whose samples were successfully sequenced. (I) Sanger sequencing confirms the *STAT3-JAK2* breakpoint identified by RNA sequencing. (J) Protein domain structure of STAT3, JAK2, and predicted STAT3-JAK2 fusion protein. The amino acid numbers at the breakpoints are shown as well as the location of $STAT3^{Y705}$. Using immunohistochemistry showed that the cells lack staining for (K) $pSTAT3^{Y705}$ but are positive for (L) $pSTAT5^{Y694}$ (patient 2; original magnification ×1000). CCD, coiled coil domain; DBD, DNA binding domain; FERM, four-point-one, ezrin, radixin, moesin homology domain; JH1, JAK homology 1 (tyrosine kinase) domain; JH2, JAK homology 2 (pseudokinase) domain; LD, linker domain; NTD, N-terminal domain; SH2, Src homology 2 domain; TAD, transcription activation domain.

the functionally critical Y705 amino acid of STAT3 was present in the predicted STAT3-JAK2 fusion protein, the loss of the C-terminal portion of the STAT3 transcription activation domain or conformational alterations might inhibit Y705 phosphorylation. Notably, however, the N-terminal domain essential for formation of unphosphorylated STAT3 dimers[17] was also retained in the predicted STAT3-JAK2 fusion protein. Interestingly, *STAT3-RARA* fusions recently were described in acute promyelocytic leukemia, and coimmunoprecipitation studies confirmed the ability of the resultant fusion proteins to homodimerize.[18] JAK2 fusion partners such as TEL and PCM1 have been shown to contribute functionally to oncogenic signaling by facilitating fusion protein dimerization, leading to STAT5 activation.[4,5,7] Therefore, we examined pSTAT5 by immunohistochemistry and indeed 3 of 4 GI TLPD samples with *STAT3-JAK2* fusions were positive (Figure 1L) compared with only 1 of 6 evaluable samples without fusions. The patient sample that was negative for pSTAT5 was also negative for pSTAT1, as were samples from 6 of 8 additional patients tested.

Although limited follow-up data were available, 2 patients developed overt T-cell lymphomas. Patient 4 had a $CD4^+$ GI TLPD with *STAT3-JAK2* fusion and subsequently developed a large-cell transformation that shared the same fusion (supplemental Figure 1). The WHO classification notes that GI TLPDs expressing CD4 seem to have a higher risk of progression than those expressing CD8.[1,19] Interestingly, FISH in the large-cell component from patient 4 also showed copy number gains of *STAT3*, present in a previously reported $CD4^+$ GI TLPD with large-cell transformation.[19] Patient 9 had a $CD8^+$ GI TLPD that lacked *JAK2* rearrangement and subsequently developed systemic anaplastic lymphoma kinase–negative anaplastic large-cell lymphoma. Polymerase chain reaction studies identified different clonal T-cell receptor gene rearrangements in the 2 specimens (not shown), suggesting that the anaplastic large-cell lymphoma arose as a second, distinct process.

Taken together, our findings conclusively demonstrate *STAT3-JAK2* fusions in GI TLPD, representing the first recurrent genetic abnormality reported in this disease. Fusions were seen in 4 of 5 patients with $CD4^+$ phenotypes but not in other phenotypes, suggesting that the molecular pathogenesis of GI TLPD may vary on the basis of cell of origin and that distinct molecular subentities may exist. FISH for *JAK2* rearrangements might help distinguish GI TLPD from reactive processes because GI TLPDs are cytologically bland and may lack phenotypic aberrancy. Furthermore, *STAT3-JAK2* fusions represent a candidate therapeutic target in GI TLPD. A variety of strategies to inhibit JAK-STAT signaling are clinically available or in preclinical development for lymphoma, other malignancies, and nonneoplastic conditions.[20,21] T-cell lymphoma cell lines with *PCM1-JAK2* fusion are sensitive to the selective JAK2 inhibitor TG101348,[7] whereas clinical responses to the JAK1/2 inhibitor ruxolitinib have been reported in myeloid neoplasms with *PCM1-JAK2*.[22-24] Thus, the activity of JAK2 inhibitors in GI TLPD with *STAT3-JAK2* fusion merits further study.

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Authorship

Contribution: A.S., J.A.M., and A.L.F. designed the study; N.O., G.H., H.K.B., D.L.K., and S.M.K.-N. performed experiments; A.S., K.N.L., N.N.B., G.S.N., and J.A.M. interpreted clinical data; N.O., T.-T.W., and A.L.F. interpreted pathology data; R.L.B., A.A.N., S.D., and A.L.F. interpreted genetic findings; R.P.K. and P.T.G. interpreted FISH results; R.H. interpreted T-cell receptor gene rearrangement studies; B.W.E., J.J., J.I.D., and S.D. interpreted RNA quality control and sequencing data; A.L.F. drafted the manuscript; and all authors approved the final manuscript.

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ORCID profile: A.L.F., 0000-0001-5009-4808.

Correspondence: Joseph A. Murray, Division of Gastroenterology and Hepatology, Mayo Clinic, 200 First St SW, Rochester, MN 55905; e-mail: murray.joseph@mayo.edu; and Andrew L. Feldman, Department of Laboratory Medicine and Pathology, Mayo Clinic, 200 First St SW, Hilton Building, Room 800F, Rochester, MN 55905; e-mail: feldman.andrew@mayo.edu.

Footnotes

*J.A.M. and A.L.F. contributed equally to this work.

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TO THE EDITOR:

RUNX1 mutations in pediatric acute myeloid leukemia are associated with distinct genetic features and an inferior prognosis

Genki Yamato,[1-3] Norio Shiba,[3,4] Kenichi Yoshida,[5] Yusuke Hara,[2,3] Yuichi Shiraishi,[6] Kentaro Ohki,[7] Jun Okubo,[1] Myoung-ja Park,[1] Manabu Sotomatsu,[1] Hirokazu Arakawa,[2] Nobutaka Kiyokawa,[7] Daisuke Tomizawa,[8] Souichi Adachi,[9] Takashi Taga,[10] Keizo Horibe,[3] Satoru Miyano,[6,11] Seishi Ogawa,[5,12] and Yasuhide Hayashi[1,3,13]

[1]Department of Hematology/Oncology, Gunma Children's Medical Center, Gunma, Japan; [2]Department of Pediatrics, Graduate School of Medicine, Gunma University, Gunma, Japan; [3]Clinical Research Center, National Hospital Organization Nagoya Medical Center, Aichi, Japan; [4]Department of Pediatrics, Yokohama City University Hospital, Kanagawa, Japan; [5]Department of Pathology and Tumor Biology, Graduate School of Medicine, Kyoto University, Kyoto, Japan; [6]Laboratory of DNA Information Analysis, Human Genome Center, Institute of Medical Science, The University of Tokyo, Tokyo, Japan; [7]Department of Pediatric Hematology and Oncology Research, National Research Institute for Child Health and Development, Tokyo, Japan; [8]Children's Cancer Center, National Center for Child Health and Development, Tokyo, Japan; [9]Department of Human Health Science, Graduate School of Medicine, Kyoto University, Kyoto, Japan; [10]Department of Pediatrics, Shiga University of Medical Science, Shiga, Japan; [11]Laboratory of Sequence Analysis, Human Genome Center, Institute of Medical Science, The University of Tokyo, Tokyo, Japan; [12]Center for Hematology and Regenerative Medicine, Department of Medicine, Karolinska Institute, Stockholm, Sweden; and [13]Japanese Red Cross Gunma Blood Center, Gunma, Japan

Acute myeloid leukemia (AML) is a complicated disease characterized by the uncontrolled proliferation of hematopoietic precursors and the loss of differentiation ability caused by various genetic alterations. Recent advances in massively parallel sequencing technologies have identified several gene mutations associated with the pathogenesis of AML, including mutations in *NPM1*, *DNMT3A*, *IDH1/2*, and *TET2*.[1-6] However, the rarity of some of mutations, such as *DNMT3A*, *IDH1/2*, and *TET2*, in pediatric AML[7,8] necessitates exploring additional biomarkers to stratify pediatric patients with AML. Remarkably, the 2017 European LeukemiaNet recommendations incorporated *RUNX1* mutations to the group of markers, suggesting adverse risks in adult AML.[9] However, the low frequency of *RUNX1* mutations renders the prognosis of pediatric patients with AML uncertain.[10-12] Thus, this study aims to investigate *RUNX1* mutations and their correlation with other gene aberrations to elucidate the prognostic impact in 503 pediatric patients with de novo AML.

In this retrospective cohort study, we recruited patients with de novo AML (age, <18 years) who participated in either the AML99 clinical trial of the Japanese Childhood AML Cooperative Study (January 2000 to December 2002) or the AML-05 clinical trial of the Japanese Pediatric Leukemia/Lymphoma Study Group (November 2006 to December 2010).[13,14] Overall, we enrolled 503 patients with available leukemic samples in this study comprising 134 of 280 from the AML99 trial and 369 of 485 from the AML-05 trial (supplemental Table 1, available on the *Blood* Web site). We observed no significant differences in the overall survival (OS) between the available and unavailable samples in the AML99 or AML-05 trial (supplemental