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

A Sharma, et al.

Supplemental Material

Supplemental Methods

Patients and clinical samples

The study was approved by the Mayo Clinic Institutional Review Board. Using an electronic database search and review of medical records and pathology slides from patients with T- or NK-cell lymphomas or lymphoproliferative disorders involving the gastrointestinal tract, we identified 10 patients with conditions meeting current WHO criteria for indolent T-cell lymphoproliferative disorder of the gastrointestinal tract (GI TLPD) and 1 patient with NK enteropathy diagnosed at Mayo Clinic in Rochester, MN between 2006 and 2016.

Fluorescence *in situ* hybridization (FISH) and immunohistochemistry

Rearrangements involving the *JAK2* locus on 9p24.1 were assessed using a previously published breakapart FISH probe.[1] A dual-fusion probe to detect t(9;17)(p24.1;q21.2) translocations involving the *JAK2* and *STAT3* loci was developed by labeling bacterial artificial chromosomes with SpectrumGreen dUTP (Vysis; *JAK2*: RP11-980L14, RP11-927H16, and CTD-2506A8) or SpectrumOrange dUTP (Vysis; *STAT3*: RP11-358B23 [AC099811.7], RP11-1120O4, and RP11-194N12). Interphase FISH was performed and scored on formalin-fixed, paraffin-embedded (FFPE) tissue sections as previously described.[2,3] Immunohistochemistry was performed on FFPE tissue sections as previously described[4] using rabbit monoclonal antibodies (Cell Signaling Technology) to pSTAT1^{Y701} (clone 58D6; 1:800), pSTAT3^{Y705} (clone D3A7; 1:400) and pSTAT5^{Y694} (clone C11C5; 1:300). Other antigens indicated in Table 1 were assessed

diagnostically using antibodies and methods previously published.[5] Stains were reviewed by two pathologists (N.O. and A.L.F.). Staining in ≥30% of tumor cells was considered positive. Photomicrographs were taken using an Olympus (Melville, NY) DP71 camera, Olympus BX51 microscope, and Olympus cellSens image acquisition software.

T-cell receptor (TCR) gene rearrangement analysis

Polymerase chain reaction was performed for TCR Vβ and Vγ using the BIOMED-2 assay (Invivoscribe), subjected to electrophoresis, and evaluated using standard criteria as previously published.[6,7]

RNA sequencing and Sanger validation

RNA was extracted from FFPE tissue sections using the AllPrep DNA/RNA FFPE kit (Qiagen) following the manufacturer's protocol. RNA library preparation was performed using TruSeq Stranded Total RNA and RNA Access capture (Illumina) and sequencing was performed as previously published.[8,9] Candidate fusion transcripts were identified using two versions of the Mayo Analysis Pipeline for RNA sequencing (MAP-RSeq) based on STAR and TopHat software, respectively.[10-12] For Sanger sequencing confirmation of the breakpoint, cDNA was amplified using either two rounds of PCR (1st round: forward primer, 5'-TAGATTCATTGATGCAGTTTGG-3' and reverse primer, 5'-AAAACTCCATTTGTCTGTTGCC-3'; 2nd round: forward primer, 5'-GGAAATAATGGTGAAGGTGCTG-3' and reverse primer, 5'-AAAACTCCATTTGTCTGTTGCC-3') or a single round of PCR (forward primer, 5'-GGAAATAATGGTGAAGGTGCTG-3' and reverse primer, 5'-TTTCAATGCATTCAGGTGGTAC-3'). PCR conditions for all reactions were: 95°C for 15 min, (94°C for 15 sec, 58°C for 30 sec, and 72°C for 30 sec) x 35 cycles, and final extension at 72°C for 10 min.

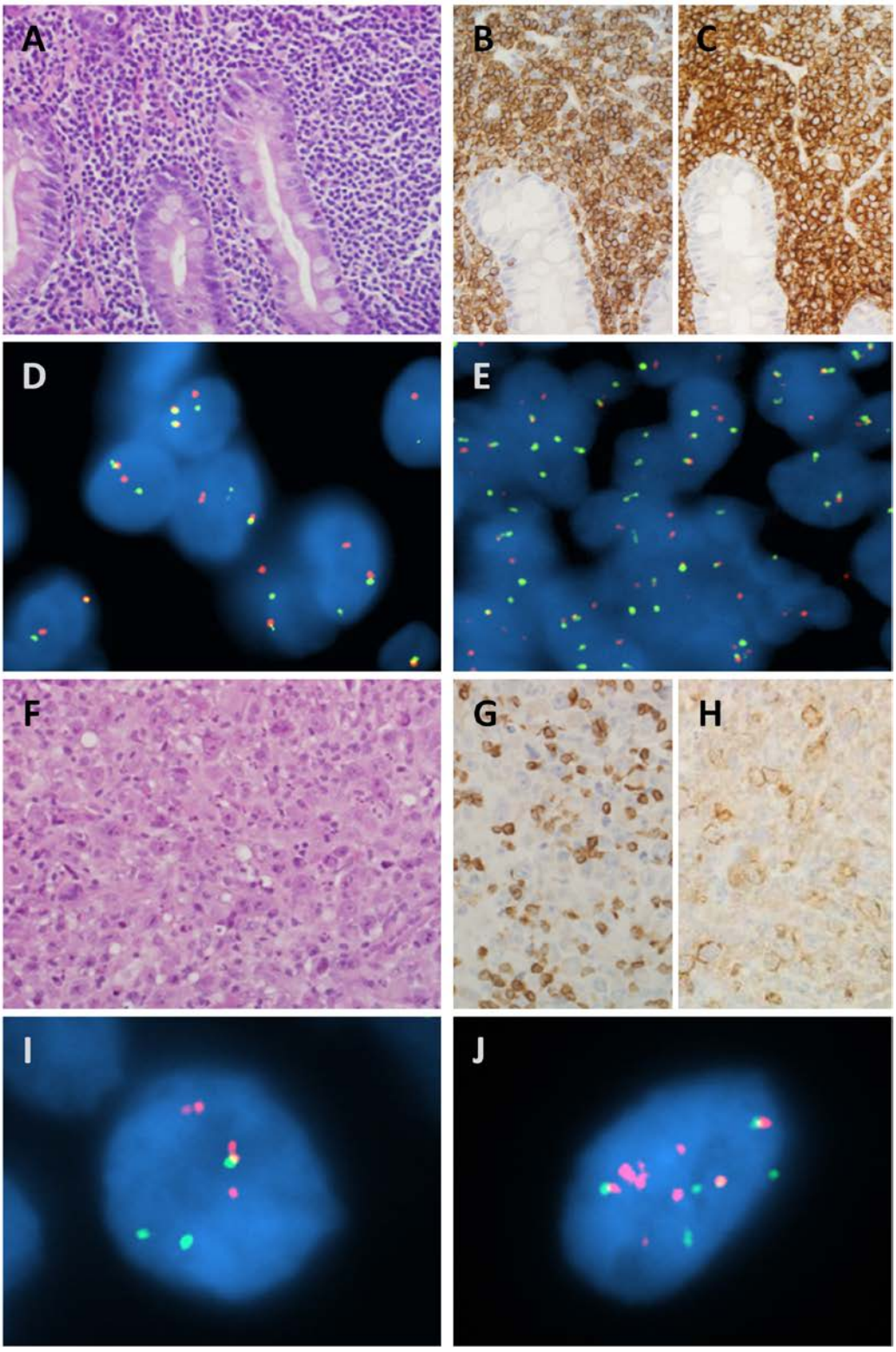
Supplemental Table

Supplemental Table 1. Results of FoundationOne Heme Testing in Indolent Gastrointestinal T-cell Lymphoproliferative Disorder or NK-cell Enteropathy

Patient	FoundationOne Heme Findings
1	*STAT3-JAK2* fusion
2	n.d.
3	n.d.
4	n.d.
5	*ARID1A* L2029fs*7
6	n.d.
7	n.d.
8	*CHEK2* splice site 793-1G>A
9	n.d.
10	n.d.
11	*NRAS* G12D, *RAD50* Q833fs*11

n.d., not done.

Supplemental Figure



Supplemental Figure 1. Large cell transformation of gastrointestinal T-cell lymphoproliferative disorder with *STAT3-JAK2* fusion. (A) Duodenal biopsy shows a

monotonous population of small lymphocytes in the lamina propria with preservation of the glands (H&E stain, patient 4). By immunohistochemistry, the cells are positive for CD3 (B) and CD4 (C). (D) FISH using a breakapart probe to the *JAK2* gene region shows separation of red and green signals, indicating the presence of a rearrangement. (E) FISH using a dual-fusion probe for the *STAT3* (red) and *JAK2* (green) gene regions shows red-green fusion signals, indicating the presence of fusion of these two regions. (F) Biopsy of an inguinal lymph node shows an infiltrate of large atypical mononuclear cells. These cells show loss of CD3 (G; positive cells represent admixed small reactive T cells) with weak expression of CD4 (H). (I) Breakapart FISH shows rearrangement of *JAK2*. (J) Dual-fusion FISH shows *STAT3-JAK2* fusion. Additional copy number abnormalities also are present in the large cell transformation. Original magnifications: H&E and immunohistochemical stains, ×400; FISH images, ×600.

Supplemental References

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