Genetic and phenotypic characterization of indolent T-cell lymphoproliferative disorders of the gastrointestinal tract

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Supplementary Methods

Immunohistochemistry

Immunohistochemical (IHC) staining was performed using the following primary antibodies: CD3, CD5, CD8, CD20 and CD30 (DAKO, Carpinteria, CA); CD2, CD7, CD25 and CD56, (Vector, Burlingame, CA); CD4 (BioGenex, San Ramon, CA); TCRγ (ThermoFisher, Waltham, MA); perforin (Novocastra, Newcastle Upon Tyne, UK); granzyme-B (Chemicon, Temecula, CA); T-cell intracellular antigen-1 (TIA-1) (Beckman Coulter, Fullerton, CA); Ki-67 (Ventana, Tucson, AZ); PD1 (Cell Marque, Rocklin, CA); CD103, FoxP3, H3K36me3, H3K36me2 (Abcam, Cambridge, MA), GATA3 (Biocare Medical, Pacheco, CA), T-bet, p-STAT3(Tyr705)(clone D3A7) (Cell Signaling Technologies, Danvers, MA), p-STAT5(Tyr694/9)(clone Y694/99) (Advantex BioReagents, Houston, TX), MATK(LSK)(clone H-1) (Santa Cruz Biotechnology, Dallas, TX) and SETD2 (Sigma, Darmstadt, Germany). Staining for pSTAT5 was performed on a Benchmark Ultra autostainer (Ventana). After heat-induced epitope retrieval (Tris based pH = 9.0), slides were incubated with the antibody (1:50 dilution) for 32 minutes at 37°C; Optiview DAB kit (Ventana) was used for visualization. Staining for pSTAT3 was performed on a Discovery Ultra autostainer (Ventana). After heat-induced epitope retrieval (Tris based pH = 9.0), slides were incubated with antibody (1:25 dilution) for 2 hours at room temperature; ChromoMap DAB kit (Ventana) was used for visualization. MATK staining was performed on a Bond Max autostainer (Leica Biosystems, Bannockburn, IL, USA). After heat-induced epitope retrieval (EDTA based pH = 9.0), slides were incubated with antibody (dilution of 1:200) for 15 minutes; Bond Polymer Refine detection kit (Leica) was used for visualization. For SETD2 and H3K36, IHC was performed manually after heat-induced antigen retrieval (Tris/EDTA pH = 9.0) as previously described[17]. All other IHC staining was performed according to standard protocols on a Bond III autostainer (Leica) after online-automated heat epitope induced retrieval and the Bond Polymer Refine detection kit was used for visualization.

Flow cytometry

Four or eight color flow cytometry was performed on cell suspensions prepared from tissue samples (FACScan; Becton Dickinson, San Diego, CA) and data were analyzed using FCS Express software (De Novo Software, Los Angeles, CA) according to standard procedures. The antigens evaluated included CD45, CD2, CD3 (cytoplasmic and surface), CD4, CD5, CD7, CD8, CD30, CD56, CD20, CD25, CD43, CD103, PD1, TCRαβ and TCRγδ.

Validation of chromosomal structural alterations

PCR amplification of the DNA breakpoints and Sanger sequencing was performed on the tumor and matched normal samples. M13-tailed case-specific primers were designed to span the identified breakpoints. PCR was performed using 50 ng sample DNA, 30 ng of each specific primer, and Platinum Taq High Fidelity DNA polymerase (Invitrogen, ThermoFisher Scientific, Pittsburgh, PA). Thermal cycling conditions were one cycle at 94 C for five minutes; followed by 35 cycles at 94 C for 60s, 55 C for 90s, 68 C for 60s; followed by 68 C for 5 min. Products were used as templates for bidirectional BigDyeTerminator V1.1 Sanger sequencing (Applied Biosystems, ThermoFisher Scientific, Pittsburgh, PA).

Supplementary Tables

Supplementary Table 1: Quantification of T-bet and GATA3 expression.

Case	*Phenotype*	*T-bet (% positive)*	*GATA3 (% positive)*
1	CD4+	100%	20%
2	CD4+	100%	50%
3	CD4+	20%	100%
4	CD4+	100%	100%
5	CD4+/CD8+	50%	100%
6	CD4-/CD8-	5%	100%
7	CD8+	20%	100%
8	CD8+	60%	100%
9	CD8+	10%	100%
10	CD8+	20%	100%

Supplementary Table 2: Variants of Uncertain Significance

Case	Gene	Transcript	Chrm	Position	Total Reads	Alt Reads	Ref Base	Alt Base	AA Change	Base Change
4	FAT1	NM_005245	4	187628173	601	45	G	A	R937X	c.2809C>T
4	FOXL2	NM_023067	3	138665048	213	16	C	G	A173P	c.517G>C
4	KAT6A	NM_001099412	8	41790108	1397	109	C	T	R1877H	c.5630G>A
4	KDM5C	NM_004187	X	53223826	626	122	G	A	S1178L	c.3533C>T
4	KDM5C	NM_004187	X	53223844	587	119	G	T	P1172H	c.3515C>A
4	KMT2D	NM_003482	12	49415596	276	24	A	C	C5527W	c.16581T>G
4	RBM10	NM_001204468	X	47032564	291	53	G	A	R222Q	c.665G>A
6	MED12	NM_005120	X	70339981	684	117	G	A	E172K	c.514G>A
6	TET2	NM_001127208	4	106164741	438	130	C	G	S1203R	c.3609C>G
7	PDGFRA	NM_006206	4	55143621	491	26	C	A	S618Y	c.1853C>A
7	POLE	NM_006231	12	133256236	488	25	G	T	P142Q	c.425C>A
8	FAT1	NM_005245	4	187524362	737	42	C	T	R3773H	c.11318G>A
9	ROS1	NM_002944	6	117714432	1146	253	G	A	P406L	c.1217C>T

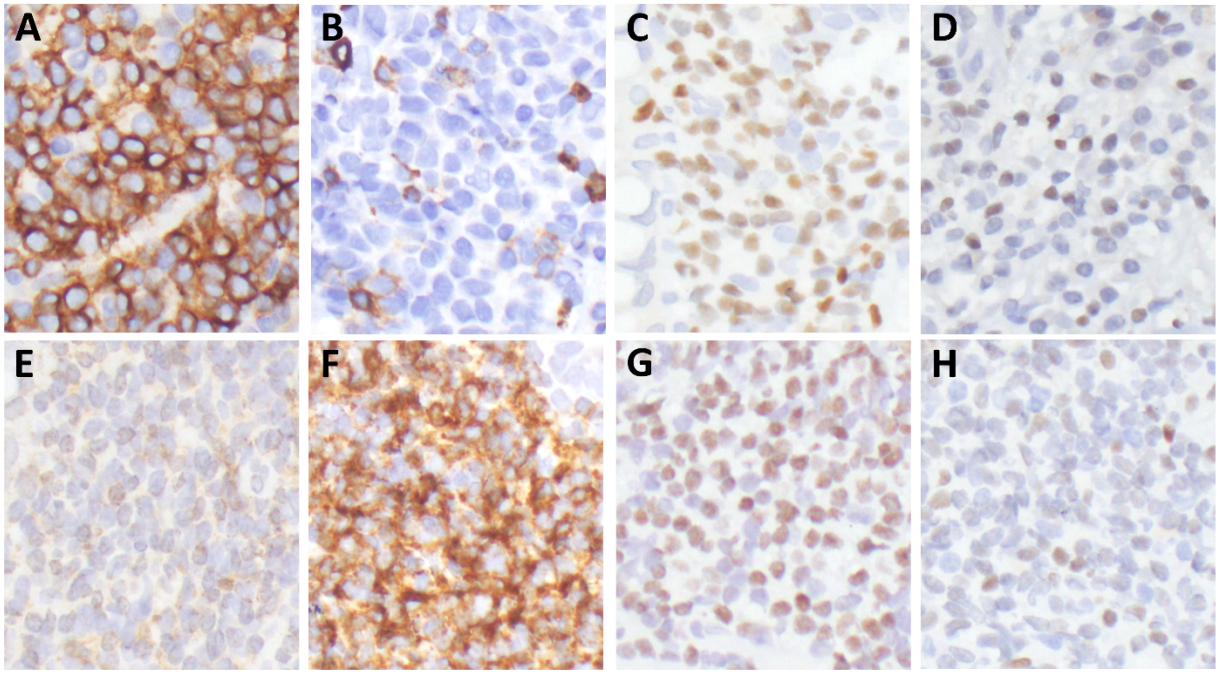
Supplementary Table 3: SETD2-H3K36me3 and pSTAT3/pSTAT5 Immunohistochemical Analysis

Case	*SETD2 FISH*	*SETD2 IHC*	*H3K36me2*	*H3K36me3*	*pSTAT3 (Y705)*	*pSTAT5 (Y694)*
1	-	+	+	+	-	-
2	-	+	+	+	-	-
3	NP	NP	NP	NP	-	-
4	-	+	+	+	-	-
5	NP	NP	NP	NP	-	-
6	-	+	+	+	-	NP
7	-	+	+	+	-	-
8	-	+	+	+	-	-
9	-	+	+	+	-	-
10	NP	NP	NP	NP	NP	NP
TOTAL	0/7 0%	7/7 (100%)	7/7 (100%)	7/7 (100%)	0/9 (0%)	0/8 (0%)
CD4+	0/3 (0%)	3/3 (100%)	3/3 (100%)	3/3 (100%)	0/4 (0%)	0/4 (0%)
DP	NP	NP	NP	NP	0/1 (0%)	0/1 (0%)
DN	0/1 (0%)	1/1 (100%)	1/1 (100%)	1/1 (100%)	0/1 (0%)	NP
CD8+	0/3 (0%)	3/3 (100%)	3/3 (100%)	3/3 (100%)	0/3 (0%)	0/3 (0%)

+, positive; -, negative; DN, Double-negative; DP, Double-positive; NP, Not performed

Supplementary Figures

Supplementary Figure 1: T-bet and GATA3 immunohistochemistry.



Case 1 is positive for **(A)** CD4 and negative for **(B)** CD8. **(C)** T-bet is expressed by the majority and **(D)** GATA3 is expressed by 20% of cells.

Case 9 is negative for **(E)** CD4 and positive for **(F)** CD8. **(G)** GATA3 is expressed by virtually all the cells and **(H)** T-bet is expressed by 10% of cells.