IGH sequencing identifies multiple EBV+ mucosal ulcers and may distinguish from recurrent malignant DLBCL

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History and Presentation

• 66 y.o. Cuban male with no history of malignancy.

• Lymphadenopathy 4 years prior, “atypical lymphoid proliferation” with increased EBV+ cells (reviewed at Mayo Clinic and Univ. Rochester)

• Began experiencing B. symptoms, anemia, severe fatigue, and rapid growth of lymph nodes (May 2015).

• PET scan demonstrated significant hypermetabolic LAD as well as uptake in the spleen, lungs, and bones.

• Axillary lymph node biopsy (June, 2015).
Flow cytometry:
93% T-cells, ~60% atypical
Positive: CD2, CD4, CD5
Negative: CD3, CD7, CD30.

PCR:
Positive TCRg (clonal)
Negative IGK (polyclonal)

No evidence of TFH derivation
Negative: PD-1, CD10, CD23
Bcl-6: scattered cells.
CD21: sparse FDC remnants.
Diagnosis

Axillary lymph node:

Peripheral T-cell lymphoma, not otherwise specified, with EBV positive Hodgkin/Reed-Sternberg-like cells.

EBV+ RS-like cells occur in PTCL

Both EBV+ and negative HRS variants exist in PTCL (F_{HT}-T-cell derivation).
Subsequent Course

- Rapid disease progression; within <1 month (July 2015) readmitted for upper GI bleed, prior to treatment.

- Endoscopy identified ulcerative gastric nodule, biopsied:
Negative: CD20, Pax5, CD138, CD2, CD3, CD4, CD5

PCR:
- Positive IGK (clonal)
- Positive IGH (clonal)
- Negative TCRg (weak signal)
Diagnosis #2:

Gastric nodule:

**EBV+ diffuse large B cell lymphoma.**

Comment: potential relationship to concurrent T cell NHL.

- **EBV+ B-LPD in AITL and PTCL**

- **Histologic evolution of AITL: insights into natural history and disease progression.**

- **B-cell lymphomas in AITL (EBV+ early proliferations).**

- **Targeting intratumoral B cells with Rituximab in addition to CHOP in AITL**
Treatment

• Six (6) full cycles CHOP.

• Negative PET scan (Nov, 2015) = complete remission.

• One month later (Dec 2015), repeat upper endoscopy for surveillance - blind gastric biopsies (antrum and body):
Negative: CD138

PCR:
  - Positive IGH (clonal)
  - Positive IGK (clonal)
  - Negative TCRg
Diagnosis #3:

Gastric biopsy, antrum and body:

**EBV+ B cell lymphoproliferative disorder**

*Comment: most consistent with recurrent DLBCL*

- Short course of lenalidomide was poorly tolerated and stopped.
- Felt well with good performance status.
- Monitored for “active disease”. 
Additional Course

• One year later (Dec 2016), with no additional treatment, lower GI bleeding recurred and colonoscopy revealed ulcerative lesion(s).

• Colonic biopsy performed:
EBER

PCR:
IGH weak positive (polyclonal background)
IGK positive oligoclonal/clonal
Diagnosis #4:

Colon, mass/ulceration (40 cm):

**EBV+ lymphoproliferative disorder**

Comment: defer to additional B cell clonality studies for further evaluation
Axillary lymph node (June 2015) – PTCL-NOS w/ EBV+ RS-like cells
- Polyclonal IGH pattern

NGS sequencing: IGH gene rearrangements
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**First gastric Bx (July 2015):**
- Monoclonal
- Single dominant VDJ recombination (two sequences differing by 1 base)
- VH3-J4 rearrangement (V3-73, J4, D4-23)
- Total combined reads ~55%.

**Second gastric Bx (Dec 2015):**
- Monoclonal
- Single dominant VDJ recombination (two sequences differing by 1 base)
- **DIFFERENT** VH4-J4 rearrangement (V4-59, J4, D3-10)
- Total combined reads ~56%.

**Third colonic Bx (Dec 2016):**
- Oligoclonal
- At least 4 **DIFFERENT** VDJ clones
- Each ranging ~2-8% total reads
- Evidence of some specific VDJ rearrangements that were also found in earlier specimens at low level (≤1% of total combined reads above)
Proposed Diagnosis:

Multiple independent clonal EBV+ mucocutaneous ulcers, status-post PTCL-NOS with EBV+ RS-like cells.

Panel Diagnosis:

EBV+ lymphoproliferative disorder (mucocutaneous ulcer versus DLBCL)
Follow up:
August/September 2017 (26 months).

Recent onset fatigue and weight loss

Persistent GI bleeding:

Negative upper endoscopic biopsy

Lower endoscopy identified TWO (2) distinct ulcerative lesions/masses

- Transverse colon

- Sigmoid colon (40cm – tattoo ink)
Diagnosis #5:
Morphologically c/w Diffuse large B cell lymphoma (DLBCL)

Diagnosis #6:
Morphologically c/w Peripheral T cell lymphoma (PTCL)
Diagnosis #5:
Morphologically c/w Diffuse large B cell lymphoma (DLBCL)

PCR:
- IGH: POSITIVE
- IGK: POSITIVE
- TCRg: POSITIVE

Diagnosis #6:
Morphologically c/w Peripheral T cell lymphoma (PTCL)

PCR:
- IGH: POSITIVE
- IGK: POSITIVE
- TCRg: POSITIVE
Diagnosis #5: Morphologically c/w Diffuse large B cell lymphoma (DLBCL)

PCR:
- IGH: POSITIVE
- IGK: POSITIVE
- TCRg: POSITIVE

None are the same size rearrangement

Diagnosis #6: Morphologically c/w Peripheral T cell lymphoma (PTCL)

PCR:
- IGH: POSITIVE
- IGK: POSITIVE
- TCRg: POSITIVE
Discussion:

Concurrent PTCL and EBV+ DLBCL at diagnosis and relapse.

Intervening multiple GI biopsies highly concerning for EBV+ DLBCL, but indolent/smoldering clinical course.

Positive PCR testing for clonality supported such an interpretation.

IGH sequencing, however, demonstrates entirely unique dominant B cell clone(s) (i.e. VDJ sequences) from each specimen.

Thus, we exclude “clonal evolution” of a single B cell line (i.e. single VDJ recombination event).

Next-gen deep sequencing of the IGH locus shows a complex relationship with varying dominant B cell clones over time.
Discussion (cont’d):

Among the low level, or “subclonal” recombination sequences detected in each specimen, there is evidence that same clone (or clones) can be minimally detected at different points in time.

In a patient with abnormal immune system, the pathology within the GI tract, over >1 year, may represent multiple independent EBV+ lymphoproliferative disorders

Clonal dynamics may change or shift over time in response to, or as a cause of (?), recurrent T cell lymphoma.
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Approach: IGH NGS Platform

- LymphoTrack IGH Assay (Invivoscribe)
  - Illumina MiSeq
- Framework 1 primers – unique VDJ sequences
- Sequencing depth: >$10^6$ reads
- 200 top unique sequences analyzed
- Negative (polyclonal) control
- Positive control: example (5% spike)
- Clonal signal: unique sequence $\geq 2\%$ total reads