Case#: SH2017-0111

**STAT3**-mutated T-cell Large granular lymphocytic leukemia (T-LGL)
donor-derived, status-post cord transplant for T lymphoblastic leukemia

David Wu, Lori Soma, Jonathan Fromm, Katherine Tarlock, and Brent Wood
Univ. of Washington, Seattle

Session 2: Genetic Testing in the Diagnosis of Lymphoid Neoplasms
Chairs: Miguel Piris and Rebecca King
History

- 6 year old female with outside diagnosis of Precursor T lymphoblastic leukemia (T-ALL) submitted for routine flow cytometry MRD detection
- Original diagnostic sample not provided, but day 15 post-induction sample showed abnormal T-cell population
Day 15 Post induction T-ALL

- Persistent disease with 25% leukemic blasts, she underwent salvage chemotherapy with complete remission
  - Received 4/6 mismatched cord blood transplant with ex vivo expanded cord blood unit
  - Developed acute grade IIB GVHD and flare of gastrointestinal GVHD which was treated with steroids
- Subsequent Day 28 and 1 year samples were negative for MRD
- However, ~4 years and 2 months post-transplant, developed neutropenia progressing to persistent cytopenia
4 years post-cord transplant: Persistent LGL expansion

Peripheral blood, 21% of total leukocytes, LGL population in teal

Bone marrow, 39% of total leukocytes, LGL population in teal
Molecular analysis of TRG was clonal

- Cytopenia was persistent over months

- Clonal TRG gene rearrangement identified in the post-transplant bone marrow:

Findings c/w a clonal expansion; however, reactive T cells may be clonal
Differential of T-cell LGL

• Reactive T/NK-cell proliferation
• Malignant T/NK-cell proliferation
  – T prolymphocytic leukemia
  – Hepatosplenic T-cell lymphoma
  – EBV+ T-cell lymphoproliferative disorder
  – Peripheral T-cell lymphoma, not otherwise specified
NGS showed *STAT3* mutation in unsorted PB sample (NM_139276.2:c.1919A>T, p.Y640F)

*STAT3* (c.1919A>T, p.Y640F)
hg19, Chr17:40474482 A>T variant 360, reference reads 2812
VAF 11%
LGL by flow cytometry at 20.9%
Donor-derived origin

- Female patient had received male donor cord-transplant
  - Based on NGS sequencing data, informal copy analysis of genes on X chromosome suggested male karyotype (data not shown)
  - Short-tandem repeat analysis (data not shown) on flow cytometry cell sorted LGL population from peripheral blood confirmed full chimerism
**STAT3 mutations common in LGL**

- Koskela *et al.* used NGS to identify index *STAT3* heterozygous SH2 mutation T>A (p.D661V) in 70 year old M
- In 31 of 77 additional patients (40%), additional *STAT3* mutations identified in exon 21, encoding Src-like homology 2 (SH2) domain
- Mutations (dimerization surface) result in a more hydrophobic surface and activation
- *In vitro* work demonstrated STAT3 pathway activation and confirmed by phospho-STAT3 IHC in patient samples

**STAT3** mutations common in LGL

- **STAT3** mutations present in 1/3 of LGL and chronic NK cell lymphoproliferative disorders

*Jerez et al., Blood. 2012;120(15):3048-3057*
**STAT3 mutations may occur outside of SH2 domain**

- Samples from LGL leukemia patients (n=106) without STAT3 and STAT5 SH2-domain mutations
  - 88 patients had T-LGL leukemia / 18 cases NK-LGL leukemia.
- Targeted deep amplicon sequencing of all 23 coding exons of the STAT3 gene
- 3.8% (4 of 106 patients) had non-SH2 mutations

Anderrson et al., Leukemia. 2016 May;30(5):1204.
**STAT5b mutations in LGL**

- Exome/transcriptome sequencing of two STAT3-wild-type patients identified Y665F in Src-like homology 2 domain of STAT5b gene
- Targeted sequencing of 211 LGL leukemia revealed 2 additional patients with STAT5b mutation (N642H); 2% (4 of 211)
- Both Y665F and N642H mutations result in increased STAT5b transcriptional activity

Raja et al., Blood 2013 121:4541-4550
STAT3 mutations in other T-cell lymphomas

- Ohgami et al., Leukemia (2013) 27, 2244–2247
  - 95 cases: ALK-negative ALCL (n=12), ALK-positive ALCL (2), CD30 lymphoproliferative disorders in the skin (3), Sezary syndrome (13), T-LGL leukemia (36) or PTCLs (including angioimmunoblastic T-cell lymphoma and peripheral T-cell lymphoma, NOS; 29)
  - 2/12 cases of ALK-negative CD30+ ALCL and 2/8 cases CD30+ PTCLs positive for STAT3 mutation (N647I, D661H, Y640F, same as LGL)

  - Activating mutations of STAT3 and STAT5B in NK/T-cell lymphomas (n = 51),
Conclusion

- *STAT3* mutations are present in ~30 to 40% of T-LGL cases
- Rare mutations outside of SH2-like domain in *STAT3* and in *STAT5b* may occur in T-LGL
Proposed and Panel Diagnosis

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• T-cell Large granular lymphocytic leukemia, donor-derived
• STAT3 mutation testing aided in diagnosis
Limited literature on LGL post-transplantation

• Gill et al., Bone Marrow Transplant, 47(7):952 (2012):
  – 4 women, 3 men after allogeneic (n=4) and autologous (n=3) HSCT had increased T-LGLs; clonal TCR gene confirmed
  – In allogeneic cases, T-LGL were donor-derived in 3 patients

  – 16 year old male s/p allo-SCT for PTCL-NOS presented with neutropenia and splenomegaly 9 months after SCT
  – No STAT3 mutation in exon 21

• Le Bris et al., Bone Marrow Transplant 2017 Jan 30. doi:10.1038/bmt.2016.364: Considered 85 recipients of unrelated cord blood allo-HSCT
  – 25% (n=21) had sustained lymphocytosis, median duration 12 months, with onset at 12.6 months
  – Flow cytometry analysis showed CD8+ expansion and/or polyclonal B-cell expansions;
  – 3 had monoclonal process; STAT3 mutation not reported; patients with lymphocytosis had better outcome