Case#: SH2017-0111

STAT3-mutated T-cell Large granular lymphocytic leukemia (T-LGL)

donor-derived, status-post cord transplant for
T lymphoblastic leukemia

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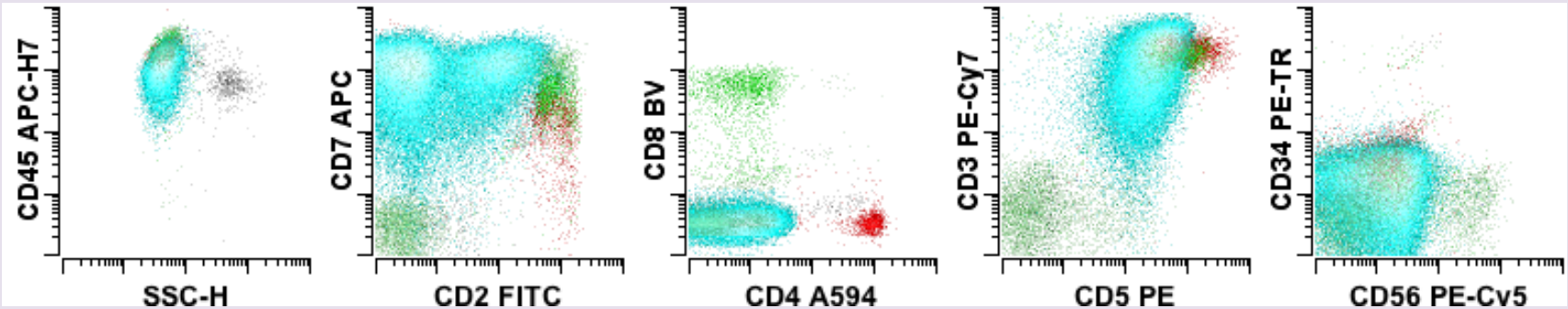
Session 2: Genetic Testing in the Diagnosis of Lymphoid Neoplasms

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

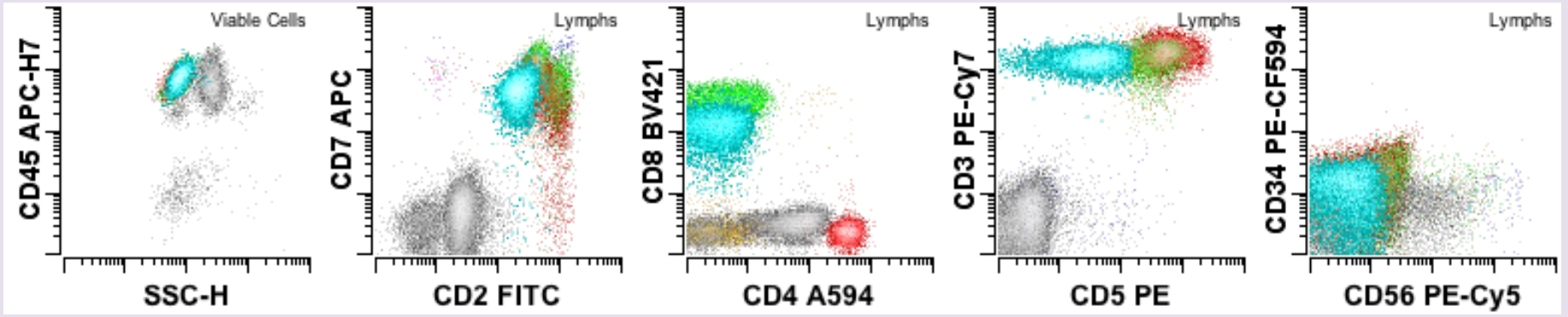
Day 15 Post induction T-ALL



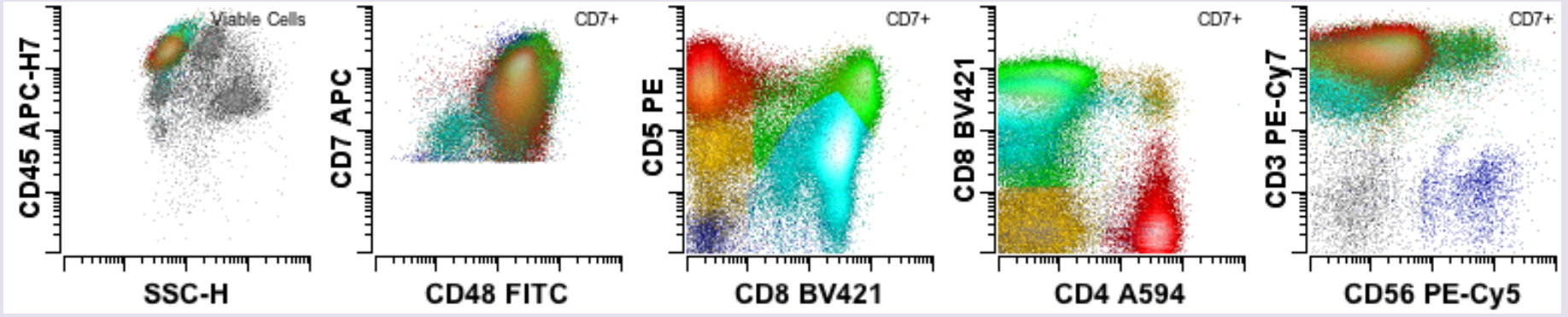
- Persistent disease with 25% leukemic blasts, she underwent salvage chemotherapy with complete remission
  - Received 4/6 mismatched cord blood transplant with ex vivo expanded cord blood unit
  - Developed acute grade IIB GVHD and flare of gastrointestinal GVHD which was treated with steroids
- Subsequent 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

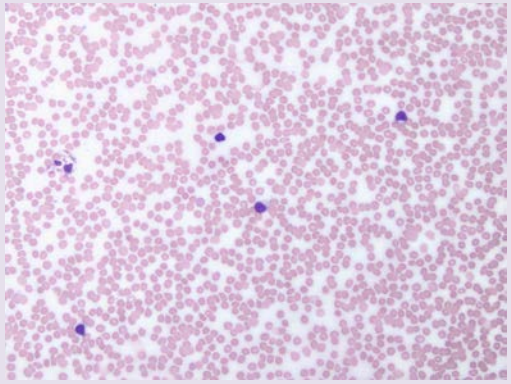
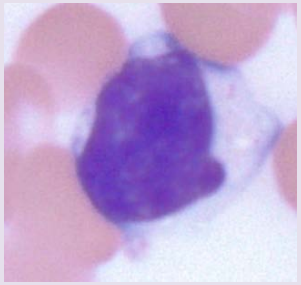
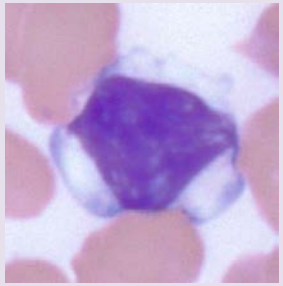
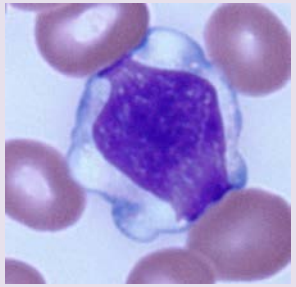


Bone marrow, 39% of total leukocytes, LGL population in teal

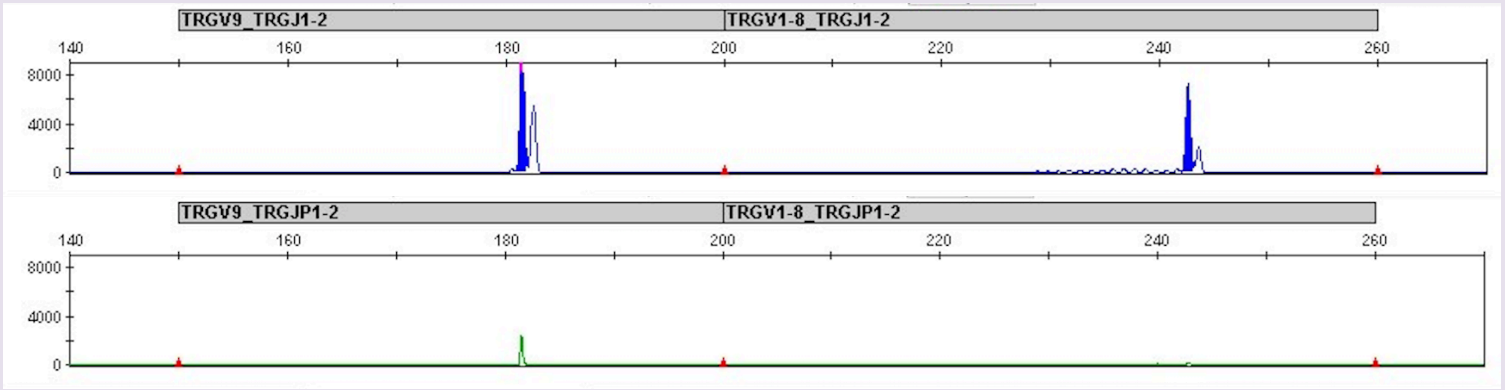


Molecular analysis of *TRG* was clonal

- Cytopenia was persistent over months

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



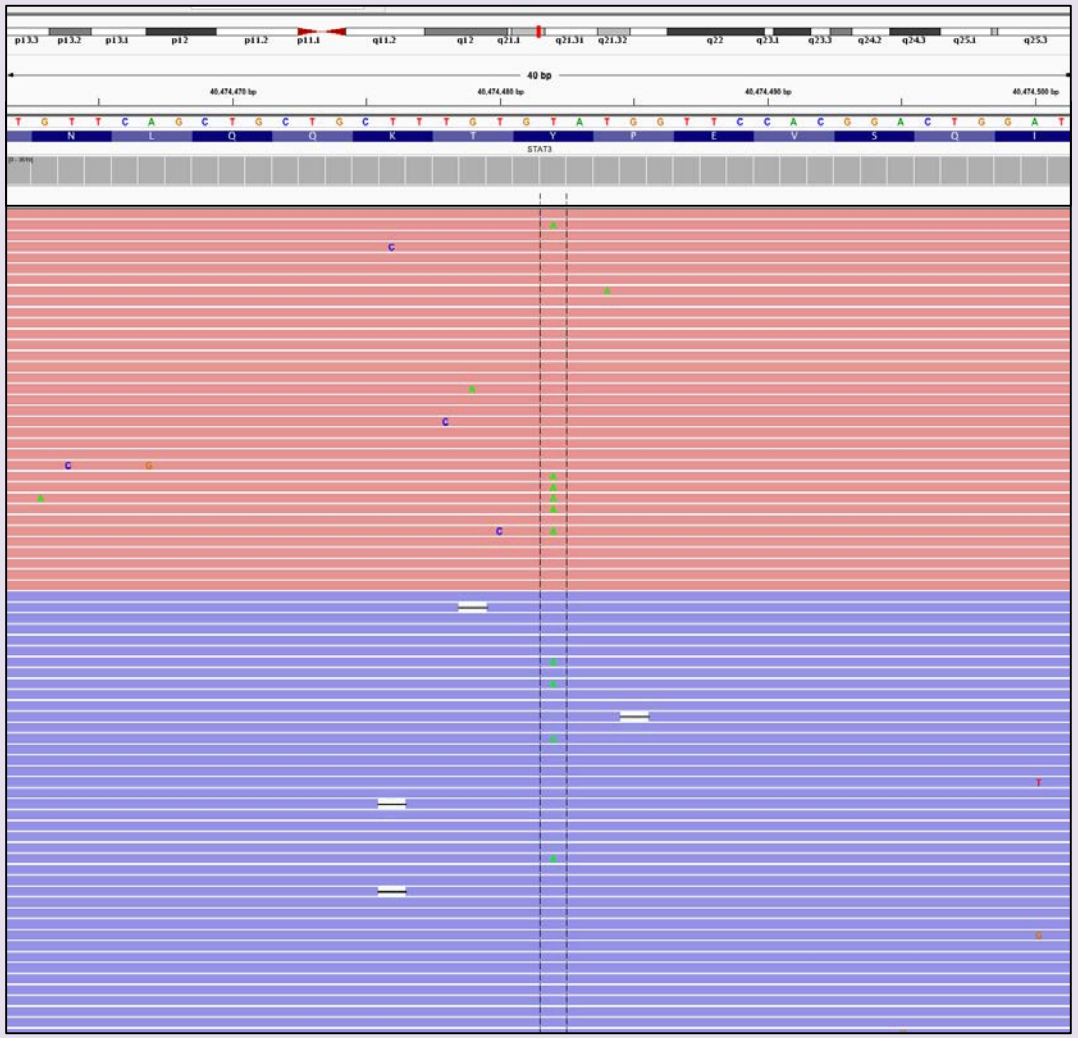
Findings c/w a clonal expansion; however, reactive T cells may be clonal

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%

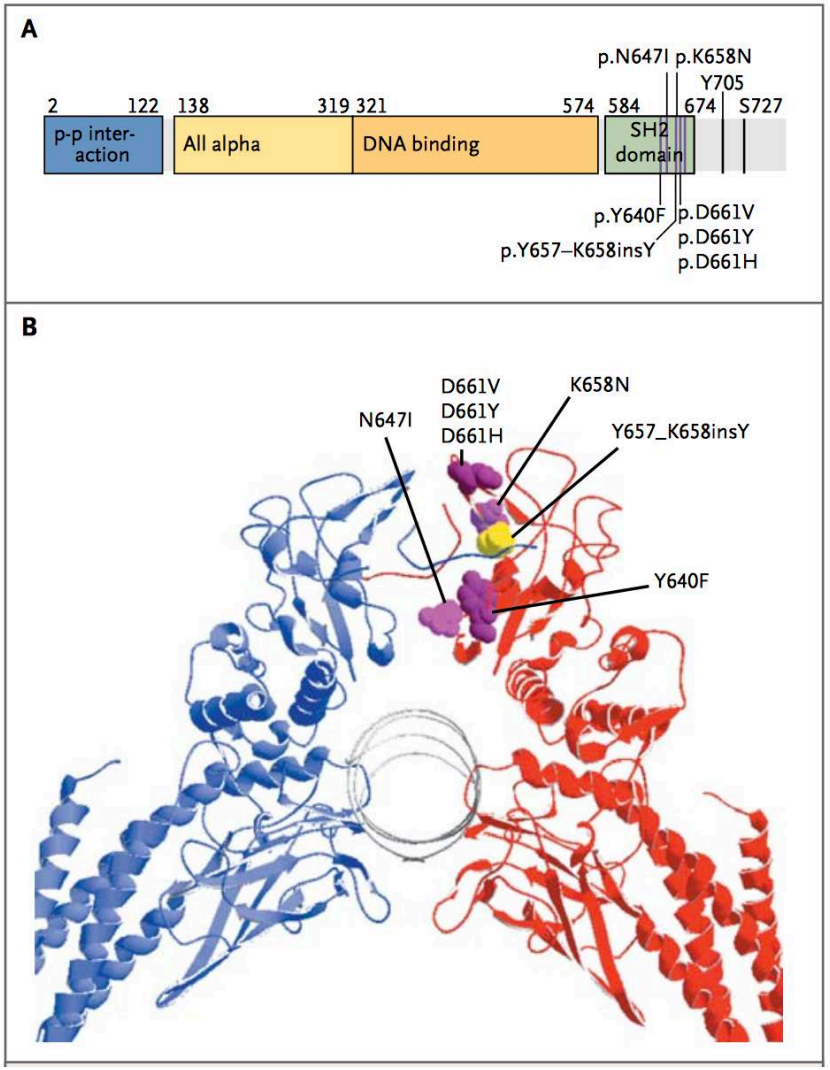


?Donor-derived origin

- 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

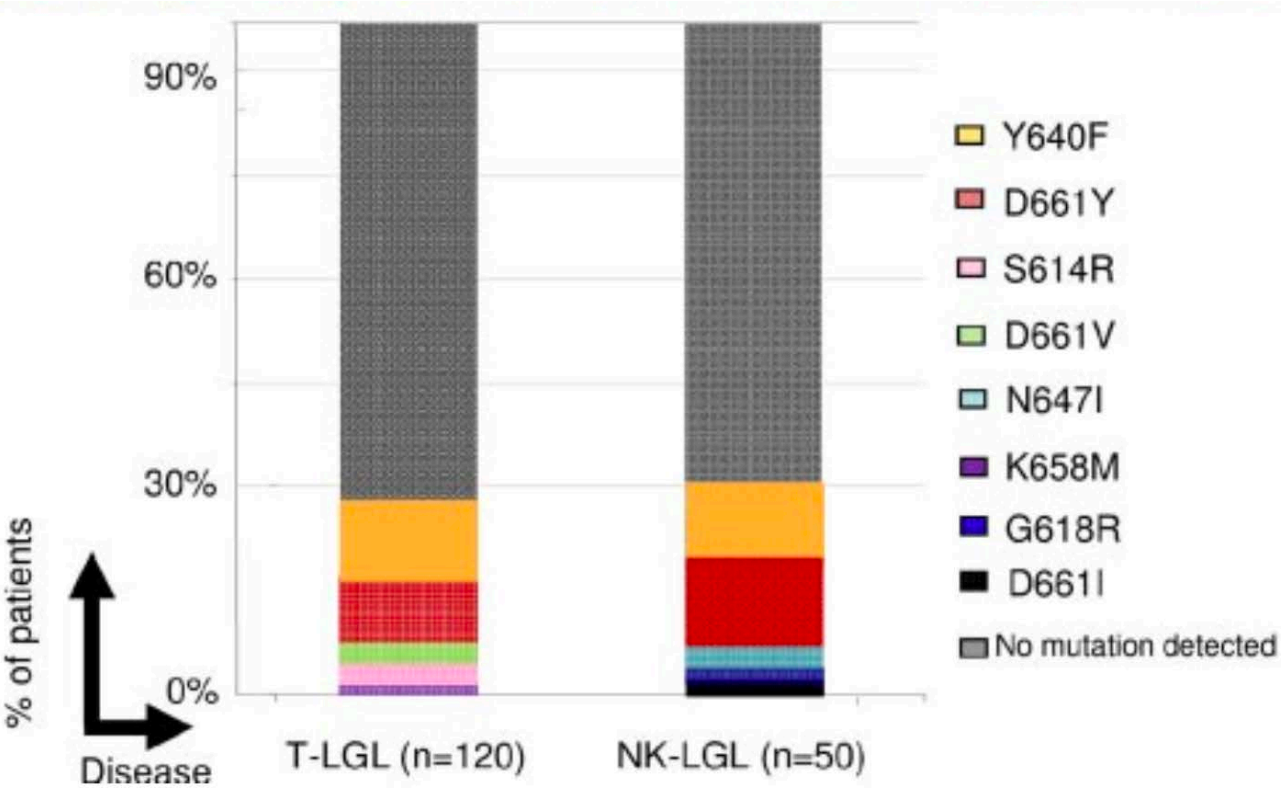
- Koskela *et al*. used NGS to identify index *STAT3* heterozygous SH2 mutation T>A (p.D661V) in 70 year old M
- In 31 of 77 additional patients (40%), additional *STAT3* mutations identified in exon 21, encoding Src-like homology 2 (SH2) domain
- Mutations (dimerization surface) result in a more hydrophobic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



N Engl J Med. 2012;366(20):1905- 1913.

STAT3 mutations common in LGL

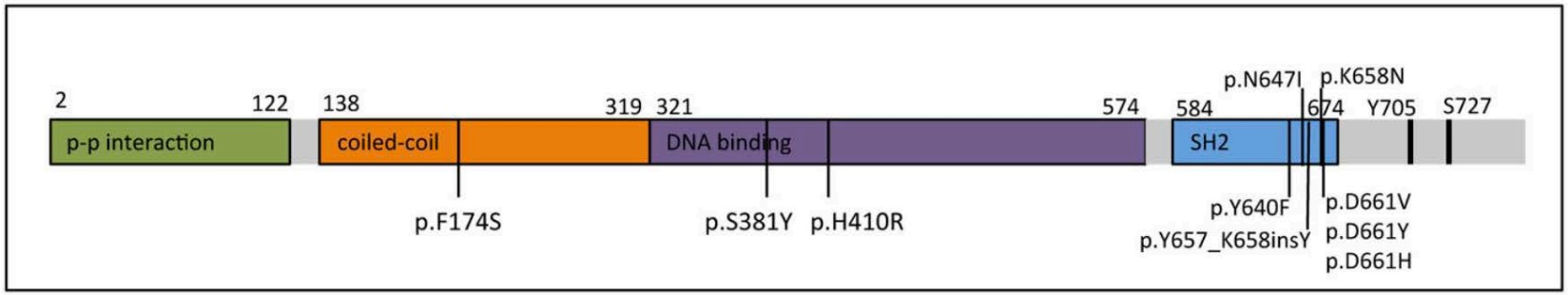
- *STAT3* mutations present in 1/3 of LGL and chronic NK cell lymphoproliferative disorders



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 phosphorylation

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)
- Kucük et al., Nat. Comm. (2015) 6:6025 DOI: 10.1038/ncomms7025
  - 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
- Hidalgo Lopez et al., J. Natl Compr Canc Netw., 14(8):939-44 (2016):
  - 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