blood advances

American Society of Hematology
2021 L Street NW, Suite 900,
Washington, DC 20036
Phone: 202-776-0544 | Fax 202-776-0545
bloodadvances@hematology.org

Distinguishing STAT3/STAT5B Mutated Large Granular Lymphocyte Leukemia from Myeloid Neoplasms by Genetic Profiling

Tracking no: ADV-2022-008192R2

Mark Kavesh (University of Utah Health Care, United States) Maedeh Mohebnasab (Oregon Health and Science University, United States) Marcela Angel (Oregon Health and Science University, United States) Wei Xie (Oregon Health & Science University, United States) Philipp Raess (Oregon Health & Science University, United States) Wei Cui (University of Kansas Medical Cener, United States) Richard Press (Oregon Health & Science University, United States) Guang Yang (Hospital of the University of Pennsylvania, United States) Peng Li (Genomics Laboratory, ARUP Laboratories, United States)

Abstract:

Conflict of interest: No COI declared

COI notes:

Preprint server: No;

Author contributions and disclosures: PL designed the study, and PL and MK performed data and statistical analysis. MK drafted the manuscript. PL, MM, MRA, WX, PWR, GY and WC collected patients' clinical, flow cytometric, cytogenetic and molecular data. PL, MM, MRA, WX, PWR, RDP, GY and WC interpreted and classified all variants by NGS testing. All authors reviewed and approved the final manuscript.

Non-author contributions and disclosures: No;

Agreement to Share Publication-Related Data and Data Sharing Statement: Emails to the corresponding author

Clinical trial registration information (if any):

Distinguishing *STAT3/STAT5B*-mutated large granular lymphocyte leukemia from myeloid neoplasms by genetic profiling

Mark Kavesh[1], Maedeh Mohebnasab[2], Marcela Riveros Angel[2], Wei Xie[2], Philipp W. Raess[2], Wei Cui[3], Richard D. Press[2], Guang Yang,[4] Peng Li[1,5,*]

[1]Division of Hematopathology, Department of Pathology, University of Utah Health, Salt Lake City, Utah 84108; [2]Department of Pathology, School of Medicine, Oregon Health and Science University, 3181 SW Sam Jackson Park Rd, Portland, OR 97237; [3]Department of Pathology and Laboratory Medicine, University of Kansas Medical Center, University of Kansas, 3901 Rainbow Blvd, Kansas City, KS 66160; [4]Department of Pathology and Laboratory Medicine, Perelman School of Medicine, University of Pennsylvania, 3400 Spruce Street, Philadelphia, PA 19104; [5]Genomics Laboratory, ARUP Laboratories, 500 Chipeta Way, Salt Lake City, UT 84108

***Corresponding author:** Peng Li, M.D., Ph.D., University of Utah, Salt Lake City, UT 84108; Phone, 801-583-2787 ext. 2593; E-mail, pengl.li@aruplab.com

Running title: **Distinguishing LGLL from MN by Genetic Profiling**

Key words: *STAT3*, *STAT5B*, pathogenic variants, T-cell large granular lymphocytic leukemia, chronic lymphoproliferative disorder of NK cells, myeloid neoplasm, large granular lymphocyte expansion

Data sharing: Contact the corresponding author for data sharing: pengl.li@aruplab.com.

Content:

Figures: 2

Supplementary tables: 4

Text word count: 1168 words

References: 24

Next-generation sequencing (NGS) has demonstrated the existence of recurrent activating mutations in *STAT3* and *STAT5B* in approximately 40-50% of patients with T-cell large granular lymphocytic leukemia (T-LGLL) and 20-30% of patients with NK cell granular lymphocytic leukemia (NK-LGLL, also known as chronic lymphoproliferative disorder of NK cells).[1-3] *STAT3/5B* mutations alone are not specific to T/NK-LGLL, as they have been frequently found in other lymphoid neoplasms (LNs), as well as in myeloid neoplasms (MNs), aplastic anemia and other autoimmune disorders, and rarely in asymptomatic individuals with clonal hematopoiesis of indeterminate potential.[1,4-6] Compounding the issue of specificity is that clonal LGL expansions are frequently observed in MNs and frequent *STAT3/5B* mutations have been reported in MNs with associated LGL expansion.[4,7,8] Rarely, have LGLLs been diagnosed concomitantly in patients with otherwise typical MNs.[9,10]

Based on morphology, flow cytometry, and T-cell clonality studies alone, it may be challenging to classify a marrow process with both LGL expansion[11-14] and morphologic dysplasia[7] (Fig. S1) as an LGLL versus an MN or both.[7] To present further challenges, both manifest with varying degrees of cytopenias and heterogenous lymphocyte counts, and are frequently found in the elderly. Genetic profiling using NGS supports an accurate diagnosis in challenging cases and is an area of investigation as cytopenic patients with different underlying causes require different therapy.[10]

In this multi-institutional study, we examined how a targeted myeloid NGS panel can distinguish T/NK-LGLLs from MNs. We retrospectively identified 118 hematologic neoplasm patients (66 LNs, 50 MNs and 2 cases of concomitant LGLL and myelodysplastic syndrome, MDS, Fig. 1a), all with pathogenic/likely-pathogenic (P/LP) *STAT3*/*5B* variants as well as 18 MDS patients with clonal LGL expansions[8,15] in the absence of *STAT3/5B* mutations (Fig. 1a)

amongst an unselected cohort of 6690 patients who underwent NGS testing (Tab. S1-2). We then analyzed the demographic and genomic profiles, clonal metrics of *STAT3/5B* variants, T-cell clonality, and flow cytometric studies (Fig. 1a and Tab. S3). Further details on material and methods are available in the Supplementary Materials.

There was a significant male predominance in T-LGLL (70%) and NK-LGLL (100%) as well as in all other LNs (91%), whereas it was not observed to the same extent in MNs (52% male, Tab. S4). The median age at the time of LGLL diagnosis was 71-73 years old (range 19-89 years), which was similar to those of patients with other lymphoproliferative disorders (LPDs) or MNs (Tab. S4).

LNs showed predominantly *STAT3* variants as compared to *STAT5B* (Fig. 1b-c, 92% in LN versus 65% in MN, P=0.0003), and variants appeared concentrated in the SH2 domain of both genes. Amongst *STAT3* variants, similar to those reported in the literature,[2,4] the D661 and Y640F variants were more prevalent in LNs, and S614R and G618R variants were common in both lymphoid and myeloid neoplasms (Fig. 1b-c). p.N642H was the most common variant in *STAT5B* in MNs, comparable to those reported in the literature.[5,16] In contrast to MNs, *STAT5B* variants were rare in LGLLs in this retrospective study (Fig. 1a, 4% in *STAT3/5B* mutant LGLLs versus 34% in *STAT3/5B* mutant MNs, P<0.0001), similar to previous case series.[2,4,17-20] The median VAF of *STAT3*/*5B* variants[21] was 8.8 (range: 1.4 to 48.6) amongst LGLLs and 12.0 in MNs (range: 1.1 to 65.2, P=0.01, Fig.1d-e). There appeared a bimodal and broader distribution in MNs with two predominant populations of around 10% or between 40 and 50% (Fig. 1e), whereas most VAFs were between 5 and 18% in LGLLs (Fig. 1d, dashed lines),

Further, there was a robust correlation (Fig. 2a-b) between the VAFs of *STAT3/5B* variants and the percentage of aberrant T-/NK large granular lymphocytes detected by flow

cytometry in LGLLs (Fig. 2a), whereas such a correlation did not exist in MNs (Fig. 2b). These findings suggested *STAT3/5B* variants were the founder clone in LGLLs driving neoplastic cell proliferation. Amongst LGLLs, concomitant mutations were uncommon (Fig. 2c, 35% in LGLL vs 92% in MN, P<0.0001) and most occurred in genes involved in epigenetic regulation (light green in Fig. 1a). In contrast, MNs showed more complex genetic profiles (Fig. 1a and Tab. S3) demonstrated by a greater number (Fig. 2d, 1.65 in LGLL vs 4.17 variants in MN per case, P<0.0001), greater diversity of concomitant variants (Fig. 1a) and greater VAFs of the leading non-*STAT3/5B* variants (Fig. 2e) in comparison to LGLLs. In LGLLs, a strong correlation arose between the VAFs of *STAT3/5B* variants and those of the leading non-*STAT3/5B* variant/clone (Fig. 2f, R^2=0.90), suggesting *STAT3/5B* variants as the leading clones in all LGLLs (Fig. 4f, 100% in LGLL vs 52% in MN, P<0.0001). In MNs, 48% of *STAT3/5B* variants appeared subclones (Fig. 2g-h). The complex genetic profiles in MNs were also reflected by the otherwise similar genetic characteristics in MNs with LGL expansions, detected by flow cytometry, in the absence of *STAT3*/*5B* mutations (Fig. 1a, 18 columns on the right). These findings may be the result of the myeloid-directed NGS panel used in this study, and should not be interpreted to mean that LGLLs generally harbor fewer total mutations.[22]

Cytogenetics showed predominantly normal or low-risk karyotypes amongst LGLLs (64%) and a greater proportion of complex karyotypes (CK) amongst MNs (Fig. 1a, 36% in LGLL vs 64% in MN with CK, P=0.01). T-cell clonality was uniformly positive amongst T-LGLL and other T-LPD cases, and uniformly negative in NK-LGLL and MN cases in which it was performed, with two exceptions of composite T-LGLL and MDS cases (patients 66 and 67 in Tab. S3 and Fig. 1a). Rare cytopenia cases with *STAT3* variants (patients 69 – 76 in Tab. S3 and Fig. 1a) had mutational profiles more like LGLLs, among which T-cell clonality was

negative in two and unknown in the remaining five. Given that mutations involving epigenetic regulation are common in both clonal cytopenia of undetermined significance and in LGLLs,[22,23] diligent T-cell clonality evaluation, accompanied by flow cytometric studies, could be helpful to avoid missed diagnoses of LGLL in such cases.

This study was limited by the lack of a subgroup analysis of LGLLs versus *STAT3/5B* mutant MDS cases with known, persistent clonal LGL expansions[8,15] since only three such cases were identified (patients #89, 90, and 107 in Tab. S3) while attempts of T-cell clonality provided no further supports. Such a study would require performance of T-cell clonality and possibly cell sorting in all *STAT3*/*5B*-mutated MNs with clonal LGL expansions prospectively.[10,23,24] Although a myeloid NGS panel is often sufficient when evaluating cytopenias, use of an expanded panel that includes genes whose mutations are common in LNs[22] would provide more information for classification, especially in cases with composite LGLLs and MNs.[10,15]

In conclusion, this is the first study to characterize the molecular features of *STAT3/5B* mutant LGLLs and MNs using a myeloid malignancy-targeted NGS panel. This study supports NGS as a necessary test for facilitating an accurate diagnosis of LGLL versus MN in challenging cases. Summarizing genetic features distinguishing LGLLs from MNs include more frequent *STAT3* mutations, a lower median VAF of *STAT3/5B* variants, the absence or reduced diversity of concomitant somatic variants, and *STAT3/5B* variants being the founder and leading clonal driver variant.

Authorship

Contributions: PL designed the study, and PL and MK performed data and statistical analysis. MK drafted the manuscript. PL, MM, MRA, WX, PWR, GY and WC collected patients' clinical, flow cytometric, cytogenetic and molecular data. PL, MM, MRA, WX, PWR, RDP, GY and WC

93 interpreted and classified all variants by NGS testing. All authors reviewed and approved the
94 final manuscript.
95 COI: None of the authors has a relevant conflict of interest.
96

References

1 Jerez, A. *et al.* STAT3 mutations unify the pathogenesis of chronic lymphoproliferative disorders of NK cells and T-cell large granular lymphocyte leukemia. *Blood* **120**, 3048-3057, doi:10.1182/blood-2012-06-435297 (2012).

2 Koskela, H. L. *et al.* Somatic STAT3 mutations in large granular lymphocytic leukemia. *N Engl J Med* **366**, 1905-1913, doi:10.1056/NEJMoa1114885 (2012).

3 Kim, D. *et al.* STAT3 activation in large granular lymphocyte leukemia is associated with cytokine signaling and DNA hypermethylation. *Leukemia* **35**, 3430-3443, doi:10.1038/s41375-021-01296-0 (2021).

4 Qu, S. *et al.* STAT3 and STAT5B mutations have unique distribution in T-cell large granular lymphocyte proliferations and advanced myeloid neoplasms. *Leuk Lymphoma* **62**, 1506-1509, doi:10.1080/10428194.2020.1869964 (2021).

5 de Araujo, E. D. *et al.* Structural and functional consequences of the STAT5B(N642H) driver mutation. *Nat Commun* **10**, 2517, doi:10.1038/s41467-019-10422-7 (2019).

6 Steensma, D. P. Clinical consequences of clonal hematopoiesis of indeterminate potential. *Blood Adv* **2**, 3404-3410, doi:10.1182/bloodadvances.2018020222 (2018).

7 Fattizzo, B., Bellani, V., Pasquale, R., Giannotta, J. A. & Barcellini, W. Large Granular Lymphocyte Expansion in Myeloid Diseases and Bone Marrow Failure Syndromes: Whoever Seeks Finds. *Front Oncol* **11**, 748610, doi:10.3389/fonc.2021.748610 (2021).

8 Komrokji, R. S. *et al.* Characterization of myelodysplastic syndromes (MDS) with T-cell large granular lymphocyte proliferations (LGL). *Leukemia* **34**, 3097-3099, doi:10.1038/s41375-020-0928-4 (2020).

9 Huh, Y. O. *et al.* T-cell large granular lymphocyte leukemia associated with myelodysplastic syndrome: a clinicopathologic study of nine cases. *Am J Clin Pathol* **131**, 347-356, doi:10.1309/AJCP6YHI1JEXAWAP (2009).

10 Sauntharajah, Y. *et al.* Coincident myelodysplastic syndrome and T-cell large granular lymphocytic disease: clinical and pathophysiological features. *Br J Haematol* **112**, 195-200, doi:10.1046/j.1365-2141.2001.02561.x (2001).

11 Polyatskin, I. L., Artemyeva, A. S. & Krivolapov, Y. A. [Revised WHO classification of tumors of hematopoietic and lymphoid tissues, 2017 (4th edition):lymphoid tumors]. *Arkh Patol* **81**, 59-65, doi:10.17116/patol20198103159 (2019).

12 Morice, W. G., Kurtin, P. J., Tefferi, A. & Hanson, C. A. Distinct bone marrow findings in T-cell granular lymphocytic leukemia revealed by paraffin section immunoperoxidase stains for CD8, TIA-1, and granzyme B. *Blood* **99**, 268-274, doi:10.1182/blood.v99.1.268 (2002).

13 Lundell, R., Hartung, L., Hill, S., Perkins, S. L. & Bahler, D. W. T-cell large granular lymphocyte leukemias have multiple phenotypic abnormalities involving pan-T-cell antigens and receptors for MHC molecules. *Am J Clin Pathol* **124**, 937-946 (2005).

14 Pandolfi, F. *et al.* Clinical course and prognosis of the lymphoproliferative disease of granular lymphocytes. A multicenter study. *Cancer* **65**, 341-348, doi:10.1002/1097-0142(19900115)65:2<341::aid-cncr2820650227>3.0.co;2-2 (1990).

15 Durrani, J. *et al.* Large granular lymphocytic leukemia coexists with myeloid clones and myelodysplastic syndrome. *Leukemia* **34**, 957-962, doi:10.1038/s41375-019-0601-y (2020).

16 Cross, N. C. P. *et al.* Recurrent activating STAT5B N642H mutation in myeloid neoplasms with eosinophilia. *Leukemia* **33**, 415-425, doi:10.1038/s41375-018-0342-3 (2019).

17 Rajala, H. L. *et al.* Discovery of somatic STAT5b mutations in large granular lymphocytic leukemia. *Blood* **121**, 4541-4550, doi:10.1182/blood-2012-12-474577 (2013).

18 Couronne, L. *et al.* STAT3 mutations identified in human hematologic neoplasms induce myeloid malignancies in a mouse bone marrow transplantation model. *Haematologica* **98**, 1748-1752, doi:10.3324/haematol.2013.085068 (2013).

19 Funakoshi-Tago, M., Tago, K., Abe, M., Sonoda, Y. & Kasahara, T. STAT5 activation is critical for the transformation mediated by myeloproliferative disorder-associated JAK2 V617F mutant. *J Biol Chem* **285**, 5296-5307, doi:10.1074/jbc.M109.040733 (2010).

20 Greenfield, G., McMullin, M. F. & Mills, K. Molecular pathogenesis of the myeloproliferative neoplasms. *J Hematol Oncol* **14**, 103, doi:10.1186/s13045-021-01116-z (2021).

21 Galli, A. *et al.* Relationship between clone metrics and clinical outcome in clonal cytopenia. *Blood* **138**, 965-976, doi:10.1182/blood.2021011323 (2021).

22 Cheon, H. *et al.* Genomic landscape of TCRalphabeta and TCRgammadelta T-large granular lymphocyte leukemia. *Blood* **139**, 3058-3072, doi:10.1182/blood.2021013164 (2022).

23 Raess, P. W. *et al.* Concurrent STAT3, DNMT3A, and TET2 mutations in T-LGL leukemia with molecularly distinct clonal hematopoiesis of indeterminate potential. *Am J Hematol* **92**, E6-E8, doi:10.1002/ajh.24586 (2017).

24 Lewis, N. E. *et al.* Clonal hematopoiesis in angioimmunoblastic T-cell lymphoma with divergent evolution to myeloid neoplasms. *Blood Adv* **4**, 2261-2271, doi:10.1182/bloodadvances.2020001636 (2020).

Figure legends

Figure 1. Comprehensive genetic profiles, the hotspots and VAF distribution of *STAT3* and *STAT5B* variants in both lymphoid and myeloid neoplasms. (a) Lymphoid neoplasms (LN) were displayed on left (light blue, top row) and myeloid neoplasms (MN) on right (light orange, top row). The WHO entities in each group were listed on the second row. The concomitant variants were grouped into six categories based on their gene function: epigenetic, epigenetic regulators, genes involving DNA methylation or histone acetylation and deacetylation (light green); SFs, RNA splicing factors (purple); TFs, transcription factors (orange); Signaling, molecules in tyrosine kinase pathway or RAS/MAPK pathways (pink); C, cohesins (light purple); Others, genes with functions beyond the above categories (variable colors). Each column represented one patient. Each bar represented one variant and split bars indicated two or more variants in the same gene. LN, lymphoid neoplasm; MN, myeloid neoplasm; T-LGLL, T-cell large granulocytes lymphocytic leukemia; NK-LGLL, NK cell large granulocytes lymphocytic leukemia or chronic lymphoproliferative disorder of NK cells; LPD, lymphoproliferative disorders; MDS, myelodysplastic syndrome; MPN, myeloproliferative neoplasm; AML, acute myeloid leukemia; APL, acute promyelocytic leukemia; NL, normal; NI, no information. (**b-c**) LNs showed predominantly *STAT3* variants (**b**) as compared to *STAT5B* (**c**) variants (P = 0.0003), and variants appeared concentrated in in the SH2 domain of both genes. (**b**) Amongst *STAT3* variants, the D661 variants were most prevalent in lymphoid neoplasms including both D661V and D661Y, followed by Y640F. In contrast in MNs, D661Y, not D661V was the most common hotspot mutation followed by F614R and Y640F. (**c**) *STAT5B* N642H variant was particularly represented amongst those in MNs, and only rarely detected in LNs. SH2, Src homology 2 domain ; TAD, topologically associating domain. (**d**) The distribution of

VAFs in amongst LGLL peaked at a median of 8.8% (solid line; range: 1.4 to 48.6%). (**e**) The VAF distribution in MNs appeared wider with a median VAF at 12.0% (solid line; range: 1.1 to 65.2 ; *, P=0.01 by unpaired t-test) and significant second population concentrated around 48-50% (P=0.006 by Chi-square test for trend). The dashed lines represented the 25th and 75th percentile. The relative density of VAF distribution was displayed as previously descripted.[21]

Figure 2. Correlation of *STAT3/5B* variants to neoplastic cells identified by flow cytometry and genetic features in *STAT3/5B* mutant LGLLs and MNs. (a) VAFs of *STAT3/5B* variants correlated with their corresponding percentage of atypical T/NK cells detected by flow cytometry (R^2=0.92) in LGLL, **(b)** while there appeared no such a correlation in those in myeloid neoplasms (R^2=0.12). (**c**) In LGLLs, 35% of all cases had concomitant somatic pathogenic and likely pathogneic variants, whereas 92% (P<0.0001) of MNs were accompanied by at least one concomitant variants beyond *STAT3/5B* variants. (**d**) The average number of somatic variants was significantly lower in LGLLs (median 1.7 per case, range 1 to 4), than that in MNs (median 4.1 per case, range 1 to 8 ; ****, P<0.0001). (**e**) The VAFs of the leading concomitant, beyond *STAT3/5B*, variant in LGLLs (mean ± SEM, 11.9 ± 2.5) appeared significant smaller that in MNs (37.5 ± 4.0, P=0.0003). (**f**) In LGLLs, there appeared a significant correlation (R^2=0.90) between the VAFs of *STAT3/5B* variants to the VAF of the leading non-*STAT3/5B* variants, suggesting *STAT3/5B* variants being the leading variants in all LGLL cases (100% in **h,** blue). (**g**) This correlation did not exist in MNs (R^2=0.20), and indeed the data suggested 48% of all *STAT3/5B* variants (above the dashed line in **g**) being a subclone (**h**, orange, P<0.0001). LGLL, T/NK cell large granular lymphocytic leukemia; MN, myeloid neoplasm.

Figure 1

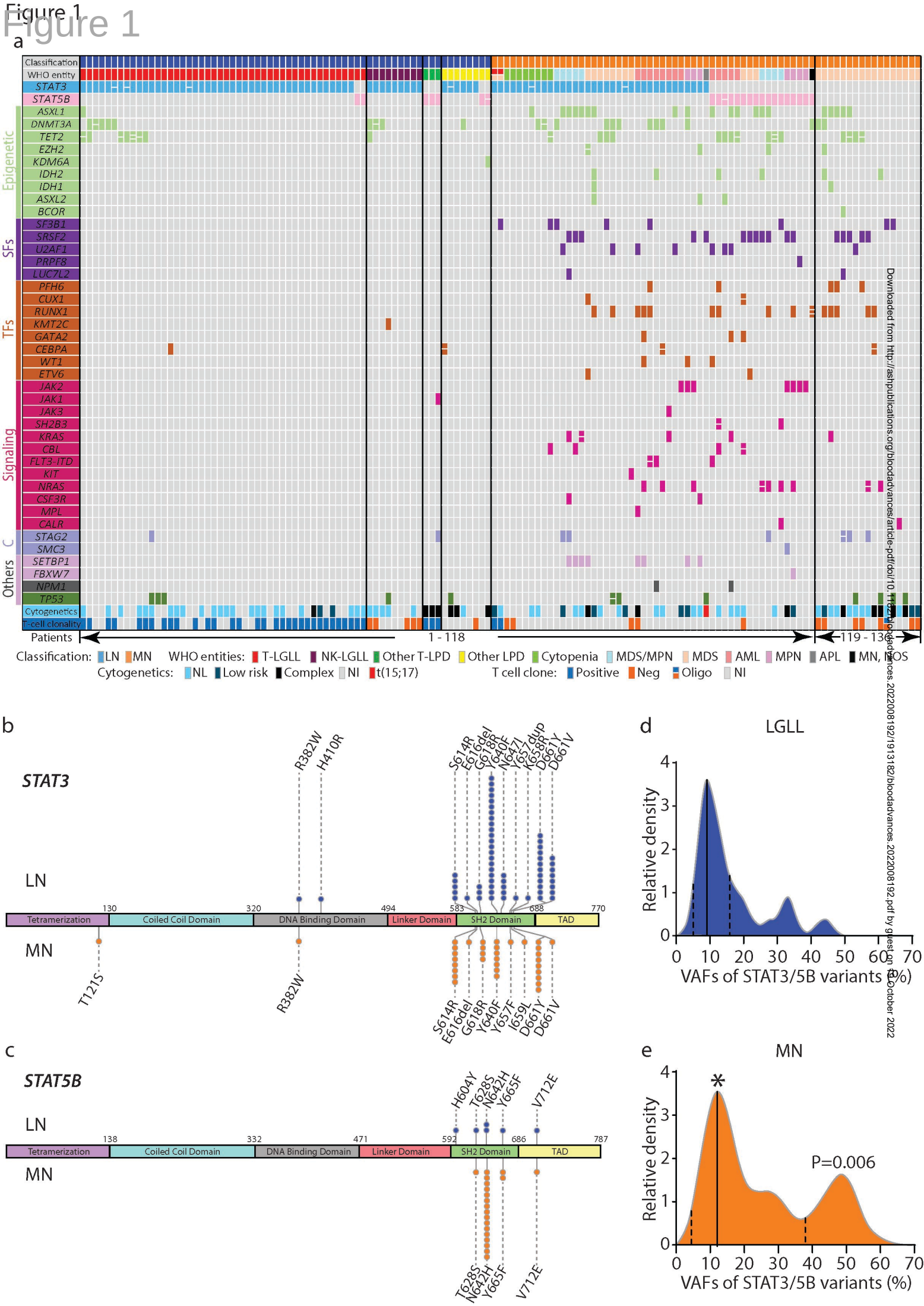

Figure 2