Oregon Health & Science University
School of Medicine

Scholarly Projects Final Report

Title *(Must match poster title; include key words in the title to improve electronic search capabilities.)*

Molecular and Flow Cytometric Findings in T-cell Large Granular Lymphocytic Leukemia

Student Investigator's Name

Lauren Raymond

Date of Submission *(mm/dd/yyyy)*

03/14/2022

Graduation Year

2022

Project Course *(Indicate whether the project was conducted in the Scholarly Projects Curriculum; Physician Scientist Experience; Combined Degree Program [MD/MPH, MD/PhD]; or other course.)*

Conducted in the Scholarly Projects Curriculum

Co-Investigators *(Names, departments; institution if not OHSU)*

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All co-investigators are in the Department of Pathology at OHSU

Mentor's Name

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Concentration Lead's Name

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Project/Research Question

What is the frequency of abnormal findings in cases with clinical or hematopathologic suspicion for T-LGLL and therefore the relative importance of each of these diagnostic entities?

Type of Project *(Best description of your project; e.g., research study, quality improvement project, engineering project, etc.)*

Research study

Key words *(4-10 words describing key aspects of your project)*

T-cell large granular lymphocytic leukemia (T-LGLL); lymphoproliferative disorder; small lymphocytic leukemia; flow cytometry; hematopathology; T-cell clonality; T-cell receptor gene rearrangement; *STAT3* mutation

Meeting Presentations

If your project was presented at a meeting besides the OHSU Capstone, please provide the meeting(s) name, location, date, and presentation format below (poster vs. podium presentation or other).

College of American Pathologists Annual Meeting; Las Vegas, NV; 10/10/2020; Poster Presentation

Publications *(Abstract, article, other)*

If your project was published, please provide reference(s) below in JAMA style.

Raymond L, Fan G, Wang Y, Dunlap J, Press R, Raess P. Molecular and Flow Cytometric Findings in T-Cell Large Granular Lymphocytic Leukemia. Archives of Pathology & Laboratory Medicine. 2020 Sep;144(9s1):e2-e212. doi: 10.5858/arpa.2020-0991-AB.

Submission to Archive

Final reports will be archived in a central library to benefit other students and colleagues. Describe any restrictions below (e.g., hold until publication of article on a specific date).

None

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Next Steps

What are possible next steps that would build upon the results of this project? Could any data or tools resulting from the project have the potential to be used to answer new research questions by future medical students?

One possible next step would be to identify whether or not diagnostic clusters exist within the patient group that received a diagnosis of T-LGLL to determine if there are possible sub-classifications, which could lead to simplification of the current diagnostic criteria. Additionally, these patients could be further analyzed to see whether or not another diagnostic marker might be present that could further simplify this diagnosis. My work included building a database of all patients with clinical or hematopathologic suspicion for T-LGLL, which could certainly be utilized by future medical students to continue to work towards a simplified diagnostic work-up for patients with concern for T-LGLL.

Please follow the link below and complete the archival process for your Project in addition to submitting your final report.

https://ohsu.ca1.qualtrics.com/jfe/form/SV_3ls2z8V0goKiHZP

Student's Signature/Date *(Electronic signatures on this form are acceptable.)*
This report describes work that I conducted in the Scholarly Projects Curriculum or alternative academic program at the OHSU School of Medicine. By typing my signature below, I attest to its authenticity and originality and agree to submit it to the Archive.

X _____

Student's full name

Mentor's Approval *(Signature/date)*

X _____

Mentor Name

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Report: *Information in the report should be consistent with the poster, but could include additional material. Insert text in the following sections targeting 1500-3000 words overall; include key figures and tables. Use Calibri 11-point font, single spaced and 1-inch margin; follow JAMA style conventions as detailed in the full instructions.*

Introduction *(≥250 words)*

T-cell large granular lymphocytic leukemia (T-LGLL) is a chronic lymphoproliferative disorder of mature cytotoxic T-cells. The underlying etiology of T-LGLL is unknown, but some hypotheses propose that T-LGLL arises in the setting of sustained immune stimulation due to its frequent association with autoimmune disorders. This immune response to a chronic, persistent stimulus can lead to clonal selection with possible acquisition of oncogenic mutations allowing for further expansion and establishment of a monoclonal population, ultimately resulting in T-LGLL[1]. However, reactive conditions also show chronically elevated large granular lymphocytes with a T-cell clone and cytopenias, making the distinction of whether there is a reactive expansion of T-cells versus a significant neoplastic clonal expansion of T-cells difficult to distinguish[2].

Accurate diagnosis of T-LGLL is challenging. The diagnosis of T-LGLL requires integration of morphologic, immunophenotypic, molecular, and clinicopathologic findings. The 2017 World Health Organization defines T-LGLL as a "heterogeneous disorder characterized by a persistent (> 6 months) increase in the number of peripheral blood large granular lymphocytes (LGLs)…without a clearly identified cause"[1]. However, the absolute level of lymphocytosis required for a diagnosis of T-LGLL is not defined. Furthermore, while there is a typical immunophenotypic profile for T-LGLL, variants exist and each antigen can show a range of normal to aberrant phenotypes. The only firm diagnostic criterion is documentation of T-cell clonality via T-cell receptor gene rearrangement studies by PCR[1]. Given that T-cell clonality can be detected in a variety of non-neoplastic settings including viral infection or normal aging, this is a sensitive but non-specific measure for T-LGLL diagnosis.

Although these diagnostic criteria are important to guide accurate diagnosis of T-LGLL, each individual criterion exists in multiple different clinical scenarios. As previously stated, LGL expansion and T-cell clonality can be seen in reactive as well as other neoplastic conditions. Additionally, hepatosplenic T-cell lymphoma can have similar peripheral blood and bone marrow manifestations as T-LGLL[3]. *STAT3* and *STAT5B* mutations are only present in a minority of T-LGLL patients[4]. Furthermore, this is a diagnosis of exclusion with regard to the patient's underlying cytopenia(s). Given that T-LGLL might arise in the setting of sustained immune stimulation, it would be impossible for the clinician to determine whether the cytopenia was due to a reactive or neoplastic process.

The focus of this study is to examine the frequency of abnormal findings in cases with clinical or hematopathologic suspicion for T-LGLL to investigate the relative importance of each of these diagnostic entities. An improved understanding of how to accurately diagnose T-LGLL will allow for earlier treatment initiation and greater consideration of additional genetic underpinnings or personalized therapeutic approaches.

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Methods *(≥250 words)*

A retrospective cohort review was performed of clinicopathologic data from all patients with flow cytometric evaluation for T-LGLL at a single institution in Portland, Oregon from January 1, 2017 to December 31, 2019. These patients were identified through stored KIR testing data from a secured folder on the password-protected secured Oregon Health & Science University server (Oregon Health & Science University has maintained a prospective database of T-LGLL patients since 2017, which is maintained and updated by study staff). The population included all patients who underwent KIR testing at Oregon Health & Science University during the study period. Clinicopathologic data were abstracted in the form of continuous (age, Complete Blood Count data) and discrete (race, gender, ethnicity, clinical status, flow cytometric evaluation of KIRs and other antigen aberrancy information, and molecular data including T-cell clonality and mutational analysis) variables from the clinical and laboratory electronic medical record. Subgroups included those who were ultimately diagnosed with T-LGLL and those who were not. Patients who opted out of using their genetic data for research were excluded from the study. Descriptive statistics, including means and frequencies, were performed to better characterize demographic and clinical variables. This study was approved by the Oregon Health and Science University Institutional Review Board. The specific aims of this project were to determine the utility of flow cytometric data in the diagnosis of T-LGLL, to determine the factors associated with reactive versus neoplastic causes of LGL expansion, and to determine the molecular drivers underlying LGL expansion and T-LGLL.

Results *(≥500 words)*

Of the 145 patients with clinicopathologic suspicion for T-LGLL, there were twenty-six patients diagnosed with T-LGLL. Ten patients had a clinical work-up resulting in suspicion for T-LGLL, but these patients had not yet met full diagnostic criteria. Additionally, seventeen patients had incomplete work-ups leading to an indeterminate diagnosis where T-LGLL could not definitively be excluded. Another large granular lymphocyte disorder known as chronic lymphoproliferative disorder of natural killer cells (CLPD-NK) is clinically very similar to T-LGLL, albeit more rare, with a similar diagnostic work-up. In this cohort of patients with clinical or hematopathologic suspicion for T-LGLL, two patients were ultimately diagnosed with CLPD-NK and four patients had a clinical work-up resulting in suspicion for CLPD-NK. Lastly, eighty-six patients had a negative work-up for T-LGLL and CLPD-NK. Within the negative cohort, the majority of patients were determined to have a reactive T-LGL population or increased NK cells without evidence of antigen aberrancy. A minority of the negative patient population were determined to have other hematologic or immune conditions including acute myeloid leukemia, myelodysplastic syndrome, plasma cell myeloma, T-cell lymphoma, and acute Epstein-Barr virus infection. Clinicopathologic details are presented in Table 1.

A diagnosis of T-LGLL was more common in males and occurred across a wide age range. However, notably a number of patients in this cohort were direct consults from the adjacent Veteran Health Administration as this center does not have flow cytometry and next generation sequencing. 64% of patients with T-LGLL presented with at least one cytopenia, most commonly anemia followed by neutropenia. All cases demonstrated immunophenotypic aberrancy in T-cells. Within the T-LGLL population, dim/absent CD5 expression was the most common antigen aberrancy. Additionally, the typical immunophenotypic presentation of T-LGLL was represented in this population including CD3+, CD8+, and CD57+ expression. Uniform KIR expression was seen only in a minority of cases with subgroups CD158a and CD158i as the most frequently identified aberrancy. All tested cases in patients with a diagnosis of T-LGLL, suspicion for T-

LGLL, and an indeterminate diagnosis showed positive T-cell receptor gene rearrangement studies by PCR.

With regards to next generation sequencing, 43% of patients with T-LGLL demonstrated *STAT3* mutations and 5% demonstrated *STAT5B* mutations. In comparison, 11% of negative cases presented with *STAT3* mutations and 3% had *STAT5B* mutations. However, this difference was not statistically significant. But looking at the specific mutations in each subgroup, the p.Y640F mutation was common among T-LGLL patients and not present in the negative group. 56% of patients with T-LGLL who had *STAT3* mutations also had mutations in other genes. Additionally, of the three patients with a diagnosis of CLPD-NK or suspicion for CLPD-NK who underwent next generation sequencing, only one patient presented with a *STAT3* mutation and the point mutation of p.K685R was not seen in either the T-LGLL cohort nor the negative patient population. 38% of patients with T-LGLL had a pre-existing autoimmune condition. Of these patients with a history of autoimmune diseases, 50% had a history of rheumatoid arthritis.

Of the 26 patients diagnosed with T-LGLL, the majority presented with: monoclonality by TCR gene rearrangement, genetic mutations, immunophenotypic aberrancy in T-cells, and at least one cytopenia.

Table 1. Clinicopathologic characteristics of patients with clinical or hematopathologic suspicion for T-LGLL

Parameter	T-LGLL	Suspicious for T-LGLL	CLPD-NK	Suspicious for CLPD-NK	Indeterminate	Negative
Number of patients	26	10	2	4	17	86
Sex (M/F)	18/8	7/3	2/0	4/0	11/6	52/34
Median age (IQR)	64 (27-89)	71 (51-78)	42.5 (37-48)	61 (46-67)	66 (21-82)	60.5 (1-91)
Hematologic manifestations % (n)						
Anemia (Hb < 11 g/dL)	42% (11)	0% (0)	50% (1)	25% (1)	6% (1)	35% (30)
Thrombocytopenia (platelets <150 x 10^9/L)	30% (8)	20% (2)	100% (2)	50% (2)	35% (6)	51% (44)
Neutropenia (ANC <1.5 x 10^9/L)	39% (7)*	38% (3)*	100% (2)	0% (0)*	23% (3)*	39% (27)*
Severe neutropenia (ANC <0.5 x 10^9/L)	22% (4)*	13% (1)*	50% (1)	0% (0)*	0% (0)*	9% (6)*
Lymphocytosis (ALC >2.9 x 10^9/L)	50% (9)*	50% (4)*	0% (0)	33% (1)*	15% (2)*	26% (18)*
STAT3 mutations	9/21 p.Y640F (4), p.D661Y (3), p.D661V (1), p.S614R (1)	0/2	1/2 p.K685R (1)	0/1	2/8 p.D661Y (1) p.Y640F (1)	4/38 p.H694Q (1), p.P714L (1), p.R382W (1), p.S614R (1)
STAT5B mutations	1/21	0/2	0/2	0/1	1/8	1/38
Other mutations	11/21 *TET2* (3), *MPL* (2), *KRAS* (2), *ASXL1* (2), *PTPN11* (2), *PAX5* (2), others (17)	2/2 *DNMT3A* (2)	2/2 *TET2* (2), others (4)	1/1 other (3)	5/8 *TET2* (2), *DNMT3A* (2), *ATM* (2), *NOTCH1* (2), *ABL1* (2), others (19)	33/38 *TET2* (8), *DNMT3A* (5), *IDH1* (5) *TP53* (4), *SRSF2* (4) others (54)

TCR monoclonality	24/24	10/10	1/1	1/2	11/11	20/30
Autoimmune conditions	10**	1**	1	None**	4**	16**

Legend: T-LGLL: T-cell large granular lymphocytic leukemia; CLPD-NK: chronic lymphoproliferative disorder of natural killer cells; IQR: interquartile range; Hb: hemoglobin; ANC: absolute neutrophil count; ALC: absolute lymphocyte count; TCR: T-cell receptor gene rearrangement polymerase chain reaction; *Some patient's complete blood counts did not include a white cell count differential. The patients without absolute neutrophil and absolute lymphocyte counts were excluded in the calculation of the percentage of patients with neutropenia, severe neutropenia, and lymphocytosis. **Detailed clinical histories not available for all patients.

Discussion *(≥500 words)*

Heterogeneity exists in certain features of T-LGLL, including the presence of cytopenias, *STAT3/STAT5B* mutations, and uniform expression of a KIR isotype. The most commonly observed abnormalities in T-LGLL cases were monoclonal TCR gene rearrangements and immunophenotypic aberrancy by flow cytometry, which is expected, as these are key features of the current diagnostic criteria for T-LGLL. *STAT3/STAT5B* and other mutations occurred at similar frequencies in patients with T-LGLL and those without. Intriguingly, many patients with *STAT3* mutations also had mutations in other genes. The diagnosis of T-LGLL requires integration of morphologic, immunophenotypic, and molecular findings and cannot rely on the presence of specific mutations. Additionally, in this clinicopathologic context, monoclonal TCR rearrangements are sensitive, but not specific, for a diagnosis of T-LGLL.

The primary limitation of this study is missing data. With the current guidelines for a diagnosis of T-LGLL in place, the standard diagnostic criterion necessitates multiple points of clinical follow-up and extensive and costly laboratory analysis. This requires a patient to have continued access to the health care system and requires the health care system to have adequate testing resources in order to allow for further work-up. This issue is further compounded by the practice of outsourcing for specific resources at different medical centers, which can lead to patients falling through the cracks of our health care system and not receiving the proper follow-up or care that they deserve. Given that pathology only serves as one data point for this patient, the treating clinician must integrate the data presented in a pathology report with additional studies to determine whether or not a patient will meet the criteria for a diagnosis of T-LGLL. A clear example of this is that only 47% of indeterminate cases and 20% of cases with suspicion for T-LGLL resulted in next generation sequencing to determine whether or not a *STAT3* or *STAT5B* mutation might be present. This is drastically lower than the 81% of patients who received a diagnosis of T-LGLL and also underwent next generation sequencing.

The other limitations of this work is limited external validity as there is a skew towards more men in this study and this is likely a result of the direct connection between the academic institution and the Veteran Health Administration. This led to outsourcing of certain diagnostic studies to the academic center and the generation of numerous consults. As the Veteran population is fairly unique and comprises a fairly significant number of the cases represented in this study, this may in turn limit the study's external validity.

In summary, patients presenting with unexplained cytopenias have a broad differential diagnosis that includes conditions with high morbidity and mortality. If patients with T-LGLL are not worked-up correctly, there could be an enormous cost to the health care system for missed diagnoses. With improvement to the diagnostic criteria for T-LGLL, patients could be diagnosed sooner, treated more effectively, and allow for more judicious use of our medical resources. Additional studies can focus on whether or not the co-

mutations seen in patients with concurrent *STAT3* mutations are within the neoplastic T-cell clone or markers of an independent process such as clonal hematopoiesis in order to potentially narrow down to fewer diagnostic entities.

Conclusions *(2-3 summary sentences)*

The most commonly observed abnormalities in T-LGLL cases were monoclonal TCR gene rearrangements and immunophenotypic aberrancy by flow cytometry. Diagnosis of T-LGLL requires integration of morphologic, immunophenotypic, and molecular findings and cannot rely on the presence of specific mutations. Additionally, in this clinicopathologic context, monoclonal TCR rearrangements are sensitive, but not specific, for a diagnosis of T-LGLL. Further research is needed.

References *(JAMA style format)*

1. Swerdlow SH, Campo E, Harris NL, Jaffe ES, Pileri SA, Stein H, Thiele J (Eds): WHO Classification of Tumours of Haematopoietic and Lymphoid Tissues (Revised 4th edition). IARC: Lyon 2017.
2. Ohgami R, Ohgami J, Pereira I. et al. Refining the diagnosis of T-cell large granular lymphocytic leukemia by combining distinct patterns of antigen expression with T-cell clonality studies. Leukemia. 2011; 25,1439–1443. doi:10.1038/leu.2011.107.
3. Jaffe ES, Arber DA, Campo E, Harris NL, Quintanilla-Martinez L. (2017). Hematopathology.
4. Lamy T, Moignet A, Loughran TP. LGL leukemia: from pathogenesis to treatment. Blood. 2017; 129 (9): 1082–1094. doi:10.1182/blood-2016-08-692590.
5. Morgan EA, Lee MN, DeAngelo DJ, Steensma DP, Stone RM, Kuo FC, et al. Systematic STAT3 sequencing in patients with unexplained cytopenias identifies unsuspected large granular lymphocytic leukemia. Blood Adv. 2017; 1 (21): 1786–1789. doi:10.1182/bloodadvances.2017011197.