Published in final edited form as:

Blood Rev. 2014 May ; 28(3): 87–94. doi:10.1016/j.blre.2014.02.001.

The pathogenesis and treatment of large granular lymphocyte leukemia

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Abstract

Large granular lymphocyte (LGL) leukemia is a spectrum of rare lymphoproliferative diseases of T lymphocytes and natural killer cells. These diseases frequently present with splenomegaly, neutropenia, and autoimmune diseases like rheumatoid arthritis. LGL leukemia is more commonly of a chronic, indolent nature; however, rarely, they have an aggressive course. LGL leukemia is thought to arise from chronic antigen stimulation, which drives long term cell survival through the activation of survival signaling pathways and suppression of pro-apoptotic signals. These include Jak-Stat, Mapk, Pi3k-Akt, sphingolipid, and IL-15/Pdgf signaling. Treatment traditionally includes immunosuppression with low dose methotrexate, cyclophosphamide, and other immunosuppressive agents; however, prospective and retrospective studies reveal very limited success. New studies surrounding Jak-Stat signaling suggest this may reveal new avenues for LGL leukemia therapeutics.

Keywords

Large granular lymphocyte leukemia; pathogenesis; cell signaling

1. Introduction

Large granular lymphocyte leukemia embodies a spectrum of rare clonal lymphoproliferative disorders, all which involve inappropriate expansion of large granular lymphocytes (LGLs); either cytotoxic T-lymphocytes (CTLs) or natural killer (NK) cells^{1,2}. In normal adults, LGLs represent 10–15% of peripheral blood mononuclear cells

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Conflicts of interest: None.

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(PBMcs) and can be classified into two distinct lineages as either CD3+ CTLs or CD3- NK cells. Both cell types play important roles in the immune system. LGLs become activated through antigen recognition and undergo significant expansion with subsequent death by apoptosis upon antigen clearance. In LGL leukemia, these LGLs persist³. In 1985, the term *LGL leukemia* was first introduced as a disorder involving clonal invasion of the blood, marrow and spleen⁴. In 1993, the distinction between CD3+ T-cell and CD3- NK-cell lineage subtypes of LGL leukemia was proposed⁵. In 1999, the WHO classification included T- and NK-cell LGL leukemia in the mature peripheral T-cell neoplasms subgroup⁶. In 2008, a provisional entity of chronic NK-cell lymphoproliferative disorder (CLPD-NK) was created by the WHO to separate it from the more aggressive NK-cell leukemia⁷ (Table 1). This review paper will cover topics regarding clinical presentation, diagnosis and treatment possibilities along with providing a future perspective on the LGL spectrum of disorders.

2. Epidemiology

The frequency of LGL leukemia has not been accurately determined but is estimated to account for 2–5% of chronic lymphoproliferative disorders in North America and up to 5–6% in Asia¹. Indolent T-cell LGL leukemia represents the most frequent LGL disorder in Western countries, accounting for 85% of all cases. The median age at diagnosis is 60 years and both sexes are affected equally. Aggressive T-cell LGL leukemia is a rare disorder that has been suggested to be a separate clinicopathologic entity within the spectrum of LGL disorders^{8,9}. It is possible that it arises from clonal evolution of indolent T-cell LGL leukemia but more likely it develops *de novo*. It typically affects a younger population with median age of 41 years. Most cases are poorly understood and have been reported in the literature using different terms^{10,11}. CLPD-NK is an indolent disease comprising 5% of all LGL disorders. The median age at diagnosis is 58 years¹². Finally, aggressive NK-cell leukemia is an extremely malignant disease with poor prognosis, early presentation (median age of 39 years) and predominantly in patients of Asian descent¹². Approximately 10% of LGL disorders can be classified as aggressive NK-cell leukemia. An association with Epstein-Barr virus (EBV) has been demonstrated in these patients, and an initiating role for EBV in aggressive NK-cell leukemia has been suggested^{13,14}.

3. Clinical Features

3.1 T-LGL Leukemia

Most cases of T-LGL leukemia have an overall indolent behavior, but up to two-thirds will become symptomatic. Nearly 80% of symptomatic patients will develop neutropenia and 45% will develop a severe neutropenia (absolute neutrophil count below $0.5 \times 10^9 / L$)⁵. A range of hematologic manifestations are seen in patients with LGL leukemia. Neutropenia is the most common cytopenia that is seen in LGL leukemia, resulting in an increased frequency of bacterial infection. The mechanism of neutropenia is most likely multifactorial but includes secretion of pro-inflammatory cytokine, Fas ligand^{15,16}. Pure red cell aplasia (PRCA) has been seen in 8 to 19% of patients and aplastic anemia has also been reported^{17,18}. Thrombocytopenia occurs in about 20% of patients, and immune thrombocytopenic purpura (ITP) is seen at increased frequency in LGL patients. Myelodysplastic syndrome (MDS) has been reported in patients with T-cell LGL disease¹⁹.

Some experts suggest that autoimmune disorders comorbid with T-LGL leukemia can occur in up to 40% of patients^{1, 20}. Rheumatoid arthritis appears to be the most frequent autoimmune disease in patients with LGL; it has been reported in up to 36% of cases¹. Serologic abnormalities (Rheumatoid Factor, anti-nuclear antibody, and polyclonal hypergammaglobulinemia) are frequent²⁰.

3.2 Chronic Lymphoproliferative Disorder of NK Cells

Clinical features of CLPD-NK are similar to those of T-LGL leukemia. Generally, CLPD-NK is an indolent disorder with a good prognosis. In most cases, CLPD-NK is detected on routine blood studies with persistent elevated circulating LGLs. Like T-LGL leukemia, CLPD-NK patients can be neutropenic, anemic or thrombocytopenic and can also present with a wide array of autoimmune conditions, though this occurs at a lower frequency²⁰.

3.3 Aggressive NK-cell leukemia

Aggressive NK-cell leukemia is a highly aggressive hematological malignancy with a poor prognosis, younger age of presentation (median age of 39 years) and is most often seen in patients of Asian descent⁶. Aggressive NK-cell leukemia patients generally experience fulminant B symptoms, hepatosplenomegaly and a wide range of cytopenias. EBV appears linked to pathogenesis.

4. Pathogenesis of LGL Leukemia

Research into LGL leukemia is focused on understanding the pathogenesis and etiology of the disease, with the idea that if we can understand the pathogenesis, we can develop drugs to combat these dysregulations. Activation of survival pathways and evasion of apoptosis are major dysregulations seen in LGL leukemia. These include dysregulated Fas and Fas ligand (FasL) signaling, growth factor signaling, Map kinases (Mapk), Pi3k-Akt, nuclear factor kappa-light-chain-enhancer of activated B cells (Nfkb), and Jak-Stat signaling. A summary of these dysregulations is included in Table 2.

4.1 Fas and FasL

Fas and FasL signaling normally induces apoptosis and plays a fundamental role in regulation of the immune system. Fas binding FasL produces the death inducing signaling complex (DISC), leading to activation of caspase-dependent apoptosis. This is a major mechanism through which cytotoxic T cells (including LGLs) induce cell death in infected or foreign cells. Fas/FasL signaling also plays a role in T cell homeostasis. During T cell activation and clonal expansion, T cells are resistant to apoptosis, but as the infection resides, activated cytotoxic cells are eliminated through the Fas/FasL pathway in a process known as activation-induced cell death (AICD). AICD limits an excessive immune response and T cells that respond to self-antigens. Humans and mice with mutations in the Fas/FasL pathway have been known to develop auto-immune diseases like systemic lupus erythematosus. Unlike normal activated T cells, leukemic LGLs are resistant to AICD through the Fas/FasL pathway, although leukemic LGLs express high levels of FasL and have not been found to have any mutations in the Fas receptor gene²¹. Further research has demonstrated that two proteins FADD and c-FLIP are over-expressed in leukemic LGLs.

These proteins normally inhibit DISC formation and are believed to be the mechanism through which leukemic LGLs become resistant to apoptosis²². Why FADD and c-FLIP are over-expressed is not entirely clear. A cleaved form of Fas called soluble Fas can block Fas-dependent apoptosis by interfering with normal binding of FasL to its receptor. FasS has been shown to be elevated in LGL patient sera²³.

4.2 Interleukin-15 and Platelet-derived growth factor

Interleukin-15 (Il-15) is a growth factor that regulates normal T cell and NK cell activation and proliferation and has been demonstrated as a survival signal in LGL leukemia. Il-15 signals through a heterotrimeric receptor to activate downstream signals including Jak-Stat and Mapk²⁴. This signal acts to suppress pro-apoptotic factors like Bid and Bim and to induce the Bcl2 family of anti-apoptotic proteins²⁵. *In vitro* and mouse models targeting Il-15 demonstrated that blocking Il-15 signaling was able to suppress LGL leukemia^{25, 26}; however Phase I trials of a humanized antibody to Il-15 demonstrated no therapeutic efficacy^{27, 28}. A computational systems biology approach identified Il-15 and Pdgf as master regulators of leukemic LGL survival. This study demonstrated that dysregulation of these cytokines could explain all known molecular features of LGL leukemia and posed that survival required concomitant Pdgf and Il-15 dysregulation²⁹, which could explain failure of the Phase I trials of a humanized antibody to Il-15²⁸. Pdgf signals through a receptor tyrosine kinase (Pdgfr) to activate survival pathways including Pi3k-Akt and Mapk. An autocrine loop has been demonstrated to regulate survival in leukemic LGLs³⁰.

4.3 Map Kinase

Mapk signaling is a pathway that links extracellular signals to the internal regulation of cell proliferation, cell division, differentiation, and apoptosis. As such, dysregulation of this pathway is one of the most common features of cancer³¹. Constitutively active Mapk signaling has been shown to be a critical survival mechanism in the natural killer cell subset of leukemic LGLs in multiple disease models³². Furthermore, Mapk blockade via chemical inhibition or a dominant negative form of the Mek1 protein suppresses the anti-apoptotic protein Survivin and leads to apoptosis^{32, 33}.

4.4 Pi3k-Akt Signaling

Pi3ks constitute a family of kinases downstream of growth factor receptor tyrosine kinases and leads to the activation of Akt through phosphorylation. Activated Akt modulates downstream components related to survival signaling, cell proliferation, and growth, all processes that are critical to tumorigenesis³⁴. The role of Pi3k-Akt signaling in tumorigenesis has been extensively implicated in human cancer³⁵. In leukemic LGLs, the Pi3k pathway is constitutively activated by Src family kinases. Activated Pi3K signaling leads to inhibition of DISC formation and inhibition of pro-apoptotic signaling³⁶. Treatment with a Pi3k inhibitor (LY294002) that targets Pim1 and the Pi3k catalytic subunit gamma isoform^{29, 36}.

4.5 Sphingolipid rheostat

The sphingolipids sphingosine-1-phosphate (S1P) and ceramide are interconvertible metabolites that maintain a balance (a so-called “rheostat”) controlling the cellular decision of survival versus apoptosis. Gene expression profiling in LGL patients demonstrated that the sphingolipid rheostat was shifted toward the pro-survival S1P and away from the pro-apoptotic ceramide and sphingosine. Specifically, acid ceramidase, which converts ceramide into sphingosine, was shown to be upregulated in LGL leukemia³⁷. Inhibition of acid ceramidase and delivery of C6-ceramide into a rat model of NK-LGL leukemia led to apoptosis of leukemic LGLs³². Additionally, sphingosine kinase 1 (SphK1), which converts sphingosine to S1P, is over-expressed in LGL leukemia patients. Pharmacological inhibition of SphK1 induced apoptosis in leukemic LGLs³⁸. Furthermore, the S1P receptor, S1PR5 is over-expressed in leukemic LGLs³⁷.

4.6 Nuclear-factor κ B

Nfkb is a transcription factor that plays an important role in the survival of immune cells. In unstimulated cells, Nfkb remains bound to a family of inhibitors called inhibitors of κ B (Ikb). Activation of Nfkb can translocate to the nucleus to regulate gene expression. Nfkb, specifically the c-Rel protein, has been shown to be activated in leukemic LGLs and promotes the expression of anti-apoptotic Bcl-2 proteins. Nfkb activation in leukemic LGLs has been shown to be downstream of Akt signaling and independent of Stat3 signaling²⁹.

4.7 Jak-Stat signaling

Jak-Stat signaling, like Mapk and Pi3k-Akt signaling is an intracellular signaling cascade that transmits the effects of extracellular signals (e.g. Il-15) into the cell. Ligand binding to a receptor causes Jak, which has intrinsic tyrosine kinase activity, to phosphorylate the receptor, creating sites for binding of proteins with SH2 domains, mainly Stat proteins. Stat proteins are then recruited to the receptors, where they are phosphorylated by Jak proteins, leading to homo and hetero-dimerization of Stat proteins. Stat dimers then enter the nucleus to modulate gene expression, importantly, genes related to cell survival. Dysregulated Jak-Stat signaling has been observed in numerous cancers. Constitutive activation of Stat3 has been demonstrated as a unifying feature in LGL leukemia, promoting LGL survival through regulation of anti-apoptotic proteins³⁹. Additionally, loss of the signaling inhibitor suppressor of cytokine signaling-3 (SOCS3) has been shown to cooperate with Interleukin-6 to maintain Jak-Stat pathway activation⁴⁰. More recently, it has been demonstrated that about 30–40% of NK and T-LGL patients have somatic activating mutations in the SH2 dimerization and activation domain in the *STAT3* gene^{41, 42}. Treatment with Stat3 inhibitors in both wildtype and mutant Stat3 patients, results in apoptosis in leukemic LGLs. In patients, mutant Stat3 is predictive of an earlier time to treatment failure⁴² and may contribute to the auto-immune phenotype in LGL leukemia and related diseases⁴³. Interestingly, our recently conducted Eastern Cooperative Oncology Group (ECOG) trial, the first prospective clinical trial in LGL leukemia, suggested that a Y640F Stat3 mutation was predictive of favorable methotrexate response⁴⁴. These results suggest screening for Stat3 mutations in the clinic may be important for improving patient outcomes; however our preliminary findings certainly need validation in a larger cohort of patients. Stat5b mutations

were discovered for the first time in human cancer in LGL leukemia. These mutations lead to over-active Stat5b and seem to occur at a low frequency in the LGL population (~2%); however, the clinical course in patients with the N642H mutation was aggressive and fatal. The aggressive form, marked by this mutation, differs from typical LGL leukemia, which generally has a chronic indolent course⁴⁵.

5. Diagnosis

Clinical presentation, cellular morphology, immune phenotype, and TCR rearrangement are important diagnostic criteria for LGL leukemia. A clinical algorithm for the diagnosis of LGL leukemia is included in Figure 1. Clinical features (described in more detail in section 3) frequently include splenomegaly, neutropenia, anemia, lymphocytosis, and some kind of autoimmune condition (e.g. rheumatoid arthritis). Patients presenting with these features should be under high suspicion for LGL leukemia⁴⁶.

LGLs are large, about 15–18 μm in diameter. LGLs have a round to reniform nucleus and a large cytoplasm and azurophilic granules, which contain perforin and granzyme B for cell killing. Normal LGLs exist at 0.25×10^9 LGLs/L in the peripheral blood⁴. Initial diagnostic criteria suggested that $2\text{--}10 \times 10^9$ LGLs/L was required for a diagnosis of LGL leukemia^{5, 47}. It is now recognized that clonal LGL may occur with lower levels of circulating LGL. In such cases, a bone marrow aspirate with IHC staining can be helpful to establish the diagnosis by demonstrating linear arrays of cytotoxic cells⁴⁸. It is next important to determine whether clonal LGLs exist⁴⁹. If clonality does not exist, the abnormal lymphocytic expansion could be a reactive process (e.g. infection). The identification of clonality in T-LGL is straightforward; however, it is more difficult in CLPD-NK (see below).

T-LGL cells have a mature, post-thymic immune phenotype: $\text{TCR}\alpha\beta^+$, $\text{CD}3^+$, $\text{CD}4^-$, $\text{CD}5^{\text{dim}}$, $\text{CD}8^+$, $\text{CD}27^-$, $\text{CD}28^-$, $\text{CD}45\text{RO}^-$, $\text{CD}57^+$. These markers support a constitutively active T cell phenotype^{50, 51}. $\text{CD}45$ and $\text{CD}62\text{L}$ markers can be used to distinguish naïve T cells, central memory, effector memory, and effector T cell populations⁵². Leukemic T-LGL cells have a $\text{CD}45\text{RA}^+$, $\text{CD}62\text{L}^-$ phenotype, representative of a terminal effector memory phenotype²². A small number of cases are $\text{CD}4^+/\text{CD}8^{\text{dim}}$. Less than 10% of patients express $\text{TCR-}\gamma\delta^+$ instead of $\text{TCR}\alpha\beta$ and are associated with a favorable prognosis (85% survival at 3 years)^{53, 54}. T-using antibodies to the TCR, as there is antibody coverage of approximation 75% of the variable regions^{55, 56}. NK activating receptors may be expressed in T-LGL including $\text{CD}94$ and killer immunoglobulin-like receptors ($\text{CD}158$). Monotypic expression of $\text{CD}158\text{a}$, $\text{CD}158\text{b}$, and $\text{CD}158\text{e}$ occurs in more than half of the patients^{57, 58}.

CLPD-NK has an NK cell phenotype: $\text{TCR}\alpha\beta^-$, $\text{CD}2^+$, $\text{CD}3^+$, $\text{CD}3^+$, $\text{CD}4^-$, $\text{CD}8^+$, $\text{CD}16^+$, $\text{CD}56^+$, $\text{CD}57$ is variably expressed^{5, 12}. No TCR rearrangement exists, as NK cells do not express the TCR; thus, clonality is difficult to assess. If a gross chromosomal abnormality exists, this can aid in the diagnosis; however, this is a rare occurrence. $\text{CD}94$ and monotypic expression of activating KIRs can aid in diagnosis as well^{59, 60}.

5.1 LGL-related diseases

Benign, reactive increases in LGLs are seen in association with splenectomy and viral causes including HIV infection⁶¹. Rare cases of T-LGL leukemia have been seen after renal⁶² and liver transplantation⁶³ as well as allogeneic hematopoietic stem cell transplantation (HSCT)^{64, 65}. Interestingly, these leukemias were of donor origin, suggesting that these cells could be an abnormal proliferation to a foreign antigen (e.g. by graft-versus host disease (GVHD)). In a series of 418 patients that received HSCT nearly 20% of patients developed LGL lymphocytosis. This proliferation of LGLs after HSCT was associated with three clinical factors: CMV seropositivity of recipients, CMV reactivation in recipients and chronic GVHD. Transplant patients that developed LGL lymphocytosis showed a higher overall survival, lower non-relapse mortality and lower relapse incidence⁶⁶.

Many auto-immune conditions are associated with LGL leukemia and have a very similar clinical picture. Felty's syndrome is the combination of rheumatoid arthritis (RA), neutropenia, and splenomegaly and is a rare complication of RA but may be seen in up to 40% of patients with LGL leukemia. Evidence suggests that Felty's syndrome and LGL leukemia have a common pathogenesis⁶⁷. Furthermore, data have strongly suggested that LGL leukemia is antigen-driven and evidence for a viral or bacterial cause for RA is a long-standing hypothesis⁶⁷⁻⁶⁹. Other auto-immune conditions, including Hashimoto's thyroiditis, Sjogren's syndrome, Evan's syndrome, Graves' disease, Cushing's syndrome, hyperparathyroidism, multiple sclerosis, ulcerative colitis, uveitis, and psoriasis have been infrequently associated with LGL leukemia⁷⁰.

6. Treatment

The institution of treatment for both T-LGL leukemia and CLPD-NK, both of which follow chronic clinical courses, generally share the same indications. These indications include severe neutropenia (absolute neutrophil count [ANC] < 500), moderate neutropenia (ANC > 500) with symptoms from recurrent infections, symptomatic or transfusion-dependent anemia, and associated autoimmune conditions such as RA.

There is no standard treatment for LGL leukemia patients. This stems from a lack of prospective trials. Each of the 6 largest series published in the literature so far are retrospective. These series are often incomplete and do not contain time to response, treatment duration or time to treatment failure. However, a mainstay mode of treatment is immunosuppressive therapy with agents including methotrexate (MTX), oral cyclophosphamide and cyclosporine (CyA) (Table 3 and Figure 2). Since disease response is slow, a minimum of four months of therapy should be administered before assessing response.

6.1 Steroids

The use of prednisone as a first-line treatment has provided disappointing. There have been temporary improvements in neutropenia but no permanent response. The most complete series shows a 9% (2 of 22 patients) overall response rate (ORR) with no patients reaching clinical remission²⁰. Prednisone may be better served as an adjunct therapy with first-line immunosuppressive agents such as MTX, cyclophosphamide or CyA.

6.2 Methotrexate

MTX has been an effective treatment for RA and has contributed to the improvement of rheumatologic symptoms and RA-associated neutropenia in these patients. LGL patients demonstrate RA in approximately 40% of cases and this led to the institution of methotrexate as a first-line treatment in 1994⁷¹. In this limited uncontrolled prospective study, ten patients with T-LGL leukemia were treated with low dose (10mg/m²) MTX with or without prednisone. Complete clinical responses (CR) were observed in five of ten patients with an additional one patient having partial clinical response (PR). Of the five that demonstrated complete response, three of these had molecular response (MR) with disappearance of the T-LGL clone. A larger series of 62 patients treated with methotrexate demonstrated an overall response rate of 55%. This expanded study was noteworthy for demonstrating a significant proportion of patients relapsing on MTX. Of the 18 patients followed for more than one year, 12 (67%) relapsed²⁰. Such a high relapse rate is not the experience of this author (TPL). We recently completed the first prospective trial with MTX. Our ECOG trial demonstrated a 39% response rate to MTX as initial therapy⁴⁴.

6.3 Cyclophosphamide

An alkylating agent, cyclophosphamide, has been used successfully in both NK and T-LGL leukemia. In the French cohort study, when used orally at dosages of 50 to 100mg, there was an ORR of 66% comparing similarly to MTX^{17, 20, 72–74}. It has been used preferentially in the treatment of LGL patients with pure red cell aplasia with success^{73, 74}. Additionally, 11 of 15 patients who had failed MTX as a first-line therapy responded to cyclophosphamide as a second-line treatment²⁰. Treatment is usually limited to 6–12 months for responders because of risk of bone marrow toxicity and cancer development (MDS and AML)

6.4 Cyclosporine

CyclosporineA has shown an ORR of approximately 60%^{20, 72, 74–79} and response has been linked to HLA-DR4 expression⁷⁷. This treatment can be proposed as an alternative first/second line treatment to MTX/cyclophosphamide.

6.5 Other Treatment Modalities

Though the foundation of treatment is broad non-specific immunosuppressive agents; other treatment options have been pursued. Purine analogues (Eg: fludarabine in combination with dexamethasone) have been used in less than 40 patients and have had impressive response rates (79% ORR)^{20, 72, 78, 80–83}. However, purine analogue use should be cautioned because they have only been employed in a very limited numbers of cases. Alemtuzumab (Campath), a humanized monoclonal antibody against CD52 has been approved for use in chronic lymphocytic leukemia patients and is the standard initial therapy for T-prolymphocytic leukemia. Its use in LGL leukemia has been limited (less than 30 patients, mostly with PRCA) but has an ORR of more than 60%^{84–89}. Patients that have been refractory to immunosuppressive therapy or with aggressive clinical presentation have been treated with CHOP-like regimens with limited success⁹⁰.

7. Future directions: The emergence of personalized medicine in LGL

Leukemia

The discovery of Stat3 and Stat5b mutations in patients with LGL leukemia may pave the road for personalization of medicine in this patient population. The initial preliminary finding suggesting that patients harboring the Y640F mutation in Stat3 respond better to methotrexate is one example of how knowing the genetics of an individual patient or the patient's leukemia could improve disease treatment and outcomes⁴⁴. Whether this leads to decreased morbidity or increased survival in patients with LGL leukemia remains to be addressed and is always an important question to ask when making therapeutic decisions. Additional studies are needed to validate these preliminary findings associating methotrexate response to the STAT3 Y640F mutation. Furthermore it will be interesting to determine whether therapeutic response to immunosuppressive agents other than methotrexate are associated with STAT3 mutational status. . Furthermore, although STAT3 is activated in all LGL leukemia patients (reference JCI 2001), activation by mutation occurs in only about one third of these patients. Therefore, it is conceivable that other genes may be mutated that contribute to STAT3 activation; such genomic studies are ongoing. Lastly, aggressive cases of T-LGL leukemia seem to be characterized by a specific mutation (N642H) in STAT5B. Again, this finding needs validation in a larger number of patients with the aggressive variant of LGL leukemia. Such patients do not appear to respond to combination chemotherapy and better therapeutic approaches are needed. Perhaps, inhibitors of STAT pathway may have a role in future treatment regimens. Taken together, a comprehensive mutation profile for LGL leukemia and the corresponding best treatment options based on personalized genetics might improve patient outcomes in LGL leukemia. A hypothetical algorithm is proposed to incorporate this approach in the treatment of LGL leukemia (Figure 3).

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Figure 1. A proposed diagnostic algorithm for the diagnosis of T-LGL leukemia and CLPD-NK

The peripheral blood smear should be examined in patients with signs and symptoms suggesting LGL leukemia. LGL count can be derived from the total WBC and differential as well as by flow cytometry. The immune phenotype and clonality should be determined to differentiate T-LGL from CLPD-NK.

RA = rheumatoid arthritis; LGL = large granular lymphocyte; CLPD of NK = chronic lymphoproliferative disease of natural killer cells; TCR = T-cell receptor; KIR = Killer-cell immunoglobulin-like receptor.

Figure 2. Proposed Treatment Algorithm for LGL Leukemia

It should be noted that response to treatment with methotrexate or cyclophosphamide is slow. Treatment should be continued for at least 4 months with evaluation of response determined by blood counts.

ANC: Absolute neutrophil count; CR: Complete response; CyA: Cyclosporine A; MTX: Methotrexate; PR: Partial Response; RA: Rheumatoid Arthritis; TCR: T-cell Receptor.

* Pulse steroid dosing could be considered for patients with severe neutropenia (<200/ μ L)

Figure 3.

Adopting a personalized medicine approach to improve outcomes in patients with LGL leukemia. After the diagnosis of LGL leukemia, patients' leukemic LGLs can be extracted and their DNA screened for an array of mutations found to alter treatment decisions in LGL leukemia. Treatment decisions could then be made based on the best treatment for that genotype, in order to improve patient outcomes in LGL leukemia.

Table 1

Summary of LGL Leukemia Disorders

Type	Median Age	Clinical Features	Markers
T-LGL, indolent	60	Asymptomatic OR Symptomatic with <ul style="list-style-type: none"> • Neutropenia • Anemia • Thrombocytopenia • Recurrent bacterial infections Autoimmune conditions <ul style="list-style-type: none"> • Eg. RA, PRCA, AIHA, ITP 	CD3 ⁺ CD8 ⁺ CD16 ⁺ CD56 ⁻ CD57 ⁺ TCRαβ ⁺ (10% are TCRγδ ⁺)
T-LGL, aggressive (rare)	41	Cytopenias Acute B-symptoms, Hepatosplenomegaly	CD3 ⁺ CD8 ⁺ CD57 ⁺ TCR-αβ
CLPD-NK	39	Similar to T-LGL, indolent Less prevalent autoimmune disease	CD3 ⁻ CD16 ⁺ /CD56 ⁺ CD57 ⁺
Aggressive NK-cell Leukemia	58	Fulminant B symptoms Cytopenias Hepatosplenomegaly	CD3 ⁻ CD16 ⁺ CD56 ⁺ CD57 ⁺ EBV ⁺

Table 2

Summary of known dysregulations in LGL leukemia

Pathway	Details	Potential as treatment modality
Fas and FasL and inhibition of activation-induced cell death (AICD)	Leukemic LGLs have elevated Fas-FasL levels in sera and are resistant to Fas-FasL mediated apoptosis AICD. Soluble Fas (FasS) is elevated in patient sera and can block AICD	
Interleukin-15 and Platelet-derived growth Factor (Pdgf) signaling	Computational network modeling suggested that constitutive activation of Interleukin-15 (IL-15) and Pdgf are sufficient to reproduce all known dysregulations in LGL leukemia.	Phase I clinical trials targeting IL-15 with a humanized antibody (Mikβ1) does not appear to be an effective treatment.
Map kinase signaling	Constitutively activated Map kinase signaling has been demonstrated as a critical survival mediator in CLPD-NK.	<i>In vitro</i> pharmacologic Erk inhibition using PD098059 led to apoptosis in the NKL cell line.
Pi3k-Akt signaling	Pi3k-Akt signaling is constitutively active in T-LGL leukemia, due to overactive Src family kinases, which leads to inhibition of pro-apoptotic signaling.	<i>In vitro</i> treatment with Pi3K inhibitor LY 294002 significantly inhibited the activity of NFκB and induced apoptosis in patient T-LGL leukemia PBMCs
Sphingolipid rheostat	Imbalance of sphingolipids has been demonstrated in LGL leukemia. Pro-apoptotic ceramide is decreased and anti- apoptotic sphingosine-1- phosphate is elevated (S1P). S1P receptor 5 is over-expressed in leukemic LGLs	<i>In vivo</i> inhibition of acid ceramidase and delivery of C6- ceramide into a rat model of NK-LGL leukemia led to apoptosis of leukemic LGLs. <i>In vitro</i> pharmacological inhibition of Sphingosine kinase 1 via FTY750 led to apoptosis in leukemic LGLs and remission of leukemia in a rat model.
Nuclear-factor κB signaling	Nfκb has been shown to be activated in leukemic LGLs downstream of Akt and promotes the expression of anti- apoptotic Bcl-2 proteins.	Pharmacologic inhibition of NF-κB with BAY 11-7082 in T-LGL cells led to apoptosis <i>in vitro</i> . Bortezomib proteasome inhibitor has shown promise in preclinical studies in T-LGL leukemia cells.
Jak-Stat signaling	Jak-Stat pathway activation has been demonstrated in LGL leukemia. 30–40% of NK and T-LGL patients have somatic activating mutations in the SH2 dimerization and activation domain in the <i>STAT3</i> gene.	<i>In vitro</i> treatment with Stat3inhibitors in both wildtype and mutant Stat3 patients results in apoptosis in leukemic LGLs. Mutant Stat3 is predictive of an earlier time to treatment failure and may contribute to the auto-immune phenotype in LGL leukemia and related diseases. Y640F Stat3 mutation was predictive of favorable methotrexate response

LGLs = large granular lymphocyte cells; PBMCs = peripheral blood mononuclear cells;

Table 3

Major Treatment Modalities in LGL Leukemia.

Treatment	Pros	Cons	Duration of Treatment	Pitfalls to be Avoided	Comments
Methotrexate	~40% overall response rate If well-tolerated, can continue indefinitely	Potentially high relapse rate	Undefined TTR: 2–12 weeks Do not pursue Tx after 4 mo in non-responders	Recurrence of neutropenia has been seen Increased liver enzymes may occur and hepatic function should be monitored	
Cyclophosphamide	~60–70% overall response rate Effective as second-line therapy in patients who have failed MTX	Potential for cyclophosphamide-induced AML	Do not pursue Tx after 4 mo in non-responders and 6–12 months in responders	Due to risk of bone marrow toxicity and cancer development, tx should be limited to 6–12 mos for responders	
Cyclosporine A	~ 60% overall response rate Potential first/second-line treatment in MTX/cyclophosphamide patients	No eradication of leukemic LGL clone in clinical responders Tx must be continued indefinitely Side effects often lead to discontinuation	Indefinite in responders	Monitor renal function and blood pressure	HLA-DR4 may be predictive of response

AML: Acute Myeloid Leukemia; MTX: Methotrexate; TTR: Time to response; Tx: Treatment;