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T cell large granular lymphocyte leukemia and chronic NK lymphocytosis

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ABSTRACT

Large Granular Lymphocyte Leukemia (LGLL) is a rare chronic lymphoproliferative disorder characterized by the clonal expansion of Large Granular Lymphocytes (LGLs). Among LGLL, the 2016 WHO classification recognizes two different entities, i.e. T-LGLL and the provisional entity Chronic Lymphoproliferative disorder of NK cells (CLPD-NK). In both subtypes neutropenia represents the hallmark of the disease and is frequently regarded as the leading reason to start treatment. Leukemic LGLs are characterized by the up-regulation of several pro-survival signaling pathways, the most relevant being the JAK-STAT axis, whose constitutive activation is partly explained by somatic mutations in *STAT3* and *STAT5b*. In addiction, in the last few years, a relationship between STAT3 mutations/activation and the development of neutropenia was found. Given that backbone treatment relying on immunosuppressive agents is generally unsatisfactory, novel agents targeting the JAK/STAT pathway can represent a turning point in LGLL treatment.

Introduction

Large Granular Lymphocyte Leukemia (LGLL) is a rare chronic lymphoproliferative disorder characterized by the clonal expansion of Large Granular Lymphocytes (LGLs) [1]. Since its original description, LGLL has represented a matter of controversy, because LGL proliferations include a large spectrum of disorders ranging from polyclonal, usually self-limiting lymphocytosis, to indolent clonal expansions, until symptomatic aggressive treatment requiring disease [2–6].

Among LGLL, the 2016 World Health Organization (WHO) classification recognizes two different and well separated entities, namely T-LGLL and the provisional entity Chronic Lymphoproliferative disorder of NK cells (CLPD-NK) [7]. Besides the more common indolent subtypes of LGLL, rare aggressive variants of T/NK disorders have been reported, namely Aggressive NK cell Leukemia and NK lymphoblastic leukemia, but these entities are out of the scope of this review. Although derived from distinct cell lineages, both T-LGLL and CLPD-NK are morphologically similar and are characterized by the accumulation of large granular lymphocytes related to a mature cytotoxic effector type. Besides, in both disorders, cytopenias and other comorbidities are often associated and the management is actually superimposable, entailing a careful observation of chronic lymphocytosis or immunosuppressive regimen [8]. The etiopathogenesis of the disease is still unknown but most authors hypothesize that a viral or autoantigen is responsible for an initial lymphocyte proliferation then sustained by the aberrant activation of anti-apoptotic signaling

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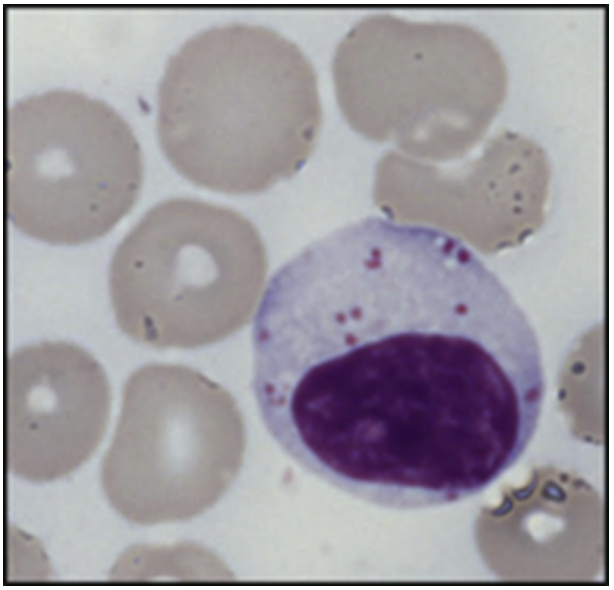

Fig. 1. Peripheral blood smear representing classical Large Granular Lymphocyte morphology detected in T-LGLL and CLPD-NK.

pathways.

Given the similarities in terms of pathogenesis, clinical features and treatment, traditionally LGL disorders were considered two sides of the same disease. However, recent evidence supports the idea that the pathogenesis of the disease is even more complex than expected.

According to the 2016 WHO classification mentioned above, T-LGLL, sustained by the proliferation of $CD3^+$ T-LGLs, is the most frequent variant of the disease and represents approximately 85% of LGLs proliferations. CLPD-NK and ANKL are regarded as two distinct clinical pathological entities within the $CD3^-$ NK-cells lymphoproliferative diseases. CLPD-NK (~10% of cases) affects mainly the western population, while ANKL (< 5% of LGLs disorders) has a distinct geographic distribution, with the most prevalence among East Asian populations.

T-LGLL and CLPD-NK share an indolent and chronic disease, with similar clinical and biological features. Despite arising from different cell lineages, the discovery of the same genetic lesions, both in leukemic T-LGLs and NK cells, suggests a common pathogenetic mechanism unifying these two lymphoproliferative disorders. In contrast, ANKL is characterized by a highly aggressive clinical course and poor prognosis.

LGLs proliferations account for 2%–5% of chronic lymphoproliferative disorders in North America and Europe and for 5%–6% in Asia [4]. Recently, the incidence of LGLL has been published from the Dutch and the American registry, which reported 0.72 cases and 0.2 cases per 1 million individuals per year, respectively [9,10]. T-LGLL and CLPD-NK are commonly diagnosed in elderly patients, with a median age of 60–70 years.

Diagnosis

The diagnosis of LGLL requires the demonstration of a clonal lymphocytosis of LGLs with the typical morphological features lasting for at least 6 months (Fig. 1). Immunophenotypical analysis is mandatory to define proliferation of LGLs, either $CD3^+$ (T-LGLL) or $CD3^-$ (CLPD-NK), as they express characteristic cytotoxic markers like CD16, CD56 and CD57 [1]. T-LGLL is generally sustained by a TCR α/β CD4-/CD8+ although a CD4+/$CD8^{dim/neg}$ variant is described in about 30% of cases [11]. The immunophenotype is also characterized by the expression of CD2, CD45RA, CD122 with LGLs usually displaying a cytotoxic phenotype with the features of terminal effector memory T-lymphocyte (CD45RA+, $CD27^-$, $CD28^-$, CD62L-, CCR7-) [12]. In a minority of cases, the T cell expansion is derived from TCR γδ cells displaying a Vδ1/2 and Vγ9 profile.

At variance, CLPD-NK is characterized by a $CD3^-$ LGLs proliferation $CD16^+$, $CD56^+$, with variable CD57 expression. NK LGLs typically display an aberrant NK receptor (NKR) pattern. As a matter of fact, a restricted pattern of Killer Immunoglobulin Like receptor (KIR) is usually present, which is characterized either by a dominant expression of a relevant KIR, or by a lack of KIR expression [13,14]. NK receptors of the CD94-NKG2 family are found at high level on patients' NK cells, usually associated with the inhibitory subunit NKG2A, although in some cases the association with the activating form NKG2C has been reported [15].

The formal proof for diagnosis of LGL leukemia is given by the demonstration of clonality. In T-LGLL this is provided by TCRγ gene rearrangement analysis, allowing to distinguish reactive LGL proliferations from truly leukemic disease [16]. Moreover, deep sequencing of TCR has demonstrated a restricted diversity of TCR repertoire [17]. Flow cytometry analysis with monoclonal antibodies (MoAb) against the various Vβ repertoire of the TCR can be used as a surrogate for clonality by showing the preferential use of one or two TCR-Vβ segments [18]. The current Vβ panel covers almost 75% of Vβ repertoire and is strongly associated with

monoclonal CD3 region, although fluctuations in clonal dominance referred to as clonal drift is described up to one third of cases [19].

Given the lack of TCRγ rearrangement in NK cells, clonality assessment of CLPD-NK is more complex and can only be provided by the analyses of chromosomal abnormalities or restricted fragment polymorphism of X-linked genes. However, a restricted pattern of KIR expression detected by low cytometry is generally accepted as a surrogate marker of clonality [13,14,20,21].

The diagnosis of LGLL is established by peripheral blood analysis, so bone marrow aspirate and/or biopsy is not recommended and routinely performed as a part of the initial diagnostic work-up. However, in some cases in which the diagnosis is not specified or the etiology of cytopenias is not clear, a bone marrow evaluation is suggested to exclude other bone marrow failure syndromes frequently associated to LGLL such as Myelodisplastic Syndrome (MDS), Aplastic Anemia and Paroxysmal Nocturnal Hemoglobinuria. A classical feature of bone marrow infiltration by LGLL is the presence of lymphoid clusters of CD8+/TIA1+ or granzyme B+ lymphocytes that sometimes can support the diagnosis in ambiguous settings [22].

Clinical features

As stated before, LGLL usually affects elderly people with a median age of 60 years at diagnosis [4,23,24]. The disease is initially asymptomatic in about 30% of patients, with LGL lymphocytosis being the unique abnormality [2,4], usually within 1.0–6.0 $\times 10^9$/L. Isolated neutropenia (Absolute Neutrohil Count $< 1.500/mm^3$) represents the clinical hallmark of the disease, affecting 39–62% of patients, with severe neutropenia (ANC $< 500/mm^3$) characterizing 19–26% of the population [25–27]. Neutropenia favors the onset of oral ulcerations and infections, usually bacterial, involving skin, oropharynx, lung and perirectal areas, blood stream infections may also occur [28]. Acute viral and fungal infections are less common.

Anemia, even transfusion dependent, can be detected in even more variable amounts of patients, ranging from 25 to 49% of cases, with autoimmune hemolytic anemia and Pure Red Blood Cell Aplasia (PRCA) involving a not negligible percentage of patients.

Finally thrombocytopenia is less frequent, observed approximately in 20% of cases [25–27]. Fatigue and B symptoms (fever, night sweats, weight loss) are observed in nearly 25% of patients. Splenomegaly is reported in 20–50% of cases while lymphoadenopathy is rare [29].

A peculiar feature of the disease is the association with autoimmune disorders, both hematological (autoimmune hemolytic anemia, immune thrombocytopenia) and non-hematological (like rheumatoid arthritis, detected up to 30% of patients). The disease frequently coexists with secondary neoplasm, mostly hematological as plasma cell discrasias, non-Hodgkin lymphomas and Myelodysplastic Syndromes (Table 1).

Etiology

The effector-memory phenotype of leukemic LGLs suggests that the initial step triggering the expansion of these cells could be represented by a chronic antigenic stimulation, likely mediated by an auto-antigen or a viral peptide [11,30]. According with this hypothesis, a peculiar association with autoimmune disorders [31,32] or the concurrence of chronic viral infections such as HCV, CMV and EBV, have been documented in LGLL patients [33,34].

In addition, serological studies demonstrated a cross-reactivity to HTLV epitopes, more specifically toward the envelope protein BA21, in approximately 30–50% of patients [35–37]. Although HTLV infection has been described only occasionally [38], the high incidence of BA21 sera reactivity observed in these patients suggests that the exposure to a protein containing a homology domain to BA21 may have a role in the pathogenesis of this lymphoproliferative disorder. Moreover, these data support the hypothesis of a potential link between viral infection and LGLL development.

In an effort to elucidate the putative inciting agents involved in LGLs activation, the TCR-β complementarity determining region 3 (CDR3) repertoire has been analyzed [39]. Although the data suggest a restricted use of the TCR repertoire in T-LGLL, no common antigens have been recognized, suggesting that a unique antigen driving LGL leukemia may be unlike. Therefore, the etiology of the disease still remains elusive.

Pathogenesis

Despite the pathogenesis of LGLL is still not completely understood, it is supposed to result from a sequential multi-step process

Table 1
LGLL associated hematological and non-hematological diseases.

Hematological diseases	%	Non Hematological diseases	%
B cell Non Hodgkin Lymphomas	5	Solid neoplasms	5
Plasma Cell Discrasias	10–20	Rheumatoid Arhtritis	10–30
Myelodisplastic Syndromes	3–4	Sjögren Syndrome	2
AIHA = autoimmune hemolytic anemia	5–10	Endocrinopathies	4
ITP = immune thrombocytopenia	5	Vasculitis	3–4
PRCA	3	Polymiositis	< 1
Acute Myeloid Leukemia	< 1	Pulmonary Hypertension	< 1

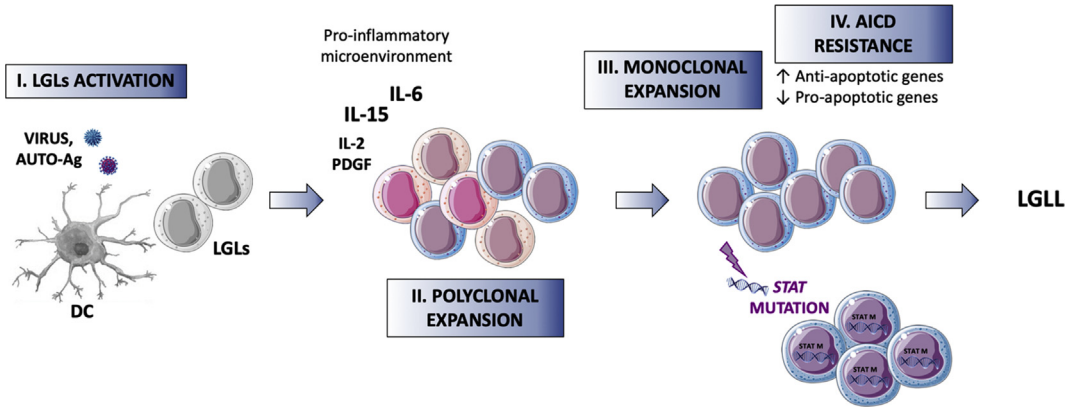

Fig. 2. Synthetic representation of LGLL pathogenesis. The first step of large granular lymphocytes (LGLs) proliferation is related to a chronic viral or auto-antigen stimulation, leading to LGLs activation and, firstly, to an oligoclonal expansion. LGLs proliferation is sustained by the chronic stimulation of pro-inflammatory cytokines (IL-2, IL-15, IL-6, IL-18) and platelet-derived growth factor (PDGF). A third event leads to a monoclonal expansion that obtains a resistance to the physiological mechanism of activation induced cell death (AICD). The escape to AICD could be related to a dysregulation of pro- and anti-apoptotic genes expression and to the insurgence of genetic lesions, i.e. STATs mutations, which promote the survival and the maintenance of the neoplastic clone, leading to the establishment of a chronic LGLs proliferation, referred as LGLL.

(Fig. 2). The triggering event of LGLs proliferation is likely related to a chronic antigenic stimulation, leading to an initial polyclonal LGLs expansion [40]. Immunohistochemical analysis, performed on bone marrow (BM) biopsies of LGLL patients, showed the presence of a direct contact between leukemic LGLs and dendritic cells (DC), in contrast to healthy controls in which these cells present a random distribution. Moreover, the proliferative response of discrete LGL populations to DC stimulation suggested the presence of a specific antigen within BM DC. These data support the role of DC in the pathogenesis of this disease and lead to hypothesize that the BM environment represents the site where the pathological proliferation is triggered [41].

Activated LGLs proliferate, giving rise to an initial polyclonal expansion. LGLs proliferation is subsequently sustained by a pro-inflammatory microenvironment, which plays a central role in LGLs survival by promoting the activation of several pro-survival signaling pathways. This chronic stimulation is mediated by pro-inflammatory cytokines, including interleukin (IL)-2, IL-6, IL-15, IL-18 and platelet-derived growth factor (PDGF) [42–47].

A computational network model suggested that constitutive activation of IL-15 and PDGF is sufficient to reproduce all known molecular alterations in LGLL [46]. In detail, IL-15 is crucial for LGL survival and proliferation, since it triggers the transcription of anti-apoptotic protein, like Bcl-2, and the down-modulation of the pro-apoptotic protein Bid through proteasomal degradation [48]. The relevance of this cytokine in the pathogenesis of LGLL has been demonstrated also *in-vivo*, since transgenic mice lacking post-transcriptional control of IL-15 gene expression develop a CD3+TCRα/β+ T-cell leukemic expansion [49]. Moreover, it has been shown that an *in-vivo* prolonged IL-15 stimulation lead to the development of LGLL in mice [43].

A third event might be responsible for the selection of LGLs monoclonal expansion. In physiological conditions, upon antigen clearance, activated cytotoxic cells are selectively eliminated through a process referred as activation induced cell death (AICD), which represents a crucial mechanism to maintain the immune homeostasis. In LGLL patients, instead, the AICD process is dysfunctional and activated LGLs cannot undergo apoptosis efficiently [30,50]. One of the mechanisms implicated in AICD resistance deals with the Fas-mediated apoptosis escape. Indeed, activation of LGLs induces the up-regulation of Fas and Fas Ligand (FasL) proteins, which are involved in the induction of AICD. However, despite the abundant and constitutive expression of Fas and FasL on their surface, leukemic LGLs are resistant to Fas-mediated apoptosis. Interestingly, this resistance cannot be attributed to loss of function mutations in *FAS/FASL* genes [50,51] but is rather related to elevated levels of a soluble form of Fas (s-Fas) detected in patients' sera, which may act as a decoy receptor for FasL, resulting in the Fas-resistant phenotype of leukemic LGLs [52]. An impaired death-inducing signaling complex (DISC) formation was also reported in leukemic LGLs. Usually, DISC formation represents the immediate downstream event of Fas–FasL cross-linking and is a prerequisite for Fas-mediated apoptosis. However, in pathological LGLs, the over-expression of the DISC inhibitory protein FLIP is thought to impair DISC formation, thus preventing Fas-mediated apoptosis [30].

In addition to the Fas/FasL axis, other signaling pathways, including JAK/STAT, NF-κB, Ras/MEK/ERK, PI3K/AKT pathways and the sphingolipid rheostat, are reported to be dysregulated in leukemic LGLs. Consistently, gene-profiling analyses have shown that the acquired resistance to AICD results from the up-regulation of pro-apoptotic genes and to the down-regulation of anti-apoptotic genes, compared to non-leukemic activated LGLs [53,54].

Among the several deregulated pathways described in leukemic LGLs, one of the most investigated is the JAK/STAT axis, a pro-survival signaling pathway involved in the different processes, like apoptosis, cell cycle and proliferation, cellular transformation, and inflammation [55]. Indeed, STAT3 constitutive activation represents a hallmark of leukemic LGLs being demonstrated that the inhibition of its transcriptional activity leads to a decrease in MCL-1 expression and restores apoptosis of leukemic cells [56]. The discovery, in 2012, of activating *STAT3* somatic mutations that confer increased transcriptional activity contributed to uncover the pathogenesis of this disease. Precisely, in patients harboring these mutations all downstream target genes of the STAT3 pathway were

up-regulated [57]. In 2017, the 2016 WHO classification has been updated with the inclusion of information related to the mutations of the JAK-STAT pathway, more specifically in *STAT3* and *STAT5b* genes. Moreover, it is highlighted that *STAT5B* genetic lesions are associated to a more aggressive disease [58].

Although these genetic lesions were reported to induce a constitutive STAT3 activation, different studies have shown that almost all LGLL patients are characterized by the activation of the JAK/STAT pathway, even without the presence of somatic mutations in the *STAT3* gene. Exome sequencing study performed on three *STAT3* wild-type patients, showed an activated STAT3 pathway even without the presence of mutations, indicating that LGLL patients are characterized by the activation of STAT3 responsive genes [59]. Among the non-mutational mechanisms accounting for STAT3 activation, persistent stimulation by IL-6, which is the most important STAT3 activating factor, has been reported to play a crucial role. Indeed, high levels of this cytokine were detected in LGLL patients' plasma, as compared with healthy controls [44]. Leukemic LGLs are also characterized by an epigenetic down-regulation of SOCS3, leading to the lack of the physiological inhibitory feedback mechanism controlling STAT3 activation. This finding suggests that both intrinsic and extrinsic mechanisms cooperate to keep up STAT3 activation even in *STAT3* wild-type patients, thus playing a role in LGLL pathogenesis [44].

Genetic landscape

Karyotype abnormalities in T-LGLL and CLPD-NK are not currently detected, also as consequence of the difficulty to growth these cell *in vitro*. Less than 10% of patients display distinct chromosomal aberrations, including 12p and 14q inversion, 5q deletion, and trisomy of 3, 8, and 14 chromosomes [24].

Notably, the presence of *STAT3* mutations has been documented in 11–73% of patients with T-LGLL and in approximately 8–30% of CLPD-NK patients [25,44,57,60,61], the most frequent mutations being Y640F and D661Y, located in the SH2 domain. Activating mutations were also discovered outside the SH2 domain, more precisely in the coiled-coil and DBD domains of *STAT3* [62]. Moreover, some patients harbor *STAT5b* mutations, initially discovered in a small subset (2%) of T-LGLL patients with aggressive behavior but subsequently detected also in 55% of CD4+/CD8$^{dim/neg}$ T-LGLL patients [63,64].

Although *STAT* mutations represent the most distinctive genetic lesions described in LGLL patients, they are not sufficient to induce LGLL in mice models, suggesting that they do not play a causal role in development of the disease [65]. Consistently, the demonstration has been recently provided that *STAT* mutations represent a secondary event arising either within an already pre-expanded clonotype, or simultaneously with the clonal expansion of the immunodominant TCR Vβ clonotype [66].

In terms of clinical impact of *STAT* mutations, a significant correlation between the presence of *STAT3* mutations and neutropenia/symptomatic disease has been highlighted in several studies [25,60,61,67].

At variance *STAT5b* mutations are reported to have different clinical impacts. According to the immunophenotype of the mutated clone, they represent a signature of aggressive clinical course with a poor prognosis in aggressive CD8$^+$ T-LGLL and ANKL patients, while they are actually devoid of negative prognostic significance in CD4$^+$ T-LGLL patients [63,64].

Pathogenesis of neutropenia

Isolated neutropenia represents the hallmark of the disease, observed in 39–62% of patients, with approximately half of them developing severe neutropenia. Its pathogenesis is still unclear and it is regarded as a multifactorial event, both humoral and cytotoxic mechanisms being involved in this condition.

One of the most supported mechanisms accounting for the development of neutropenia is neutrophils' destruction via Fas-mediated apoptosis. As a matter of fact, leukemic T-LGLs, constitutively express FasL on their surface [51] that plays a central role in the regulation of neutrophil lifespan [68]. Intriguingly, FasL expression itself can be driven by STAT3 activation [25]. Consistently, a soluble form of FasL (s-FasL) has been found elevated in the sera of LGLL patients and sera from LGLL patients were shown to induce apoptosis *in-vitro* in neutrophils. In addition, the resolution of neutropenia in treated patients was associated to a reduction in s-FasL levels. Taken together, these data suggest a role of FasL in the pathogenesis of neutropenia in LGLL patients [52]. Furthermore, it was recently demonstrated that neutropenic CD8$^+$ T-LGLL patients were characterized by the CD16+/CD56-immunophenotypic signature and by distinctive molecular features, i.e. high levels of STAT3 activation and FasL expression. More recently it has been shown that microRNA(miR)-146b down-regulation, a molecular signature of neutropenic patients, led to the translation of Human antigen R (HuR), an essential FasL mRNA stabilizer. It has been suggested that HuR protein mediated FasL mRNA stabilization, triggering increased FasL production, in turn leads to neutropenia development. These novel data suggest a direct role of miR-146b in the development of neutropenia reported in a subset of T-LGLL patients, thus representing potential targets for an individualized therapeutic approach [69].

As demonstrated in T-LGLL, a distinctive immunophenotype was identified also in neutropenic CLPD-NK patients. More specifically, dominant cytotoxic NK cells expansions with CD56$^{dim/neg}$/CD16^{high}/CD57- phenotype characterize a subset of patients with symptomatic disease and high frequency of severe neutropenia [61]. However, the heterogeneous level of STATs activation and the very low incidence of *STAT* mutations observed in these patients lead to hypothesize that different molecular mechanisms might play a role in neutropenia development in CLPD-NK (Fig. 3), including the impairment of granulopoiesis due to BM infiltration by leukemic LGLs, a direct cytotoxicity mediated by NK receptors and TNF-α and IFN-γ release [6].

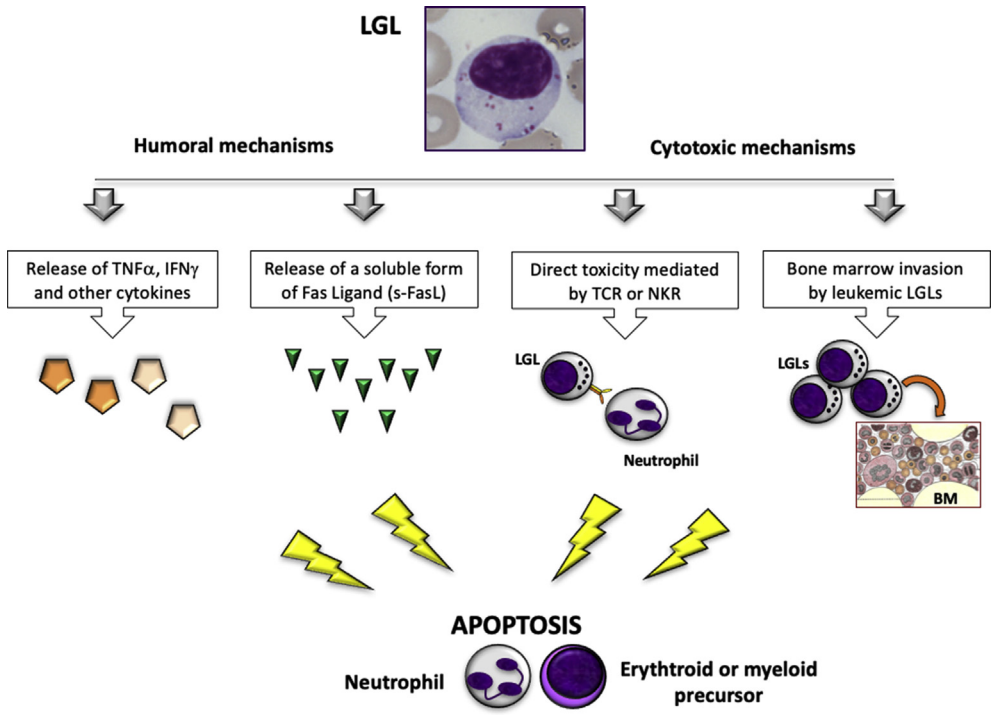

Fig. 3. Schematic representation of the putative mechanisms responsible of the development of cytopenias in LGLL. LGL: Large Granular Lymphocyte. TNF: Tumor Necrosis Factor. INFγ: Interferon gamma. TCR: T cell receptor.

Treatment

Treatment of LGLL is required in presence of symptomatic neutropenia, severe neutropenia, symptomatic or transfusion dependent anemia or presence of concomitant symptomatic autoimmune diseases [8]. Standard treatment of T-LGLL and CLPD-NK is similar and based on immunosuppressive therapy, although the supporting evidence for this approach is limited due to the lack of perspective clinical trials. A minimum of 4–6 months of therapy is mandatory to assess the response. A schematic treatment algorithm is reported in Fig. 4.

First line therapy relies on oral immunosuppressive drugs i.e. methotrexate (MTX) 10 mg/m^2 per week or cyclophosphamide (CTX) 50–100 mg/die. Most of the overall response (OR) and complete response (CR) rates are variable and based on small retrospective studies [8,70].

MTX oral therapy was initially found effective for the treatment of LGLL, with OR and CR rates of 60% and 50% respectively [70]. These data were partially confirmed by the French and Cleveland group experience, with OR rates of 40–50% [26,27].

Oral CTX as first line therapy showed efficacy in a retrospective French-Italian-American experience of 45 patients, with 71% ORR and 47% CR, with 3 patients obtaining a molecular response [71]. Deep sequencing data indicate that CTX retains potential ability to eradicate the clone, reflecting in durable responses [72]. Treatment should be continued no more than 9–12 months to avoid potential Myelodisplastic Syndrome/Acute Myeloid Leukemia complications.

Recently, the results of the first perspective trial of immunosuppressive therapy in LGLL were reported [73]. Fifty-five patients underwent MTX as first line therapy, with 38% ORR. Not responding patients switched to CTX therapy with 64% ORR. Of notice, STAT3 Y640F mutation seemed to predict MTX response, with an ORR of 73%. A randomized trial (NCT01976182) investigating first line therapy with MTX vs CTX is ongoing in France with the aim of determining the best first line choice of therapy in this setting.

Cyclosporine A (CyA) at the 3–5 mg/kg dose is preferentially used for the second line treatment or for patients with severe anemia, with variable ORR (21–100%). Duration of treatment is undefined, as disease usually relapses. In one study, HLA-DR4 haplotype was predictive of CyA response [74].

With the exclusion of immunosuppressive drugs, responses with other agents are erratic and limited to small case series.

Anti CD52 monoclonal antibody Alemtuzumub (Campath®) monotherapy has been recently investigated in relapsed/refractory disease with an ORR of 74% and 47% CR rates [75] but the concerns about toxicities and the difficulties to get the drug limit the use of this therapy in selected cases.

Anti CD20 monoclonal antibody Rituximab has been used in few patients with concomitant RA and T-LGLL, with unexpected responses. Authors suggest that the elimination of B cell expansion and auto-antigens is responsible of LGL clone suppression [76].

Purine analogs like fludarabine, cladibrine, pentostatine or bendamustine has been used in less than 50 patients with promising ORR of about 70–80% [26,77,78].

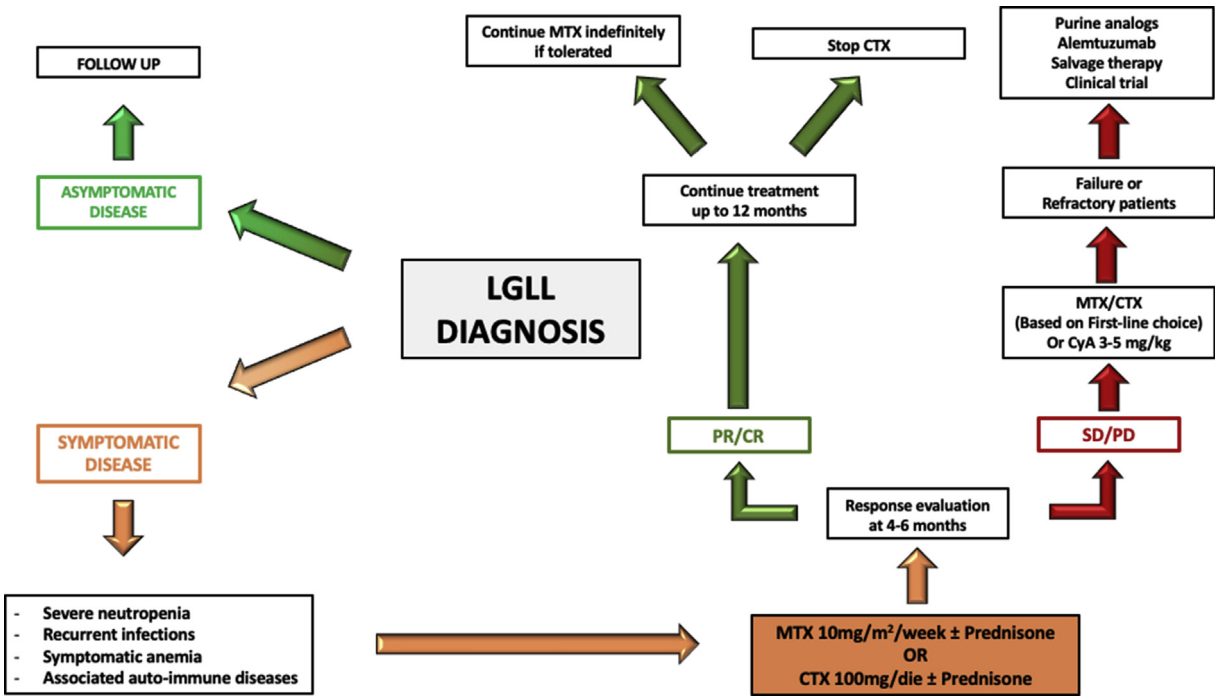

Fig. 4. Treatment algorithm generally accepted in LGLL. MTX: Methotrexate. CTX: Cyclophosphamide. CyA: Cyclosporin A. CR: complete response. PR: partial response. SD: stable disease. PD: progressive disease.

Considering the low quality responses with standard therapies and the several pathways constitutively activated in LGLL, a lot of efforts have been made to find more effective targeted therapies.

Given the crucial role of IL-15 stimulation in LGLL pathogenesis, a phase 1 trial tested the effect of monoclonal antibody blocking IL-15 trans presentation to T cells that express IL-2/IL-15Rb (CD122). Monoclonal antibody Hu-Mikβ1 infusion was safe, but no relevant clinical benefits were achieved [79].

RAS-MAPK constitutive activation was targeted with farnesyltransferase inhibitor (tipifarnib). In a small phase 2 study, 8 patients were treated with tipifarnib without significant clinical benefit, despite bone marrow and hematopoiesis improvement [80].

The discovery of *STATs* mutation point out the possibility of targeting JAK-STAT pathway in LGLL. Tofacitinib, a JAK3 inhibitor approved for the treatment of refractory RA, was tested in 9 patients (four carrying *STAT3* mutations) affected by concomitant refractory LGLL and RA, with hematological response in 6 patients and neutropenia improvement in 5 out 7 patients [81].

Among the novel therapies, the multicytokine inhibitor BNZ-1 seems to be promising. The drug is a pegylated peptide that specifically binds to the gamma chain receptor, thus inhibiting IL-2, IL-15 and to a lesser extent IL-9 signaling, and demonstrated remarkable activity in cell lines and *ex vivo* patients' LGLs [82].

Finally, the proteasome inhibitor bortezomib, could represent another promising agent for LGLL, given its efficacy in *ex vivo* patients' cells and in LGL model IL-15 transgenic mouse [43,48,83].

Summary

LGLL represents a spectrum of clinically and biologically heterogeneous disorders arising from both T Cytotoxic Lymphocytes and NK cells. Among the broad range of disease manifestations, neutropenia is considered the hallmark of the disease and, in most cases, the leading reason to start the treatment. However, responses obtained with standard immunosuppressive agents are unsatisfactory and incomplete, with the disease always invariably relapsing. Moreover, up to now predictive markers of response to immunosuppressive regimens are lacking, therefore the choice of a specific therapy in most cases is more likely to fall randomly.

In the last years, the new insights on the pathogenesis of the disease and, more specifically on the JAK-STAT pathway with the discovery of *STAT*s mutation, seem to be able to overturn the current situation. More specifically, the identification of a connection between STAT3 activation and neutropenia development, allows for the first time to address toward a targeted therapy in LGLL, supported by the preliminary encouraging results of JAK inhibitor treatment. Additional studies are needed to confirm these data and the integration of biological and clinical knowledge is required to achieve a personalized and effective treatment of LGLL.

Practice points

- Large Granular Lymphocyte Leukemia encompasses a spectrum of clinically and biologically heterogeneous disorders.

- Neutropenia represents the hallmark of the disease and frequently the leading reason to start treatment.
- In the last years, a relationship between STAT3 mutations/activation and the development of neutropenia was found.
- Treatment relies on immunosuppressive therapy but responses are generally unsatisfactory and the disease usually relapses.

Research agenda

- Further efforts are required to identify new effective targeted therapy. Drugs targeting the JAK/STAT pathway can potentially assume a leading role in the treatment of this disease.
- Aside *STAT3* mutations, *STAT5b* mutations were recently discovered and apparently associated with both rare aggressive T-LGLL variant and indolent $CD4^+$ T-LGLL. Additional studies should clarify the pathogenetic role of these mutations in LGLL.
- The frequency of *STATs* mutations in CLPD-NK is lower towards its $CD3^+$ counterpart, thus hypothesising a secondary role of JAK-STAT pathway in this variant. A deepen molecular dissection of CLPD-NK represents an attractive subject of study.

Conflicts of interest

The authors declare no competing financial interests.

References

[1] Semenzato G, Zambello R, Starkebaum G, Oshimi K, Loughran Jr. TP. The lymphoproliferative disease of granular lymphocytes: updated criteria for diagnosis. Blood 1997;89:256–60.
[2] Semenzato G, Pandolfi F, Chisesi T, De Rossi G, Pizzolo G, Zambello R, et al. The lymphoproliferative disease of granular lymphocytes. A heterogeneous disorder ranging from indolent to aggressive conditions. Cancer 1987;60:2971–8.
[3] Loughran Jr. TP, Starkebaum G. Large granular lymphocyte leukemia. Report of 38 cases and review of the literature. Medicine (Baltim) 1987;66:397–405.
[4] Loughran Jr. TP. Clonal diseases of large granular lymphocytes. Blood 1993;82:1–14.
[5] Dhodapkar MV, Li CY, Lust JA, Tefferi A, Phyliky RL. Clinical spectrum of clonal proliferations of T-large granular lymphocytes: a T-cell clonopathy of undetermined significance? Blood 1994;84:1620–7.
[6] Zambello R, Semenzato G. Large granular lymphocyte disorders: new etiopathogenetic clues as a rationale for innovative therapeutic approaches. Haematologica 2009;94:1341–5.
[7] Swerdlow SH, Campo E, Pileri SA, Harris NL, Stein H, Siebert R, et al. The 2016 revision of the World Health Organization classification of lymphoid neoplasms. Blood 2016;127:2375–90.
[8] Lamy T, Loughran Jr. TP. How I treat LGL leukemia. Blood 2011;117:2764–74.
[9] Dinmohamed AG, Brink M, Visser O, Jongen-Lavrencic M. Population-based analyses among 184 patients diagnosed with large granular lymphocyte leukemia in The Netherlands between 2001 and 2013. Leukemia 2016;30:1449–51.
[10] Shah MV, Hook CC, Call TG, Go RS. A population-based study of large granular lymphocyte leukemia. Blood Canc J 2016;6:e455.
[11] Garrido P, Ruiz-Cabello F, Barcena P, Sandberg Y, Canton J, Lima M, et al. Monoclonal TCR-Vbeta13.1 + /CD4 + /NKa + /CD8-/ + dim T-LGL lymphocytosis: evidence for an antigen-driven chronic T-cell stimulation origin. Blood 2007;109:4890–8.
[12] Wlodarski MW, Nearman Z, Jankowska A, Babel N, Powers J, Leahy P, et al. Phenotypic differences between healthy effector CTL and leukemic LGL cells support the notion of antigen-triggered clonal transformation in T-LGL leukemia. J Leukoc Biol 2008;83:589–601.
[13] Zambello R, Falco M, Della Chiesa M, Trentin L, Carollo D, Castriconi R, et al. Expression and function of KIR and natural cytotoxicity receptors in NK-type lymphoproliferative diseases of granular lymphocytes. Blood 2003;102:1797–805.
[14] Epling-Burnette PK, Painter JS, Chaurasia P, Bai F, Wei S, Djeu JY, et al. Dysregulated NK receptor expression in patients with lymphoproliferative disease of granular lymphocytes. Blood 2004;103:3431–9.
[15] Lima M, Almeida J, Santos AH, dos Anjos Teixeira M, Alguero MC, Queiros ML, et al. Immunophenotypic analysis of the TCR-Vbeta repertoire in 98 persistent expansions of CD3(+)/TCR-alphabeta(+) large granular lymphocytes: utility in assessing clonality and insights into the pathogenesis of the disease. Am J Pathol 2001;159:1861–8.
[16] Lamy T, Moignet A, Loughran Jr. TP. LGL leukemia: from pathogenesis to treatment. Blood 2017;129:1082–94.
[17] Clemente MJ, Przychodzen B, Jerez A, Dienes BE, Afable MG, Husseinzadeh H, et al. Deep sequencing of the T-cell receptor repertoire in CD8 + T-large granular lymphocyte leukemia identifies signature landscapes. Blood 2013;122:4077–85.
[18] Langerak AW, van Den Beemd R, Wolvers-Tettero IL, Boor PP, van Lochem EG, Hooijkaas H, et al. Molecular and flow cytometric analysis of the Vbeta repertoire for clonality assessment in mature TCRalphabeta T-cell proliferations. Blood 2001;98:165–73.
[19] Clemente MJ, Wlodarski MW, Makishima H, Viny AD, Bretschneider I, Shaik M, et al. Clonal drift demonstrates unexpected dynamics of the T-cell repertoire in T-large granular lymphocyte leukemia. Blood 2011;118:4384–93.
[20] Fischer L, Hummel M, Burmeister T, Schwartz S, Thiel E. Skewed expression of natural-killer (NK)-associated antigens on lymphoproliferations of large granular lymphocytes (LGL). Hematol Oncol 2006;24:78–85.
[21] Scquizzato E, Teramo A, Miorin M, Facco M, Piazza F, Noventa F, et al. Genotypic evaluation of killer immunoglobulin-like receptors in NK-type lymphoproliferative disease of granular lymphocytes. Leukemia 2007;21:1060–9.
[22] Morice WG, Kurtin PJ, Tefferi A, Hanson CA. Distinct bone marrow findings in T-cell granular lymphocytic leukemia revealed by paraffin section immunoperoxidase stains for CD8, TIA-1, and granzyme B. Blood 2002;99:268–74.
[23] Lamy T, Loughran Jr. TP. Clinical features of large granular lymphocyte leukemia. Semin Hematol 2003;40:185–95.
[24] Sokol L, Loughran Jr. TP. Large granular lymphocyte leukemia. Oncol 2006;11:263–73.
[25] Teramo A, Barila G, Calabretto G, Ercolin C, Lamy T, Moignet A, et al. STAT3 mutation impacts biological and clinical features of T-LGL leukemia. Oncotarget 2017;8:61876–89.
[26] Bareau B, Rey J, Hamidou M, Donadieu J, Morcet J, Reman O, et al. Analysis of a French cohort of patients with large granular lymphocyte leukemia: a report on 229 cases. Haematologica 2010;95:1534–41.
[27] Sanikommu SR, Clemente MJ, Chomczynski P, Afable 2nd MG, Jerez A, Thota S, et al. Clinical features and treatment outcomes in large granular lymphocytic leukemia (LGLL). Leuk Lymphoma 2018;59:416–22.
[28] Alekshun TJ, Sokol L. Diseases of large granular lymphocytes. Cancer Contr. 2007;14:141–50.
[29] Mohan SR, Maciejewski JP. Diagnosis and therapy of neutropenia in large granular lymphocyte leukemia. Curr Opin Hematol 2009;16:27–34.
[30] Yang J, Epling-Burnette PK, Painter JS, Zou J, Bai F, Wei S, et al. Antigen activation and impaired Fas-induced death-inducing signaling complex formation in T-large-granular lymphocyte leukemia. Blood 2008;111:1610–6.
[31] Zhang R, Shah MV, Loughran Jr. TP. The root of many evils: indolent large granular lymphocyte leukaemia and associated disorders. Hematol Oncol 2010;28:105–17.

[32] O'Malley DP. T-cell large granular leukemia and related proliferations. Am J Clin Pathol 2007;127:850–9.
[33] Poullot E, Bouscary D, Guyader D, Ghandour C, Roussel M, Fest T, et al. Large granular lymphocyte leukemia associated with hepatitis C virus infection and B cell lymphoma: improvement after antiviral therapy. Leuk Lymphoma 2013;54:1797–9.
[34] Kimura H, Ito Y, Kawabe S, Gotoh K, Takahashi Y, Kojima S, et al. EBV-associated T/NK-cell lymphoproliferative diseases in nonimmunocompromised hosts: prospective analysis of 108 cases. Blood 2012;119:673–86.
[35] Starkebaum G, Loughran Jr. TP, Kalyanaraman VS, Kadin ME, Kidd PG, Singer JW, et al. Serum reactivity to human T-cell leukaemia/lymphoma virus type I proteins in patients with large granular lymphocytic leukaemia. Lancet 1987;1:596–9.
[36] Loughran Jr. TP, Hadlock KG, Perzova R, Gentile TC, Yang Q, Foung SK, et al. Epitope mapping of HTLV envelope seroreactivity in LGL leukaemia. Br J Haematol 1998;101:318–24.
[37] Sokol L, Agrawal D, Loughran Jr. TP. Characterization of HTLV envelope seroreactivity in large granular lymphocyte leukemia. Leuk Res 2005;29:381–7.
[38] Loughran Jr. TP, Coyle T, Sherman MP, Starkebaum G, Ehrlich GD, Ruscetti FW, et al. Detection of human T-cell leukemia/lymphoma virus, type II, in a patient with large granular lymphocyte leukemia. Blood 1992;80:1116–9.
[39] Sandberg Y, Kallemeijn MJ, Dik WA, Tielemans D, Wolvers-Tettero IL, van Gastel-Mol EJ, et al. Lack of common TCRA and TCRB clonotypes in CD8(+)/TCRalphabeta(+) T-cell large granular lymphocyte leukemia: a review on the role of antigenic selection in the immunopathogenesis of CD8(+) T-LGL. Blood Canc J 2014;4:e172.
[40] Zambello R, Semenzato G. Large granular lymphocytosis. Haematologica 1998;83:936–42.
[41] Zambello R, Berno T, Cannas G, Baesso I, Binotto G, Bonoldi E, et al. Phenotypic and functional analyses of dendritic cells in patients with lymphoproliferative disease of granular lymphocytes (LDGL). Blood 2005;106:3926–31.
[42] Zambello R, Facco M, Trentin L, Sancetta R, Tassinari C, Perin A, et al. Interleukin-15 triggers the proliferation and cytotoxicity of granular lymphocytes in patients with lymphoproliferative disease of granular lymphocytes. Blood 1997;89:201–11.
[43] Mishra A, Liu S, Sams GH, Curphey DP, Santhanam R, Rush LJ, et al. Aberrant overexpression of IL-15 initiates large granular lymphocyte leukemia through chromosomal instability and DNA hypermethylation. Cancer Cell 2012;22:645–55.
[44] Teramo A, Gattazzo C, Passeri F, Lico A, Tasca G, Cabrelle A, et al. Intrinsic and extrinsic mechanisms contribute to maintain the JAK/STAT pathway aberrantly activated in T-type large granular lymphocyte leukemia. Blood 2013;121:3843–54. S1.
[45] Yang J, Liu X, Nyland SB, Zhang R, Ryland LK, Broeg K, et al. Platelet-derived growth factor mediates survival of leukemic large granular lymphocytes via an autocrine regulatory pathway. Blood 2010;115:51–60.
[46] Zhang R, Shah MV, Yang J, Nyland SB, Liu X, Yun JK, et al. Network model of survival signaling in large granular lymphocyte leukemia. Proc Natl Acad Sci U S A 2008;105:16308–13.
[47] Kothapalli R, Nyland SB, Kusmartseva I, Bailey RD, McKeown TM, Loughran Jr. TP. Constitutive production of proinflammatory cytokines RANTES, MIP-1beta and IL-18 characterizes LGL leukemia. Int J Oncol 2005;26:529–35.
[48] Hodge DL, Yang J, Buschman MD, Schaughency PM, Dang H, Bere W, et al. Interleukin-15 enhances proteasomal degradation of bid in normal lymphocytes: implications for large granular lymphocyte leukemias. Cancer Res 2009;69:3986–94.
[49] Fehniger TA, Suzuki K, Ponnappan A, VanDeusen JB, Cooper MA, Florea SM, et al. Fatal leukemia in interleukin 15 transgenic mice follows early expansions in natural killer and memory phenotype CD8+ T cells. J Exp Med 2001;193:219–31.
[50] Lamy T, Liu JH, Landowski TH, Dalton WS, Loughran Jr. TP. Dysregulation of CD95/CD95 ligand-apoptotic pathway in CD3(+) large granular lymphocyte leukemia. Blood 1998;92:4771–7.
[51] Perzova R, Loughran Jr. TP. Constitutive expression of Fas ligand in large granular lymphocyte leukaemia. Br J Haematol 1997;97:123–6.
[52] Liu JH, Wei S, Lamy T, Li Y, Epling-Burnette PK, Djeu JY, et al. Blockade of Fas-dependent apoptosis by soluble Fas in LGL leukemia. Blood 2002;100:1449–53.
[53] Shah MV, Zhang R, Irby R, Kothapalli R, Liu X, Arrington T, et al. Molecular profiling of LGL leukemia reveals role of sphingolipid signaling in survival of cytotoxic lymphocytes. Blood 2008;112:770–81.
[54] Shah MV, Zhang R, Loughran Jr. TP. Never say die: survival signaling in large granular lymphocyte leukemia. Clin Lymphoma Myeloma 2009;9(Suppl 3):S244–53.
[55] Leblanc F, Zhang D, Liu X, Loughran TP. Large granular lymphocyte leukemia: from dysregulated pathways to therapeutic targets. Future Oncol 2012;8:787–801.
[56] Epling-Burnette PK, Liu JH, Catlett-Falcone R, Turkson J, Oshiro M, Kothapalli R, et al. Inhibition of STAT3 signaling leads to apoptosis of leukemic large granular lymphocytes and decreased Mcl-1 expression. J Clin Investig 2001;107:351–62.
[57] Koskela HL, Eldfors S, Ellonen P, van Adrichem AJ, Kuusanmaki H, Andersson EI, et al. Somatic STAT3 mutations in large granular lymphocytic leukemia. N Engl J Med 2012;366:1905–13.
[58] Matutes E. The 2017 WHO update on mature T- and natural killer (NK) cell neoplasms. Int J Lab Hematol 2018;40(Suppl 1):97–103.
[59] Andersson EI, Rajala HL, Eldfors S, Ellonen P, Olson T, Jerez A, et al. Novel somatic mutations in large granular lymphocytic leukemia affecting the STAT-pathway and T-cell activation. Blood Canc J 2013;3:e168.
[60] Jerez A, Clemente MJ, Makishima H, Rajala H, Gomez-Segui I, Olson T, et al. STAT3 mutations indicate the presence of subclinical T-cell clones in a subset of aplastic anemia and myelodysplastic syndrome patients. Blood 2013;122:2453–9.
[61] Barila G, Teramo A, Calabretto G, Ercolin C, Boscaro E, Trimarco V, et al. Dominant cytotoxic NK cell subset within CLPD-NK patients identifies a more aggressive NK cell proliferation. Blood Canc J 2018;8:51.
[62] Andersson E, Kuusanmaki H, Bortoluzzi S, Lagstrom S, Parsons A, Rajala H, et al. Activating somatic mutations outside the SH2-domain of STAT3 in LGL leukemia. Leukemia 2016;30:1204–8.
[63] Rajala HL, Eldfors S, Kuusanmaki H, van Adrichem AJ, Olson T, Lagstrom S, et al. Discovery of somatic STAT5b mutations in large granular lymphocytic leukemia. Blood 2013;121:4541–50.
[64] Andersson EI, Tanahashi T, Sekiguchi N, Gasparini VR, Bortoluzzi S, Kawakami T, et al. High incidence of activating STAT5B mutations in CD4-positive T-cell large granular lymphocyte leukemia. Blood 2016;128:2465–8.
[65] Dutta A, Yan D, Hutchison RE, Mohi G. STAT3 mutations are not sufficient to induce large granular lymphocytic leukaemia in mice. Br J Haematol 2018;180:911–5.
[66] Kerr CM, Clemente MJ, Chomczynski PW, Przychodzen B, Nagata Y, Adema V, et al. Subclonal STAT3 mutations solidify clonal dominance. Blood Adv 2019;3:917–21.
[67] Kawakami T, Sekiguchi N, Kobayashi J, Yamane T, Nishina S, Sakai H, et al. STAT3 mutations in natural killer cells are associated with cytopenia in patients with chronic lymphoproliferative disorder of natural killer cells. Int J Hematol. 2019;109:563–71.
[68] O'Donnell JA, Kennedy CL, Pellegrini M, Nowell CJ, Zhang JG, O'Reilly LA, et al. Fas regulates neutrophil lifespan during viral and bacterial infection. J Leukoc Biol 2015;97:321–6.
[69] Calabretto G, Teramo A, Mariotti B, Rossato M, Castellucci M, Barilà G, et al. Insights into the molecular mechanism accounting for neutropenia in T-large granular lymphocytes leukemia. Blood. 60th ASH annual meeting. San Diego. 2018. p. 1575. Poster Abstract 622.
[70] Loughran Jr. TP, Kidd PG, Starkebaum G. Treatment of large granular lymphocyte leukemia with oral low-dose methotrexate. Blood 1994;84:2164–70.
[71] Moignet A, Hasanali Z, Zambello R, Pavan L, Bareau B, Tournilhac O, et al. Cyclophosphamide as a first-line therapy in LGL leukemia. Leukemia 2014;28:1134–6.
[72] Rajala HL, Olson T, Clemente MJ, Lagstrom S, Ellonen P, Lundan T, et al. The analysis of clonal diversity and therapy responses using STAT3 mutations as a molecular marker in large granular lymphocytic leukemia. Haematologica 2015;100:91–9.
[73] Loughran Jr. TP, Zickl L, Olson TL, Wang V, Zhang D, Rajala HL, et al. Immunosuppressive therapy of LGL leukemia: prospective multicenter phase II study by the Eastern Cooperative Oncology Group (E5998). Leukemia 2015;29:886–94.
[74] Battiwalla M, Melenhorst J, Saunthararajah Y, Nakamura R, Molldrem J, Young NS, et al. HLA-DR4 predicts haematological response to cyclosporine in T-large granular lymphocyte lymphoproliferative disorders. Br J Haematol 2003;123:449–53.

[75] Dumitriu B, Ito S, Feng X, Stephens N, Yunce M, Kajigaya S, et al. Alemtuzumab in T-cell large granular lymphocytic leukaemia: interim results from a single-arm, open-label, phase 2 study. Lancet Haematol 2016;3:e22–9.
[76] Cornec D, Devauchelle-Pensec V, Jousse-Joulin S, Marhadour T, Ugo V, Berthou C, et al. Long-term remission of T-cell large granular lymphocyte leukemia associated with rheumatoid arthritis after rituximab therapy. Blood 2013;122:1583–6.
[77] Ma SY, Au WY, Chim CS, Lie AK, Lam CC, Tse E, et al. Fludarabine, mitoxantrone and dexamethasone in the treatment of indolent B- and T-cell lymphoid malignancies in Chinese patients. Br J Haematol 2004;124:754–61.
[78] Zaja F, Baldini L, Ferreri AJ, Luminari S, Grossi A, Salvi F, et al. Bendamustine salvage therapy for T cell neoplasms. Ann Hematol 2013;92:1249–54.
[79] Waldmann TA, Conlon KC, Stewart DM, Worthy TA, Janik JE, Fleisher TA, et al. Phase 1 trial of IL-15 trans presentation blockade using humanized Mikbeta1 mAb in patients with T-cell large granular lymphocytic leukemia. Blood 2013;121:476–84.
[80] Epling-Burnette PK, Sokol L, Chen X, Bai F, Zhou J, Blaskovich MA, et al. Clinical improvement by farnesyltransferase inhibition in NK large granular lymphocyte leukemia associated with imbalanced NK receptor signaling. Blood 2008;112:4694–8.
[81] Bilori B, Thota S, Clemente MJ, Patel B, Jerez A, Afable Ii M, et al. Tofacitinib as a novel salvage therapy for refractory T-cell large granular lymphocytic leukemia. Leukemia 2015;29:2427–9.
[82] Wang TT, Yang J, Zhang Y, Zhang M, Dubois S, Conlon KC, et al. IL-2 and IL-15 blockade by BNZ-1, an inhibitor of selective gamma-chain cytokines, decreases leukemic T-cell viability. Leukemia 2019;33:1243–55.
[83] Yang J, LeBlanc FR, Dighe SA, Hamele CE, Olson TL, Feith DJ, et al. TRAIL mediates and sustains constitutive NF-kappaB activation in LGL leukemia. Blood 2018;131:2803–15.