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Review

Not all LGL leukemias are created equal

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ABSTRACT

Large Granular Lymphocyte (LGL) Leukemia is a rare, heterogeneous even more that once thought, chronic lymphoproliferative disorder characterized by the clonal expansion of T- or NK-LGLs that requires appropriate immunophenotypic and molecular characterization. As in many other hematological conditions, genomic features are taking research efforts one step further and are also becoming instrumental in refining discrete subsets of LGL disorders. In particular, *STAT3* and *STAT5B* mutations may be harbored in leukemic cells and their presence has been linked to diagnosis of LGL disorders. On clinical grounds, a correlation has been established in CD8+ T-LGLL patients between *STAT3* mutations and clinical features, in particular neutropenia that favors the onset of severe infections. Revisiting biological aspects, clinical features as well as current and predictable emerging treatments of these disorders, we will herein discuss why appropriate dissection of different disease variants is needed to better manage patients with LGL disorders.

1. Introduction and LGLL classifications over time

Chronic lymphoproliferative disorders of large granular lymphocytes (LGL) comprise a wide spectrum of conditions ranging from reactive polyclonal, usually self-limited lymphocytoses, to asymptomatic clonal LGL expansions, until manifest symptomatic leukemic diseases characterized by dismal outcome. These abnormal proliferations result from the expansion of cytotoxic lymphocytes, the cells typically involved in immune responses against pathogens as well as in the control of neoplastic growth. Cytotoxic responses to exogenous stimuli are actually mediated by two highly professional although extremely different players, i.e. cytotoxic T lymphocytes (CTL) and natural killer (NK) cells. A conventional polyclonal, sometimes oligoclonal, expansion of LGL is a feature currently occurring in patients with a variety of infections (Epstein-Barr virus, cytomegalovirus and hepatitis C virus, among others), following splenectomy or organ transplantation.

Large Granular Lymphocyte Leukemia (LGLL) is a rare disease originating from the clonal expansion of LGLs whose diagnosis, classification and treatment have been hampered over time by its remarkable phenotypic, genotypic and clinical heterogeneity as well as their geographic diversity. Given the morphological appearance of mature lymphocytes, the first cases of LGL expansions reported in literature were classified as chronic lymphocytic leukemia (CLL). When immunophenotyping was made available on a routine basis in the mid-70s, these disorders were included among type T-CLL [1–3] most of these lymphocytoses expressing CD3/CD8 determinants; only a minority of them exhibited markers related to NK cell lineage [4].

However, it soon became evident that many patients with lymphocytosis did not show any feature of overt clinical malignancy, raising the question of whether we were dealing with a reactive process or a neoplastic condition [5–7]. Given the virtually identical morphological features of reactive and leukemic LGLs, their distinction has been a long time dilemma, most patients presenting with indolent clinical course, similarly to CLL in very early stage.

In the mid-80s, the discovery of T Cell Receptor (TCR), and its widespread use on clinical grounds to demonstrate T cell clonality thanks to the T-cell repertoire diversity [8], represented a significant step for the study of LGL lymphocytoses [9,10]. In fact, evaluation of clonality made the distinction possible between the end of normality and the beginning of disease. This possibility obviously opened new clues into the nature of these cell proliferations and first case series of LGL disorders were published around the world [11–13]. Due to the increasingly refined high throughput molecular technologies to study the TCR, including next generation sequencing (NGS), the proof of

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clonality is easily detectable for T cell expansions. The clonality of NK-LGLs is more difficult to assess because these cells are not equipped with the TCR, thus lacking a reliable readout of clonality. In these cases, chromosomal abnormalities or a restricted fragment length polymorphism at the phosphoglycerate kinase loci on the X chromosome can provide evidence of clonality [14,15]. However, the lack of obtaining metaphases impedes to easily assess chromosomal lesions in LGLL patients and the genes' polymorphism is technically difficult to test, its evaluation also being gender-related (i.e., females who are heterozygous for the above X-linked loci). Waiting for wider applicable methods to define NK cell clonality, a killer immunoglobulin like receptors (KIR, i.e. CD158 molecules, see below) restricted pattern of expression demonstrated by flow cytometry analysis (either a dominant expression of a relevant KIR or lack of them) is accepted as a surrogate marker of clonal NK cell expansion [16,17]. In addition, the presence of mutations (including *STAT3*, *STAT5B*, *TET2*, *TNFAIP3* and *CCL22)* have been recently integrated in the diagnostic algorithm to further differentiate reactive NK expansions from NK-LGL leukemias [18].

Many designations have been used in the nineties to label these lymphocytoses including Abnormal Expansions of Granular Lymphocytes, T Cell Lymphocytosis, T8 Hyperlymphocytosis Syndrome, T-gamma Proliferations, Monoclonal Lymphocytosis of Undetermined Significance [19], until the early 2000s when the WHO classification of lymphoid neoplasms definitively brought clarity subdividing chronic LGL disorders simply relying on immunophenotypic properties (T or NK lineage) of proliferating cells [20,21]. In the most updated 5th WHO classification [22] these disorders are designated as:

- T-Large Granular Lymphocyte Leukemia (T-LGLL)
- NK-Large Granular Lymphocyte Leukemia (NK-LGLL), that nowadays replaces the former provisional entity (named Chronic Lymphoproliferative disease of NK cells, CLPD-NK)
- Aggressive NK Leukemia (ANKL).

2. Epidemiology

LGL proliferations account for 2-3% of chronic lymphoproliferative disorders in North America and Europe [23] and for 5-6% in Asia [24]. T-LGLL and NK-LGLL are commonly diagnosed in elderly patients, with a median age of ~60 years with no gender, racial or genetic predisposition; they unusually occur in individuals under 30 years. ANKL is more prevalent in East Asian populations and is frequently associated with Epstein-Barr virus (EBV) [25,26]. ANKL usually affects young to middle-aged adults (~40 years).

3. Morphologic assessment and immunophenotyping

Leukemic cells in T-LGLL and NK-LGLL share the morphological appearance of LGL with typical variable sized azurophilic granules in their cytoplasm containing the weapons they are equipped with to accomplish the cytotoxic functions mentioned above. Peripheral blood smears demonstrate large size cells (15-20μM) with a round or reniform nucleus, irregular nuclear contour, coarse chromatin and abundant pale-staining cytoplasm containing variable amount of granules (Fig. 1A). On May-Grunwald Giemsa staining of smear specimens, LGL cytologic features in T-LGLL and NK-LGLL are not distinguishable, not even from normal reactive cytotoxic lymphocytes. By contrast, proliferating cells in ANKL are characterized by atypical irregular nuclei, open chromatin with the presence of prominent nucleoli and by slightly basophilic cytoplasm containing coarse granules, sometimes hardly recognized (Fig. 1B) [27].

The immunophenotype is central to distinguish different LGLL subtypes (Fig. 2). T-LGLL accounts for approximately 80% of LGL expansions. Leukemic T-LGLs exhibit a post-thymic terminal effector memory phenotype (CD3+CD8+CD57+ CD45RA+CD62L-) along with a variable expression of CD16, CD56, KIRs and CD94/NKG2 determinants, indicating that these cells are late stage fully differentiated cytotoxic T-lymphocytes [28,29].

Beyond LGLL characterized by the above phenotype, referred to as CD8+ T-LGLL and accounting for approximately 60% of LGLL cases, a less abundant percentage of patients (~30%) exhibits the CD4 determinant either alone or in association with dimly expressed CD8 [30,31]; these cases are referred to as CD4+ T-LGLL. The preferential usage of one TCR-Vβ segment (most frequently Vβ13 in CD4+ T-LGLL [32]) through high sensitive flow cytometry analyses of the TCR repertoire can be regarded as a surrogate for molecular assessment [33,34]; of note, current anti-Vβ antibodies cover approximately 70% of all Vβ domains. Flow cytometric evaluation of the constant region 1 of the T-cell receptor β chain (TCRBC1) has recently been proposed as an easy and reliable method for assessing Tαβ clonality [35,36]. However, this type of analysis cannot be of use with the Tγ/δ disease variant mentioned below. Rare cases equipped with the CD3+CD8+CD56+ phenotype (Fig. 2) have been reported to present with very aggressive

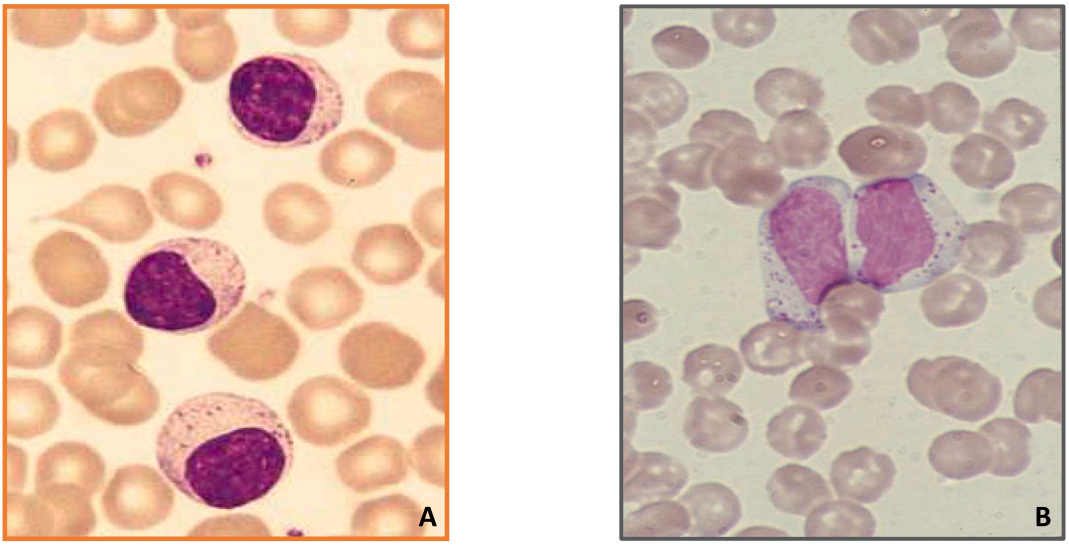

Fig. 1. Morphological appearance of LGLL. May-Grunwald Giemsa staining 1000x of
A) LGL cytologic features in T-LGLL and NK-LGLL which are not distinguishable, not even from normal reactive cytotoxic lymphocytes.
B) Cells from a patient with ANKL. Courtesy of Kazuo Oshimi (Kushiro, Hokkaido, Japan).

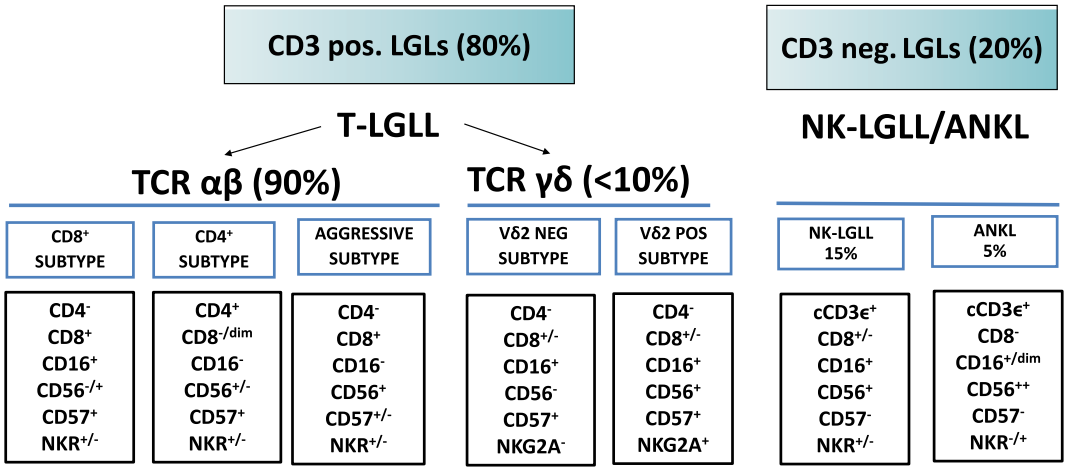

Fig. 2. Immunophenotype in different subsets of LGL disorders. Major LGLL subsets are reported, according to surface phenotype. See text for details. The figure does not include other less frequent immunophenotypic patterns, such as the rare phenotypical variants identified by a concomitant Tαβ/Tγδ or T/NK LGL proliferations [40].

disease [37,38].

To emphasize their heterogeneity, depending of the type of heavy chain of the surface TCR (α/β or γ/δ) being expressed, T-LGLL can be further subdivided with Tα/β-LGLL (either CD8+ or CD4+) and Tγ/δ-LGLL subsets [39,40]. Tγ/δ cells stand at the intersection between innate and adaptive immunity and, unlike conventional Tα/β cells, they preferentially dwell in non-lymphoid peripheral tissues and respond to ligands in a MHC-independent manner [41]. The rare patients (around 10% of T-LGLL) whose cells belong to the group referred to as Tγ/δ-LGLL are characterized by CD57 and CD16 markers, can express CD8 (~70%) and lack CD4 molecules. These cells partially (~30%) express CD56 and NK receptors (NKR) and preferentially display the Vγ9/Vδ2 rearrangement profile [39,42].

Conversely, NK-LGLL (accounting for approximately 20% of LGL disorders) is characterized by a CD3-CD16+CD56+ LGL expansion, with variable CD57 expression. NK LGLs typically display aberrant NKR and a restricted pattern of KIR is present, which is characterized either by the dominant expression of a relevant KIR, or by the lack of KIR expression [16,17,29,43]. Two monoclonal antibodies, i.e. EB6 (CD158a) and GL183 (CD158b), have been reported to recognize two different antigens expressed on NK cell subsets belonging to the same 58 KDa molecular family [44,45]. Using these reagents, normal CD3-CD16+CD56+ NK cells can be further separated into four subsets: single positive phenotypes (either EB6+GL183- or EB6-GL183+), double positive phenotype (EB6+GL183+) and double negative phenotype (EB6-/GL183-). Since the expression (or lack of expression) of each of these antigens is a stable property of NK cells, that is not modified by cell activation, proliferation or cloning [44,45], the analysis of distribution of these antigens in expanding NK cells of LGLL patients can provide information on the dominant subset. The eventuality that two different KIRs can be simultaneously expressed (or both lacking) on the same cells is a possible, already reported occurrence [46–47] that suggests clonality provided the cells represent the large majority of expanding lymphocytes.

NKR of the CD94-NKG2 family are also found at high level on patients' NK cells, usually coupled with the inhibitory subunit NKG2A; in some cases the association with the activating form NKG2C has been reported [48]. Two major NK disease subsets can be identified, i.e. patients with $CD56^{neg/dim}CD16^{dim}$ NK cells (less frequent, ~20%) and patients with $CD56^{neg/dim}CD16^{high}$ NK cells (more frequent, ~80%). Within the latter subset, the presence of CD57 has been reported in about half of the cases [40,49] and its expression discriminates two patients' subgroups characterized by CD57 negativity and positivity that were identified as "Cytotoxic" and "Memory" NK subgroups, respectively, with the more symptomatic cases and the presence of *STAT3* mutations being included among the CD57 negative subset [50].

The presence of *CCL22* somatic mutation has been recently reported in 16% of a series of 59 NK-LGLL patients, being mostly exclusive with *STAT3* mutation and preferentially harbored in $CD57^{dim/neg}$ patients [51]. These features point to the mechanistic role of the *CCL22* activating mutation in favoring the IL-15 mediated proliferating activity in NK-LGLL. Finally, the value of the combination of KIR phenotyping and targeted high-throughput sequencing was tested in a cohort of 114 consecutive patients with NK cell proliferations. A NK-cell clonality score was proposed, combining flow cytometry and molecular profiling (namely *STAT3* and *TET2* mutations) with a positive predictive value of 93%, thus contributing to a more stringent diagnosis of disease [18,52].

The immunophenotype of cells in ANKL (about 5% of LGL disorders) is not completely divergent from that of NK-LGLL and to some extent similar to the aggressive CD3+CD8+ T-LGLL [37]. More in detail, in this disorder leukemic cells are devoid of the TCR and express surface CD2+CD3-CD5-CD7+$CD16^{+/dim}$ CD56+CD57- [26,53,54].

4. Genetics

Molecular genetics nowadays dictates the classification of many hematological malignancies and LGL disorders are not an exception [55]. A genetic characterization of LGL disorders is also becoming steadily more relevant on clinical grounds for the possibility to distinguish discrete disease subsets on this basis, thereby lending the possibility of informing the categorization as well as the management of LGLL patients [56–58]. However, we want to emphasize the difficulty in having comparable data on the real median incidence of mutations associated to LGLL since different methods were used, ranging from Sanger and targeted amplicon sequencing (mainly focused on the hotspot regions of the genes) to whole exome/genome sequencing.

The discovery of *STAT* mutations has made a substantial contribution to the LGLL field not only for expanding our understanding of LGL leukemogenesis but also having improved the classification of these disorders [55,59]. Mutations on *STAT3, STAT5B* and *CCL22* have been recognized as the commonest gain-of-function genetic lesions up to now identified in LGLL patients [51,60–67]. As discussed later in the section of etiopathogenesis, it is worth mentioning that the above gene mutations are unlikely to be the initial inciting trigger of leukemic process; they are rather believed to represent an acquired event during the disease that confers a competitive growth advantage on clone development [68]. We herein put emphasis on the use of *STAT* mutations screening as a hallmark of disease, and in particular in terms of clinical correlations

and disease subtypes. Together with an appropriate immunophenotypic analysis, the assessment of mutational landscape is therefore now recommended for accurate characterization of LGLL patients [22].

STAT3 and *STAT5B* mutations occur in phenotypically distinct T-LGLLs and Fig. 3 shows the frequency of *STAT* mutations in LGL subtypes. Before getting into details, it must be specified that the reported median incidence, according to the references mentioned in the figure, has the limitation that available data in different studies have been performed with different methods (also note the wide range). That being stated, *STAT3* mutations are a discrete feature of CD8+ T-LGLL and of some Tγ/δ-LGLL, with an incidence ranging from 20 up to 70% across different case series (approximate mean 40%). *STAT3* mutations are not detectable in the CD4+ T-LGLL subset, with only rare exceptions [65,69]. *STAT5B* mutations are mostly associated with the indolent CD4+ T-LGLL disease subtype [62] (with an incidence of ~66% using NGS analysis [63]) or with the aggressive variant of CD8+ T-LGLL [38]. In CD8+ T-LGLL *STAT5B* mutations are very rare and, when present, configure a severe disease [38]. Pretty rare *STAT5B* mutations have been also identified among some Tγ/δ-LGLL [70].

As for NK-LGLL, *STAT3* mutations have a lower incidence (~25%, again with a wide range) in this disease as compared to T-LGLL (Fig. 3) [52,60,65,67,71–74]. In addition to *STAT3*, *TET2* mutations were also detected in NK-LGLL with a similar incidence, whereas *PIK3CD* and *TNFAIP3* mutations resulted less recurrent [72,73]. Opposite to CD4+ T-LGLL, NK-LGLL appears to be strikingly devoid of *STAT5B* genetic lesions, with the only exception of the aggressive case reported by Rajala and coworkers, who subsequently developed ANKL [38].

Mutations of the JAK/STAT pathway were also detected (~20%) in the aggressive ANKL in association with histone modifying molecules (i.e. *TET2* [~20%], *MLL2* and *CREBBT*) [75–77].

Y640F and D661Y are the most frequent *STAT3* genetic lesions, whereas in *STAT5B* gene the most recurrent mutations are N642H and Y665F. Other activating abnormalities, albeit at much lower frequencies, include both point mutations and insertion or deletions and are usually found in SH2 domain, although they can be located also in other gene domains [78]. In addition, in T-LGLL other mutated genes have been found, including *TNFAIP3* and less frequently *BCL11B*, *FLT3* and *PTPN23*, and in particular recurrent mutations in chromatin and epigenetic modifying genes have been recently discovered in LGLL [52,72–74]. The above mentioned recent study by Baer and co-investigators [51] demonstrated that somatic mutations in the chemokine gene *CCL22* is typical of a subset of NK cell proliferations (27%). This mutation is not shared by T-LGLL and, being mutually exclusive of *STAT3* and *STAT5B* mutations, has been proposed as the hallmark of NK-LGLL. This genetic lesion has been regarded as a deregulating event of microenvironmental crosstalk in this disease [51] but the actual significance of all new mutations needs further studies.

A variety of cytogenetic abnormalities, but without a consistent pattern of specific changes, have been occasionally reported in patients with T-LGLL and NK-LGLL [6,19,79] whilst ANKL is characterized by 7p and 17p losses and 1q gains [80].

5. Etiopathogenesis

The etiology of LGL leukemia still remains unknown, but some cornerstones to figure out disease's development have been elucidated. Fig. 4 summarizes the current knowledge on etiopathogenesis of LGL disorders.

The proliferation and persistence of T or NK cell clones result from a repeated hitherto unrecognized antigenic stimulation (auto-antigens or foreign infective antigens) in association with dysregulated LGL homeostasis related to intrinsic (mutations) and extrinsic (microenvironment) factors.

As for the chronic antigenic stimulation, likely of viral origin hitting inside or outside the LGL population [81], serologic reactivity against the recombinant BA21 epitope of type one human T-lymphotropic virus (HTLV-I) envelope protein p21 has been detected in several LGLL patients, although an association with a prototypical HTLV infection has not been established [82–85]. Focusing on NK-LGLL patients, analysis of whole exome sequencing data failed to detect viral sequences, thus denying a direct role of an integrated or episomal viral agent in NK cells. However, merged literature data allowed to hypothesize the presence of retroviral agents located outside the hematopoietic compartment that might contribute to activate NK cells and, in turn, to sustain cell expansion [81,86–89]. At variance, EBV infection has long been associated with ANKL, with a higher prevalence among people from Asia and Central/South America [25,26].

The bone marrow (BM) has been proposed as the setting where the putative antigen presentation takes place and dendritic cells (DC) have been hypothesized to represent the target of infection in these patients. In fact, a co-localization of DCs and leukemic LGLs has been demonstrated both in T-LGLL and in NK-LGLL [90,91].

Once established, the survival of founding LGL clones is powered up by several pro-inflammatory cytokines, mostly related to immune cytotoxic response after viral infections, including IL-1β, IL-1Rα, IL-6, IFNγ, CCL5, CCL4, IL-18, IL-8, CXCL10, and CXCL9, some produced by leukemic LGLs themselves, others by non leukemic cells [92–96].

IL-15 is another key cytokine involved in LGLL pathogenesis and

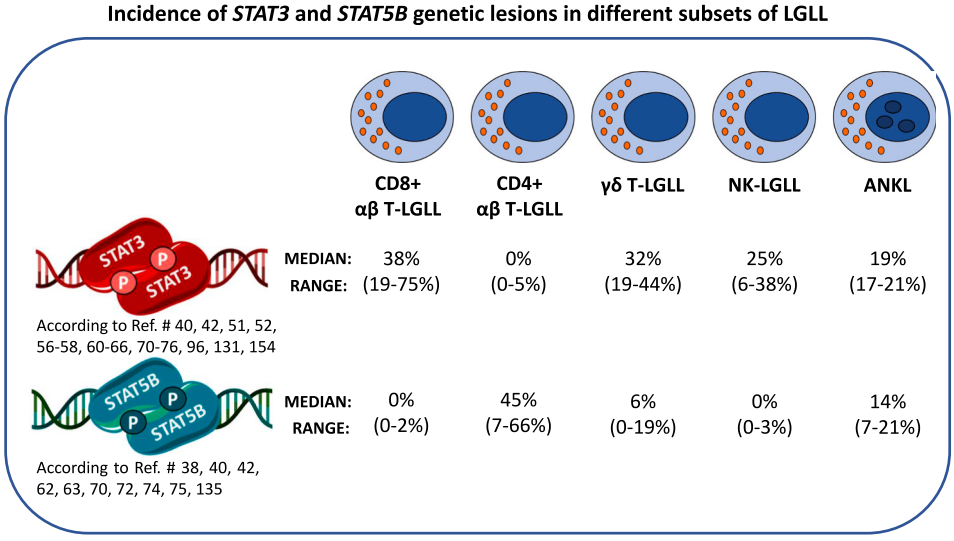

Fig. 3. Incidence of *STAT3* and *STAT5B* genetic lesions in the different LGLL subsets. The figure shows the median incidence (percentage) and the range (percentage) of *STAT3* and *STAT5B* genetic lesions in LGLL subtypes. Data included in the figure were collected from studies (the number of references shown in the figure relates to the list of references reported in the manuscript) based on cohorts of patients larger than 10 patients. A limitation of the reported percentages lies in the fact that the frequency of *STAT* mutations has been evaluated with different methods in different cohorts (see text).

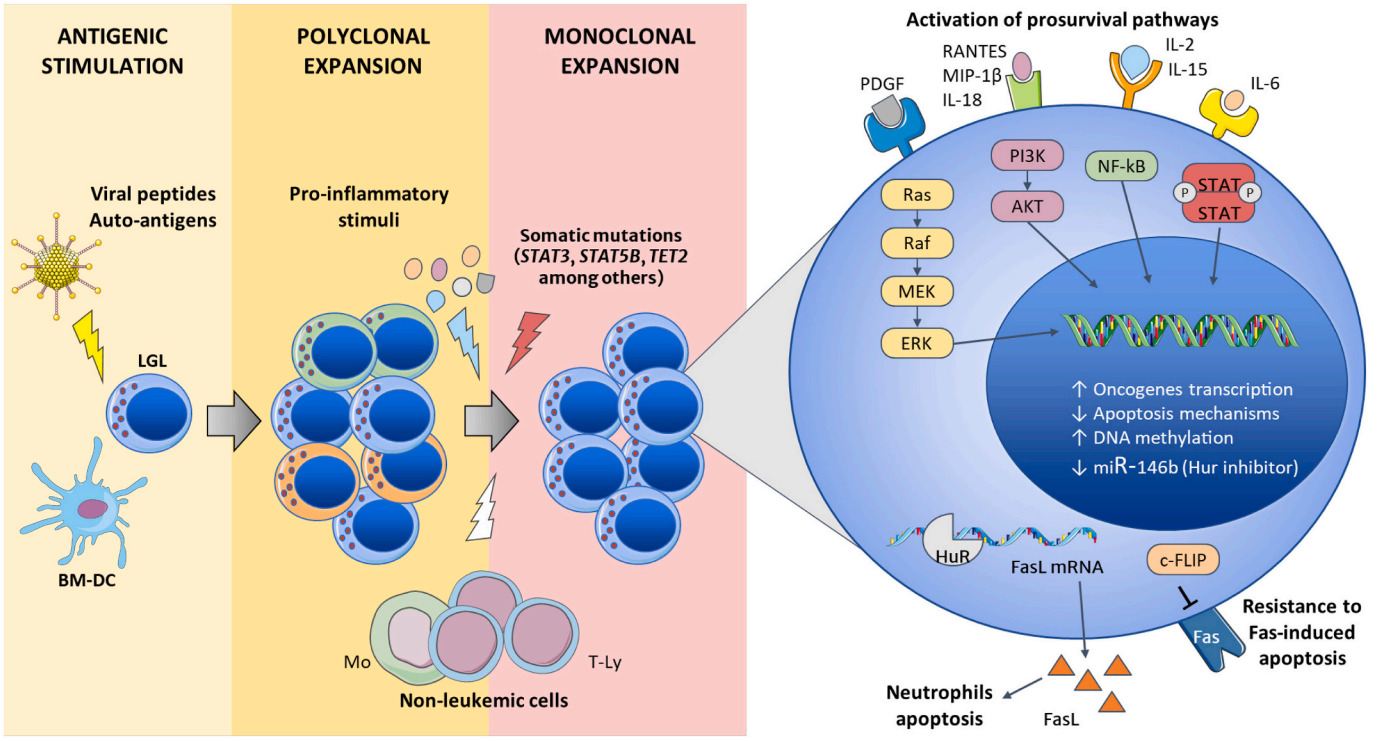

Fig. 4. Schematic representation of LGLL etiopathogenesis. Three main pathogenetic steps are shown: 1. the antigenic stimulation, 2. the polyclonal expansion, and 3. the monoclonal expansion. The lightning bolts indicate the putative key triggers (in the 1st phase) and then subsequent events (in the 2nd and 3rd phases) that sustain LGL proliferation and the disease progression.
LGL, large granular lymphocyte; BM-DC, bone marrow dendritic cell; Mo, monocytes; T-Ly, T lymphocytes; PDGF, platelet-derived growth factor; RANTES: regulated on activation, normal T cell expressed and secreted; MIP-1β: macrophage inflammatory proteins-1β; IL, interleukin; MEK, mitogen activated protein kinase kinase; ERK, extracellular-regulated kinase; PI3K, phosphatidylinositol-3-kinase; NF-kB, nuclear factor kappa B; STAT, signal transducer and activator of transcription; HuR, human antigen R; c-FLIP, cellular FLICE-like inhibitory protein; Fas, first apoptosis signal; FasL, Fas ligand. Parts of the figure were drawn by using pictures from Servier Medical Art.

there is a lot that goes to that: (i) the generation of IL-15 transgenic mice (IL-15tg) developing a fatal clonal NK and memory CD8+ expansion [97], (ii) the possibility to elicit LGL cytotoxicity and proliferation through proteasomal degradation of the pro-apoptotic protein Bid [98,99], and (iii) the induction of chromosomal instability and DNA hypermethylation *via* repression of mir-29b and the induction of a Myc/NF-kB/DNMT3a axis [100]. Further supporting the pathogenetic role of IL-15, several groups identified IL-15, together with PDGF, as the master survival signaling switch that has a relevant impact on all known de-regulations in T-LGLL [18,101,102].

The contribution of microenvironment to LGLL pathogenesis is also provided by the non-leukemic cells that are likely to be triggered by the same antigens prompting the expansion of the leukemic clone. In fact, a recent paper demonstrated that non-leukemic T cells in T-LGLL patients are mature, cytotoxic, clonally restricted and the non-leukemic immune cells are interconnected with leukemic cells *via* costimulatory cell–cell interactions, monocyte-secreted proinflammatory cytokines, and T-LGL-clone-secreted IFNγ [103].

In terms of intrinsic factors, leukemic LGLs are characterized by a dysfunctional activation-induced cell death (AICD) mechanism being LGLs not sensitive to Fas-induced apoptosis [104] and equipped with high levels of c-FLIP, a inhibitory protein of the death-inducing signaling complex [105].

Several cell signaling networks have been reported to play a role in LGL survival. The hyperactivity of STAT3, a transcription factor of several oncogenes, results from overactivation of STAT proteins as well as by the effects of gain-of-function mutations found in *STAT3*. As mentioned earlier, this finding is regarded as disease hallmark in LGLL patient. Beyond its role in cell survival, STAT3 has been also shown to be central in the pathogenesis of neutropenia through the STAT3-miR146b-FasL axis. In fact, neutropenic T-LGLL patients show large amounts of plasmatic soluble FasL leading to neutrophils' Fas-mediated apoptosis [56,106,107]. The increased FasL production is due to Human antigen R (HuR), an essential FasL mRNA stabilizer and target gene of miR-146b, that in neutropenic patients is down-regulated as a consequence of STAT3-dependent miR-146b promoter hypermethylation [108]. The above effects of STAT3 activation are amplified by *STAT3* mutations that stabilize the protein in its activated form and substantially foster clone development [68]. Kim and coworkers also showed that in leukemic LGLs *STAT3* mutations modify the autoregulation of p-STAT3 and drive changes in epigenetic regulator levels, global DNA hypermethylation, and ROS production [109].

Other than STAT3 hyperactivity, an increased function of the PI3K-AKT pathway contribute to inhibit the apoptotic program in T-LGLs [110] and the high levels of RANTES, MIP-1beta and IL-18 demonstrated in LGLL patients' plasma [94], with the evidence of frequent somatic mutations on PI3K family members [74], are consistent with this finding. Acting downstream the PI3K-Akt pathway, and independently from STAT3, a crucial role to prevent apoptosis is played by NF-kB through Mcl-1. Neoplastic LGLs express increased amount of c-Rel, a member of the NF-kB family, and exhibit higher NF-kB activity than normal peripheral blood mononuclear cells [101]. In NK-LGLL, the activation of Ras/MEK/ERK pathway contributes to the accumulation of NK cells caused by a constitutive stimulation of both extracellular-regulated kinase (ERK) and Ras. Consistently, Ras and ERK inhibition causes the reduction of the survival of patients' NK cells [111]. In addition, ERK1/2 signaling can be activated also by a dysregulation of sphingolipid rheostat [112,113].

6. Future challenges of research

Exhaustive answers to the many remaining questions in terms of genomics and immunopathogenic mechanisms are expected [114]. Much is still to be learned in terms of the role of mutations already known and of possible additional ones as well as about the permissive influences of epigenetic mechanisms. Furthermore, the study of the effects of TCR sharing on both pathogen escape and disease should be prioritized. Towards that end, in depth tracking of the clonal and non-clonal T-cell repertoires as well as the non-leukemic immune landscape are currently being exploited in LGLLs taking advantage of the unprecedented improvement of high throughput sequencing and multi-omics single-cell analysis [103,115].

Since also *STAT* unmutated patients are equipped with a hyperactive STAT machinery, other than somatic mutations, additional mechanisms are likely to finely tune the STAT axis network pathway in LGLL, including non coding RNA (microRNA, circular RNA, long non coding RNA). Development of recurrent pervasive mutations in chromatin and epigenetic modifying genes could represent the additional event entailing progression. A genome-wide DNA methylation profile analysis might be central to further investigate this issue. In addition, the crosstalk contribution of myeloid cells to signals between myeloid clonal hematopoiesis of indeterminate potential (CHIP) and LGL leukemia [114] has been up to now much less characterized and warrants further assessment.

Another relevant issue to be further investigated rests on the immune environment that is paramount for development and progression of LGL disorders [96]. The microenvironment is a dynamic and complex process that can provide both a negative role by promoting the immune escape of tumor cells as well as a positive task by limiting tumor growth through the activation of antitumor immunity. These investigations, including the interplay between leukemic cells and the different players occurring in the immune environment, should be aimed not only at better uncover the mechanisms underpinning the pathogenesis of these proliferations but could also represent another important step to specify the *primum movens* that triggers the disease's onset ultimately aiding to shed some light on the etiology of LGL leukemias. Hopefully, these researches will also set the stage for discovering new markers that may connote how patient is going to behave or define prognostic models to estimate the disease progression risk. We anticipate that a comprehensive understanding of the interactions between tumor cells and the immune environment, of their function and spatial organization, nowadays made possible thanks to the forthcoming single cell transcriptomic analysis, will also help lay the groundwork for better immunotherapies.

7. Clinical features

Most T- and NK-LGLL are indolent and chronic diseases. Conversely, ANKL is characterized by a highly aggressive clinical course typically refractory to therapeutic intervention.

CD8+ T-LGLL is initially asymptomatic in about 30% of patients, with LGL lymphocytosis being the unique abnormality [4,5,22], usually within ~2 to 20 x 10^9 granular lymphocytes/L. Fatigue and B symptoms (fever, night sweats, weight loss) are seldom observed. On exam, lymphadenopathy and hepatomegaly are uncommon although splenomegaly may occur (~20%). Isolated neutropenia (Absolute Neutrophil Count [ANC] <1.5 x 10^9/L) represents a clinical hallmark of the disease, with severe neutropenia (ANC <0.5 x 10^9/L) affecting 19–26% of the population [82]. Neutropenia favors the onset of aphthous, oral ulcerations and infections, usually bacterial, involving skin, oropharynx, lung and perirectal areas; blood stream infections may also happen. Acute viral and fungal infections are less common. During the natural history of the disease, patients tend to accumulate complications mainly due to recurrent infections.

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, myelodysplastic neoplasms and Pure Red Blood Cell Aplasia (PRCA) involving a not negligible percentage of patients [4,5,29,116–120]. Of notice, the incidence of LGLL associated PRCA is highly variable, ranging from 7.3% to 68.2% in the reported cases series [121]. Most importantly, anemia due to PRCA seems to be a more common hematologic complication in Asian patients as compared to Western patients, suggesting the role of a genetic background in the pathogenesis of this disorder. It is well established that clonal T cells are detected in PRCA patients [122], but recent evidence suggests that *STAT3* mutated CD8+ T cells expansion can be recognized both in PRCA patients with LGLL and without LGLL, even if with higher frequency in the first subgroup [123–124]. These findings postulate a common pathogenetic mechanism in erythroid impairment in different PRCA subsets and a crucial role of *STAT3* mutated CD8+ T cells clones in erythroid suppression. Thrombocytopenia is less frequent, observed in approximately 20% of cases.

A peculiar feature of the disease is the association with autoimmune disorders [29,125,126], both hematological (autoimmune hemolytic anemia, immune thrombocytopenia) and non-hematological like rheumatoid arthritis (RA), detected up to 30% of patients, including "gray-zone" cases [127]. The disease frequently coexists with secondary neoplasm, mostly hematological including plasma cell dyscrasias, non-Hodgkin lymphomas and Myelodysplastic Neoplasms [29,118–120,128].

CD4+ T-LGLL is usually an indolent disease and patients, unlike those affected by the CD8 counterpart, do not present autoimmune symptoms and cytopenias [30]; it is rather associated with concurrent solid tumors [65]. CD4+ T-LGL clones have been reported to recognize CMV antigens [129], with other viruses having a role in the pathogenesis [130].

According to the largest series today covering more than two decades and including 127 patients at 9 sites across 5 countries on 3 continents [70], Tγδ-LGLL represents a subset of T-LGLL characterized by more frequent symptoms, need for treatment and reduced survival as compared to Tαβ-LGLL. Neutropenia and anemia are the most relevant clinical features, being present in approximately half of cases, including severe neutropenia and anemia in around 20% of patients. The absence of the Vγ9/Vδ2 rearrangement profile, together with an infrequent expression of CD56 and NKG2A, is correlated to a more symptomatic disease [42].

In terms of clinical correlations between mutations and clinical features, following the first preliminary series of cases [60,61,131,132], the presence of *STAT3* mutation has been convincingly linked to CD8+ T-LGLL patients characterized by neutropenia, to the CD16+CD56- phenotype regardless of the CD57 presence, and to symptomatic disease [40,56,58]; more specifically, the evidence of neutropenia in *STAT3* mutated patients correlates with a worse survival. Suggested mechanisms accounting for neutropenia development include the trigger of a STAT3-miR-146b-FasL axis [108]. Furthermore, the majority of patients harboring *STAT3* genetic aberrancies are neutropenic and exhibit the largest LGL clonal expansion [40].

Besides neutropenia, several other clinical features turned out to be more frequent in patients with *STAT3* mutations, including different cytopenias or autoimmune diseases and treatment requiring disease [40,67,74,116,117,133]. Interestingly, T-LGLL patients with multiple *STAT3* mutations have been reported concomitantly with rheumatic diseases, in particular with RA [125,126], a disorder that has long been associated with LGL leukemias [11–13]. Furthermore, recent data support the hypothesis that somatic mutations in leukemia driver genes contribute to autoimmune disease. In fact, Masle-Farquhar et al. in mice with germline *STAT3* gain-of-function mutations demonstrated that the resulting effector CD8+ T cell clonal accumulation contributes to autoimmune pathology [134].

At variance with *STAT3*, *STAT5B* mutations have been associated with a different clinical behavior [40,61,62]. Depending on the immunophenotype of the mutated clone, the presence of *STAT5B* mutations in

the same hotspot position represents a signature of poor prognosis in aggressive CD8+CD56+ T-LGLL patients [37,38], while it is devoid of negative prognostic significance in CD4+ T-LGLL and Tγδ-LGLL patients [62,70].

Reasoning on future potential utilities of the application of molecular profiling in LGLL, the question of whether treatment decisions might be guided by molecular features prior to the emergence of clinical indications still remains unanswered and needs detailed longitudinal studies.

Despite the different role of *STAT* mutations, the clinical features of NK-LGLL are basically not divergent to those seen in T-LGLL, but these patients are usually less symptomatic. Similar to T-LGLL, NK-LGLL often occurs in association with other disease states but less frequently with autoimmune diseases [65]. Dissimilarly from ANKL, evidence of EBV infection is lacking in T-LGLL and NK-LGLL.

ANKL belongs to NK related chronic disorders, but it manifests as a rapidly progressing life-threatening systemic disease, with patients often dying within weeks or months of onset. Patients present with fever, lymphadenopathy, enlarged spleen and liver, constitutional symptoms and lymphocytosis. ANKL sometimes can resemble the hepatosplenic T cell lymphoma. Based on the fact that leukemic cells are equipped with an immunophenotype similar to NK-LGLL (Fig. 2), clinical evaluation is necessary to make the diagnosis [22,26,135]. Bone marrow involvement is an ever-present finding. The evidence of large nucleoli in leukemic NK cells (Fig. 1B), associated with systemic symptoms, lymph node swelling, hepatosplenomegaly and EBV positivity (EBERs detectable by immunohistochemistry) strongly points to the diagnosis of ANKL. EBV negative patients are known to occur but their morphological and clinical features are similar to the EBV positive counterpart.

8. Diagnosis of LGLL

Prerequisite for the diagnosis of LGLL is the exclusion of conditions mounted or perpetuated by highly specific polarized immune responses to strong antigenic stimuli, thereby the demonstration of cell clonality is mandatory. T and NK cell expansions are engendered as a part of a reactive immune response but following the clearance of the relevant stimuli, these proliferating cells undergo AICD, this way maintaining immune homeostasis. These cell expansions can be either polyclonal, oligoclonal or monoclonal, the latter conditions sometimes making the differential diagnosis with T/NK malignancies difficult [114,136,137]. In fact, an immunodominant clonal expansion may be so significant to be detectable above the polyclonal background. For this reason, the persistence of the abnormal population over time (>6 months) is required.

The number of LGL in the peripheral blood is usually greater than 2.0 x 10^9/L but also >0.5 x 10^9 clonal lymphocytes/L are accepted for LGLL diagnosis, particularly when associated with an unexplained cytopenia.

The decision making process to meet the diagnosis of LGLL is summarized in Fig. 5. The following criteria need to be diligently fulfilled

(i) the presence of an abnormal LGL expansion characterized by T (CD3+CD8+ or CD3+CD4+, and associated cytotoxic markers) or NK (CD3-, CD16+, KIR and associated cytotoxic markers) cells; see Fig. 2.

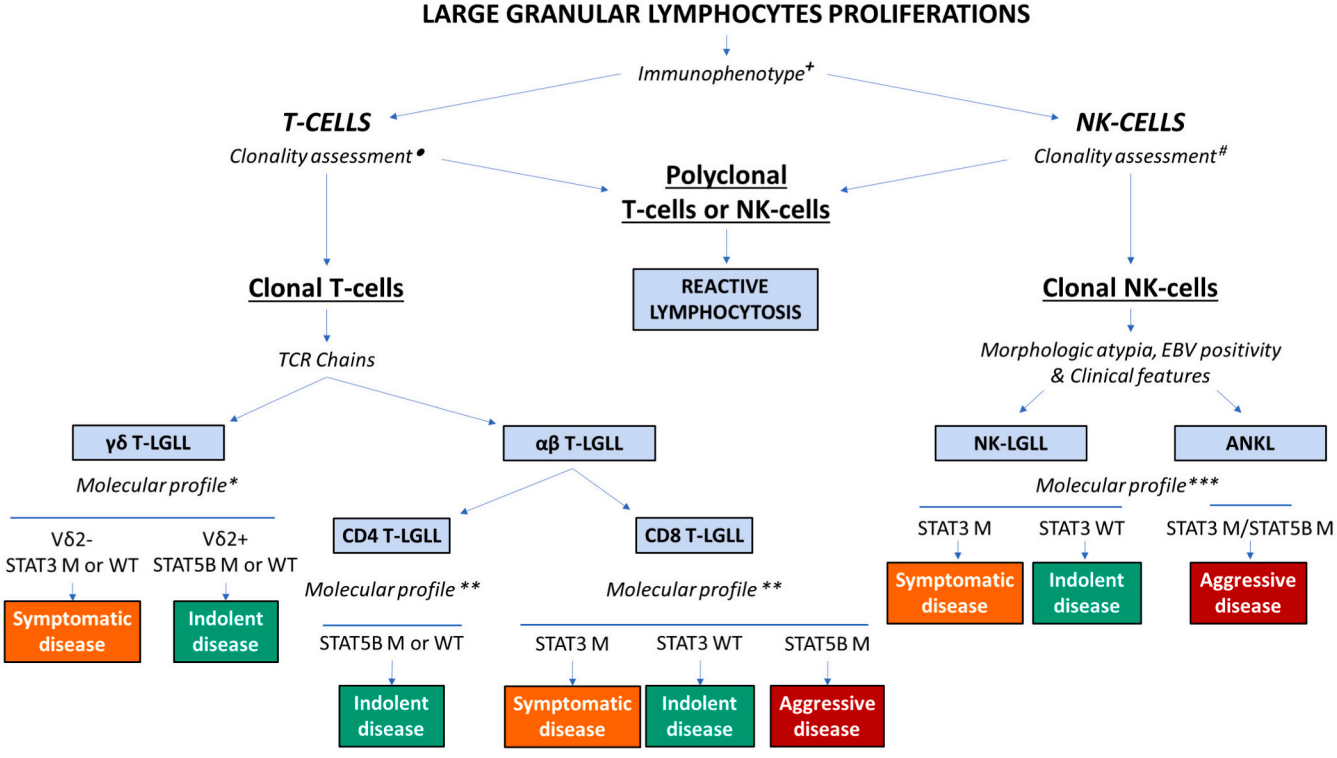

Fig. 5. Algorithm to proceed to diagnosis of LGL disorders. As mentioned in the legend of Fig. 2, additional less frequent possible immunophenotypic patterns might occur.
+ Immunophenotype with monoclonal antibodies against CD3, CD4, CD8, CD16, CD56, CD57, KIR;
• T cell clonality assessment by evaluation of the TCR;
NK cell clonality assessment by evaluation of KIR restriction (p58 molecules);
* molecular profile by *STAT3* and *STAT5B* mutations and Vδ2 assessment;
** molecular profile by *STAT3* and *STAT5B* mutations;
*** molecular profile by *STAT3*, *TET2* and *CCL22* mutations.
M, mutated; WT, wild-type.

(ii) the presence of a clonal T cell population detected by molecular techniques (Polymerase chain reaction, Spectratyping, Sanger sequencing or NGS) or by the proof of a discrete TCR Vβ usage (in T cell expansions [32–36,138]) or KIR restriction (in NK cell expansions [16,17]) at flow cytometry analysis; see Fig. 5. NGS profiling is nowadays the forefront of standard of methods [64,139] but this technique is still expensive and requires high quality DNA and bioinformatics skill for the analysis of data to ensure their appropriate interpretation.

(iii) the persistence of the abnormal population over time (>6 months) particularly when associated with an unexplained cytopenia, in the absence of positive serology for common viruses, thus ruling out reactive conditions [43,57].

(iv) the presence of *STAT3* or *STAT5B* mutations might contribute to confirm the clonality in discrete patients (e.g., oligoclonal LGL expansions with no restricted immunophenotype) and especially help to predict the outcome [40,58,66]. Their evaluation is an important complement in the differential diagnostic work-up and is considered one of the major findings that can aid the diagnosis of LGLL [56–62]. However, the diagnosis of T-LGLL can be made also in *STAT3* wild-type patients.

The BM evaluation is sometimes suggested to exclude other potential causes of cytopenias, including myelodysplastic neoplasms and pure red cell aplasia. BM appearance in LGLLs does not present a specific pattern in terms of cellularity; BM biopsy shows variable in size interstitial and intrasinusoidal infiltrates of lymphocytes, sometimes associated to nodular aggregates of lymphoid cells with azurophilic granules often hardly recognizable [140]. In patients with neutropenia, a decrease in granulocyte precursors and left-shift maturation is usually observed but the degree of marrow infiltration by LGL does not correlate with the degree of cytopenia [141]. The evidence of interstitial and intrasinusoidal CD8+/TiA1+, Granzyme B lymphoid infiltrates with altered immunophenotype may confirm the diagnosis [140]. BM biopsy in patients with mutant *STAT3* often revealed hypercellular marrow with a higher percentage of T-LGLL tumor burden, and intrasinusoidally distributed cytotoxic T cells, relative to patients with wild-type *STAT3* [57].

Once the above criteria are met, the distinction between T-cell clones of uncertain significance (T-CUS) and LGLL is mandatory to rule out incidental non-malignant clonal T cell expansions, first of all ascertaining whether the clone is stable, progressive, or transient. CUS defines T-cell clones of uncertain significance exhibiting immunophenotypic features closely resembling those of T-LGLL but the individuals are devoid of clinical or laboratory features supporting a diagnosis of T-cell malignancy [114,136,142]. Given the fact that T-CUS is a potential precursor of T-LGLL, some overlap between non-malignant clonal T cell expansions and indolent T-LGLL may occur. The differential diagnosis is usually based in the context of clinical features mentioned above, mainly by cytopenias and related events including aphthous oral mucosa and recurrent bacterial infections, by bone marrow and other tissues involvement, by the evidence of a CD8+/CD57+/CD16± phenotype, as well as by the presence of mutations [29,114,136,143–145]. Of course, the number of circulating abnormal cells is central, the threshold commonly accepted for LGLL diagnosis being 0.5 x 10^9 clonal lymphocytes/L [40,43,64].

Tγ/δ−LGLL sometimes may resemble hepatosplenic T cell lymphoma since they can both display Tγ/δ profile, CD56 and NKR markers, however the latter generally lacks CD8 and CD57 expression [146,147]. Also in this disorder discrete mutations (*STAT5B* [31%] *STAT3* [9%] and *PIK3CD* [9%]) have been detected, thus not helping in the differential diagnosis [148].

9. Current treatment options and future directions: the challenge of innovative therapies

Unfortunately, the lack of deep understanding of etiopathogenesis of the disease prevents the design of effective target therapies, leaving current patients with limited therapeutic options. Reasoning that leukemic LGL are activated cytotoxic lymphocytes, standard treatment of T-LGLL and NK-LGLL today mostly relies on immunosuppressive backbone (Methotrexate, Cyclophosphamide and Cyclosporin A) [149]. Supporting evidence for this approach is limited due to the lack of prospective clinical trials and we have long been hoping for better appropriate therapeutic, possibly targeted, options.

The decision on when and if a LGLL patient should undergo therapy is critical. Treatment of LGLL is usually required in presence of symptomatic neutropenia, transfusion dependent anemia or presence of concomitant symptomatic autoimmune diseases, but the results are not exciting [29,65]. At least 4-6 months of therapy are required to evaluate the response [127,149,150].

Methotrexate (MTX) 10 mg/m^2 per week and Cyclophosphamide (CTX) 50-100 mg/day are the generally recommended standards of care for the first line treatment while Cyclosporine A (CyA) is usually favored for patients at relapse [151]. Overall response (OR) and complete response (CR) rates are variable (40-70% and 47-50%, respectively) depending on the reported retrospective case series [152–153]. Nevertheless, in the first prospective trial of immunosuppressive therapy in LGLL, the MTX OR rate (ORR) was found slightly lower (38%) [154]. Of notice, while MTX therapy can be pursued until progression or unacceptable toxicity, CTX therapy should be continued not more than 12 months to avoid secondary myelodysplastic neoplasms or acute myeloid leukemia. The first prospective randomized trial (NCT01976182) evaluating MTX or CTX as first line treatment for LGLL is currently ongoing and preliminary results are up to now not available.

CyA at 3-5 mg/kg/day dose administered until progression is generally used in patients failing the first line therapy, with variable ORR (21 to 100%) [152]. However, the largest multicenter Tγδ series of patients today showed better response with first line CyA with respect to MTX and CTX, suggesting a potential benefit upfront in this rare subtype of T-LGLL [70].

By contrast, ANKL requires intensive chemotherapy even though the optimal regimen has not yet been established and unfortunately complete remissions are rare and the outcome is poor [25]. Nevertheless, L-asparaginase-based regimens such as SMILE (dexamethasone, methotrexate, ifosfamide, etoposide and L-asparaginase), AspaMetDex (L-asparaginase, methotrexate, dexamethasone) or VIDL (etoposide, ifosfamide, dexamethasone, L-asparaginase) have improved the outcome of ANKL patients; in particular SMILE chemotherapy and hematopoietic stem cell transplantation (HSCT) were suggested to be the key components of the therapeutic strategies of ANKL [155,156].

For relapse/refractory disease, only few agents are available with limited experience and variable responses. Monotherapy with the anti-CD52 monoclonal antibody Alemtuzumab has been evaluated in 25 patients and proved effective with an ORR of 74% and 47% CR rates, respectively [157]; the presence of a *STAT3* mutation doesn't seem to impact on ORR [158]. Purine analogs (fludarabine, cladribine, pentostatine) and bendamustine have been used in limited cases series with variable OR rates [159–161]. Finally, in patients with concomitant RA and T-LGLL, rituximab monotherapy induced unexpected responses, including CR [162]. Splenectomy may be considered in cases of symptomatic splenomegaly or refractory cytopenias particularly anemia [163]; in this series of cases, although transfusion independence improved, neutropenia did not ameliorate. HSCT has been reported for a series of patients [164–166]; among the 15 patients reported by EBMT, five underwent autologous HSCT (three obtaining CR, still alive and two with progressive disease), and ten allogeneic HSCT (5 still alive at last follow up). This indicates that bone marrow transplantation for LGL leukemia is not without risks and should be limited to well selected

patients.

In this landscape, the desperate demand for more effective and possibly personalized therapies represents an unmet need in LGLL patients. The adverse impact of *STAT3* mutations in patients' survival [40,58] suggests that this pathway as well as other emerging networks should be regarded as a potential target to be exploited to help direct the design of specific new compounds for innovative, molecularly tailored therapeutic approaches, even subset-specific at least leading to prolonged survival. Available correlative studies argue that a gene signature and mutated *STAT3* Y640F genotype are potential predictors of response to MTX [56,65,80] but data to that effect are still immature and further validation in larger studies is warranted. In this context, the JAK3 inhibitor Tofacitinib has been evaluated in 9 patients (four of them being *STAT3* mutated) with relapse/refractory T-LGLL and concomitant RA. Hematological response was observed in 6 cases with increase in neutrophils' count in 5 patients [167]. Moignet and colleagues reported promising results with the JAK inhibitor Ruxolitinib in two cases of refractory LGL leukemia [168].

IL-15 plays a crucial role in LGLL pathogenesis [98–100] and several attempts to inhibit this pathway have been pursued, thus offering the proof of concept for further drug developments. A phase 1 trial evaluating the monoclonal antibody Hu-Mikβ1 which blocks IL-15 trans-presentation failed to prove a significant clinical benefit [169]. More recently, emphasis has been put on BNZ-1, a novel multi-cytokine inhibitor that has been claimed as a new promising drug in LGLL [170]. BNZ-1 is a pegylated peptide that blocks IL-2, IL-9 and IL-15 binding to the common γ chain receptor. *In vitro* studies on T-LGLL cell lines and patients' samples showed increased apoptosis following BNZ-1 treatment; moreover, this agent blocked *in vivo* T-LGLL development in IL-15 transgenic mice. Based on these findings, a phase I/II multicenter clinical trial evaluating BNZ-1 treatment in T-LGLL patients is ongoing with promising results (enrolled 14 cases, with results indicating 20% of hematological response) [171] but we are waiting for maturity of data.

Despite the high incidence of *STAT* genetic lesions [57–66], additional gene mutations [51,52,72–74], or deregulation of other signaling molecules including Fas ligand axis [108,105,172] might be involved in association with *STAT* mutations thus contributing to pathogenesis of these disorders. Increased DNA methylation [173] and the development of recurrent pervasive mutations in chromatin and epigenetic modifying genes [174] are likely to represent additional events involved in disease onset also in relationship to the influences on microenvironment immunocompetent cells and their control of neoplastic growth [103]. This is clearly going to be another option to help us make further effective treatments.

Defining new putatively druggable targets [175], these emerging studies might offer the rationale to direct the design of specific innovative compounds (STAT pathway's inhibitors, demethylating agents, etc.) providing hope for new interventions that could lead to prolonged survival of these patients. This challenge of genomics will be made possible only by an appropriate disease subtypes' categorization and subsequent recruitment of discrete patients' subgroups.

10. Relevance to physician and patient of disease categorization

A final remark in closing. Why make such great efforts to dissect disease's subsets and look for correlations between immunophenotype (Fig. 2), molecular analysis (Fig. 3) and clinical features with the ultimate goal to appropriately characterize and overall differentiate these rare diseases one from the other and from irrelevant T/NK cell expansions (Fig. 5)? Inherent in this question is to what extent this endeavor might facilitate the doctor-patient relationship. The achievement of a correct disease's categorization will provide comfort both to physicians and their patients. Physicians can juggle better in the maze of these rare conditions more properly managing patients with LGLL. Patients, understandably anxious about a disorder termed as leukemia, a definition that invokes the specter of relentless lethal process, and constantly reminded of the insidious nature of their disease at follow-up visits, can in this setting benefit of confidence that physician can convey explaining the indolent clinical course of their leukemia in most these disorders.

The issue of clonality and its relationship with cancer is a controversial issue to address and we should never forget that mutations themselves do not denote malignancy [114,176]. In fact, the presence of a clone is not the sole defining characteristic of neoplasia and several additional factors, the progress to metastasis and/or compromise of normal tissue function among others, must be taken into account, including indolent disease that causes no harm during the patient's lifetime. In this regard, as research continues to progress, we believe that further knowledge on the biology of these disorders might also lead to the reassessment of the semantic distinction of these conditions. Their renames might not only influence the patients' self-perception but also have practical repercussions in terms of regulatory prerogatives, such as health care resource allocation and cancer-specific indemnity policy payouts; this represents a must for the patient.

Practice Points

- Need to ensure that patients undergo accurate immunophenotyping to differentiate discrete disease subsets.
- Evaluation of *STAT3* and *STAT5B* somatic mutations is recommended to aid to envisage the prognosis of patients.
- The association with accompanying diseases must always be investigated.
- Therapy is required in the presence of symptomatic neutropenia, transfusion dependent anemia or presence of concomitant symptomatic autoimmune diseases.

Research Agenda

- Learn more on pathogenetic mechanisms of disease, the precise role of mutational profiles, including newly discovered mutations.
- Assess clinical and laboratory parameters that may define prognostic models to estimate the disease progression risk.
- Develop selected novel agents based on mechanisms of disease to improve the treatment efficacy.
- Design randomized controlled trials evaluating new therapeutic strategies based on the rationale provided by genetic molecular profiling and functional studies.

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Declaration of Competing Interest

The authors declare no competing financial interests.

References

[1] Brouet JC, Flandren G, Sasportes M, Preud'Homme J-L, Seligman M. Chronic lymphocytic leukemia of T-cell origin: Immunological and clinical evaluation in eleven patients. Lancet 1975;ii:890–3.
[2] McKenna RW, Parkin J, Kersey JH, Gajl-Peczalska KJ, Peterson L, Brunning RD. Chronic lymphoproliferative disorder with unusual clinical, morphologic, ultrastructural and membrane surface marker characteristics. Am J Med 1977;62:588–96.
[3] Pandolfi F, De Rossi G, Semenzato G, Quinti I, Ranucci A, De Sanctis G, et al. Immunologic evaluation of T chronic lymphocyte leukemia cells: correlations among phenotype, functional activities, and morphology. Blood 1982;59:688–95.
[4] Chan WC, Link S, Mawle A, Check I, Brynes RK, Winton EF. Heterogeneity of large granular lymphocyte proliferations: delineation of two major subsets. Blood 1986;68:1142–53.
[5] Semenzato G, Pizzolo G, Ranucci A, Agostini C, Chilosi M, Quinti I, et al. Abnormal expansions of polyclonal large to small size granular lymphocytes: reactive or neoplastic process? Blood 1984;63:1271–7.

[6] McKenna RW, Arthur DC, Gajl-Peczalska KJ, Flynn P, Brunning RD. Granulated T cell lymphocytosis with neutropenia: malignant or benign chronic lymphoproliferative disorders? Blood 1985;66:259–66.
[7] Gastl G, Rumpold H, Kraft D, Gattringer C, Schuler G, Margreiter R, et al. Abnormal expansion of granular lymphocytes: Reactive lymphocytosis or chronic leukemia? Case report and literature review. Blut 1986;52:73–89.
[8] Maini MK, Casorati G, Della Bona P, Wack A, Beverley PC. T-cell clonality in immune responses. Immunol Today 1999;20:262–6.
[9] Rambaldi A, Pelicci P, Allavena P, Knowles 2nd DM, Rossini S, Bassan R, et al. T cell receptor beta chain gene rearrangements on lymphoproliferative disorders of granular lymphocytes/natural killer cells. J Exp Med 1985;162:2156–62.
[10] Foà R, Pelicci PG, Migone N, Lauria F, Pizzolo G, Flug F, et al. Analysis of T-cell receptor beta chain (T beta) gene rearrangements demonstrates the monoclonal nature of T-cell chronic lymphoproliferative disorders. Blood 1986;67:247–50.
[11] Loughran Jr TP, Kadin ME, Starkebaum G, Abkowitz JL, Clark EA, Disteche C, et al. Leukemia of large granular lymphocytes: association with clonal chromosomal abnormalities and autoimmune neutropenia, thrombocytopenia, and hemolytic anemia. Ann Intern Med 1985;102:169–75.
[12] Semenzato G, Pandolfi F, Chisesi T, De Rossi G, Pizzolo G, Zambello R, et al. The lymphoproliferative disease of granular lymphocytes. A heterogeneous disease ranging from indolent to aggressive conditions. Cancer 1987;60:2971–8.
[13] Oshimi K. Granular lymphocyte lymphoproliferative disorders: report of 12 cases and review of the literature. Leukemia 1988;2:617–27.
[14] Vogelstein B, Fearon ER, Hamilton SR, Treisinger AC, Willard HF, Michelson AM, et al. Clonal analysis using recombinant DNA probes from X-chromosome. Cancer Res 1987;47:4806–13.
[15] Nash R, McSweeney P, Zambello R, Semenzato G, Loughran Jr TP. Clonal studies of CD3-negative lymphoproliferative disease of granular lymphocytes. Blood 1993;81:2363–8.
[16] 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.
[17] Barcena P, Jara-Acevedo M, Tabernero MD, Lopez A, Sanchez ML, Garcia-Montero AC, et al. Phenotypic profile of expanded NK cells in chronic lymphoproliferative disorders: a surrogate marker for NK-cell clonality. Oncotarget 2015;6:42938–52.
[18] Drillet G, Pastoret C, Moignet A, Lamy T, Marchand T. Toward a better classification system for NK-LGL disorders. Front Oncol 2022;12:821382.
[19] Loughran Jr TP. Clonal diseases of large granular lymphocytes. Blood 1993;82:1–14.
[20] Jaffe ES, Harris NL, Stein H, Vardiman J, (eds).. World Health Organization classification of tumours. Pathology and genetics of tumours of haematopietic and lymphoid tissues. 3. ed. Lyon, France: IARC; 2001.
[21] Swerdlow SH, Campo E, Harris NL, Jaffe ES, Pileri SA, Stein H, et al. WHO Classification of tumours of haematopoietic and lymphoid tissues, ed 4. Lyon, France: IARC; 2008.
[22] Alaggio R, Amador C, Anagnostopoulos I, Attygalle AD, Barreto De Oliveira Araujo I, et al. The 5th edition of the World Organization classification of haematolymphoid tumours: lymphoid neoplasms. Leukemia 2022;36:1720–48.
[23] Poullot E, Zambello R, Leblanc R, Bareau B, De March E, Roussel M, et al. Chronic natural killer lymphoproliferative disorders: characteristics of an international cohort of 70 patients. Ann Oncol 2014;25:2030–5.
[24] Shah MV, Hook CC, Call TG, Go RS. A population-based study of large granular lymphocyte leukemia. Blood Cancer J 2016;6:e455–9.
[25] Tang YT, Wang D, Luo H, Xiao M, Zhou HS, Liu D, et al. Aggressive NK-cell leukemia: clinical subtypes, molecular features, and treatment outcomes. Blood Cancer J 2017;7:660–5.
[26] Suzuki R, Suzumiya J, Nakamura S, Aoki S, Notoya A, Ozaki S, et al. Aggressive natural killer-cell leukemia revisited: large granular lymphocyte leukemia of cytotoxic NK cells. Leukemia 2004;18:763–70.
[27] Ishida F, Ko YH, Kim WS, Suzumiya J, Isobe Y, Oshimi K, et al. Aggressive natural killer cell leukemia: Therapeutic potential of L-aparaginase and allogenic hematopoietic stem cell transplantation. Cancer Sci 2012;103:1079–83.
[28] Baesso I, Pavan L, Boscaro E, Miorin M, Facco M, Trentin L, et al. T-cell type lymphoproliferative disease of granular lymphocytes (LDGL) is equipped with a phenotypic pattern typical of effector cytotoxic cells. Leuk Res 2007;31:371–7.
[29] Lamy T, Moignet A, Loughran Jr TP. LGL leukemia: from pathogenesis to treatment. Blood 2017;129:1082–94.
[30] 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.
[31] 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.
[32] Zambello R, Trentin L, Facco M, Cerutti A, Sancetta R, Milani A, et al. Analysis of the T cell receptor in the lymphoproliferative disease of granular lymphocytes: Superantigens activation of clonal CD3+ granular lymphocytes. Cancer Res 1995;55:6140–5.
[33] 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.
[34] Morice WG, Kimlinger T, Katzmann JA, Lust JA, Heimgartner PJ, Halling KC, et al. Flow cytometric assessment of TCR-Vbeta expression in the evaluation of peripheral blood involvement by T-cell lymphoproliferative disorders: A comparison with conventional T-cell immunophenotyping and molecular genetic techniques. Am J Clin Pathol 2004;121:373–83.
[35] Shi M, Jevremivic D, Otteson GE, Timm MM, Olteanu H, Horna P. Single antibody detection of T-cell receptor ab clonality by flow cytometry rapidly identifies mature T-cell neoplasms and monotypic small CD8-positive subsets of uncertain significance. Cytom Part B 2020;98B:99–107.
[36] Muñoz-Garcìa N, Moran-Plata FJ, Villamor N, Lima M, Barrena S, Mateos S, et al. High-sensitive TRBC1-based flow cytometric assessment of T-cell clonality in Tαβ-large granular lymphocytic leukemia. Cancers 2022;14:408–17.
[37] Gentile TC, Uner AH, Hutchinson RE, Wright J, Ben-Ezra J, Russel EC, et al. CD3+, CD56+ aggressive variant of large granular lymphocyte leukemia. Blood 1994;84:2316–21.
[38] Rajala HLM, Eldfors S, Kuusanmaki H, van Adrichem AJ, Olson T, Langstrom S, et al. Discovery of somatic STAT5b mutations in large granular lymphocyte leukemia. Blood 2013;121:4541–50.
[39] Bourgault-Rouxel AS, Loughran TP, Zambello R, Epling-Burnette PK, Semenzato G, Donadieu J, et al. Clinical spectrum of gammadelta T cell LGL leukemia: analysis of 120 cases. Leuk Res 2008;32:45–8.
[40] Barilà G, Teramo A, Calabretto G, Vicenzetto C, Gasparini VR, Pavan L, et al. Stat3 mutations impact on overall survival in large granular lymphocyte leukemia: a single-center experience of 205 patients. Leukemia 2020;34:1116–24.
[41] Silva-Santos B, Serre K, Norell H. γδ T cells in cancer. Nat Rev Immunol 2015;15:683–91.
[42] Teramo A, Binatti A, Ciabatti E, Schiavoni G, Tarrini G, Barilà G, et al. Combined immunophenotypic and molecular evaluation contributes to better define TCRγδ lymphoproliferative disorders. Nat Commun 2022;13:3298–308.
[43] Semenzato G, Zambello R, Starkebaum G, Oshimi K, Loughran Jr TP. The lymphoproliferative disease of granular lymphocytes: updated criteria for the diagnosis. Blood 1997;89:256–60.
[44] Moretta A, Tambussi G, Bottino C, Tripodi G, Merli A, Ciccone E, et al. A novel surface antigen expressed by a subset of human CD3-CD16+ natural killer cells. J Exp Med 1990;171:695–714.
[45] Moretta A, Bottino C, Pende D, Tripodi G, Tambussi G, Viale O, et al. Identification of four subsets of human CD3-CD16+ natural killer (NK) cells by the expression of clonally distributed functional surface molecules: correlation between subset assignment of NK clones and ability to mediate specific alloantigen recognition. J Exp Med 1990;172:1589–98.
[46] Zambello R, Trentin L, Ciccone E, Bulian P, Agostini C, Moretta A, et al. Phenotypic diversity of natural killer (NK) populations in patients with NK-type lymphoproliferative disease of granular lymphocytes. Blood 1993;81:2381–5.
[47] 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.
[48] Bigouret V, Hoffmann T, Arlettaz L, Villard J, Colonna M, Ticheli A, et al. Monoclonal T-cell expansions in asymptomatic individuals and in patients with large granular leukemia consist of cytotoxic effector T cells expressing the activating CD94:NKG2C/E and NKD2D killer cell receptors. Blood 2003;101:3198–204.
[49] Morice WG, Jevremovic D, Olteanu H, Roden A, Nowakowski G, Kroft S, et al. Chronic lymphoproliferative disorder of natural killer cells: a distinct entity with subtypes correlating with normal natural killer cell subsets. Leukemia 2010;24:881–4.
[50] Barilà 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 Cancer J 2018;8:51–5.
[51] Baer C, Kimura S, Rana MS, Kleist AB, Flerlage T, Feith DJ, et al. CCL22 mutations drive natural killer cell lymphoproliferative disease by deregulating microenvironmental crosstalk. Nat Genet 2022;54:637–48.
[52] Pastoret C, Desmots F, Drillet G, Le Gallau S, Boulland M-L, Thannberger A, et al. Linking the KIR phenotype with STAT3 and TET2 mutations to identify chronic lymphoproliferative disorders of NK cells. Blood 2021;137:3237–50.
[53] Li Y, Jia Wei J, Mao X, Gao Q, Liu L, Cheng P, et al. Flow cytometric immunophenotyping is sensitive for the early diagnosis of de novo aggressive natural killer cell leukemia (ANKL): A multicenter retrospective analysis. PLoS One 2016;11:e0158827–45.
[54] Pu Q, Cao X, Liu Y, Yan D, Tan R, Li J, et al. Comprehensive analysis and summary of the value of immunophenotypes of mature NK cell tumors for differential diagnosis, treatment, and prognosis. Front Immunol 2022;13:918487–500.
[55] De Leval L, Alizadeh AA, Bergsagel PL, Campo E, Davies A, Dogan A, et al. Genomic profiling for clinical decision making in lymphoid neoplasms. Blood 2022;140:2193–227.
[56] Teramo A, Barilà 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.
[57] Shi M, He R, Feldman AL, Viswanatha D, Jevremovic D, Chen D, et al. STAT3 mutations and its clinical and histopathologic correlation in T-cell large granular lymphocytic leukemia. Hum Pathol 2018;73:74–81.
[58] Muñoz-Garcìa N, Jara-Acevedo M, Caldas C, Barcena P, Lopez A, Puig N, et al. STAT3 and STAT5B mutations in T/NK-chronic lymphoproliferative disorders of large granular lymphocytes (LGL): association with disease features. Cancers 2020;12:3508–30.

[59] Semenzato G, Zambello R. Interrogating molecular genetics to refine LGLL classification. Blood 2022;139:3002–4.
[60] Jerez A, Clemente MJ, Makishima H, Koskela H, LeBlanc F, Ng KP, et al. STAT3 mutations unify the pathogenesis of chronic lymphoproliferative disorders of NK cells and T-cell large granular lymphocyte leukemia. Blood 2012;120:3048–57.
[61] 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.
[62] Andersson EI, Tanahashi T, Sekiguchi N, Gasparini VR, Bortoluzzi S, Tawakami T, et al. High incidence of activating STAT5B mutations in CD4-positive T-cell large granular lymphocyte leukemia. Blood 2016;128:2465–8.
[63] Bhattacharya D, Teramo A, Gasparini VR, Huuhtanen J, Kim D, Theodoropoulos J, et al. Identification of novel *STAT5B* mutations and characterization of TCRβ signatures in CD4+ T large granular lymphocyte leukemia. Blood Cancer J 2022;12:31–43.
[64] Fasan A, Kern W, Grossmann V, Haferlach C, Haferlach T, Schnittger S. STAT3 mutations are highly specific for large granular lymphocytic leukemia. Leukemia 2013;27:1598–600.
[65] Riviero A, Mozas P, Jiménez L, Lòpez-Guerra M, Colomer D, Bataller A, et al. Clinicobiological characteristics and outcomes of patients with **T**-cell large granular lymphocytic leukemia and chronic lymphoproliferative disorder of natural killer cells from a single institution. Cancers 2021;13:3900–11.
[66] Kristensen T, Larsen M, Rewes A, Frederiksen H, Thomassen M, Møller MB. Clinical relevance of sensitive and quantitative STAT3 mutation analysis using next-generation sequencing in T-cell large granular lymphocytic leukemia. J Mol Diagn 2014;16:382–92.
[67] Kurt H, Jorgensen JL, Amin HM, Patel KP, Wang SA, Pei Lin P, et al. Chronic lymphoproliferative disorder of NK-cells: A single-institution review with emphasis on relative utility of multimodality diagnostic tools. Eur J Haematol 2018;100:444–54.
[68] Kerr CM, Clemente MJ, Chomczynski PW, Przychodzen B, Nagata Y, Adema V. Subclonal STAT3 mutations solidify clonal dominance. Blood Adv 2019;3: 917–21.
[69] Hafeez S, Mnayer LO, DiGiuseppe AJ. CD4+ T-cell large granular lymphocytic leukemia with STAT3 mutation and neutropenia. Cytometry B Clin Cytom 2022; 102:168–70.
[70] Barilà G, Grassi A, Cheon HJ, Teramo A, Calabretto G, Chahal J, et al. Tγδ large granular lymphocyte leukemia identifies a subset of patients with more symptomatic disease: analysis of a large international cohort of 127 patients. Blood 2022. https://doi.org/10.1182/blood.2021013489. Online ahead of print.
[71] 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.
[72] Gasparini VR, Binatti A, Coppe A, Teramo A, Vicenzetto C, Calabretto G, et al. A high definition picture of somatic mutations in chronic lymphoproliferative disorder of natural killer cells. Blood Cancer J 2020;10:42–52.
[73] Olson TL, Cheon H, Xing JC, Olson KC, Paila U, Hamele CE, et al. Frequent somatic TET2 mutations in Chronic NK-LGL Leukemia with distinct patterns of cytopenias. Blood 2021;138:662–73.
[74] Cheon H, Xing JC, Moosic KB, Ung J, Chang VW, Chung DS, et al. Genomic and transcriptomic sequencing reveals the landscape of large granular lymphocyte leukemia. Blood 2022;139:3058–72.
[75] Dufva O, Kankainen M, Kelkka T, Sekiguchi N, Award SA, Eldfors S, et al. Aggressive natural killer-cell leukemia mutational landscape and drug profiling highlight JAK-STAT signaling as therapeutic target. Nat Commun 2018;9: 1567–78.
[76] Huang L, Liu D, Wang N, Ling S, Tang Y, Jun Wu J, et al. Integrated genomic analysis identifies deregulated JAK/STAT-MYC-biosynthesis axis in aggressive NK-cell Leukemia. Cell Res 2018;28:172–86.
[77] El Hussein S, Patel KP, Fang H, Thakral B, Loghavi S, Kanagal-Shamanna R, et al. Genomic and immunophenotypic landscape of aggressive NK-cell leukemia. Am J Surg Pathol 2020;44:1235–43.
[78] Andersson E, Kuusanmäki H, Bortoluzzi S, Lagström S, Parsons A, Rajala H, et al. Activating somatic mutations outside the SH2-domain of STAT3 in LGL leukemia. Leukemia. 2016;30:1204–8.
[79] Brito-Babapulle V, Matutes E, Parreira L, Catovsky D. Abnormalities of chromosome 7q and Tac expression in T cell leukemias. Blood 1986;67:516–21.
[80] Nakashima Y, Tagawa H, Suzuki R, et al. Genome-wide array-based comparative genomic hybridization of natural killer cell lymphoma/leukemia: different genomic alteration patterns of aggressive NK-cell leukemia and extranodal Nk/T-cell lymphoma, nasal type. Genes Chromosom Cancer 2005;44:247–55.
[81] Giussani E, Binatti A, Calabretto G, Gasparini VR, Teramo A, Vicenzetto C, et al. Lack of viral load within chronic lymphoproliferative disorder of natural killer cells: What is outside the leukemic clone? Front Oncol 2020;10:613570–5.
[82] Zambello R, Teramo A, Barilà G, Gattazzo C, Semenzato G. Activating KIRs in Chronic lymphoproliferative disorder of NK Cells: Protection from viruses and disease induction? Front Immunol 2014;5:72–6.
[83] Thomas A, Perzova R, Abbott L, Benz P, Poiesz MJ, Dube S, et al. LGL leukemia and HTLV. AIDS Res Hum Retrovir 2010;26:33–40.
[84] 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.
[85] Nyland SB, Feith DJ, Poss M, Olson TL, Krissinger DJ, Poiesz BJ, et al. Retroviral sero-reactivity in LGL leukaemia patients and family members. Br J Haematol 2020;188:522–7.
[86] Teruya H, Tomita M, Senba M, Ishikawa C, Tamayose M, Miyazato A, et al. Human T-cell leukemia virus type I infects human lung epithelial cells and induces gene expression of cytokines, chemokines and cell adhesion molecules. Retrovirology 2008;5:86–96.
[87] Lamy T, Bauer FA, Liu JH, Li YX, Pillemer E, Shahidi H, et al. Clinicopathological features of aggressive large granular lymphocyte leukaemia resemble Fas ligand transgenic mice. Br J Haematol 2000;108:717–23.
[88] 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.
[89] Li W, Yang L, Harris RS, Lin L, Olson TL, Hamele CE, et al. Retrovirus insertion site analysis of LGL leukemia patient genomes. BMC Med Genet 2019;12:88–99.
[90] 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.
[91] Balsamo M, Zambello R, Teramo A, Pedrazzi M, Sparatore B, Scordamaglia F, et al. Analysis of NK cell/DC interaction in NK-type lymphoproliferative disease of granular lymphocytes (LDGL): role of DNAM-1 and NKp30. Exp Hematol 2009; 37:1167–75.
[92] Makishima H, Ishida F, Ito T, Kitano K, Ueno S, Ohmine K, et al. DNA microarray analysis of T cell-type lymphoproliferative disease of granular lymphocytes. Br J Haematol 2002;118:462–9.
[93] Choi YL, Makishima H, Ohashi J, Yamashita Y, Ohki R, Koinuma K, et al. DNA microarray analysis of natural killer cell-type lymphoproliferative disease of granular lymphocytes with purified CD3-CD56+ fractions. Leukemia 2004;18: 556–65.
[94] Kothapalli R, Nyland SB, Kusmartseva I, Bailey RD, McKeown TM, Loughran TP. Constitutive production of proinflammatory cytokines RANTES, MIP-1β and IL-18 characterizes LGL leukemia. Int J Oncol 2005;26:529–35.
[95] Momose K, Makishima H, Ito T, Nakazawa H, Shimodaira S, Kiyosawa K, et al. Close resemblance between chemokine receptor expression profiles of lymphoproliferative disease of granular lymphocytes and their normal counterparts in association with elevated serum concentrations of IP-10 and MIG. Int J Hematol 2007;86:174–9.
[96] 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: 3643–54.
[97] Fehniger TA, Suzuki K, VanDeusen JB, Cooper MA, Freud AG, Caligiuri MA. Fatal leukemia in interleukin-15 transgenic mice. Blood Cells Mol Dis 2001;27:223–30.
[98] 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.
[99] 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.
[100] 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.
[101] 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.
[102] 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.
[103] Huuhtanen J, Bhattacharya D, Lonnberg T, Kancainen M, Kerr C, Theodoropoulos J, et al. Single-cell transcriptomics identifies synergistic role of leukemic and non-leukemic immune repertories in CD8+ T cell large granular lymphocytic leukemia. Nat Commun 2022;13:1981–96.
[104] 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.
[105] 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.
[106] Liu JH, Wei S, Lamy T, Epling-Burnette PK, Starkebaum G, Djeu JY, et al. Chronic neutropenia mediated by fas ligand. Blood 2000;95:3219–22.
[107] Lamy T, Bauer FA, Liu JH, Li YX, Pillemer E, Shahidi H, et al. Clinicopathological features of aggressive large granular lymphocyte leukaemia resemble Fas ligand transgenic mice. Br J Haematol 2000;108:717–23.
[108] Mariotti B, Calabretto G, Rossato M, Teramo A, Castellucci M, Barilà G, et al. Identification of a miR-146b-FasL axis in the development of neutropenia in T-Large Granular Lymphocyte Leukemia. Haematologica 2020;105:2040–52.
[109] Kim D, Park G, Huuhtanen J, Ghimire B, Rajala H, Moriggl R, et al. STAT3 activation in large granular lymphocyte leukemia is associated with cytokine signaling and DNA hypermethylation. Leukemia 2021;35:3430–43.
[110] Schade AE, Wlodarski MW, Maciejewski JP. Pathophysiology defined by altered signal transduction pathways: the role of JAK-STAT and PI3K signaling in leukemic large granular lymphocytes. Cell Cycle 2006;5:2571–4.
[111] Epling-Burnette PK, Bai F, Wei S, Chaurasia P, Painter JS, Olashaw N, et al. ERK couples chronic survival of NK cells to constitutively activated Ras in

lymphoproliferative disease of granular lymphocytes (LDGL). Oncogene 2004;23:9220–9.
[112] LeBlanc FR, Liu X, Hengst J, Fox T, Calvert V, Petricoin 3rd EF, et al. Sphingosine kinase inhibitors decrease viability and induce cell death in natural killer-large granular lymphocyte leukemia. Cancer Biol Ther 2015;16:1830–40.
[113] Kothapalli R, Kusmartseva I, Loughran TP. Characterization of a human sphingosine-1-phosphate receptor gene (S1P5) and its differential expression in LGL leukemia. Biochim Biophys Acta 2002;1579:117–23.
[114] Semenzato G, Teramo A, Calabretto G, Gasparini VR, Zambello R. All that glitters is not LGL leukemias. Leukemia 2022;36:2551–7.
[115] Gao S, Wu Z, Arnold B, Diamon C, Batchu S, Giudice V, et al. Single-cell RNA sequencing coupled to TCR profiling of large granular lymphocyte leukemia T cells. Nat Commun 2022;13:1982–94.
[116] Lundgren S, Keranen MAI, Kankainen M, Huuhtanen J, Walldin G, Kerr CM, et al. Somatic mutations in lymphocytes in patients with immune-mediated aplastic anemia. Leukemia 2021;35:1365–79.
[117] Salama Y, Zhao F, Oliveira JL, Yuan J, Jevremovic D, Go RS, et al. Isolated anemia in patients with large granular lymphocytic leukemia (LGLL). Blood Cancer J 2022;12:30–6.
[118] Calabretto G, Attardi E, Teramo A, Trimarco V, Carraro S, Mossuto S, et al. An Italian multi-center experience of hypocellular Myelodysplastic Syndromes (h-MDS): from clinical description to immunological characterization. Leukemia 2022;36:1947–50.
[119] Durrani J, Awada H, Kishtagari A, Visconti V, Kerr K, Adema V, et al. Large granular lymphocytic leukemia coexists with myeloid clones and myelodysplastic syndrome. Leukemia 2020;34:957–62.
[120] Ai K, Li M, Wu P, Deng C, Huang X, Ling W, et al. Concurrence of Myelodysplastic syndromes and large granular lymphocyte leukemia: clinicopathological features, mutational profile and gene ontology analysis in a single center. Am J Cancer Res 2021;11:1616–31.
[121] Qiu ZY, Qin R, Tian GY, Wang Y, Zhang YQ. Pathophysiologic mechanisms and management of large granular lymphocytic leukemia associated pure red cell aplasia. Onco Targets Ther 2019;12:8229–40.
[122] Masuda M, Teramura M, Matsuda A, Bessho M, Shimamoto T, Ohyashiki K, et al. Clonal T cells of pure red-cell aplasia. Am J Hematol 2005;79:332–3.
[123] Kawakami T, Sekiguchi N, Kobayashi J, Imi T, Matsuda K, Yamane T, et al. Frequent *STAT3* mutations in $CD8^+$ T cells from patients with pure red cell aplasia. Blood Adv 2018;2:2704–12.
[124] Kawakami F, Kawakami T, Yamane T, Maruyama M, Kobayashi J, Nishina S, et al. T cell clonal expansion and STAT3 mutations: a characteristic feature of acquired chronic T cell-mediated pure red cell aplasia. Int J Hematol 2022;115:816–25.
[125] Savola P, Kelkka T, Rajala HL, Kuuliala A, Kuuliala K, Eldfors S, et al. Somatic mutations in clonally expanded cytotoxic T lymphocytes in patients with newly diagnosed rheumatoid arthritis. Nat Commun 2017;8:15869–83.
[126] Couette N, Jarjour W, Brammer JE Jonathan E, Meara AS. Pathogenesis and treatment of T-Large Granular Lymphocytic Leukemia (T-LGLL) in the setting of rheumatic disease. Front Oncol 2022;12:854499–508.
[127] Gorodetskiy V, Sidorova Y, Bideman B, Kupryshina N, Ryzhikova N, Sudariko A. STAT3 mutations in “gray zone” cases of T-cell large granular lymphocyte leukemia associated with autoimmune rheumatic diseases. Front Med 2022;9:1000265–74.
[128] Viny AD, Linchtin A, Pohlman B, Loughran T, Maciejewski J. Chronic B-cell dyscrasias are an important clinical feature of T-LGL leukemia. Leuk Lymphoma 2008;49:932–8.
[129] Rodríguez-Caballero A, García-Montero AC, Bárcena P, Almeida J, Ruiz-Cabello F, Tabernero MD, et al. Expanded cells in monoclonal TCR-alphabeta+/ CD4+/NKa+/CD8-/+dim T-LGL lymphocytosis recognize hCMV antigens. Blood 2008;112:4609–16.
[130] Zambello R, Loughran Jr TP, Trentin L, Pontisso P, Battistella L, Raimondi R, et al. Serologic and molecular evidence for a possible pathogenetic role of viral infection in CD3-negative NK-type lymphoproliferative disease of granular lymphocytes. Leukemia 1995;9:1207–11.
[131] Tanahashi T, Sekiguchi N, Matsuda K, Takezawa Y, Ito T, Kobayashi H, et al. Cell size variations of large granular lymphocyte leukemia: Implication of a small cell subtype of granular lymphocyte leukemia with STAT3 mutations. Leuk Res 2016;45:8–13.
[132] Qiu Z, Fan L, Wang R, Gale RP, Liang H, Wang L, et al. Methotrexate therapy of T-cell large granular leukemia impact of STAT3 mutation. Oncotarget 2016;7:61419–25.
[133] Calabretto G, Teramo A, Barilà G, Vicenzetto V, Gasparini VR, Semenzato G, et al. Neutropenia and Large Granular Lymphocyte Leukemia: from pathogenesis to therapeutic options. Cells 2021;10:2800–15.
[134] Masle-Farquhar E, Jackson KJL, Peters TJ, Al-Eryani G, Singh M, Payne KJ, et al. STAT3 gain-of-function mutations connect leukemia with autoimmune disease by pathological $NKG2D^{hi}$ $CD8^+$T cell dysregulation and accumulation. Immunity 2022;55:2386–404.
[135] Gao L-M, Zhao S, Liu W-P, Zhang W-Y, Li G-D, Küçük C, et al. Clinicopathologic characterization of aggressive natural killer cell leukemia involving different tissue sites. Am J Surg Pathol 2016;40:836–46.
[136] Shi M, Olteanu H, Jevremovic D, He R, Viswanatha D, Corley H, et al. T-cell clones of uncertain significance are highly prevalent and show close resemblance to T-cell large granular lymphocytic leukemia. Implications for laboratory diagnostics. Mod Pathol 2020;33:2046–57.
[137] Calabretto G, Attardi E, Gurnari C, Semenzato G, Voso MT, Zambello R. LGL clonal expansion and unexplained cytopenia: two clues don't make an evidence. Cancers 2022;14:5236–51.
[138] Novikov ND, Griffin GK, Dudley G, Drew M, Rojas-Rudilla V, Lindeman NI, et al. Utility of a simple and robust flow cytometry assay for rapid clonality testing in mature peripheral T-cell lymphomas. Am J Clin Pathol 2019;151:494–503.
[139] Haferlach T. The time has come for next-generation sequencing in routine diagnostic workup in hematology. Haematologica 2021;106:659–61.
[140] 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.
[141] Burks EJ, Loughran Jr TP. Pathogenesis of neutropenia in large granular lymphocyte leukemia and Felty syndrome. Blood Rev 2006;20:245–66.
[142] Singleton TP, Yin B, Teferra A, Mao JZ. Spectrum of clonal large granular lymphocytes (LGLs) of αβ T Cells: T-cell clones of undetermined significance, T-cell LGL leukemias, and T-cell immunoclones. Am J Clin Pathol 2015;144:137–44.
[143] Zang R, Shah MV, Loughran TP. The root of many evils: indolent large granular lymphocyte leukemia and associated disorders. Hematol Oncol 2010;28:105–17.
[144] Chin-Yee B, Suthakaran A, Hedley BD, Howlett C, Stuart A, Sadikovic B, et al. T-cell clonality testing for the diagnosis of T-cell large granular lymphocytic leukemia: Are we identifying pathology or incidental clones ? Int J Lab Hematol 2022;44:1115–20.
[145] Barilà G, Calabretto G, Teramo A, Vicenzetto C, Gasparini VR, Semenzato G, et al. T cell large granular lymphocyte leukemia and chronic NK lymphocytosis. Best Pract Res Clin Haematol 2019;32:207–16.
[146] Yabe M, Medeiros LJ, Tang G, Wang SA, Ahmed S. Nieto Y prognostic factors of hepatosplenic T-cell lymphoma: clinicopathologic study of 28 cases. Am J Surg Pathol 2016;40:676–88.
[147] Pro B, Allen P, Behdad A. Hepatosplenic T-cell lymphoma: a rare but challenging entity. Blood 2020;136:2018–26.
[148] McKInney M, Moffit AB, Gaulard P, Travert M, De Leval L, Nicolae A, et al. The genetic basis of hepatosplenic T cell lymphoma. Cancer Discov 2017;7:369–79.
[149] Lamy T, Loughran TP. How I treat LGL leukemia. Blood 2011;117:2764–74.
[150] Loughran Jr TP, Kidd PG, Starkebaum G. Treatment of large granular lymphocyte leukemia with oral low-dose methotrexate. Blood 1994;84:2164–70.
[151] 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 of 229 cases. Haematologica 2010;95:1534–41.
[152] 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.
[153] 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.
[154] Loughran Jr TP, Zickl L, Olson TL, Wang V, Zhang D, Rajala HLM, et al. Immunosuppressive therapy of LGL leukemia: prospective multicenter phase II study by the Eastern Cooperative Oncology Group (E5998). Leukemia 2015;29:886–94.
[155] Ishida F. Aggressive NK-cell leukemia. Front Pediatr 2018;6:292–7.
[156] Fujimoto A, Maeda T, Fukuhara N, Miyazaki K, Yamaguchi M, Ishida F, et al. Improving prognosis of aggressive natural killer cell leukemia in Japan. Blood 2022:139. Annual ASH Meeting, New Orleans LA. Abs n. 4247.
[157] Dimitriu B, Ito S, Feng X, Stephens N, Yunce M, Kajigaya S, et al. Alemtuzumab in T-cell large granular lymphocytic leukemia: interim results from a single-arm, open-label, phase 2 study. Lancet Haematol 2016;3:e22–9.
[158] Uliah F, Dima D, Gurnari C, Kumar Ballasubramanian S, Ogbue O, Awada H, et al. Intense immunosuppressive therapy (anti-thymocyte globulin, alemtuzumab) as salvage therapy in refractory T-cell large granular lymphocytic leukemia and pure red cell aplasia. Blood 2022:139. Annual ASH Meeting, New Orleans LA. Abs n. 2567.
[159] Ma SY, Au WY, Chim CS, Lie AKW, Lam CCK, 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.
[160] Osuji N, Matutes E, Tjonnfjord G, Grech H, Del Giudice I, Wotherspoon A, et al. T-cell large granular lymphocytic leukemia. A report on the treatment of 29 patients and a review of the literature. Cancer 2006;107:570–8.
[161] Zaja F, Baldini L, Ferreri AJM, Luminari S, Grossi A, Salvi F, et al. Bendamustine salvage therapy for T cell neoplasms. Ann Hematol 2013;92:1249–54.
[162] Cornec D, Devauchelle-Pensec V, Jousse-Joulin S, Marhadour T, Ugo V, Berthou C, et al. Long-term remission of T-cell large granular lymphocytic leukemia associated with rheumatoid arthritis after rituximab therapy. Blood 2013;122:1583–6.
[163] Subbiah V, Viny AD, Rosenblatt S, Pohlman B, Lichtin A, Maciejewski JP. Outcomes of splenectomy in T-cell large granular lymphocyte leukemia with splenomegaly and cytopenia. Exp Hematol 2008;36:1078–83.
[164] Marchand T, Lamy T, Finel H, Arcese W, Choquet S, Finke J, et al. Hematopoietic stem cell transplantation for T-cell large granular lymphocyte leukemia: a retrospective study of the European Society for Blood and Marrow Transplantation. Leukemia 2016;30:1201–4.
[165] Alekshun TJ, Tao J, Sokol L. Aggressive T-cell large granular lymphocyte leukemia: a case report and review of the literature. Am J Hematol 2007;82:481–5.
[166] La Nasa G, Littera R, Cocco E, Battistini L, Marrosu MG, Contu L. Allogeneic hematopoietic stem cell transplantation in a patient affected by large granular lymphocyte leukemia and multiple sclerosis. Ann Hematol 2004;83:403–5.

[167] Bilori B, Thota S, Clemente MJ, Patel B, Jerez A, Afable 2nd M, et al. Tofacitinib as a novel salvage therapy for refractory T-cell large granular lymphocytic leukemia. Leukemia 2015;29:2427–9.
[168] Moignet A, Pastoret C, Cartron G, Coppo P, Lamy T. Ruxolitinib for refractory large granular lymphocyte leukemia. Am J Hematol 2021;96:E368–70.
[169] 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 Mikβ1 mAb in patients with T-cell large granular lymphocytic leukemia. Blood 2013;121: 476–84.
[170] Wang TT, Yang J, Zhang Y, Zhang M, Dubois S, Conlon KC, et al. IL-2 and IL-15 blockade by BNZ-1, an inhibitory of selective γ-chain cytokines, decreases leukemic T-cell viability. Leukemia 2019;33:1243–55.
[171] Brammer JE, Tagaya Y, Rogers K, Mishra A, Waldmann T, Azimi N, et al. Blockade of IL-15 utilizing BNZ-1, a selective g-chain inhibiting peptide, is safe and has clinical activity in patients with T-cell large granular lymphocytic leukemia (T-LGLL): results of a phase I/II multi-center clinical trial. Blood 2019:134. Annual ASH Meeting, Orlando FL. Abs n. 4776.
[172] Assmann JLJC, Leon LG, Stavast CJ, van den Bogaerdt SE, Schilperoord-Vermeulen J, Sandberg Y, et al. miR-181a is a novel player in the STAT3-mediated survival network of TCRαβ+ CD8+ T large granular lymphocyte leukemia. Leukemia 2022;36:983–93.
[173] Gattazzo C, Miorin M, Scquizzato E, Teramo A, Cabrelle A, Balsamo M, et al. Lack of expression of inhibitory KIR3DL1 receptor in patients with NK-type lymphoproliferative disease of granular lymphocytes. Haematologica 2010;95: 1722–37.
[174] Johansson P, Laguna T, Ossowski J, Pancaldi V, Brauser M, Duhrsen U, et al. Epigenome-wide analysis of T-cell large granular lymphocytic leukemia identifies BCL11B as a potential biomarker. Clin Epigenetics 2022;14:148–60.
[175] Wahnschaffe L, Herling M. Hijacking the pathway: perspectives in the treatment of mature T-cell leukemias. HemaSfere 2021;5:e573–8.
[176] Mustjoki S, Young NS. Somatic mutations in "benign" disease. N Engl J Med 2021; 384:2039–52.