PERSPECTIVE ARTICLE

T-cell clones of uncertain significance. When is the rogue clone dangerous?

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Abstract

T-cell large granular lymphocyte clones that persist over time and that exhibit molecular and immunophenotypic features closely resembling those of T-cell large granular lymphocyte leukemia (T-LGLL) may be detectable in individuals who lack any clinical or laboratory features supporting a diagnosis of a T-cell malignancy. This condition represents a potential precursor state termed T-cell clones of uncertain significance (T-CUS). T-CUS represents the even more benign extreme of the wide spectrum of clonal T-large granular lymphocyte proliferations, emphasizing the need for an appropriate multiparametric diagnostic assessment that avoids misdiagnosis of T-cell neoplasia. This approach should overcome numerical cut-offs as the sole criteria to differentiate the benign condition from the related malignancies. In particular, genomic aberrancies might prospectively identify individuals who are at risk of progression to a full-blown T-cell malignancy. We herein discuss the significance of these T-cell clones in both healthy and disease states, suggesting molecular assays for tracking early steps of disease.

Introduction

T-cell clones of uncertain significance (T-CUS) exhibit molecular and immunophenotypic features closely resembling those of T-cell large granular lymphocytic leukemia (T-LGLL). However, individuals harboring these rogue clones lack any clinical or laboratory features supporting a diagnosis of a T-cell malignancy.[1-3]

T-CUS is actually a premalignant condition detectable in otherwise healthy individuals and it is reminiscent of other precursor states involving the B-cell branch of immunity, such as monoclonal B-cell lymphocytosis (MBL) and monoclonal gammopathy of undetermined significance (MGUS), as well as the myeloid compartment, e.g., clonal hematopoiesis of indeterminate potential (CHIP).[4] These conditions are identified by clones that manifest with an abnormal cell expansion and/or the increase of their by-products (monoclonal component).

T-CUS is being increasingly intercepted due to the availability and use of new techniques that can uncover even small clones undetectable with conventional methods. As a result, more individuals are now being diagnosed with this condition than in the past. Upon receiving this diagnosis, patients understandably experience anxiety about a disorder defined as “clonal”. Furthermore, during follow-up visits, they are constantly reminded of the insidious nature of their abnormality, and addressing the prognosis and likelihood of progression to overt malignancy remains challenging due to the limited knowledge on the matter. Only a few original papers have been published on T-CUS,[2,3,5-8] well-documented prospective cohorts are not available, and currently there are no official clinical guidelines on the management of this condition.

Oligoclonal/clonal T-cell expansions might reflect the long-lived status of effector/memory cells specific to past infections.[9-11] Otherwise, they might result from continuous antigenic stimulation by non-pathogenic infections as well as from any setting characterized by prolonged antigenic pressure, including allogeneic hematopoietic stem cell transplantation (HSCT), vaccinations and other stimuli.[12] We herein discuss how to approach these T-cell clones and their significance in both healthy and disease states. These conditions need to be carefully considered and our understanding on the matter must be translated into an

appropriate diagnostic interpretation that distinguishes T-CUS from its mimickers, in particular T-LGLL and other borderline T-cell malignancies.

T-cell clones in healthy subjects

During a primary immune response against antigens, T-cell expansions are usually polyclonal, but oligoclonal or strictly monoclonal populations might develop, which can sometimes make the differential diagnosis from T-cell malignancies challenging. The emergence of T-cell clones after cell activation is a normal occurrence under physiological conditions, with these T-cell expansions typically generated as a part of a reactive immune response. The detection of a clonal expansion can sometimes represent the extreme larger-than-expected physiological proliferation of cytotoxic clonotypes central to adaptive immunity, which does not necessarily imply neoplasia.[1,13]

Following the clearance of the relevant stimuli, proliferating cells undergo activation-induced cell death. This process helps to maintain immune homeostasis by eliminating excessive or unnecessary immune responses. Reactive T-cell expansions are transient, usually self-limited, and typically observed in association with well-defined triggering events, primarily viral infections, but not only. Since reactive T-cell expansions typically resolve once the infection is cleared, the persistence of a small abnormal population over time (usually 6 months) is required before considering the possibility of malignancy.

Given the essentially indistinguishable morphology of leukemic and reactive large granular lymphocytes (LGL), proof of clonality is mandatory. This confirmation is primarily based on the unique structure of the T-cell receptor (TCR) which is thought to carry the fine specificity for the antigen in its hypervariable complementarity-determining region 3 (CDR3). Clonality of T cells is easily detectable by conventional molecular technologies based on polymerase chain reaction, which allows the identification of lymphocyte populations derived from a single cell (clonotypes) by showing the pattern of the CDR3 length distribution and the frequency of identical sequences within CDR3 amplification products.[14] In recent years, the assessment of TCR clonality is preferably made using next-generation sequencing (NGS), which allows the detection of even small T-cell clones and subclones that would otherwise be missed by other common tests, irrespective of the clone size. Additionally, NGS enables the detection of somatic mutations characterizing clonal expansion by calculating the variant allele frequency, which is the proportion of reads supporting a mutant allele out of the total number of reads in a NGS. Table 1 summarizes the current assays used to evaluate T-cell clonality, highlighting the advantages and disadvantages of each method.[14-18]

Evidence of incidental clonal T-cell expansions in healthy individuals has been reported across all age groups,[19] but these expansions are notably more prevalent in older individuals.[20-22] This phenomenon is likely interpreted as the remnant of prior immune stimulation by virus, allo-, auto-, or tumor-associated antigens through a person's lifetime, potentially leading to a restriction in the diversity of antigen recognition. The persistence of expanded $CD8^+$ clones can be either antigen-dependent or -independent and is typically associated with immunosenescence and reduction of naïve $CD8^+$ T cells.[23] Notably, the $CD8^+CD57^+$ phenotype, particularly when combined with the absence of CD28 expression and CD45RA positivity, is regarded as a marker of late-differentiated, highly antigen-experienced cytotoxic lymphocytes. This $CD8^+CD57^+CD28^-CD45RA^+$ TEMRA (terminally differentiated effector memory T cells) phenotype has been reported in various diseases or conditions associated with persistent antigenic stimulation.[24] Consistently, studies in normal mice models have demonstrated that increasing age is the most important factor in the spontaneous development of clonal CD8 T-cell expansions.[25]

Lessons learned over the last decade from single-cell

Table 1. Current assays used to evaluate T-cell clonality: pros and cons compared.

T-cell clonality assay	Advantages	Limits
PCR-based techniques of TCR gene rearrangements	Identification of lymphocyte populations derived from a single cell (clones)	No information beyond the presence or absence of clonality
NGS of TCR gene rearrangements	Identification of all the rearrangements composing an individual's TCR repertoire and precise definition of CDR3 sequences, including dimensions of the immunodominant clonotypes (even small T-cell clones and subclones)	Expensive and not routinely used in diagnostic hematology laboratories
Flow cytometer analysis of TCRVβ expression[15,16]	Fast approach assessing the preferential usage of one TCR-Vβ segment	The repertoire identified is limited, TCRVβ antibodies cover approximately 70% of the normal human TCRVβ repertoire
Flow evaluation of the constant regions 1 and 2 of the T-cell receptor β chain (TRBC1 and TRBC2)[7,17,18]	Fast approach for evaluating TRBC restriction (the two TRBC genes are mutually exclusive and randomly used during TCR gene rearrangement)	T cell clonality cannot be identified in Tγδ lymphoproliferations

PCR: polymerase chain reaction; TCR: T-cell receptor; NGS: next-generation sequencing; TRBC: T-cell receptor β chain constant region.

technologies and high-throughput sequencing as well as from the expanding use of TRBC1/2 antibodies (Table 1)[7,17,18] indicate that the incidence of T-cell clones in elderly people, and more in general in healthy individuals, may be higher than previously thought, tentatively around 1-2%. This figure is consistent with the incidence observed in other premalignant conditions such as MBL and MGUS.[4] Their preferential occurrence with increasing age, especially in the oldest populations,[20] suggests potential overlaps with the mechanisms of immune system senescence. The obvious question then arising regards the significance of these cell expansions in different settings, both in hematologic disorders as well as in various non-hematologic disease states.

T-cell clones in patients with large granular lymphocyte leukemia

Large granular lymphocyte leukemias (LGLL) are rare diseases characterized by the clonal expansion of LGL whose diagnosis, classification and treatment have been hampered over time by their remarkable phenotypic, genotypic and clinical heterogeneity as well as their geographic diversity. To classify the different subtypes of LGL disorders precisely, appropriate immunophenotypic and molecular characterization is mandatory. In accordance with the scope of this paper, which aims to compare these disorders with T-CUS, Table 2 focuses on T-LGLL, summarizing the relevant features of this disease, especially in terms of the criteria required for diagnosis. Further details on the classification, pathogenesis, presentation, disease evolution, and treatments of T-LGLL have been extensively covered in recent reviews.[26,27] We would like to comment briefly on a couple of issues: the size of the clone and the recent evidence of somatic mutations.

The number of clonal LGL in the peripheral blood of patients with T-LGLL typically exceeds 2.0x10^9/L but the diagnosis of LGLL may also be made with LGL counts >0.5x10^9/L,[28] or even lower when a restricted LGL clone is demonstrated in an appropriate clinical context (severe symptoms not attributable to other causes) including the association with unexplained cytopenias[29] or concurrent autoimmune disease. We label this subset of patients as “low-count LGLL”. Nevertheless, this cutoff presents a diagnostic challenge that requires further insights and concerted efforts to address, as outlined below.

As in many hematologic conditions, genetic alterations have broadened our knowledge on LGL disorders and are becoming instrumental in distinguishing discrete disease subsets.[30] This achievement is also guiding the research towards precision medicine strategies for patients, allowing for more tailored and effective treatments. In LGLL, the STAT3 pathway has been claimed as the central hub of the abnormal T-cell proliferation[26,27,31] and different mutations, such as *STAT3* and *STAT5B* to mention the most relevant,[32,33] have been found to be harbored in leukemic cells. Furthermore, their detection helps in predicting the disease outcome.[32,34-37] This finding not only advances our understanding of the pathogenesis of LGLL but can also aid in the diagnostic process and, in turn, refine the classification of these disorders.[38]

T-cell clones in different non-hematologic diseases

Evidence has accumulated of associations of T-cell clones and/or T-LGLL with both non-hematologic disease states, including inflammatory/autoimmune disorders and neoplasia. Whether the presence of clonal T populations in these conditions is pure coincidence and the exact role they play

Table 2. Relevant clinical and biological features of T-large granular lymphoycte leukemia.

	Clinical and biological features
T-LGLL subtypes	T-LGLL has two distinct subtypes, Tα/β-LGLL (90%) and Tγ/δ-LGLL (10%). Among Tα/β-LGLL, the more common subset is CD8$^+$ T-LGLL while CD4$^+$ T-LGLL is less frequent[26,27]
Clinical manifestations	The disease is asymptomatic in nearly 35% of cases. Symptomatic patients exhibit cytopenias and clinical complications, mainly infections, closely linked to neutropenia[26,27]
Associated diseases	T-LGLL is frequently associated with a wide spectrum of accompanying diseases, particularly autoimmune disorders such as rheumatoid arthritis,[26,27,40] but also with other hematologic and non-hematologic diseases
LGL immunophenotype	The abnormal LGL expansion is characterized by T cells that are CD3$^+$CD8$^+$ or CD3$^+$CD4$^+$(CD8$^{-/dim}$), with variable expression of cytotoxic markers such as CD57, CD16, CD56 as well as inhibitory natural killer receptors[28]
Somatic mutations	The presence of gain-of-function mutations, mainly in the *STAT3* and *STAT5B* genes and less frequently in other genes such as *TET2*, *KMT2D*, and *TNFAIP3*, may help to confirm the clonality in discrete patients (e.g., those with oligoclonal LGL expansions with no restricted immunophenotype). The incidence of *STAT3* mutations in T-LGLL ranges from 20% to 70% across different series of patients, and their detection correlates with neutropenia[32,34-37]
Bone marrow involvement	Trephine biopsy shows variable-in-size interstitial and intrasinusoidal CD8$^+$/TIA1$^+$ cells or granzyme B$^+$ lymphoid infiltrates with altered immunophenotype[28,85]

T-LGLL: T-large granular lymphocyte leukemia; LGL: large granular lymphocyte.

pathogenically and prognostically remain elusive.

Rheumatoid arthritis is the most frequent autoimmune disorder associated with T-LGLL, being reported in approximately 20% of patients across different case series.[39,40] However, it is possible that several rheumatoid arthritis-related cases resemble but do not fully meet the diagnostic criteria[41,42] for T-LGLL. The genesis of this association is still controversial, particularly regarding whether the detectable T-cell clones in this concurrent disease are the result or the cause of the immune-inflammatory associated events.[40] Whatever their origin, these expanded clones have been shown to contribute to the pathogenesis of rheumatoid arthritis. In fact, cytotoxic $CD8^+$ lymphocytes targeting citrullinated proteins have been recently demonstrated in patients with rheumatoid arthritis.[43] These cells are clonally expanded and highly express cytotoxic and synovium-trafficking molecules, likely mediating synovitis and joint tissue destruction.

T-LGLL has also been reported to coexist with solid neoplasms, including prostate, breast, lung, melanoma, colorectal, and kidney tumors, as well as neuropathies, immunodeficiency (e.g., hypogammaglobulinemia, common variable immune deficiency, CVID) and following solid organ transplantation (allogeneic HSCT), as reported by Bareau *et al.*[44] and Viny *et al.*[45]

The question of whether these clonal proliferations represent an independent indolent form of T-LGLL or whether they come from a highly exaggerated benign T-cell response to the antigenic stimulation provided by the accompanying disorder is still under discussion. A distinctive feature of T-LGLL is its well-known association with robust immune responses, such as after viral infections (Epstein-Barr virus, hepatitis C virus, human T-cell lymphotropic virus type I/II) and rheumatoid arthritis.[46,47] In cases in which neoplasia co-occurs, the expansion of LGL is likely the result of a seemingly unstoppable stimulation by tumor antigens. This hypothesis is tantalizing and is supported by rare reports of LGL expansions resolving after the primary disease has disappeared.[45,48-50]

T-cell clones in other hematologic disorders

T-cell clones are also detectable in several hematologic conditions including bone marrow failure syndromes, acute and chronic leukemias, as well as plasma cell dyscrasias, Hodgkin and non-Hodgkin lymphomas, and immune thrombocytopenia. A retrospective systematic survey of different hematologic disorders that have been associated with T-LGLL is beyond the scope of this paper and comprehensive reviews on the matter have already been provided by Zhang *et al.*[50] and more recently by Bravo-Perez *et al.*[51] Similar to what has been discussed for non-hematologic disease states, it is conceivable that these expanded clones result from a chronic immune response triggered by immunogenic antigens or molecules expressed by neoplastic cells in relevant malignant hematopoietic disorders. We herein want to focus on a few selected circumstances that might provide insights into the intricate nature of these cell proliferations. The following examples may elucidate the stimuli that potentially drive T-cell clones and might trigger the evolution of T-CUS.

First of all, consider the setting of premalignant conditions. Interestingly, multiple precursor states are sometimes detectable concurrently, including MBL with T-LGLL,[52,53] clonal hematopoiesis with T-LGLL,[54] T-CUS with MGUS,[55] and MGUS with T-LGLL.[56,57] These co-occurrences serve as proof of concept that the entire hematopoietic system is under antigenic pressure, potentially leading to multi-compartment disorders. Fluctuations in clonal dynamics[58,59] and evidence of multi-clonal MBL,[60] MGUS,[57] and T-CUS[2] are consistent with the hypothesis of chronic antigen-driven immune responses resulting from a strong and extensive reactive process that occurs prior to the stepwise acquisition of genomic alterations. These findings also indicate that the antigen drive likely underlies cell expansions, acting in an environment-specific context that over time may be progressively established by additional, secondary pervasive mutations.

Peripheral expansion of clonal cytotoxic T lymphocytes derived from the graft in the initial stages of allogeneic HSCT immune recovery is a well-known physiological event,[61] with such clonal expansions persisting beyond the early transplantation period. The presence of persistent immunodominant T-cell clonotypes following allogeneic HSCT is significantly more frequent in those patients who developed cytomegalovirus reactivation and/or acute graft-*versus*-host disease, a finding which suggests a reactive cell expansion. The absence of *STAT3* mutations in CD3-sorted populations and the declining longitudinal kinetics further support the benign nature of these clones.[62] Moreover, Mohty *et al.*[63] demonstrated that a subset of allotransplanted patients achieved long-term complete remission concomitant with or following LGL expansion, suggesting that these cells could represent effector lymphocytes which may participate in graft-*versus*-leukemia activity.

Several studies have documented a marked peripheral large granular lymphocytosis after treatment with dasatinib,[64,65] a second-generation multi-tyrosine kinase inhibitor currently used in chronic myeloid leukemia (CML). Clonal lymphocytes were already present at diagnosis, persisted at low levels during first-generation tyrosine kinase inhibitor therapy, and expanded during dasatinib treatment.[66] In addition to the intended inhibition of BCR-ABL1 kinase, dasatinib also inhibits other kinases, including SRC and TEC that behave as major regulators of immune responses.[67] By inhibiting distinct off-target kinases in immune effector cells, dasatinib may restore the function of anergic, exhausted leukemia-specific clonal cytotoxic preexisting

long-lived effector memory cells that were already present in untreated patients at the time of CML diagnosis. Since the group of CML patients with clonal expansions had a better prognosis,[65] it has been suggested that following dasatinib therapy, LGL may mediate therapeutic activity against leukemia, either attacking CML stem cells or by eliminating residual CML cells, thus ultimately favoring the maintenance of responses. The evidence of clonotypes of cytomegalovirus-specific CD8 T cells[68] and the finding that patients with large granular lymphocytosis more easily reached a major molecular response[64] further point to the protective role of these clonal expansions.

Examples in the settings mentioned above support the hypothesis that these clonal reactive populations originate in response to discrete events, sometimes beneficial (such as the response to pathogens or graft-*versus*-leukemia activity), sometimes ineffective. They undergo malignant transformation only in exceptional cases. In fact, chronic antigen-driven stimulation of the immune system may trigger a polyclonal cytotoxic T lymphocyte response that subsequently evolves, eventually leading to the lymphoproliferative disorder, particularly upon acquisition of mutations. According to this interpretation, Awada *et al.*[69] demonstrated *STAT3* mutations in two out of 13 (15%) patients with T-LGLL after solid organ transplantation and allogeneic HSCT. These mutations, by conferring oncogenic properties to affected cells, solidify their autonomous proliferation, leading to progression towards the full-blown disease. Taken together, these observations recapitulate the stepwise model of cancer progression, in which a series of events (whether mutational or related to microenvironment) drives clonal expansions with progressively more disordered phenotypes.

The work-up of T-cell clones of uncertain significance

T-CUS, either concurrently with, or independently of other hematologic/non-hematologic diseases, is a premalignant condition that has the potential to evolve into T-LGLL or other T-cell malignancies. Similarly to other previously mentioned precursor states, T-CUS is preferentially observed in older individuals. The risk of evolution to the full-blown malignancy is estimated to be approximately 1% per year, but this figure will require more extensive evaluation. This means that most individuals will never experience a life-threatening disease.

In terms of pathogenesis, the lack in T-cell clonal expansions of a common antigen specificity,[21] of shared TCR clonotypes[70] as well as the lack of common TCRA and TCRB clonotypes in $CD8^+$ TCR$\alpha\beta$ in LGLL even among HLA-matched individuals,[71] reinforce the concept of a widespread cellular activation. Emerging evidence indicates that the inciting event may be universal to the entire immune system. In fact, recent studies in T-LGLL highlighted a considerable overlap between leukemic and non-leukemic parts of TCR repertoires via possible common triggers.[33,72] The antigen-driven clonotypes in T-LGLL patients occurred concomitantly with non-antigen-driven clones and neither shared T-LGLL clonotypes nor T-LGLL clonotypes targeting known antigens were detected.[70,72] In contrast with T-LGLL, it is likely that in T-CUS, clonal T cells do not progress towards pervasive clone overgrowth or suppression of hematopoiesis. Instead, they are maintained in a steady-state equilibrium, possibly mediated by the microenvironment.

The prevalent phenotype in T-CUS is $CD3^+CD8^+CD57^+CD28^-CD27^-$ and, to some extent, positive to NK receptors (p58 molecules, the killer immunoglobulin-like receptors), a pattern which is consistent with fully differentiated effector/memory T cells with low proliferative and high cytotoxic activity. However, based on the surface immunophenotype, T-CUS encompasses a spectrum of phenotypic variants, all competent in cytotoxicity, including CD8 T-CUS, CD4 T-CUS, and $\gamma\delta$ T-CUS. Data available in T-CUS on gain-of-function *STAT* mutations, which are regarded as the hallmark of T-LGLL, are still scanty.[1-3,54,62] Also in terms of microenvironmental features, including non-clonal T lymphocytes, macrophages and other immunocompetent cells, only a few detailed accounts are available, mainly related to the overt malignancy.[72,73]

At this time, following appropriate analysis of the history of putative conditions mounted or perpetuated by highly specific polarized immune responses to strong antigenic stimuli that might discern reactive lymphocytosis, the definition of T-CUS, incidental T-cell clones detected in other conditions and T-LGLL must take into account the following items:

- *Persistent clonality*. Evidence of persistent clonality over time, demonstrated as reported above, is a prerequisite.
- *Clone size*. In T-CUS the size of the involved clone is, by definition, smaller than the threshold required to establish a diagnosis of T-LGLL, this set threshold acting as an artificial watershed. This threshold makes a tentative distinction between the end of the physiological range and the beginning of disease. However, as in all other precursor states, the threshold by itself does not help separate individuals at risk of progression from those bound to remain in a pre-malignant condition. For the time being, the arbitrary LGL count less than $0.5x10^9$/L distinguishes T-CUS from T-LGLL[1,2,7,15,28] but this criterion might evolve. T-CUS and indolent T-LGLL likely represent a biological continuum, making the distrinction between the two entities challenging due to the presence of a gray zone (Figure 1). Sometimes the LGL count exceeds $0.5x10^9$/L and for this reason observation for at least 6 months is mandatory to ensure that LGL expansion is not a transient/benign reactive proliferation. A less pronounced Vβ skewing in T-CUS than in indolent T-LGLL might aid in differentiating T-CUS from T-LGLL. However, this distinction is largely a matter of semantics as the two

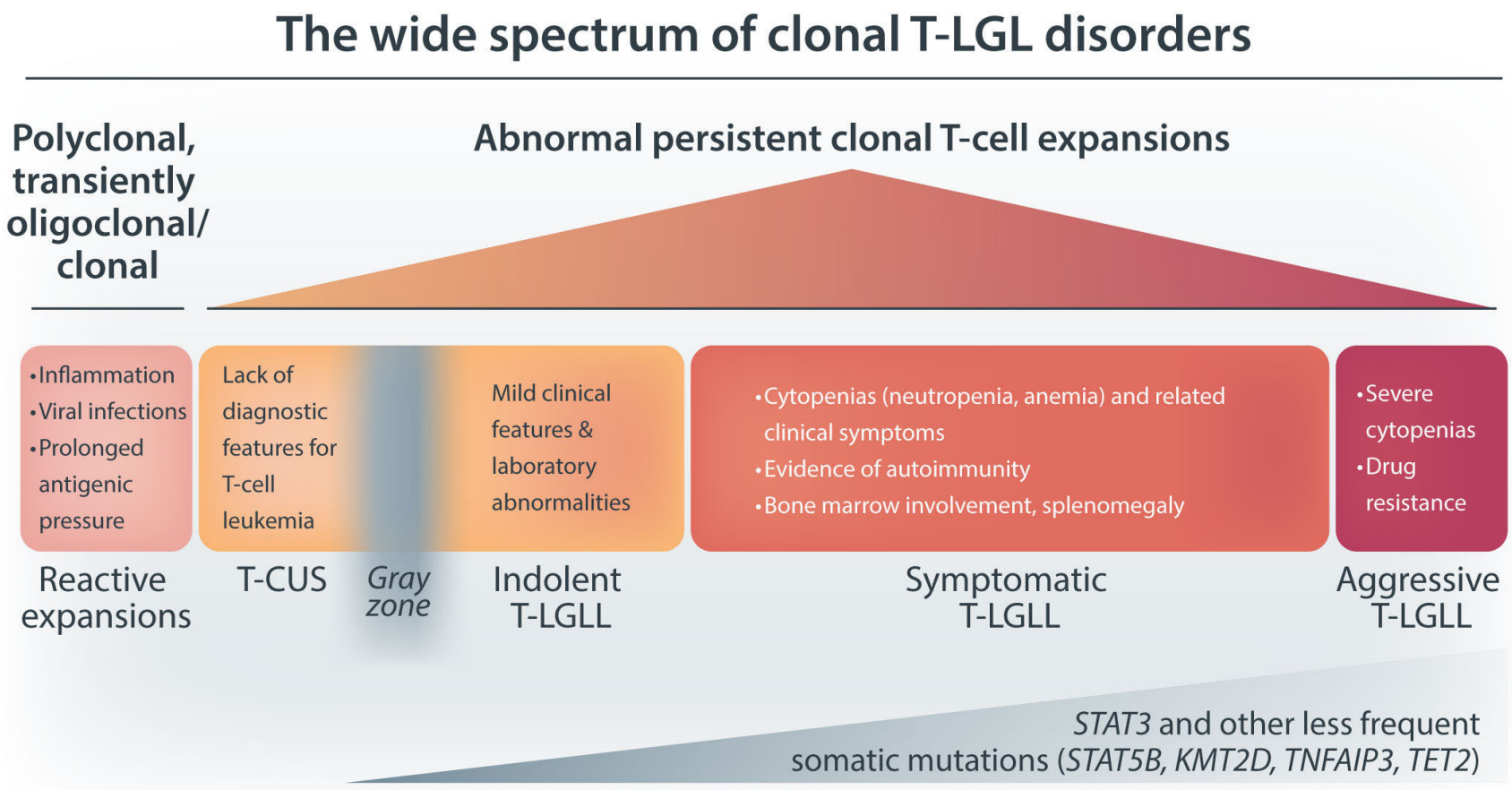

Figure 1. Landscape of clonal chronic lymphoproliferative disorders of granular lymphocytes. T-cell clonopathy of uncertain significance represents the even more benign extreme of the spectrum of clonal T large granular lymphocyte proliferations, but the gray zone must be further specified. Detection of putative somatic mutations (mentioned in the faded area in the figure) could help to identify these subsets of patients more precisely. Color shades gradually moving from yellow to red represent disease progression, with yellow indicating asymptomatic patients and red indicating more advanced stages of disease. The size of the areas tentatively reflects the prevalence of patients' subsets. T-LGL: T large granular lymphocyte; T-CUS: T-cell clones of uncertain significance; T-LGLL: T-cell large granular lymphocyte leukemia.

groups of patients do not undergo treatment. Molecularly based quantification of the clonal size[74] might also help in the distinction. Obviously, we must check whether the clone is stable, progressive, or transient.

- *Healthy state*. Evidence that the patient is devoid of other laboratory and clinical features supporting a diagnosis of malignancy is another prerequisite to define T-CUS. In particular, the context of earlier mentioned clinical features includes associated diseases, splenomegaly, cytopenias, and related symptoms.

- *Mutational screening*. Somatic mutations, particularly *STAT3*, have not been demonstrated in T-CUS up to now[1-3,54,62] and this might represent a significant differentiator from T-LGLL. However, this concept is not definitively established. In fact, it is well recognized that somatic mutations occur frequently in T cells and do not equate to malignancy.[1,13] Somatic mutations are ubiquitous in patients with oligoclonal T-cell expansions[75,76] and *STAT3* mutations have been found in $CD8^+$ T cells of healthy blood donors carrying human T-cell leukemia virus type 2.[77] In addition, since several mutations have been reported in other premalignant conditions (e.g., *TP53* mutation/deletion, *NOTCH1*, *SF3B1*, and *BIRC3* mutations in MBL;[78] *KRAS* and *NRAS* mutations, *TP53* deletion, *MYC* alterations in *MGUS*,[79] among others), it is reasonable to expect, based on systematic studies, that similar alterations could also be present in T-CUS. If this were the case, a heavy mutational burden of these purported mutations might aid in identifying patients with a reasonable likelihood of progressing to overt T-LGLL. Similarly, the tumor mutational load predicts progression to requiring therapy in high-count MBL[80] and CHIP predicts the risk of developing myelodysplastic syndromes.[81] It is hoped that evaluation of the entity of *STAT3* mutations (variant allele frequency-based analysis) could be helpful in assessing tumor burden, rather than relying solely on numerical cutoffs. This could also assist in refining the concept of clonal hematopoiesis and its translational implications. In fact, Masle-Farquhar *et al*.[82] demonstrated, in a murine model, that germline *STAT3* gain-of-function mutations, keeping lymphocyte activation of T-cell clones out of check, perpetuated tissue damage thus contributing to the development of autoimmunity.

The evidence that *STAT3* mutations are sometimes found[42] and sometimes not found[83] in patients with autoimmune-accompanying disorders suggests that we are dealing with two extremes of the same phenomenon. However, a possible bias due to the sensitivity of the technical methods used must be considered. Furthermore, given that some overlap between non-malignant clonal T-cell expansions and in-

dolent T-LGLL may occur, individuals must be considered one by one. Further prospective studies are needed to find a proper cutoff to define those patients who have a true pre-leukemic state rather than manifesting a byproduct of a nonspecific immune response with more limited impact. This is in line with the diversity established between high- and low-count MBL,[84] with the latter subset being associated with a negligible risk of progression.

- *Bone marrow*. An evaluation of the bone marrow, if performed, may be of help. In fact, the absence of interstitial and intrasinusoidal cytotoxic T-cell infiltrates, or interstitial cytotoxic T-cell clusters of lymphoid cells with azurophilic granules, favors the diagnosis of T-CUS.[28,85]

All the above are needed to bring us to the diagnosis of T-CUS. Figure 1 illustrates the clinical landscape of T chronic lymphoproliferative disorders of LGL. T-CUS stands between reactive, polyclonal or transiently clonal lymphocytosis and indolent LGLL. It represents the benign end of the broad spectrum of these disorders, but the watershed distinguishing indolent T-LGLL from T-CUS is nuanced, as emphasized early. We hope to find a biomarker that differentiates between T-CUS and indolent T-LGLL. Certainly, the putative detection of somatic mutations can prospectively bridge this knowledge gap, enabling a more timely identification of these borderline subsets of patients.

Understanding the evolution of T-cell clones is crucial for clinical management and future research directions

When can we be confident that we are dealing with a T-CUS instead of true T-LGLL (Table 3)? We believe that the selected circumstances previously discussed[45,52-56,63-66,69] should be reconsidered on a case-by-case basis taking into account the precise criteria currently in use for T-CUS/T-LGLL in the era of genomics.

Chronic exposure to specific antigens (infections, tumor antigens, protein products or graft antigens) could trigger the initial LGL proliferation and the emergence of a clone then confers a growth advantage on the clone persistence and expansion of lymphocytosis. In some cases, the acquisition over time of specific genetic alterations or permissive epigenetic changes is believed to definitely establish founding clones. This leads to clonal overgrowth and suppression of hematopoiesis, undergoing malignant transformation and then resulting in the onset of LGL leukemia. Are these further steps mediated by mutations or epigenetic alterations that grant the affected cells a growth advantage over their counterparts, making the rogue clone become dangerous by triggering cytopenias and autoimmunity? Consistent with this hypothesis, *STAT3* mutations have not been detected in persistent cytotoxic T lymphocyte expansions following allogeneic HSCT that remained stable over the time[62] nor in inflammatory myositis.[86] This absence of mutations may indicate a scenario in which an equilibrium is reached between immune system control and the triggering event(s). Should mutations disrupt this equilibrium, the clone undergoes progressive expansion with detrimental effects. These findings offer novel insights into this neglected topic, underscoring the need for future prospective studies to track early steps of disease and to determine which patients, when, and under what circumstances progress to having full-blown disease. Steps to progression are likely mediated by multiple events, which include not only mutations but also dysregulated pathways, and the influence of the microenvironment. Indeed, the absence of any LGLL phenotype in mice expressing *STAT3* mutations[87] suggests that additional gene mutations or deregulation of other signaling molecules or pathways[30] might be involved in association with *STAT3* mutations in the pathogenesis of LGLL. Taken together, these findings underscore the complexity of the disease and the need for further research to elucidate the underlying mechanisms and the discovery of molecules/pathways that may be attractive for immunotherapeutic approaches.

Table 3. Distinguishing features of T-cell clones of uncertain significance and T-large granular lymphocyte leukemia.

Variables	T-CUS	T-LGLL
Clone size	≤0.5 LGL x10^9/L	>0.5 LGL x10^9/L
Clinical manifestations	Absent	From mild to aggressive
Associated diseases	Absent	Detectable in a variable proportion of cases
Mutational pattern	Preliminary data indicate lack of somatic mutations	Somatic mutations are detected in approximately >50% of cases
Bone marrow involvement[#]	Absent	Present
Disease subtypes	$CD8^+$ Tα/β, $CD4^+$ Tα/β and Tγ/δ. Frequency to be defined	$CD8^+$ Tα/β (~65%), $CD4^+$ Tα/β (~25%) and $Tγ/δ^-$ (~10%)
Treatment	None	Indications for treatment include severe cytopenias, particularly neutropenia associated with recurrent infections

[#]Usually unnecessary in T-cell clones of uncertain significance; sometimes performed in low-count T-large granular lymphocyte leukemia (for differential diagnosis from myelodysplastic syndromes or other cytopenias). T-CUS: T-cell clones of uncertain significance; T-LGLL: T-large granular lymphocyte leukemia; LGL: large granular lymphocytes.

The discovery of genetic lesions and/or biological features universally recognized as robust and reliable markers that predict the patients who are or who are not at risk of a subsequent diagnosis of T-LGLL is an unmet clinical need. Studying the temporal longitudinal dynamics of T-CUS with aging, as well as the correlation with clone size, and possibly of subclones, is warranted. However, the small number of rogue cells typically creates a limitation to this approach. Furthermore, the discovery of new dependencies beyond the STAT pathways may reveal new vulnerabilities in leukemic cells which could be targeted by innovative strategies. Bridging these gaps would ultimately contribute to overcoming reliance on numerical cutoffs, which are currently the standard criteria to differentiate the benign condition from the T-LGLL-related malignancy. Our understanding of the matter may also be translated into an appropriate diagnostic interpretation that distinguishes T-CUS from T-cell malignancies. This translation, leveraging on specific biological or genetic markers, will bring the field closer to an answer empowering treating physicians with greater confidence in informing patients about their risk of progression and, ultimately, in improving clinical management.

Disclosures

No conflicts of interest to disclose.

Contributions

GS led the writing of the article with input from the other authors. All the authors read and approved the final manuscript.

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