PATHOGENESIS, TREATMENT, AND FUTURE DIRECTIONS FOR RARE T-CELL LEUKEMIAS

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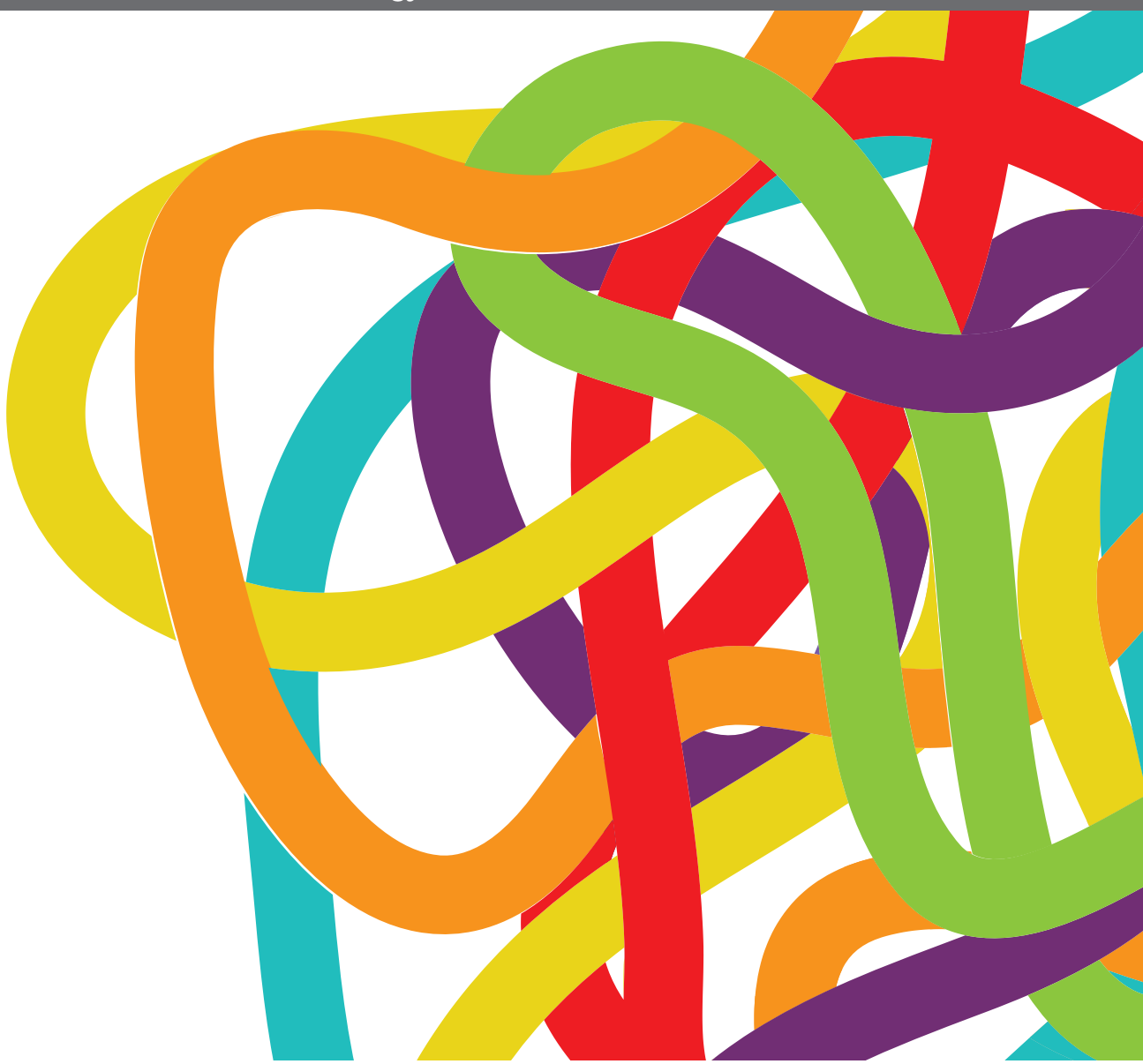

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PATHOGENESIS, TREATMENT, AND FUTURE DIRECTIONS FOR RARE T-CELL LEUKEMIAS

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Table of Contents

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Editorial: Pathogenesis, treatment, and future directions for rare T-cell leukemias

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Editorial on the Research Topic
Pathogenesis, treatment, and future directions for rare T-Cell leukemias

Mature T-cell leukemias represent rare, but increasingly recognized diseases of which, compared to their B-cell counterparts, comparatively little is established on their pathogenesis, diagnosis, and treatment. These leukemic post-thymic T-cell neoplasms range from the spectrum of chronic, sometimes debilitating disorders such as T-large granular lymphocytic leukemia (T-LGLL), and related leukemias such as NK-LGLL, to more aggressive malignancies such as T- prolymphocytic leukemia (T-PLL). In this series, entitled 'Pathogenesis, Treatment, and Future Directions for Rare T-cell Leukemias' we review the current state of the science of these important T-cell neoplasms to inform on their treatment, diagnosis, and pathophysiology.

First, in the review by El-Sharkawi et al., the diagnosis of T-cell leukemias is appraised in detail, with a practical guide to the spectrum of T-cell leukemias. Subsequently, the series can be divided between different reports on T-PLL and T-LGLL, with one paper by Yin et al., evaluating the prognostic importance of genomic mutations in patients with (immature) T-cell acute lymphoblastic leukemia.

Two papers review our current understanding of the pathogenesis and management of T-PLL. In the review by Braun et al., the authors summarize the known pathogenetic data of T-PLL and propose an intriguing model using the key molecular drivers of T-PLL to inform future translational approaches. In the second review by Varadarajan and Ballen, the authors describe the current state of cellular therapies, including allogeneic

stem cell transplantation and emerging novel strategies to treat T-PLL that will guide clinicians as they seek to provide curative therapies for these patients.

A key focus of this series is on T-LGLL in which 7 papers, ranging from original data and cases series to cross-disciplinary reviews, provide a perspective on the current understanding of this disease. Drillet et al., review recent data on the diagnosis of NK-LGL, and provide a classification system that will likely serve as the standard for categorizing this rare leukemia for future investigations. Cytokines are integral in the biology of T-LGLL, and Isabelle et al., provide the most comprehensive review of cytokines and their contribution to T-LGLL pathogenesis to date. T-LGLL often overlaps with autoimmune disorders, such as rheumatoid arthritis (RA), hence, providing a fascinating opportunity to explore the intersection between cancer and autoimmunity, with important implications for the management of both. In the review by Couette et al., the authors evaluate the pathogenesis of T-LGLL, particularly as it relates to cytokines and key molecular pathways in a broad array of autoimmune diseases. In a focused review evaluating the intersection between RA and LGLL, Moosic et al., outline the current understanding of the mechanistic links between RA and LGLL. In two reports by Pflug et al., and Schreiber et al., the authors present illustrative case catalogues of T-LGLL, with a focus on diagnosis and cross-disciplinary management of these often complex patients, with a review of current treatment strategies. Finally, in an original report by Braunstein et al., the authors present the largest series of patients with concomitant plasma cell dyscrasias and T-cell malignancies, including T-LGLL to date, raising awareness of these co-incident disorders, with important recommendations on the management of these diseases.

This Research Topic represents the current state-of-the art understanding of mature T-cell leukemias, with a focus on T-PLL and T-LGLL. The knowledge gained from recent investigations into these diseases has led to increased interest not only amongst lab-based and clinical researchers, but also among pharmaceutical companies to address these rare malignancies. In T-PLL, this has manifested in the work of the T-PLL International Study Group (TPLL-ISG), that is leading the development of novel clinical trials based on the current understanding of the pathogenesis of T-PLL, as outlined in the review by Braun et al. This group has recently published consensus criteria on the diagnosis and treatment responses for this disease, an important step in developing trials for T-PLL (1). In fact, several trials are currently enrolling for patients with T-PLL and target the pathways described in the review by Braun et al., (NCT04496349, NCT03989466). Similarly, in T-LGLL, there has been renewed interest in developing novel therapeutics, given the modest efficacy of current immune-suppressive therapies. In particular, research has targeted the cytokine IL-15, as this is thought to be the central cytokine that drives the pathogenesis of T-LGL as was elegantly outlined in the reviews by Isabelle et al. and Couette et al. A recently completed phase I/II study utilized the selective cytokine inhibiting peptide BNZ-1 in T-LGLL patients, and reported clinical efficacy and near-universal apoptosis of *in vivo* T-LGLL cells, demonstrating the cytokine dependence of T-LGLL (2). Further, using an alternate approach targeting IL-15, the only currently enrolling prospective trial in the United States (NCT05141682) uses the hypomethylating agent CC-486 to treat patients with T-LGLL based on data demonstrating its efficacy in decreasing IL-15 (3). Using these approaches and others, we hope that significant progress can be made in treating this rare disease.

Finally, the editors wish to thank all who contributed to this important Research Topic. It is our sincere hope that this Research Topic will help to educate and inspire the development of innovative treatment approaches in these rare diseases that will impact patient outcomes.

Author contributions

MH contributed to the development of the manuscript, revised the manuscript, and approved the final version WJ contributed to the development of the manuscript, revised the manuscript, and approved the final version AM contributed to the development of the manuscript, revised the manuscript, and approved the final version JB wrote the manuscript, revised the manuscript. All authors contributed to the article and approved the submitted version.

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Finally, the editors wish to thank all who contributed to this important series.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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MINI REVIEW
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Advanced Pathogenetic Concepts in T-Cell Prolymphocytic Leukemia and Their Translational Impact

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T-cell prolymphocytic leukemia (T-PLL) is the most common mature T-cell leukemia. It is a typically aggressively growing and chemotherapy-resistant malignancy with a poor prognosis. T-PLL cells resemble activated, post-thymic T-lymphocytes with memory-type effector functions. Constitutive transcriptional activation of genes of the T-cell leukemia 1 (TCL1) family based on genomic inversions/translocations is recognized as a key event in T-PLL's pathogenesis. TCL1's multiple effector pathways include the enhancement of T-cell receptor (TCR) signals. New molecular dependencies around responses to DNA damage, including repair and apoptosis regulation, as well as alterations of cytokine and non-TCR activation signaling were identified as perturbed hallmark pathways within the past years. We currently witness these vulnerabilities to be interrogated in first pre-clinical concepts and initial clinical testing in relapsed/refractory T-PLL patients. We summarize here the current knowledge on the molecular understanding of T-PLL's pathobiology and critically assess the true translational progress around this to help appraisal by caregivers and patients. Overall, the contemporary concepts on T-PLL's pathobiology are condensed in a comprehensive mechanistic disease model and promising interventional strategies derived from it are highlighted.

Keywords: T-PLL, clonal evolution, pathogenesis, TCL1A, ATM

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INTRODUCTION

T-cell prolymphocytic leukemia (T-PLL) is an aggressive peripheral T-cell malignancy (1) and represents the most common mature T-cell leukemia in Western countries (incidence ≈ 2.0/million/year) (2). Patients suffering from T-PLL typically present with exponentially rising white blood cell counts, (hepato-) splenomegaly, and small-node lymphadenopathy. CNS involvement has been described as a severe clinical manifestation in a minority of T-PLL (<5% of cases) (3, 4). The rapidly expanding and chemotherapy-refractory course is reflected by a median overall survival from diagnosis of less than 3 years (5, 6). Up to now, the humanized CD52-antibody alemtuzumab is the only substance that induces acceptably high response rates, (in >80% of patients at first line).

Notably, nearly all patients relapse within 2 years after alemtuzumab, with very limited options to salvage (4, 7).

First described in 1973 (8), the diagnosis of T-PLL was mainly based on cytomorphological characteristics (6). In the following decades, the pathogenetic concept of T-PLL was centered around cytogenetic abnormalities. *Inversions* or *translocations* of the *TCL1A* locus are the most common chromosomal aberrations and are central in establishing the diagnosis of T-PLL (9). Within the last 5-7 years, genomic and epigenomic studies have remarkably expanded our pathogenetic understanding of T-PLL. More recently, molecular hallmarks around perturbed responses to DNA damage, including repair and apoptosis, as well as alterations of cytokine signaling and epigenetic deregulations, were identified as exploitable dependencies. Here, we condense these novel advances in a comprehensive mechanistic disease concept and highlight promising interventional strategies that are being derived from it.

CELL OF ORIGIN CONCEPTS

In >95% of T-PLL, aberrant constitutive expression of the proto-oncogenes *TCL1A* or *MTCP1* by *inversions* or *translocations* are observed that juxtapose the *TCL1A* (at 14q32.1) or *MTCP1* (at Xq28) loci to the 14q11.2 locus and by that under control of highly active *TRA* gene enhancer elements. This prevents physiological downregulation of *TCL1A* or *MTCP*1 and is considered the initial event of T-PLL's leukemogenesis (10). Both oncogenes have shown their oncogenic potential in transgenic mouse models (11–13). Under physiological conditions, expression of the *TCL1A* oncogene is silenced in CD4/CD8 double-positive (dp) thymocytes (14, 15). At this stage, rearrangements of the *TRA* locus, encoding for the T-cell receptor (TCR) α-chain, take place (16). Whole-genome sequencing and breakpoint analyses identified that all T-PLL had a breakpoint involving recombination signal sequences (RSS) of the J region of the *TRA* locus. On the opposite side of the *inversion/translocation*, breakpoints were more variable, but also involved classical or cryptic RSS (17). In accordance with the finding that virtually all T-PLL express the surface TCR complex (18), the other allele of the analyzed T-PLL cases showed legitimate *TRA* rearrangements, leading to the expression of a functional TCR (17). Together, these findings suggest, that the aberrant *TRA-TCL1A/MTCP1* rearrangements occur during the opening of the *TRA* locus at the CD4/CD8 dp thymocyte stage in a RAG1/2 dependent manner (17), followed by legitimate recombination of the locus on the other allele. High TCL1A expression is associated with genomic instability (19), thereby forming the basis for additional genomic hits driving oncogenesis (9, 10). However, whether the illegitimate rearrangement is the first hit in the pathogenesis of T-PLL is uncertain. A preceding mono-allelic deletion or mutation of *ATM*, which are highly recurrent in T-PLL cells, is possible as well. This is supported by a high incidence of T-PLL in patients with germline *ATM* defects as well as its involvement in the regulation of monoallelic cleavage and genomic stability during *TRA* recombination (20).

STRUCTURAL GENOMIC ABERRATIONS

Complex karyotypes (≥3 structural or numerical cytogenetic aberrations) are seen in ~70% of T-PLL and were associated with a poorer prognosis (21). T-PLL genomes usually show complex somatic DNA copy number alterations (CNA) in array-based profiling (10, 21, 22). Generally, losses of chromosomal regions are more frequent than gains. These somatic CNA usually affect hundreds of genes in a patient and are not closely associated with altered expression of the respective genes, indicating additional modes of transcriptional dysregulation beyond CNA. Besides the above-described aberrations affecting genes of the *TCL1* family, genomic losses of chromosome 11q and gains of chromosome 8q are most recurrently observed. Losses affecting chromosome 11 involve the tumor suppressor *ATM* (11q22.3) as the minimally deleted region (6, 10, 19, 21–30). This is implicated in T-PLL development by dysregulation of proper DNA damage repair as highlighted by more complex karyotypes in *ATM* deleted cases (10). The genomic region encoding for the downstream effector of ATM, p53, is only disrupted in a minority of T-PLL (10). Gains of chromosome 8q can mainly be attributed to a trisomy of 8q, resulting from isochromosomes (8)(q10) (29). Overexpression of the proto-oncogene *MYC* (8q24.21) is not strictly associated with the presence of 8q gains and vice versa. Other genes like *AGO2* at 8q24.3 are more frequently involved in these 8q amplifications. Overexpression of AGO2, which centrally regulates RNA interference, may additionally contribute to T-PLL development (10).

At lower frequencies, genomic losses of chromosomes 6q, 8p, 12p, 13q, and 22q as well as genomic amplifications of 6p and 22q are observed in T-PLL cells (10, 21–23, 27). Up to now, the underlying target genes of these structural aberrations and their functional contributions have not been fully revealed. First promising concepts could derive from a systems biology approach (31). Genome-wide gene expression and copy number profiles of T-PLL patients could be utilized to learn a T-PLL specific gene regulatory network (32). Such a network would allow to predict potential impacts of individual CNA on known cellular signaling pathways or treatment response signatures by network propagation (32), as demonstrated for oligodendrogliomas (33) and prostate carcinomas (34). Thus, more intensified efforts on integrating available genome-wide data could help to identify new potential driver candidates and their downstream targets in T-PLL.

THE MUTATIONAL PROFILE OF T-PLL

Besides the highly prevalent structural lesions involving the oncogenes *TCL1A*, *AGO2*, and *MYC*, as well as in the tumor suppressor *ATM*, various single-nucleotide variants (SNVs) were linked to the molecular pathogenesis of T-PLL cells (10, 26, 35, 36). Generally, SNVs occur at similar rates in T-PLL as in other hematologic and solid tumors (10). Most of these primarily somatic SNVs seem to accumulate during T-PLL's leukemogenesis in the

context of high levels of oxidative damage and in the absence of efficient repair mechanisms to counteract these hazards (10). Fittingly, *ATM*, the central apical regulator of DNA integrity, shows high rates of damaging SNVs, in addition to the above-described partial inactivation by mono-allelic losses (10, 24, 26, 35–38). These missense, nonsense, or frameshift mutations of *ATM* mainly cluster within its FAT or PI3K domains (10).

Other frequently mutated genes in T-PLL are *CHEK2*, *SAMHD1*, and *MSH*, which are also involved in DNA damage repair mechanisms, which further supports a concept of T-PLL's incompetence in safeguarding mechanisms of repair or cell death execution (10, 26, 35, 36). Remarkably, *SAMHD1* and *ATM* belong to the small fraction of genes, whose mutations show variant allele fractions (VAFs) of more than 80% (10, 35), suggesting acquisition of these lesions early in leukemogenesis.

Within the last decade, genomic aberrations affecting the JAK/STAT signaling pathway emerged as an additional hallmark of T-PLL (10, 26, 35, 36, 38–42). The *JAK3* gene shows the highest frequency of such gain-of-function mutations, followed by *STAT5* and *JAK1 (*43). These primarily missense mutations target the conserved pseudokinase (*JAK1*, *JAK3*) or SH2 domains (*STAT5*) in most T-PLL cases. Notably, SNVs affecting components of the JAK/STAT signaling pathway occur at relatively low VAFs, indicating their rather sub-clonal character (10). However, the central role of deregulated JAK/STAT signaling is substantiated by genomic losses of genes that encode for negative regulators of this pathway (e.g. *DUSP4*, *SOCS* genes) (43). Together with the high frequency of *JAK*/*STAT* gene mutations, basal phosphorylation of distal STAT5 is observed in virtually every T-PLL case (10, 43). In addition, the WNT as well as the Notch signaling pathways, are disturbed by SNVs in a minority of T-PLL cases (10, 26). Rare mutations further involve cell cycle regulation (e.g. *CDC27*) and apoptosis regulation (e.g. *BCLAF1*) (10).

THE TRANSCRIPTOMIC LANDSCAPE

Analyses of the transcriptome of T-PLL cells have been performed intensively in bulk RNA samples, either by gene expression arrays or by RNA sequencing (RNA-seq). In line with rearrangements of the chromosome 14q, *TCL1A* was the most upregulated gene in virtually every cohort (10, 35, 42, 44). The other TCL1 family members, *TCL1B* and *MTCP1*, showed additional overexpression, although to a lower extent (10). In agreement with the gains at chromosome 8q, the proto-oncogene *MYC* as well as the miR-processing regulator *AGO2* showed overexpression on mRNA level (10, 42). Highlighting the importance of deregulated JAK/STAT signaling in T-PLL, downstream targets of this pathway (e.g. BCL2L1) showed a significant upregulation (42).

Among the genes with the most significantly altered expression were those involved in TCR/cytokine signaling. Prominent examples are downregulated *CTLA4* and *SLAMF6*. They are central mediators of immune signal transduction and regulation of lymphocyte activation and we implicate their loss in the activated T-cell phenotype of the T-PLL cell (10, 18, 22). Moreover, potential underlying causes for the inability of T-PLL cells to undergo cell death upon DNA damage were identified in their altered transcriptome: Pro-apoptotic genes (e.g. *GIMAP5*, various Caspases) were significantly downregulated (10, 22). Transcriptome studies can also be utilized to identify individualized treatment options for T-PLL patients. In a first case study, RNA-seq data were integrated with exome-seq and *ex vivo* single-drug sensitivities, establishing a customized platform on individual predictions of responses to drug combinations (39).

THE MIR-OME OF T-PLL CELLS

Recently, the miR-ome of T-PLL cells was analyzed by small RNA-seq in two independent cohorts (44, 45). T-PLL cells showed a global miR expression signature of ~35 significantly deregulated miRs, resembling the miR expression profile of TCR-activated healthy T-cells (45). By combining the small RNA-seq with transcriptome sequencing data, regulatory networks involving cell survival signaling and DNA repair pathways were uncovered. In both cohorts, the miR-141/200c cluster showed the strongest upregulation among all miRs and separated T-PLL cases into two major subgroups with normal vs. upregulated expression. Preliminary data revealed a role of this cluster in TGF-β signaling (44) as well as in cell cycle regulation (45). Further perturbations of miR expression include overexpression of miR-223-3p and miR-181a/miR-181 as well as downregulation of the miR-21 and the miR-29 cluster. The functional consequences of these deregulations have yet to be demonstrated in T-PLL. Nevertheless, based on the expression of miR-200a-3p, miR-223-3p, and miR-424-5p, a first overall survival score for T-PLL (miROS-TPLL) was established and might improve clinical stratifications (45).

EPIGENETIC ALTERATIONS

Gene set enrichment analyses of T-PLL transcriptomes identified pathways of epigenetic regulation as significantly altered (10). These findings were additionally highlighted by a high incidence of mutations in epigenetic modifiers (e.g. *EZH2*, *TET2*, *KMTs*) *(*10, 26, 35, 36). However, systematic analyses of DNA-methylation, profiles of histone modifications, and states of chromatin accessibility have not yet been published. First data in a small cohort of T-PLL implicate massive epigenetic reprogramming, as shown by genome-wide alterations of chromatin states at promoters and active enhancers identified *via* H3K4me3 and H3K27ac ChIP-seq (46). These alterations correlated with changes in expression of frequently deregulated genes (e.g. *TCL1A*, *MYC*, *EZH2*, *AGO2*), presenting additional ways of their deregulation beyond the described genomic aberrations. Vice versa, a role of *TCL1A/MTCP1* activation and/or *ATM* inactivation in epigenetic disturbances is also conceivable (47, 48).

THE MICROENVIRONMENT OF T-PLL CELLS

Besides (epi)genetic changes, the dependence of leukemic cells on signals from microenvironmental sources for proliferation and survival has been shown for various entities, including T-cell neoplasms (49). Such interactions are mediated by adhesion molecules, cell surface ligands, chemokines, cytokines, and their respective receptors (50). So far, little is known about the (specific) micromilieu of T-PLL cells and how they shape it. Upregulation of cytokines (e.g. TNF, IL-8), cytokine receptors (e.g. CD25 (IL-2Rα), CD122 (IL-2Rβ), CD124, or CD127), as well as of chemokine receptors (e.g. CCR3 and CCR4) provide first hints of a deregulated crosstalk between T-PLL and bystander cells (18). Furthermore, mutations of chemokine receptors (e.g. CXCR3) are described (10). The potential proactive role of the micromilieu in T-PLL's leukemogenesis is further implicated by the secretion of the Th1-associated cytokines IFN-γ, IL-2, IL-10, TNF-α/β, and IL-8 of T-PLL cells upon TCR stimulation (18). Mechanistic proof for an involvement of CCR7 in the sustenance of T-PLL cell survival derives from studies with CCR7-blocking antibodies. They impaired survival signaling pathways in T-PLL cells *in vitro* and increased the survival of mice transplanted with the T-PLL-like cell line SUP-T11 (51). More work is required to study the composition of T-PLL's microenvironment (i.e. cell types and humoral factors) and the involved molecular interactions.

ROLE OF THE T-CELL RECEPTOR

TCR signaling is the major growth regulatory system of T-cells. It shapes their maturation, differentiation, and activation, hence their effector and tolerogenic capacity (52, 53). Amplification of TCR signaling represents a feature of many T-cell malignancies, although generated by distinct mechanisms (54): (*i*) decreased input thresholds for continuous exogeneous TCR activation, (*ii*) autonomous activation of TCR-signaling intermediates, (*iii*) downregulation of inhibitory coregulators, or (*iv*) stand-ins for TCR signals, such as strong cytokine-inputs or their mimics, e.g. *via* the ALK oncogene. T-PLL cells usually express at least one surface component of the TCR/coreceptor complex and show robust TCR-signal competence when stimulated *ex vivo (*9, 18). Their gene expression profiles show prominent signatures of TCR activation (10). Notably, TCL1A acts as a physically engaging coactivator of TCR-kinases such as AKT, ZAP70, or ERK, and by that is a TCR-signal enhancer, hence, a sensitizer towards low-abundance signals. That places T-PLL into model (*i*) of the TCR-centric pathogenetic view of T-cell neoplasms (18, 54).

Enhanced TCR signaling is further established in T-PLL cells by impaired control mechanisms [model (*iii*)], e.g. by downregulation of negative coregulators such as SLAMFs or checkpoint molecules such as CTLA4 (10). The resulting activated phenotype of T-PLL cells is additionally accompanied by a TCL1A-mediated inability to execute FAS-mediated and activation-induced cell death (18).

In line with their TCR signaling competence, T-PLL cells reveal a phenotype of mature, antigen-experienced, non-conventional memory T-cells (18). As an underlying principle, it is tempting to speculate that through enhanced TCR signaling, the transition of naïve T-cells into an expanding pool of memory T-cells is accelerated. The lack of a common TCR clonotype across cases would indicate that not a specific antigen drives TCR-mediated outgrowth in T-PLL (18, 55). More likely is an MHC-dependent TCR activation through various low-avidity (auto)antigens or antigen-independent tonic signals at place, either MHC-driven or *via* TCR self-activation in enabled memory T-cells. Although treatment strategies that target TCR signaling intermediates have shown promising potential (56), the TCR dependence of T-PLL cells at the overt leukemic stage is not conclusively clarified.

DISCUSSION

Model of Clonal Evolution of T-PLL Cells

Recent advances in omics technologies over the last decade have elevated the molecular understanding of T-PLL to another level (**Figure 1**, **Supplementary Table 1**). *Translocations* and *inversions* of chromosome 14q at the dp thymocyte stage are perceived to initiate T-PLL's leukemogenesis (10, 17). These genomic aberrations lead to overexpression of the proto-oncogenes *TCL1A* and *MTCP1* and result in apoptotic resistance and genomic instability (19). TCL1 family-activating lesions form a functionally perturbing cooperation with (preceding or subsequent) lesions that impair the tumor suppressor ATM, which further incapacitate the T-PLL cell to execute safeguarding responses (10). Likely, additional perturbations are operational for this $TCL1^{up}/ATM^{def}$ leukemic precursor to finally escape T-cell homeostatic control. These are acquired by lesions that activate JAK/STAT signaling (43), by miR (processing) deregulations (44, 45), by *MYC* amplification (6, 10), and by deregulated epigenetic mechanisms (10, 36). To a lesser degree we understand, on which central functional levels, such as TCR- or cytokine signaling or autocrine forward-feeding loops, these (epi)genetic events have a direct or less immediate impact.

Overall, many questions of T-PLL's pathogenesis remain unresolved, like (*i*) the role of pro-survival signals of T-PLL's bystander cells, (*ii*) the dependence of T-PLL cells on their TCR in clonal sustenance, (*iii*) the nature of T-PLL's epigenome, and (*iv*) the mechanisms of disease progression and treatment resistance. Especially the latter aspect calls for single-cell resolved analyses to illustrate clonal oscillations.

Clinical Implications Derived From the Current Disease Model

The identification of key drivers of the molecular pathogenesis of T-PLL offers the possibility for the development of new drugs that target its crucial pathways. Here, central pathogenetic relevance is likely not equivalent to a major vulnerability, which requires more thorough interrogations. However, there

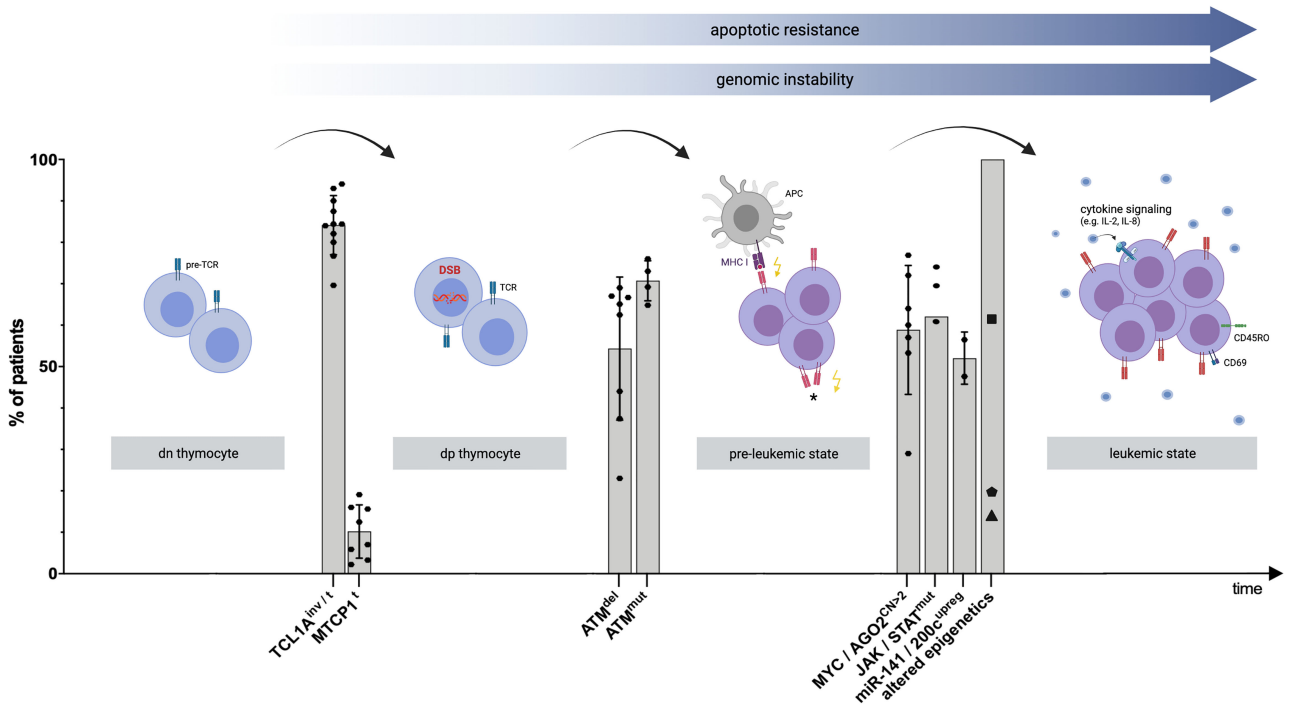

FIGURE 1 | Proposed model of clonal evolution of T-PLL cells. Schematic visualization explaining T-PLL's leukemogenesis, based on recent genomic profiling series and corresponding functional assessments. Timeline: Chronology of genomic events leading to the progression to an advanced state of (pre)malignant T-cell development. Y-Axis: Percentage of all analyzed T-PLL patients presenting the respective genomic aberration. Each dot represents a prevalence, derived from selected publications (**Supplementary Table 1**). The median, as well as standard deviation, out of these publications was calculated for each genomic event. The variability between the studies can be attributed to the different methods and cohort sizes (for more information refer to **Supplementary Table 1**). The first 'stage' involves the double-negative (dn) thymocyte, carrying the pre-T cell receptor (pre-TCR) complex. *Translocations* (*t*) and *inversions* (*inv*) of chromosome 14q at the dp thymocyte stage result in constitutive expression of the proto-oncogenes *TCL1A* or *MTCP1* in a vast majority of T-PLL cases (9, 17). These hits impair the genomic stability of the affected T-cell by reduced DNA repair capacities of DNA double-stranded breaks (DSB) or other (oxidative) insults (10). *Deletions* (*del*) and *mutations* (*mut*) involving ATM lead to a functionally hypomorphic apical regulator of repair, cell fate, and cell cycle control of the T-PLL precursor. This pre-leukemic cell becomes unable to execute such safeguarding mechanisms upon genotoxic stress (10). Among subsequent perturbations, TCL1A overexpression lowers TCR-signaling thresholds (18), enabling the cell to sustain on low-level input, either by major histocompatibility complex (MHC)-dependent (auto) antigen-presenting cells (APC), or by self-MHC drive only, or by autonomous TCR activation (*, not proven). A central distal node is the JAK/STAT transcriptional machinery. Besides major growth pathways such as the TCR and cytokine-mediated cascades feeding into it, there also is a high prevalence of hyperactivating mutations that target *JAK1*, *JAK3*, or *STAT5B* (18) and a high incidence of losses of JAK/STAT negative regulators (43). Further leukemic outgrowth and progression to an exponentially proliferating T-PLL cell are likely mediated by additional aberrations, including copy number (CN) gains on chromosome 8q, leading to *MYC* amplification and *AGO2* overexpression (10). Furthermore, deregulations of T-PLL's miR-ome, exemplarily represented by the upregulation (upreg) of the miR-141/200c family (45), and of T-PLL's epigenome in virtually all patients as shown by altered chromatin states at promoters and active enhancers (46), potentially mediated by frequent mutations in *KMTs*(■), *TET2* (⬟), and *EZH2* (▲) (10), contribute to the final leukemic outgrowth of a transformed and activated T-cell (as shown by the T-cell activation marker CD69) with memory-type effector functions (as shown by CD45RO surface expression) (18). The figure was created by the authors using Biorender.com.

is sound reason to be optimistic that we will soon see novel strategies against T-PLL cells to become the basis for future combinatorial therapies. Exemplarily, agents targeting TCR signaling or the JAK/STAT pathway (18, 56) show encouraging results, preclinically and/or in first case reports (57, 58). In addition, the inability of T-PLL cells to induce adequate responses to DNA insults was translated into therapeutic strategies to reactivate p53 *via* MDM2/MDMx inhibitors or targeting BCL2 family members (e.g. Venetoclax) (10, 59, 60). There are ongoing activities in the search for efficacious combinations of the, as single agent clinically only moderately active Venetoclax, with other classes of inhibitors in relapsed/refractory (r/r) T-PLL (59–62). In addition, epigenetic disturbances of T-PLL cells further emphasize hypomethylating agents (e.g. Cladribine) as well as inhibitors of deacetylating enzymes (e.g. Romidepsin) as options (10, 63, 64). Combining these drugs, which target molecular vulnerabilities of T-PLL cells, with the current standard therapy of alemtuzumab represents another promising approach. Another challenge to be addressed is the 'purposing' of the innate or adaptive immune system to specifically attack T-PLL cells (65).

AUTHOR CONTRIBUTIONS

TB, AD, GF, MS, JS, and MH contributed to initial and subsequent drafts of the manuscript. TB, AD, and MH designed and drew the figure and corresponding table. All

authors contributed to the article and approved the submitted version.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fonc.2021.775363/full#supplementary-material

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Case Report: Large Granular Lymphocyte Leukemia (LGLL)—A Case Series of Challenging Presentations

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Large granular lymphocyte leukemia (LGLL) represents a rare group of diseases with considerable difficulties in their correct diagnostic workup and therapy. The major challenges lie in their distinction from reactive (including autoimmune) lymphoproliferations. Moreover, monoclonal LGL proliferative diseases are in fact a heterogeneous group of disorders, as recognized by the three subtypes in the current WHO classification. It distinguishes two chronic forms (the focus of this case series), namely T-LGLL and chronic lymphoproliferative disorders of Natural Killer cells (CLPD-NK) as well as aggressive NK-cell leukemia. In the clinical routine, the variable presentations and phenotypes of T-LGLL and CLPD-NK are underappreciated. The relevant differential diagnoses range from benign reactive T-cell expansions to other mature T-cell leukemias to highly aggressive γδ-lymphomas. T-LGLL or CLPD-NK patients suffer from a wide variety of symptoms often including, but not limited to, cytopenias or classical autoimmune phenomena. They receive treatments ranging from mere supportive measures (e.g. antibiotics, growth factors, transfusions) over strategies of immunosuppression up to anti-leukemic therapies. The diagnostic pitfalls range from recognition of the subtle T-cell proliferation, repeated establishment of monoclonality, assignment to a descript immunophenotypic pattern, and interpretations of molecular aberrancies. Here, we report a series of selected cases to represent the spectrum of LGLL. The purpose is to raise awareness among the scientifically or practically interested readers of the wide variety of clinical, immunological, and phenotypic features of the various forms of LGLL, e.g. of T-cell type, including its γδ forms or those of NK-lineage. We highlight the characteristics and courses of four unique cases from two academic centers, including those from a prospective nationwide LGLL registry. Each case of this instructive catalogue serves to transport a key message from the areas of (chronic inflammatory)

contexts in which LGLL can arise as well as from the fields of differential diagnostics and of various treatment options. Implications for optimization in these areas are discussed.

Keywords: LGL leukemia, STAT-3, immunosuppression, NK, TCR, CLPD-NK

INTRODUCTION

T-cell large granular lymphocyte leukemia (T-LGLL) is a rare neoplasm, accounting for approximately 2-5% of chronic lymphoproliferative diseases in Western countries. It is characterized by clonal expansion of cytotoxic, often autoimmune reactive, mature T-cells (1). Next to T-LGLL, the 2016 World Health Organization classification of mature T- and Natural Killer (NK)-cell neoplasms, also lists the provisional entity of chronic lymphoproliferative disorders of NK cells (CLPD-NK) and aggressive NK-cell leukemia (ANKL) among monoclonal LGL proliferative diseases (2). T-LGLL, an expansion of CD3+ T-cell large granular lymphocytes (LGLs), is the most frequent variant representing ~85% of LGL proliferations and can further be subdivided into the common αβ-form and the rarer γδ-variant. Among the coreceptors $CD8^+$ is usually more commonly expressed than $CD4^+$. Additionally, mixed phenotype forms have been reported (3, 4). CLPD-NK accounts for approximately 10% and ANKL for approximately 5% of LGL proliferations. This case series excludes ANKL due to its clearly distinguished clinical and molecular features and different treatment approaches. We focus here on T-LGLL and CLPD-NK, both being referred to as LGLL.

The clinical presentation of LGLL is variable and typically includes cytopenias (particularly neutropenia and anemia), but often also symptoms of associated autoimmune disorders (mostly rheumatoid arthritis (RA), but also connective tissue diseases or vasculitis) (5). Furthermore, LGLL can be associated with secondary neoplasms, especially clonal B-cell expansions, but also solid cancers. The median patient age at diagnosis is 66 years, but approximately 15% of patients are younger than 50 years with an equal sex distribution (6, 7). Despite the course of LGLL being described as 'indolent', it is far from low-symptomatic and still associated with a shortened median overall survival (OS) of 9-10 years (8). Disease-related deaths are mainly due to severe infections. Such complications of the cytopenias and the autoimmune phenomena severely impair the quality of life of LGLL patients.

Diagnosis and management of LGLL is a challenge even for large academic centers. According to the WHO classification the diagnosis of LGLL requires a persistent (>6 months) increase in the number of peripheral blood (pB) LGL cells, usually 2-20 x 10^9/L, without a clearly identified cause (2). Clonality is mandatory to be established and usually done by T-cell receptor (TCR) gene rearrangement studies (9, 10). In CLPD-NK monoclonality can indirectly be assessed by a restricted pattern of killer-cell immunoglobulin-like receptor (KIR) expression *via* flow-cytometric immunophenotyping, which is only done in few specialized laboratories. As a molecular hallmark, many LGLL harbor a genomic lesion of the signal transducer and activator of transcription 3 (*STAT3*). The gain-of-function *STAT3* mutations D661 and Y640 account for two-thirds of such variants (9). Additionally, variants of *STAT5B* have been recognized in a minority of T-LGLL cases. Both mutations cause constitutive activation of the JAK/STAT signaling pathway (9, 11). While former studies did not find a clear impact of these lesions on clinical outcome (9, 11), a recent retrospective single-center analysis of a large LGLL cohort found an independent association of *STAT3* mutations with shorter OS (4). More recently, missense mutations of the epigenetic regulator *TET2* were identified as another major genomic hallmark in CLPD-NK (12, 13).

Overall, the diagnostic pitfalls in LGLL range from recognition of the subtle T-cell or NK-cell proliferation, repeated establishment of their clonality, distinction of the LGLL clone from normal (T-) lymphocytes by a unique immunophenotype as well as detection and interpretation of molecular aberrancies in the context of a commonly normal karyotype. Additional diagnostic challenges are imposed by a coexisting RA or by laboratory findings of an autoimmune hemolytic anemia (AIHA) or of a pure red cell aplasia (PRCA) or of myelodysplasia. Problems in differential diagnosis also expand to the differentiation from related conditions such as Felty-syndrome or from other mature T-cell leukemias/lymphomas such as T-cell prolymphocytic leukemia (T-PLL) or hepato-splenic T-cell lymphoma (HSTL) (14).

With respect to its therapeutic management, LGLL is considered incurable by currently available options, including immunosuppressive agents and low-dose chemotherapy. Treatment-defining prospective trials are hardly available. For a summary of tested strategies see (15). Furthermore, there is a great deal of uncertainty regarding the optimal timing of treatment initiation.

Here we present four challenging and instructive cases of LGLL that presented to our centers with typical as well as rare features of this heterogeneous disease. This case catalogue serves to emphasize numerous diagnostic pitfalls, unique clinical scenarios, and various therapeutic modalities. Typical characteristics and special features are presented in **Table 1**.

CASES

Patient 1

A 62-year-old Caucasian male presented in 2019 with weight loss, transfusion-dependent anemia, and thrombocytopenia with bleeding-stigmata. Three years prior to diagnosis he developed a mild anemia without signs of hemolysis, but with detection of a population of atypical NK cells in pB. Two subsequent bone marrow (BM) examinations, however, did not show any signs of a hematological disorder. The relevant medical history included living kidney donation for his wife in 2012. Possible renal causes for the anemia were excluded.

TABLE 1 | Characteristics and special features of presented patients.

	age	sex	LGLL phenotype	hepatosplenomegaly	cytopenia	autoimmune manifestations	hemoglobin (g/dl)	ANC (/µl)	platelets (/µl)	immunophenotype	percentage of LGLL cells (pB)	mutation	special features
Patient 1	62	m	CLPD-NK LGLL	yes	anemia thrombocytopenia	none	6.6	1692	62	$CD2^+CD16^+CD7^+CD3^-CD4^-CD6^-CD8^-CD56^-CD57^-$	15.3%	*STAT3*	kidney donor
Patient 2	51	m	γδ-LGLL	yes	neutropenia	urticaria, hashimoto thyreoiditis	14.3	110	189	$CD3^+CD4^-CD8^-CD45RA^+CD56^+CD57^+TCR\gamma\delta^+$	24%	*STAT3*	previous treatment with omalizumab
Patient 3	55	f	CLPD-NK LGL	no	anemia, neutropenia	None	7.7*	1230*	174*	$CD2^+CD3^-CD4^-CD8^+CD7^+CD57^{dim}CD16^+CD56^-$	82%	wildtype	alloHSCT 10 years prior to diagnosis
Patient 4	69	m	αβ / γδ T-LGLL	no	anemia, neutropenia	ulcerative colitis, pos. Coombs test	10.9*	1190*	231*	$CD3^+CD4^-CD8^+CD16^{dim}CD57^{dim}TCR\alpha/\beta^+$ & $CD3^+CD4^-CD8^-CD16^+TCR\gamma\delta^+$	74% & 16 % respectively	wildtype	PRCA

*alloHSCT, allogeneic stem cell transplantation; ANC, absolute neutrophile count; CLPD-NK, chronic lymphoproliferative disease of natural killer cells; LGLL, large granular cell leukemia; n.d., not done; pB, peripheral blood; PRCA, pure red cell aplasia; PSA, Psoriasis arthritis; WBC, white blood cells. *values at last presentation, initial blood counts unknown.*

In 2019, the repeated diagnostic work-up revealed immunophenotypic evidence of the aberrant NK-cell population ($CD2^+CD16^+CD7^+CD3^-CD4^-CD6^-CD8^-CD56^-CD57^-$) both in pB and in BM. Next generation sequencing (NGS) detected a mutation in *STAT3* (variant allele frequency [VAF] 12%, c.1847 A>G p.E616G). By PCR a clonal TRG rearrangement was detected and cytogenetics showed a normal karyotype. Computed tomography (CT) scans revealed a hepatosplenomegaly, but no lymphadenopathy. All findings were consistent with the diagnosis of NK-LGL. Treatment with cyclophosphamide at 100 mg/d was initiated and after four weeks was reduced to 50 mg/d due to neutropenia.

After six months of therapy platelet counts had improved to 70,000/µl (from 35,000/µl). Hemoglobin (Hb) levels normalized after nine months of therapy. After 10 months both pB and BM showed no signs of infiltration by NK-LGL cells. Additionally the *STAT3* mutation could no longer be detected by NGS, implicating a molecular complete remission (CR). A [^{18}F] Fluordesoxyglukose-positron emission tomography (PET-CT) confirmed a metabolic CR with normal spleen size. The patient is still in CR at one year after discontinuation of cyclophosphamide.

Patient 2

A 51-year-old Caucasian male presented with splenomegaly and grade-4 neutropenia in December 2019. His medical history included chronic urticaria, which was previously treated with omalizumab, and a euthyroid Hashimoto thyroiditis.

Flow-cytometry of the BM aspirate showed an aberrant T-cell population with a $CD3^+CD4^-CD8^-CD45RA^+CD56^+CD57^+TCR\gamma\delta^+$ phenotype. NGS revealed a mutation in *STAT3* (VAF 26%, p.G618R). Clonality of T-cells was demonstrated by consensus PCR consistent with the diagnosis γδ-T-LGLL.

The initial CT-staging revealed a splenomegaly without lymphadenopathy. Therapy with cyclophosphamide 100mg/d was initiated in January 2020, however, the dosage had to be reduced to 50 mg/d after 2 weeks. This therapy had eventually to be discontinued four months later due to worsening of neutropenia and repeated infections. Treatment was switched to cyclosporine with a targeted trough level of 150 ng/dl. Four weeks after initiation of cyclosporine, the absolute neutrophil count (ANC) started to increase and after two months on therapy a sustained improvement to a moderate neutropenia was detected.

Due to neuromuscular symptoms and exacerbated arterial hypertension, the targeted trough levels of cyclosporine were reduced to 100 ng/ml, which improved tolerance of the therapy. Six months after this the ANC had normalized. CT-based imaging further showed a normalization of spleen size and tapering of cyclosporine was started.

Flow cytometry of the BM aspirate at nine months after the start of cyclosporine showed a residual fraction of the aberrant T-cell population of 2.5% of the total lymphocyte count with the residual finding of mutated *STAT-3* at a VAF of 15%.

Patient 3

A 55-year-old Caucasian female was diagnosed with a follicular lymphoma in 2002 that subsequently transformed into an aggressive B-cell lymphoma. After several lines of therapy,

including fludarabine + rituximab (R), dexaBEAM (dexamethasone + BCNU+ etoposide + cytarabine + melphalan) followed by autologous stem cell transplantation, R+bendamustine, R-CHOP (cyclophosphamide + doxorubicin + vincristine + prednisolone) as well as radiotherapy, she received an allogeneic stem cell transplantation (alloHSCT) from a matched unrelated donor in December 2006 after conditioning with fludarabine and melphalan.

In November 2016 (10 years after alloHSCT), she developed neutropenia without signs of relapse or graft failure or infectious causes of myelosuppression. The underlying reasons could initially not be classified despite a thorough diagnostic work-up. Over the next two years she developed a transfusion dependent anemia and the diagnostic work-up was repeated. This time, flow cytometric analysis revealed an aberrant cell population (82% of total lymphocyte count) with the phenotype $CD2^+CD3^-CD4^-CD8^+CD7^+CD57^{dim}CD16^+CD56^-$ suggestive of a CLPD-NK. No mutations were found for *ATM*, *STAT3*, *STAT5b*, or *TP53* as per NGS studies. PCR analysis detected clonal *TRB* gene rearrangements. From these samples a complete donor chimerism was established, indicating the donor origin of the CLPD-NK. Due to renal insufficiency and prior exposition to cyclophosphamide, treatment with the JAK1/3 inhibitor Tofacitinib 11 mg/day, instead of methotrexate (MTX), was initiated. Under this therapy, the neutropenia improved from severe to mild within six weeks and hemoglobin levels stabilized above 10 mg/dl without further need of transfusions.

Patient 4

A 69-year-old Caucasian male presented with anemia in September of 2005. Flow cytometry of pB showed a T-cell population with an immunophenotype ($CD3^+CD4^-CD8^+CD16^{dim}CD57^{dim}TCR\alpha/\beta^+$) that was indicative of T-LGLL. Clonality of this aberrant T-cell population was proven by PCR. Cytogenetics showed a normal male karyotype and NGS revealed *STAT3* to be in wildtype configuration. His medical history included an IgG-lambda-monoclonal gammopathy of undetermined significance, ulcerative colitis, and coronary artery disease. Therapy with MTX (initially 10 mg, increased to 15 mg in March 2006) was initiated in 2006 due to declining hemoglobin levels. In 2007 a complete remission (CR) was documented, but the patient relapsed four months after discontinuation of MTX. Further therapy with four courses of fludarabine (25 mg/m2 day 1-3 every 28 days) was initiated and resulted in a second CR, which lasted for seven years until January 2014. At that time, therapy with fludarabine was repeated and the patient again achieved a clinical response that lasted until May 2019.

In June 2016, flow cytometry of pB revealed a second aberrant T-cell population, accounting for 17% of lymphocytes and presenting with the following phenotype: $CD3^+CD4^-CD8^-CD16^+TCR\gamma\delta^+$. In 2019 the patient experienced another relapse with a lymphocytosis of 5900/µl and by subsequently developing symptomatic anemia. Another cycle of fludarabine was initiated in May 2020, but without improvement in hemoglobin levels. As Coombs tests were positive, suggesting an autoimmune hemolytic etiology of the anemia, a therapeutic attempt with prednisolone (maximal dose 75 mg and subsequent tapering), followed by initiation of tofacitinib for three months, was made, but neither resulted in improvements. The trephine BM biopsy showed an isolated absence of erythropoiesis without detection of infiltration of T-LGLL cells, fulfilling the criteria of a PRCA. Treatment with cyclosporine and prednisolone was initiated in April 2021, resulting in an ongoing clinical response with stable hemoglobin levels and without further need for transfusions.

DISCUSSION

Here we present heterogeneous presentations of T-LGLL and CLPD-NK that were seen in two academic institutions, with a focus on their diagnostic and therapeutic challenges. Our patients presented with unspecific symptoms, i.e. splenomegaly, autoimmune-mediated findings, or symptoms of cytopenias with a coincidental detection of LGL cells in flow cytometry.

In LGLL, often low-level lymphocyte infiltrations are misinterpreted as reactive, which frequently delays the definitive diagnosis. A thorough algorithm in the context of a fitting set of clinical presentations should include cytomorphology/histology, flow cytometry, a molecular clonality analysis, and gene-sequencing studies (11, 14, 16, 17).

The most common phenotype of T-LGLL is $CD3^+CD4^{neg}CD5^{+/low}CD8^+CD16^+CD57^{dim}$. However, neither the described immunophenotype nor the morphological features of LGLs are entirely specific (14). Consequently, an accurate distinction from other mature T-cell disorders, e.g. early-phase (low proliferative) T-PLL, is highly relevant, prognostically and therapeutically, but can sometimes be difficult and requires the incorporation of diagnostic multi-parameter approaches. A prolymphocytic morphology is only found in ~60% of T-PLL and the post-thymic pan-T ($CD2^+CD3^+CD5^+CD7^+$) immunophenotype of T-PLL includes in a small fraction of cases also the T-LGLL-like $CD4^-CD8^+$ pattern (14, 18). However, detection of a locus rearrangement involving a *TCL1* gene (either TCL1A at chromosome 14 [mostly as an inv (14)] or *MTCP1* at chromosome X) or proof of TCL1 protein expression in T-cells are established as unique major diagnostic criteria for T-PLL (14, 19–21)

CLPD-NK typically shows a $CD3^-CD56^+CD57^{+/-}$ immunoprofile. Cases of $CD56^-$ CLPD-NK, as displayed in patients 1 and 3, have been described as well (22). CLPD-NK is associated with rather indolent courses and less often symptomatic than T-LGLL. Cytopenias and infections are characteristic as well as a higher incidence of second neoplastic diseases, however, the latter is observed across all subsets of LGLL.

There is a known association of LGLL with autoimmune conditions (23). Approximately one third of patients with LGLL suffer from rheumatoid arthritis (24) and less frequently from other autoimmune diseases like systemic lupus erythematosus, Sjögren syndrome, or autoimmune thyroid disorders (5). This association seems to be far less present in CLPD-NK (25). Consistent with the literature, patient 2 of our series had a history of chronic skin allergies and thyreoiditis while patient 4 suffered from ulcerative colitis.

Interestingly, one of our two CLPD-NK cases (patient 3) arose after an alloHSCT and the tumor cells were of donor origin. Self-limiting proliferations of LGLs after alloHSCT without

clinical relevance have been described previously (26, 27), but aggressive forms of LGLL from donor cells were reported only in single case-reports (28–30) and in a series of four patients (31).

Of note, in both cases of CLPD-NK presented here, clonal TCR gene rearrangements were detected by PCR. This may seem contradictory, however, an analysis of KIR-restricted CLPD-NKs revealed TCR rearrangements in 50% of patients at first diagnosis (32).

We further presented a case of γδ T-LGLL (patient 2) and a case (patient 4) with a mixed phenotype of αβ/γδ LGLL. Interestingly, patient 4 developed the γδ clone during the course of the disease. In a recently published series of LGLL, the γδ variant accounted for approximately 15% of cases (4). Cases of αβ/γδ mixed phenotype are extremely rare and have so far been reported only as isolated cases or in very small series (3, 4). The diagnosis of γδ T-LGLL can be challenging; especially the differentiation from HSTL, which is often of γδ type and typically shows a low-level leukemic presentation. Particularly difficult are LGLL cases with absent or very low counts of clonal LGLL cells in pB and/or with a CD4-/CD8- phenotype (33). Detection of cytogenetic aberrations (isochromosome 7q or trisomy 8), described in >50% of HSTL (34), but atypical for T-LGLL, can be of assistance. HSTL affects predominantly younger men typically in the context of (medical) immunosuppression, often for a pre-existing autoimmune disease, yet the HSTL itself has not been associated with autoimmune phenomena. Nevertheless, the invariably aggressive course of HSTL sets it well apart from LGLL.

Another challenge is to define the cause(s) of cytopenias in LGLL, especially when these can also be caused by the therapeutic strategies. Patient 4 presented with anemia and underwent treatment with MTX and fludarabine. After becoming refractory to this treatment, further diagnostic workup was necessary to discriminate between therapy-related BM toxicity, AIHA, and PRCA, and eventually the diagnosis of PRCA was made. The association of LGLL with both AIHA (1, 17, 35) and PRCA (36) has been described and the accurate discrimination of those entities is necessary for the choice of an effective therapy (37, 38).

Although most LGLL patients do not require treatment at presentation, in 2/3 of cases therapy needs to be initiated at a later stage, especially due to severe neutropenias and subsequent infections, transfusion-dependent anemias, or thrombocytopenias (24). The current treatment options in LGLL are extremely limited. Standard approaches are based on supportive measures (e.g. transfusions, hematopoietic growth factors, antibiotics) and immunosuppressive therapies like MTX, cyclophosphamide, or cyclosporine (16) with limited evidence. The optimal sequence of MTX and cyclophosphamide is yet to be determined (ongoing trial NCT01976182).

Cyclophosphamide seems to show better efficacy in the control of symptoms and cytopenias as compared to MTX, but due to associated late toxicities it should not be administered for more than 12 months (39). Both agents need a minimum of 6-12 weeks before definite response assessments (17) and they both have treatment-associated cytopenias as side effects. In our case series, cyclophosphamide was administered in two patients and proved to be an active treatment option even for a living kidney donor. Reports on responses to other substances, like rituximab (40, 41) or the JAK1/3 inhibitors tofacitinib (42) and the JAK 2 inhibitor ruxolitinib (39) are sporadic and limited to small series. Generally, the treatment responses in LGLL are usually dissatisfactory being frequently incomplete and/or short-lived.

Another relevant aspect is the disease-inherent and treatment-related immunosuppression, which is of particular focus in current contexts of the COVID-19 pandemic. Although here not represented with a particular case, it has been repeatedly shown that patients with hematologic malignancies have a higher risk of severe or fatal COVID-19 infections (43–45). A single center retrospective analysis of 835 patients hospitalized with COVID-19, recently showed a significantly increased mortality of patients previously receiving immunosuppressive therapies (46). However, due to the rarity of LGLL, the exact morbidity and mortality risks related to COVID-19 infections in LGLL patients are unknown. Moreover, we do not know how disease and immunosuppressive therapies influence the effectivity of anti-COVID-19 vaccinations in LGLL patients. In other lymphatic malignancies, such as chronic lymphocytic leukemia (CLL), responses to vaccination are influenced by disease activity, current treatments, and previous therapies, especially regarding anti-CD20-antibodies (47, 48). A pragmatic strategy might be to adopt vaccination strategies from other hematologic malignancies like CLL, but not to neglect complex aspects of both humoral and cellular immunity specific for LGLL (49).

In summary, LGLL is a heterogenous group of diseases ranging from asymptomatic presentations over cases with severe impairments of quality of life, but long survival, to cases with significantly shortened life expectancy. In this series of selected cases with unique features, we illustrate pitfalls in the diagnosis, management, and treatment of LGLL of T-cell and NK-cell nature. In agreement with the literature, the uniqueness of the individual presentations and courses seems to override potential associations of clinical features, treatment responses, or outcomes with phenotype (αβ vs. γδ or T vs. NK) or genotype.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

ETHICS STATEMENT

Written informed consent was obtained from the individual(s) for the publication of any potentially identifiable images or data included in this article.

AUTHOR CONTRIBUTIONS

MH, NP, and VV contributed to conception and design of the study. AL, AS, DB, LW, NP, and VV acquired

patient data and organized the database. NP and VV wrote the first draft of the manuscript. AL, AS, DB, and TB wrote sections of the manuscript. AD, AM, DJ, EB, MO, and TB provided diagnostic support. G-NF, MJ, SS, and UP provided administrative support. All authors contributed to the manuscript revision, read and approved the submitted manuscript.

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Toward a Better Classification System for NK-LGL Disorders

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Large granular lymphocytic leukemia is a rare lymphoproliferative disorder characterized by a clonal expansion of T-lineage lymphocyte or natural killer (NK) cells in 85 and 15% of cases respectively. T and NK large granular leukemia share common pathophysiology, clinical and biological presentation. The disease is characterized by cytopenia and a frequent association with autoimmune manifestations. Despite an indolent course allowing a watch and wait attitude in the majority of patients at diagnosis, two third of the patient will eventually need a treatment during the course of the disease. Unlike T lymphocyte, NK cells do not express T cell receptor making the proof of clonality difficult. Indeed, the distinction between clonal and reactive NK-cell expansion observed in several situations such as autoimmune diseases and viral infections is challenging. Advances in our understanding of the pathogenesis with the recent identification of recurrent mutations provide new tools to prove the clonality. In this review, we will discuss the pathophysiology of NK large granular leukemia, the recent advances in the diagnosis and therapeutic strategies.

Keywords: chronic lymphoproliferative disorders of NK cells, NK cells, KIR phenotype, STAT3, large granular lymphocyte leukemia

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INTRODUCTION

Large granular lymphocytic (LGL) leukemia is a rare disease that accounts for 2 to 5% of chronic lymphoproliferative disorders (1). Its incidence is probably underestimated in view of its indolent and often asymptomatic course and diagnostic difficulties. LGL leukemia is mainly characterized by cytopenia, primarily neutropenia predisposing to infections and is frequently associated with an array of autoimmune diseases, in particular rheumatoid arthritis. There are two main subtypes of LGL leukemia, respectively with a T or NK phenotype and a respective incidence of 85 and 15%. A provisional entity so-called chronic lymphoproliferative disorders of NK cells, or CLPD-NK, was included in the last WHO classification in 2016 (2) as a means of distinguishing it from EBV induced aggressive NK-LGL leukemia whose prognosis is quite poor.

LGL leukemia needs to be distinguished from reactive LGL proliferation, which is frequent, particularly in the context of viral infections, autoimmune diseases, after splenectomy or in post-transplant patients. Diagnosis of LGL leukemia is based on two mandatory criteria which help to

differentiate it from reactive LGL lymphocytosis: cytological identification of lymphocytes with granules > 0.5 G/L observed at least over 6 months and proof of clonality. T-LGL clonality is easily demonstrated by TCR rearrangement. On the other hand, NK-LGL clonality is far more complex to identify, as NK cells do not express CD3 on their surface and lack the T cell antigen receptor (TCR). In this review, we develop advances in the pathophysiology and understanding of NK-LGL leukemia. We review recent progresses in the development of tools for clonality diagnosis that can help to optimize nosological classification of chronic NK proliferations before finally considering therapeutic strategies.

NK CELL: A LYMPHOCYTE WITH CYTOTOXIC CAPABILITIES AND WITH COMPLEX ACTIVATION MODALITIES

NK cells have a cytotoxic and cytokinic activity close to that of the CD8+ cytotoxic T lymphocyte directed against aberrant autologous cells (infected, tumoral ou stressed) giving them antiviral and anti-tumoral functions. In contrast to T cells, NK cells do not express the TCR-CD3 complex on their surface. On the other hand, they do express the CD16a molecule, a low affinity type IIIA immunoglobulin constant fragment receptor, which enables them to bind and opsonized cells. NK cells also express CD56, or neural cell adhesion molecule (NCAM), which is more broadly expressed by other extra-hematopoietic cell types, and by a minority of activated cytotoxic T cells. They are also routinely included in CD2+/CD5-/CD7+ lymphocyte flow cytometry analysis panels (3).

Two types of NK cells with different functions have been historically identified through differential expression of CD56, CD16 in flow cytometry (4).

1) CD56high CD16low NK cells are mainly cytokine-producing NK cells such as interferon gamma. The production of interferon gamma by NK cells is stimulated by IL-12 and IL-18 in synergy with IL-2 and IL-15, which promote NK cell activation more broadly (5).

2) CD56low CD16high NK cells have a mainly cytotoxic function. These lymphocytes are the main agent of antibody-dependent cell-mediated cytotoxicity (ADCC) *via* CD16. After activation of the NK cell, targeted cells apoptosis can be mediated by two NK cell cytotoxicity mechanisms, also used by T cells, namely the perforin-granzyme pathway and the Fas/Fas ligand pathway. The perforin released by exocytosis from NK cells create pores in the plasma membrane of the targeted cell enabling granzymes entry. Granzyme B leads to the activation of the caspase cascade (6) while granzyme A induces cell death by a mitochondrial caspase-independent mechanism (7). The FAS/FASL complex or TRAIL/TRAIL-Rs induce apoptosis by pathways similar to granzyme B.

NK lymphocyte can still be considered as part of innate immunity since it uses a repertoire of surface receptors, is germline-encoded, and able to recognize stressed cells, without the need for prior sensitization and to act immediately. NK lymphocytes recognize not only MHC class I or MHC class I mimicking molecules, but also other molecules. Ligand specificity is to a variable extent dependent on the type of receptor. These receptors can induce activating or inhibitory signals to the NK and are not specific to them since they are also expressed by T lymphocytes (8).

Among the receptors that recognize the classical MHC class I (HLA-A, B, C), are the Killer Immunoglobulin-like Receptors (KIR), each of which recognizes an HLA subtype with a relatively high specificity. Every single NK lymphocyte expresses a few KIR receptors among the existing KIR, coded by 15 genes on chromosome 19, with a high polymorphism, frequent chromosomal recombinations and alternative splicing (9, 10). KIRs can induce either an inhibitory or an activating signal. The lectin-like receptors of the CD94-NKG2 heterodimer are another large NK receptor family. CD94-NKG2A induce an inhibitory signal through the non-classical MHC class I (HLA-E), which has a more restricted polymorphism than the classical MHC type I (11). Natural Cytotoxicity Receptors (NCRs), mainly represented by NKp46, NKp30, and NKp44, recognize non-MHC molecules on the cell-surface or secreted by tumoral or virus-infected cells. They also have an activating role for NK lymphocytes (12).

NK cell activation is more complex than TCR/BCR antigenic activation, which are present on T cells and B cells respectively. NK cell activation is determined by the integration of multiple signals from these different surface receptors and is only possible when the sum of activating signals exceeds that of inhibiting signals. Activation depends on the number of receptors, their affinity and the inhibitory threshold of the cell.

PATHOPHYSIOLOGY OF NK-LGL LEUKEMIA

The cytotoxic function, characteristic of both T and NK cells explains the common pathophysiological basis of T- and NK-LGL leukemia. The development of LGL leukemia is probably secondary to a chronic stimulation induced by a viral infection or a public antigen. Autocrine and paracrine interleukin 15 plays a central role in the proliferation of NK cells (13), which is initially polyclonal and then switches to monoclonal proliferation through selection of an NK clone with an activated KIR profile contrasting with the mainly inhibitory profile of KIRs observed in physiological situations (14, 15). The development of leukemia is also the consequence of dysregulated activation of several anti-apoptotic signaling and cell survival pathways (**Figure 1**). The JAK/STAT pathway plays a central role in the pathophysiology of NK-LGL leukemia. Constitutive activation of STAT3 was initially reported in 2001 (16) and an activating mutation of *STAT3* was identified in the SH2 domain on two predominant hotspots (D661 and Y640) in LGL leukemia (17), as well as in NK/T and ATLL lymphomas (18). This mutation induces constitutive phosphorylation and STAT3 unit dimerization leading to the transcription of anti-apoptotic genes such as Mcl-1 belonging to the Bcl-2 family. The *STAT3* mutation is

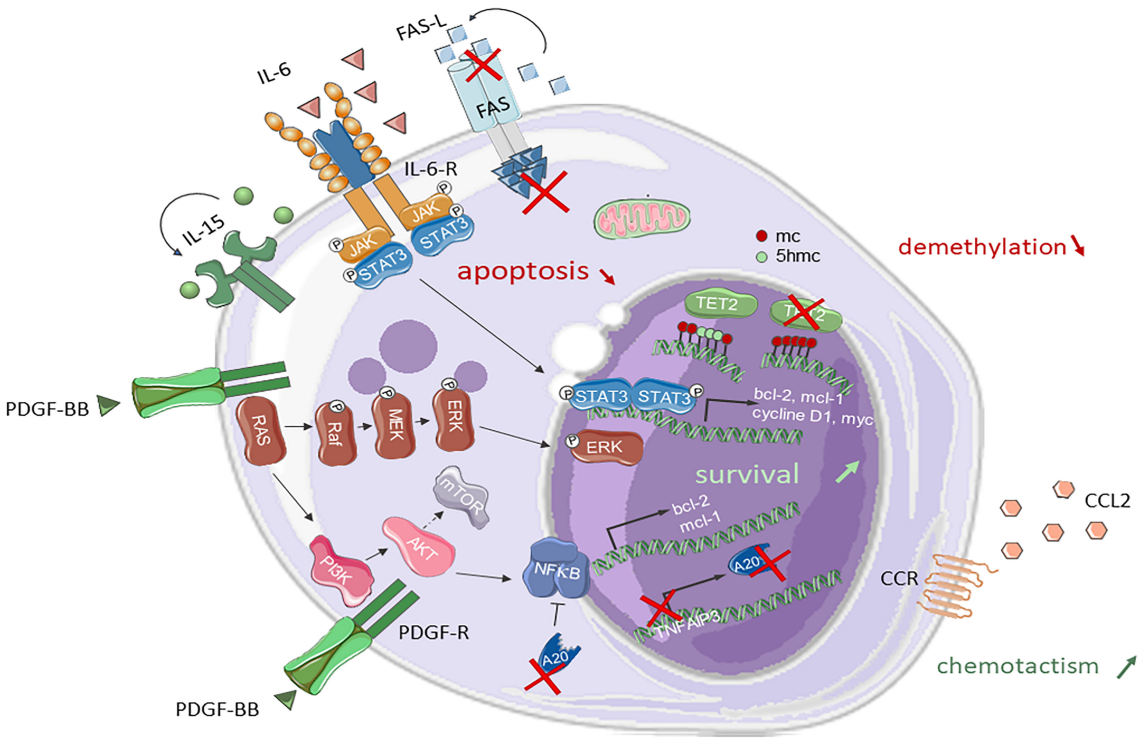

FIGURE 1 | Signaling pathways and mutations involved in NK-LGL leukemia pathogenesis. STAT3, RAS/MAPK and PI3K/AKT pathways are constitutionally activated in NK-LGL leukemia. The PI3K/AKT pathway leads to the activation of m-TOR and NFκB. STAT3 and NFκB promote the transcription of anti-apoptotic genes such as *bcl-2* ou *mcl-1*. TNFAIP3 can undergo an inactivating mutation of the A20 protein that negatively regulates NFκB. LGLs are resistant to Fas mediated apoptosis. A *TET2* loss-of-function mutation is found in 34% of NK-LGLs. The gene encoding the chemokine CCL22 is mutated in 20% of NK-LGLs. Fas, First Apoptosis Signal; FasL, FasLigand; IL, interleukin; IL-R, interleukin-receptor; Jak, Janus Kinase; STAT3, Signal transducer and activator of transcription 3; PDGF-BB, platelet-derived growth factor BB; MEK, mitogen activated protein kinase; ERK, extracellular-signal-regulated kinase; PI3K, phosphatidyl Inositol 3-Kinase; mTOR, mammalian target of rapamycin; NFκB, nuclear factor kappa B; Mcl1, Myeloid cell leukemia1; Bcl, B-cell lymphoma 2; CCL22, C-C Motif Chemokine Ligand 22; 5-mc, 5-methylcytosin; 5hmc, 5-hydroxymethylcytosin; TET2, Ten-eleven-translocation 2.

found in 30% of NK-LGL leukemia as well as in 30-40% of T-LGL leukemia, linking the two entities (17, 19). The introduction of the *in vitro* STAT3 inhibitor AG-490 or STAT3 antisense oligonucleotide treatment shows restoration of apoptosis of clonal NK LGLs apoptosis (16). A significant proportion of unmutated STAT3 LGL leukemia cases also features hyperactivation of the JAK/STAT pathway by two mechanisms (19); i) underexpression of the *SOCS3* (suppressor of cytokine signaling-3) gene, ii) excess autocrine production of interleukin-6 by NK-LGLs. Deleterious mutations of the JAK/STAT pathway were described by whole exome sequencing, such as PTK2/FAK1, PIK3R1 (20, 21), FLT3 and CD40 ligand (22). Constitutive activation of STAT3 and production of IL6 induce an increase in the transcription and expression of Fas ligand in LGL leukemia. However, NK-LGL leukemic cells show resistance to the pro-apoptotic signal of the Fas ligand (23–25) without any gene mutation being identified. Clonal LGL-NKs produce a soluble variant FAS, thought to act as a soluble FAS receptor, blocking the FAS-ligand (26). Moreover, there is a certain correlation between the soluble Fas ligand concentration and the depth of neutropenia in LGL leukemia, suggesting that the soluble Fas ligand plays a role in neutrophil apoptosis (27). The MAP kinase pathway also participates in the dysregulation of the balance between survival and apoptosis in NK-LGL leukemia (28). Suppression of ERK (extracellular signal-regulated kinase) activity by a MEK inhibitor reduces NK-LGL survival. The same phenomenon is observed with the inhibition of Ras, reported to be constitutively activated in NK-LGL leukemia patients. *KRAS, NOTCH1* and *PTEN* mutations were found in different cohorts (20, 21, 29). The PI3K-Akt complex, which can be activated by Ras and inhibited by PTEN, is also deregulated in LGL leukemia (30). Akt has numerous downstream targets involved in the cell cycle, including mTOR. Mutations in *PIK3R1*, *PIK3CD* and PIK3AP1 genes have been also identified for instance (21, 31). Another recurrent mutation affecting TNFAIP3 (tumor necrosis factor alpha–induced protein 3) was identified in 5% of LGL leukemia patients (31). This mutation results in negative regulation of NFκB signaling. The A20 protein encoded by TNFAIP3 inhibits NFB by ubiquitination mechanism. NFκB is constitutively activated in LGL leukemia (32). Downstream of the PI3K-Akt pathway, NFκB causes an increase in the anti-apoptotic factor Mcl-1, independently of STAT3. PDGF-β (Platelet-derived growth factor subunit Beta) produced in excess by clonal LGLs, forms an anti-apoptotic autocrine loop,

activating the signaling pathways mentioned above, PI3K-AKT, RAS/MEK1/ERK, and JAK/STAT. Its inhibition by a neutralizing antibody *in vitro* leads to a decrease in AKT phosphorylation (33). We recall the role of IL-15 and its receptor produced in excess in LGL-NK leukemias (34). Transgenic mice overexpressing IL-15 by post-transcriptional regulation defect developed NK lymphocyte proliferation and secondary aggressive LGL-NK leukemia rapidly lethal (35, 36). More recently, mutations in the CCL22 gene have been described in 20% of LGL-NK leukemia, and are specific to the NK subtype and exclusive of other mutations (37). The *CCL2* mutation induces *in vitro* increased CCL2 chemotaxis and decreased internalization of its Th2 T cell receptor CCR4. CCL2-mutated NK LGLs show higher CD56 expression than non-mutated ones (38).

Finally, epigenetic modifications in NK-LGL leukemia were discovered more recently. A TET2 mutation was identified in approximately 30% of patients with NK-LGL leukemia in three successive series (21, 29, 31). In Olson's 7-patient cohort, 5 times more methylated regions were observed in clonal NK-LGLs than in normal NK cells in reduced-representation bisulfite sequencing data (31), involving over a hundred RNA polymerase transcription factors or target regulatory regions. Interestingly, the gene coding for PTPRD (protein tyrosine phosphatase receptor type delta), a STAT3 inhibitor, was found to be hypermethylated compared to non-mutant *TET2* NK-LGL or normal NK cells.

TET2 mutations are common in both myeloid blood malignancies (acute myeloid leukemia/myelodysplastic syndrome, chronic myelomonocytic leukemia) and T-cell lymphoma, particularly in angioimmunoblastic T-cell lymphoma, which raises questions as to the original cell that underwent the *TET2* mutation in NK-LGL leukemia. In whole exome sequencing studies in 3 out of 6 patients analyzed, we showed that *TET2* was mutated not only in NK cells but also in myeloid precursors, suggesting a potential driver role of *TET2* mutation (29). This may explain cases of LGL leukemia association with a myelodysplastic syndrome or acute myeloid leukemia (39).

CLINICAL CHARACTERISTICS OF NK-LGL LEUKEMIA

T-LGL and NK-LGL leukemia share both pathophysiology, clinical and biological presentation (**Table 1**). The median age of LGL leukemia onset is 60 years with a sex ratio of 1:1. Its course is indolent with an overall 10-year survival rate of 70% (1). Massive hepatosplenic and bone marrow infiltration of NK-LGLs and rapidly progressive NK cell blood lymphocytosis, is related to aggressive NK cell leukemia, a rare and distinct entity with a poor prognosis (42). Symptoms are mainly due to infections (mouth ulcers, ENT or lung infections, severe sepsis) secondary to severe neutropenia which is the most common cytopenia. Neutropenia is less frequently observed in the NK subtype (29% in T LGL leukemia, as compared to 61% in NK LGL leukemia) (29, 40).

TABLE 1 | Comparison of clinical characteristics between T-LGL and NK-LGL leukemia.

	NK-LGL leukemia Poullot (n=70) [Ref: (40)]	T-LGL leukemia Bareau (n=201) [Ref: (41)]
Lymphocytes > 4G/L	56%	51%
Median LGL (G/L)	2.1	1.71
LGL <1G/L	29%	55%
LGL > 7G/L	7%	4%
Neutrophils < 1.5G/L	29%	61%
Neutrophils < 0.5%	9%	26%
Anemia < 11g/dL	18%	24%
Anemia < 8g/dL	9%	6.6%
Thrombocytopenia <150G/L	20%	19%
Thrombocytopenia < 50G/L	4%	1%
Autoimmune diseases	24%	33%
Rheumatoid arthritis	7%	17%
Seronegative arthritis	14%	8%
Polymyositis	3%	0%
Autoimmune hemolytic anemia	6%	<7%
Idiopathic thrombocytopenic purpura	7%	<7%
Vasculitis	4%	3%
Solid cancers	13%	5%
Associated blood disorder	11%	8%
B-cell lymphoma	3%	–
Myelodysplastic syndrome	3%	–
Acute myeloid leukemia	3%	–
Myeloproliferative syndrome	4%	–

Infectious complications are responsible for the majority of disease-related deaths (3-7%) (29, 40). Opportunistic infections are rare and secondary to immunosuppressive therapy. Twenty percent of patients are transfusion dependent. Thrombocytopenia is rare and moderate. LGL leukemia can be complicated by pure red cell aplasia or bone marrow aplasia. On clinical examination, splenomegaly is observed in 25% of cases (41), whereas hepatomegaly is slightly less frequent and peripheral adenopathies are rare.

LGL leukemia may be associated with autoimmune diseases, such as connective tissue disorders or vasculitis. Rheumatoid arthritis is the most common condition seen in individuals with LGL leukemia, although slightly less frequent in NK-LGL leukemia (40, 41). These diseases can precede diagnosis of LGL leukemia. In autoimmune disease settings, reactive NK cell proliferations may also be observed. Moreover, some connective tissue disorders such as lupus and Gougerot-Sjögren syndrome can have overlapping clinical characteristics such as neutropenia, pure red cell aplasia and splenomegaly that can make the diagnosis of LGL leukemia difficult. Biological markers of autoimmunity such as polyclonal hypergammaglobulinemia and presence of positive rheumatoid factors are common and the signs of a chronic antigenic stimulation mechanism (40). Moreover, there have been reports of LGL leukemia concomitant with another hematological malignancies, either of myeloid or lymphoid origin. MGUS is more common than in the general population (16%) (40, 43). LDH and beta-2 microglobulin levels are high in 36 and 66% of cases respectively (40). Concomitant association with solid cancers has also been described (44).

THE CONTRIBUTION OF FLOW CYTOMETRY AND BONE MARROW BIOPSY TO THE DIAGNOSIS OF NK-LGL LEUKEMIA

The vast majority of NK-LGL leukemia cases harbored a cytotoxic $CD16^{high}CD56^{low}CD57+/-$ profile (29). Therefore, NK leukemic cells most often display a uniform $CD16^{high}$ profile whereas normal NK cells are characterized by heterogeneous CD16 expression due to the coexistence of different NK subtypes. High CD16 expression is not sufficient to affirm NK clonality but provides an invaluable clue in the diagnostic procedure. CD56 is expressed by some activated T cells and in T-LGL leukemia and is therefore not a good marker of NK clonality. CD57 is positive in the majority of cases, associated with a memory profile (29, 31).

While normal NK cells display a CD2+/CD5-/CD7+ phenotype, clonal NK LGLs are frequently CD5dim/CD7dim. NK-LGL leukemic cells partially express CD8 with an intensity that is markedly lower than in T-LGL leukemia. However, CD8 cannot be used to distinguish NK-LGL leukemia from normal NK cells which exhibit low CD8 expression levels (3, 45). KIR phenotyping represents a major advance in NK-LGL leukemia diagnosis. However, this multiparameter analysis is complex and requires an expertise only available in some reference centers. NK-LGL leukemic cells show a restricted activated KIRs expression (15). Thus, inhibitory CD158a, CD158b and NKB1, expressed ubiquitously in normal NK cells, are very rarely expressed in NK-LGL leukemia (45). NK-LGL monoclonal proliferations express CD94 lectin with inhibitory NKG2A (15, 45), forming the CD94/NKG2A heterodimer, with a markedly higher MFI than that observed in normal or reactive NK cells. To a lesser extent, underexpression of CD161 (3) and natural cytotoxicity receptors (15), in particular NKp30 and NKp44, is more often found in NK-LGL leukemia than in NK-LGL polyclonal proliferations.

Bone marrow biopsy may contribute to ascertain the diagnosis in atypical presentations, specifically with a low LGL count (< 1G/L), an irrelevant phenotype, a marrow hypoplasia or pure cell aplasia. In paraffin sections, diffuse interstitial medullary infiltration by LGLs is found in more than 90% of cases, with a TiA1 and granzyme immunostaining. It is noteworthy that CD3 can sometimes be positive in immunofluorescence staining because of the presence of a CD3delta subunit on the NK cells, which binds to paraffin on immunolabeling. Moreover, LGLs are grouped into clusters of at least 8 TIA-1+ lymphocytes or at least 6 granzyme B+ lymphocytes. These LGL clusters may be associated with nodules of B cells surrounded by non-clonal CD4+ T cells. Intrasinusoidally, LGLs dysplay a linear TIA1+/granzyme B/+ network in close contact with antigen-presenting cells (46, 47)

CONTRIBUTION OF GENOMIC ANALYSIS. PROPOSAL FOR AN NK-CELL CLONALITY SCORE

Identification of recurrent mutations in T- and NK-LGL leukemia provided strong arguments for NK clonality, and ultimately enabled true NK-LGL leukemia to be distinguished from reactive NK-LGL proliferations. Mutational screening is more accurate than KIR receptor repertoire analysis. The frequencies of the different mutations are shown in **Table 2**.

The first major recurrent mutation initially identified in T-LGL leukemia was a *STAT3* function gain mutation found in 27-33% of NK-LGL leukemia cases (29, 31, 48). The STAT3 mutations are located in the SH2 domain within exon 20 and 21, Y640F and D661V accounting for two-thirds of mutations (17). The *STAT5B* mutation is less common, present in 5% of LGL leukemia cases (29, 49). The *TNFA1P3* mutation is particularly observed in cases of LGL leukemia associated with rheumatoid arthritis, and in 5-10% of NK-LGL leukemia cases (21, 31, 48).

In 2021, using high-throughput sequencing we and others have identified a *TET2* mutation in 28 to 34% of NK-LGL leukemia cases, constituting a new strong diagnostic marker (29, 31). *TET2* and *STAT3* mutations are generally exclusive and appear to be associated with two different NK phenotypic and functional profiles: the *STAT3* mutation is more often found in CD16high/CD57low, or cytotoxic memory NK-LGLs, while the *TET2* mutation is more commonly associated with the CD16low, or regulatory cytokine profile. The transcriptome expression profiles analyzed by C. Pastoret et al. in *STAT3*-

TABLE 2 | Phenotypic and mutational profiles of NK-LGL leukemia in the French cohort and USA cohort.

	French cohort n=46 LGL and 68 Reactive NK [Ref: (29)]			USA cohort n=63 [Ref: (31)]
	Training set N=28 LGL	Validation set N=18 LGL	Reactive NK N=68	
NK count >1G/L	68%	83%	19%	NA
KIR restricted phenotype	86%	78%	6%	NA
CD94/NKG2Ahi	68%	61%	15%	NA
STAT3	26%	28%	0%	29%
STAT5b	8%	0%	0%	0%
TNFAIP3	9%	11%	0%	10%
TET2	35%	33%	8%	28%
CCL2	NA	NA	NA	22%

NA, Not Applicable.

and *TET2*-mutated patients are quite distinct, confirming the existence of two different subgroups. Moreover, a genotype/phenotype correlation was observed, reflecting the strong impact of these mutations in the pathophysiology of LGL leukemia; *STAT3*-mutated patients have a higher incidence of neutropenia (25, 37, 48) while *TET2* mutant patients have a higher incidence of thrombocytopenia (29, 31). *STAT5B* N642H mutated patients develop more aggressive disease (50).

However, *TET2* mutation is not restricted to LGL leukemia and has been identified in angioimmunoblastic lymphoma and other T-cell lymphomas. Overall, in two-thirds of NK-LGL leukemia cases, a recurrent mutation contribute to the diagnosis. In routine practice, a high-throughput sequencing panel for T-cell lymphoma including screening for *STAT3, STAT5B, TNFAIP3, CCL22* and *TET2* mutations can thus be used for the diagnosis of NK-LGL proliferations. We proposed a prognostic score based on biological criteria ranging from 0 (low probability of clonality) to 7 (high probability of clonality) in settings suggestive of LGL leukemia (36). The criteria yielding two points each were as follows: i) NK cell count > 1G/L, ii) KIR receptor restriction defined by a low expression of at least two KIR receptors (CD158A < 9% of NK cells, CD158B < 12%, and/or NKB1 < 4%), and iii) presence of a somatic mutation of *STAT3, STAT5b, TET2* or *TNFAIP3*. A high expression of CD94 or NKG2A (>77%) carries an additional point. A score higher than or equal to 4 has a sensitivity of 83% and a specificity of 96% for NK-LGL diagnosis and a score of under 2 discounts the diagnosis with a negative predictive value of 95%. This score was validated on a cohort of 38 patients (18 LGL and 20 reactive conditions), yielding a positive predictive value of 100%. Only one LGL according conventional criteria was reclassified as reactive condition according the NK score **(Table 2).** Finally, mutations in the *CCL22* gene are also described in 20% of LGL-NK leukemias, specific to the NK subtype, and exclusive of other mutations (38). A diagnostic algorithm for LGL-NK leukemias is proposed in **Figure 2**.

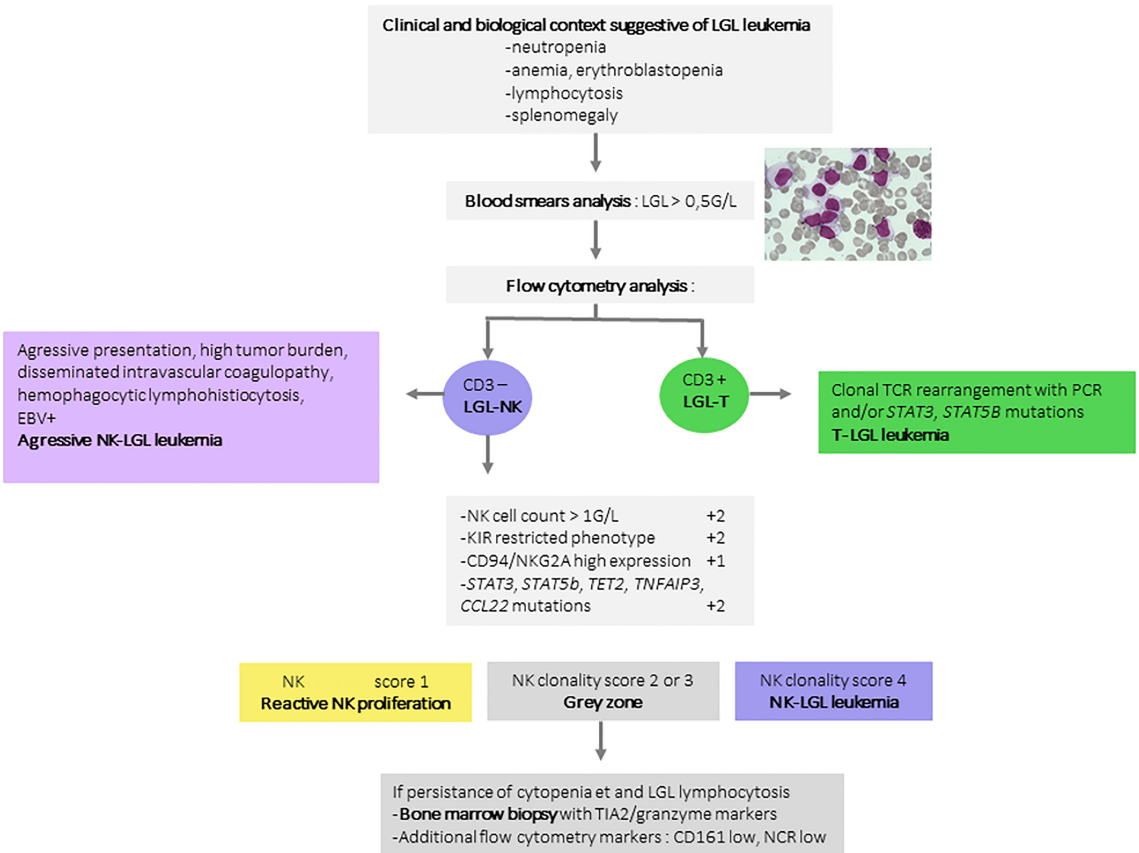

FIGURE 2 | Diagnosis algorithm for NK-LGL leukemia. A large granular lymphocyte count greater than 0.5G/L is the first element mandatory for the diagnosis of NK-LGL leukemia. T and NK-cell LGL are distinguished based on the expression of CD3. The proof of clonality is often challenging in NK-cell LGL. In these conditions, the proposed diagnostic score assigns 2 points for a restrictive KIR phenotypic profile, 1 point for CD94/NKG2A hyperexpression, 2 points for *STAT3, STAT5b, TET2, TNFAIP3*, or *CCL22* mutations. These three elements represent the most compelling arguments for clonality. In case of a score higher than 4, the diagnosis of LGL-NK leukemia can be confirmed. A score between 2 and 3 should prompt discussion of the evaluation of other NK markers such as low CD161 or low NKp30 and 44. In this situation, a bone marrow biopsy is recommended. LGL, large granular lymphocyte leukemia; KIR, Killer-cell Immunoglobulin-like receptors; STAT3, Signal transducer and activator of transcription 3; TET2, Ten-eleven-translocation 2; TNFAIP3, Tumor Necrosing Factor Alpha Induced Protein 3; CCL22, C-C Motif Chemokine Ligand 22.

THERAPEUTIC APPROACHES IN NK-LGL LEUKEMIA

The indolent course of LGL leukemia allows a watch and wait attitude at initial diagnosis in one third of patients. However, two thirds of patients will be eventually treated mainly due to neutropenia related infections or symptomatic anemia. The treatment indication can also be discussed in case of associated and symptomatic disease. It should be noted that there are no studies evaluating specific treatment of the NK-LGL leukemia subtype, as patients with NK-LGL leukemia were included with T-LGL patients with no distinction made. Immunosuppressive drugs such as methotrexate, cyclophosphamide and ciclosporin constitute the backbone of first-line treatments. Complete response rates at 4 months are low (around 16%). A prospective randomized study of first-line therapy (51), comparing methotrexate with cyclophosphamide, is currently underway. Relapse is frequent, occurring within a median time of 9 to 29 months (51, 52). Ciclosporin is more readily used in aplastic forms or pure red cell aplasia. Treatment must be maintained for at least one year in order to prevent early relapse.

In frequent cases of LGL leukemia that are refractory to immunosuppressive agents or in early relapse, alemtuzumab, an anti-CD52 antibody, alemtuzumab, which is also the treatment of choice for T-cell prolymphocytic leukemia, was tested in LGL leukemia with several response cases (53–55). A gamma chain inhibitor of the cytokine receptors IL2 and 15, BNZ-1 (56), was shown to induce *in vitro* a reduction in STAT3 and ERK phosphorylation in NK- and T-LGLs, and to induce apoptosis of T-LGLs. A phase I/II is underway (57). The use of therapies targeting the JAK/STAT pathway constitutively activated in LGLs appears promising. For example, remission was achieved with tofacitinib in a small number of refractory T LGL leukemia patients (53–55, 58), and likewise with ruxolitinib. No…..e remission rates induced with demethylating agents in cases of TET2 mutated angioimmunoblastic lymphoma (59) should prompt an assessment of their efficacy in LGL leukemia bearing the TET2 mutation.

CONCLUSION

It is now possible to propose a more precise classification of NK-LGL leukemia and discard the term chronic NK lymphocytosis. Proof of clonality of NK-LGL leukemia is crucial given the frequency of reactive NK-LGL proliferations. The identification of a phenotypic restriction in KIRs combined with identification of a *STAT3, STAT5B, TET2, TNFAIP3*, and *CCL2* mutations constitute strong arguments to confirm NK clonality in most cases. Targeted JAK/STAT pathway therapies and demethylating agents in the case of *TET2* mutation represent promising therapies that warrant assessment in prospective studies in order to reduce the relapses frequently reported after immunosuppressive therapy.

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OPINION
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T-Cell Large Granular Lymphocyte Leukemia: An Interdisciplinary Issue?

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INTRODUCTION

Large granular lymphocytic leukemia (LGLL) is an indolent and rare lymphoproliferative disorder of mature cytotoxic T-cells or Natural Killer (NK)-cells accounting for 2-5% of chronic lymphoproliferative disorders in North America and Europe (1, 2).

LGLL is associated in up to 15-40% with autoimmune disorders, with rheumatoid arthritis (RA) being the most common (10-18%). Rheumatoid factor (RF) and antinuclear antibody (ANA) are detected in about half of the patients (1). As symptoms are nonspecific, diagnosis can be delayed. A close collaboration with a specialist in hematology is recommended.

According to the WHO classification 2017 (3), LGLL is divided into T-LGL leukemia (T-LGLL, 85%), chronic lymphoproliferative disorder of NK-cells (CLPD-NK, 10%) and the more aggressive NK-LGL leukemia (ANKL, 5%). T-LGLL and CLPD-NK have a median age of 60 years and tend to have an indolent course, whereas aggressive NK-LGL leukemia more often affects younger patients and is highly associated with EBV (3–6).

LGL leukemia (LGLL) should be considered in patients with marked neutropenia, lymphocytosis, recurrent infections, anemia and autoimmune disorders. Typical "B" symptoms are seen in only 20-30% of LGLL patients (7). Most patients with T-LGLL present with chronic neutropenia resulting in recurrent infections but courses without any infections are possible (1, 8, 9). Lymphocytosis is observed in about 50%, thrombocytopenia in < 25% and anemia in 10-30% of LGL patients. Splenomegaly is seen in about a quarter of patients, whereas hepatomegaly and lymphadenopathy are rare (1, 2, 8, 10).

Diagnosis is based on cytology (blood smear), flow cytometry of peripheral blood and detection of clonality of T-cell receptor (TCR) rearrangement (see **Figure 1**).

Large granular lymphocytes represent a morphological subtype that are larger (15-18μm) than most circulating lymphocytes (7-10μm). LGL cells show an abundant cytoplasm containing prominent azurophilic granules and a round or reniform nucleus with mature chromatin (see **Figure 1**) (9).

Most patients present with a persistent increased number of circulating LGL ranging from 1-6 G/L. According to the 2017 WHO classification (12), a threshold of > 2 G/L (normal: <0.3 G/L) persistent circulating LGLs for more than 6 months is mandatory. However, numerous patients have a lower number of clonal LGLs, typically presenting with other clinical or hematologic features

such as RA or cytopenia. Accordingly, cases with LGL counts of <2 G/L meeting all other criteria are consistent with diagnosis as well (13).

The majority of T-LGL cells are CD3+, CD8+, CD16+, CD57+, CD45RA+, TCRαβ+, and CD4-, CD56-, CD27-, CD45R0-, CD28-, CD62L-, $CD5^{dim}$ and/or $CD7^{dim}$. Rarely LGLL is CD4+ with or without coexpression of CD8. NK-LGL leukemia and NK-LGL lymphocytosis are characterized by the following phenotype: CD2+, CD3−, CD3ϵ+, TCRαβ−,CD4−, CD8+, $CD16^{+}$, CD56+, $CD57^{+/-}$ (1).

Diagnosis is confirmed by detection of TCR rearrangement by PCR allowing distinguishing reactive LGL proliferation from real leukemic proliferation. The majority are αβ variants, while 10% are γδ variants (14). Clonality can also be assessed by flow cytometry for different TCR chain domains (Vβ, Vγ, Vδ) using various antibodies. The current Vβ mAbs panel covers 65% of the Vβ spectrum (15). Detection of γδTCR and its subtypes (Vδ1 and Vδ2) at protein level by flow cytometry represents a fast practical method for determining the clonality of γδ T-cells (16). As NK-LGL do not express TCR, restricted expression of activating isoforms of killer immunoglobulin-like receptor (KIR) can be used (17).

Bone marrow aspirate and/or biopsy with immunohistochemistry is not routinely recommended but can support the diagnosis in uncertain cases. Typical features observed in case of bone marrow infiltration of LGLL are hypercellularity with individual or small clusters of LGLs localized primarily in sinusoids. Often, reactive, predominantly CD20+ B-lymphoid aggregates are seen with peripherally accentuated CD3+ T-cells. Expression of cytotoxic markers TiA1, granzyme B and granzyme M are considered characteristic histopathologic findings of LGLL (18–21).

As T-LGLL can mimic other T-cell lymphoid malignancies, careful differentiation from lymphomatous and leukemic disorders affecting T-cells e.g. CLPD-NK, ANKL and from conditions with reactive LGL expansions, is required. Several conditions can lead to the development of reactive LGL proliferation, including viral infections (e.g. HIV, CMV, EBV, HBV and HCV), hemophagocytic syndrome, immune thrombocytopenia (ITP), non-Hodgkin lymphoma (NHL), solid tumors, splenectomy. These are typically poly- or oligoclonal (2, 7).

Furthermore, differentiation from Felty syndrome with typical triad of rheumatoid arthritis, neutropenia and splenomegaly might be difficult (1, 19, 20, 22, 23).

The etiology of T-LGL leukemia is still unknown. It is believed that the initial step relies on chronic antigen exposure leading to dysregulation of apoptosis, mainly due to dysregulation of the JAK/STAT pathway (1). Constitutive activation of STAT3 is often related to STAT3 gain of function mutations in 30-40% of T-LGLL (24, 25). *STAT5b* mutation is less frequent (2%) but highly prevalent in the rare subset of CD4+ T-LGL (1, 26–29). Therefore, mutations in *STAT3* and *STAT5b* were included in the 2017 WHO classification of LGL disease (3, 12). In addition, proinflammatory cytokines such as platelet-derived growth factor and IL-6, IL-12, IL-15 contribute to leukemic LGL persistence and proliferation (30). Interestingly, Felty syndrome might be associated with somatic *STAT3* mutations indicating a potential common pathogenesis (23). *STAT3* and *STAT5b* mutation might have an impact on clinical outcome, as *STAT3* mutation is associated with symptomatic disease and a specific phenotype: CD16+, CD56-, CD8+, Tγδ. Additionally, the immunophenotypic signature $CD56^{neg/dim}$/$CD16^{+}$/$CD57^{-}$ in CLPD-NK patients is associated with a more symptomatic disease and the presence of *STAT3* mutation (31). T-LGLL harboring a *STAT5b* mutation and being CD3+, CD8+, CD56+, CD16− and CD57− shows a more aggressive course with poor prognosis, whereas expression of CD4 and CD56 antigens as well as CD56, CD3, Tγδ-LGLL are often associated with a more indolent course (11, 27).

To illustrate our proposed algorithm (see **Figure 1**), we will further discuss two clinical cases of LGL-Leukemia.

CASE REPORTS

Indolent Course of a γδ T-LGL-Leukemia

A 42 year-old-male was seen by a rheumatologist for joint pain. However, no rheumatologic disease was found. Due to a leukocytosis of 17.7 G/l (3.9-10.2 G/L), the patient was referred to our clinic. B-symptoms or recurrent infections were denied. Past medical history included diabetes type 2, hypertension and obesity. The physical examination was unremarkable and the ultrasound showed neither lymphadenopathy nor hepatosplenomegaly. Laboratory findings revealed an increase

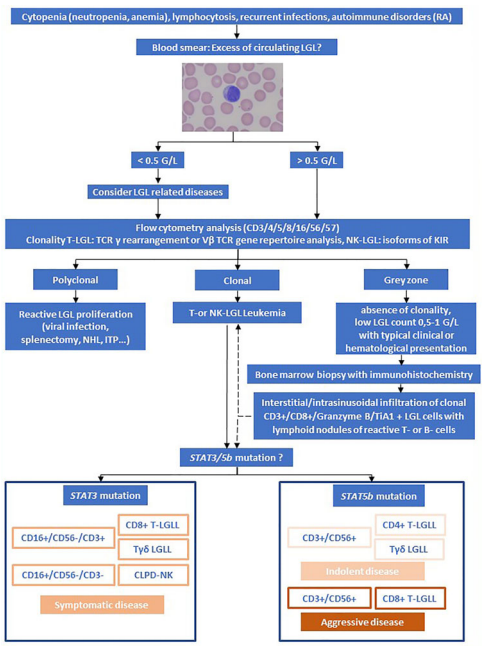

FIGURE 1 | Algorithm for the evaluation of LGL Leukemia. Adapted from Lamy et al. and Teramo et al. (1, 11). Rheumatoid arthritis (RA), Non-Hodgkin Lymphoma (NHL), immune thrombocytopenia (ITP).

of absolute lymphocytes (7.1 G/L) without neutropenia, anemia or thrombocytopenia. Serologic examination showed no viral infection or autoimmune disorder (RF, ANA negative). Peripheral blood smears demonstrated an increase of predominantly mature lymphocytes occasionally with cytoplasmic azurophilic granules. Flow cytometry revealed an increase in γδ T-cells with a CD2+, CD3+, CD16+, CD56+, CD5+, CD7+ and CD4-/CD8- phenotype, which constituted approximately 45% (2.1 G/L) of T-cells. Cytogenetic study showed a normal male karyotype and a T-cell receptor γδ gene rearrangement. In the bone marrow biopsy, a diffuse interstitial and intrasinusoidal infiltration of atypical CD3+, CD5+ T-lymphocytes with expression of cytotoxic molecules TiA1 and Granzyme B was observed. *STAT3* mutation was not detected. An asymptomatic course of T-LGLL was diagnosed, prompting a watch and wait strategy with laboratory and clinical controls every 3-6 months. After three years, the patient is in continuous observation without any symptoms.

γδ T-LGL-Leukemia Presenting With Immune Thrombocytopenia and Pure Red Cell Aplasia

A 70 year old patient presented with severe normochromic normocytic anemia with hemoglobin of 2.6 g/dL (13.5-17.2 g/dL), thrombocytopenia of 50 G/L (150-370 G/L) and normal total leukocyte and lymphocyte count. Past medical history encompassed stage II gastric carcinoma 12 years ago that was treated with gastrectomy and splenectomy, as well as perioperative chemotherapy. Thirteen months earlier to this presentation he had been admitted to the gastroenterology department due to microcytic hypochromic anemia (hemoglobin 7.5 g/dL) and thrombocytopenia (36 G/L). Bleeding as well as local recurrence were excluded by gastro-, colon- and capsule- endoscopy. Additionally, lab results showed a chronic kidney disease (CKD) with creatinine 2.31 (0.67-1.17 g/dl) and GFR 27.7 ml/min (>90 ml/min) with a concomitant iron deficiency assuming a renal anemia with substrate deficiency. The patient had received iron supplementation plus s.c. erythropoietin and had been discharged to outpatient care.

Endoscopies showed no evidence of bleeding. Next, the patient was referred to our hematology department. Neither "B" symptoms nor recurrent infections were reported. Serology revealed antibodies against glycoprotein IIb/IIa, Ib/IX confirming chronic ITP and cortisone therapy was initialized. Peripheral blood smear examination identified a slightly increased number of circulating LGL (0,985 G/L). Flow cytometry revealed an abnormal population of γδ T-cells with CD3+, CD16-, $CD57^{mid}$, $CD56^{dim}$, $CD8^{dim}$ and representing 42% of T lymphocytes. A bone marrow biopsy demonstrated selective pure red cell aplasia (PRCA), signs of dysmegakaryopoiesis, and a discrete proliferation of partially intrasinusoidal localized CD8+ CD3+ and TiA1+ T-cells. Granulocytopoiesis was largely regular. Cytogenetic and fluorescence *in situ* hybridization evaluation showed a normal karyotype (46, XY) and no chromosomal or genetic aberrations ruling out other hematological malignancies e.g. myelodysplastic syndrome. No viral (Parvovirus B19, HBV, HCV, EBV, CMV) or serological (ANA, ANCA, RF) positivity were found at the initial laboratory workup. Chest and abdominal computed tomography ruled out the presence of thymoma and other malignancies. Although *STAT3* mutation was not detected, TCR gene rearrangement showed a clonal pattern of the TCRγδ. These findings were consistent with the diagnosis of T LGL-associated PRCA. Immunosuppressive therapy was indicated because of autoimmune mediated thrombocytopenia and blood cell (RBC) transfusion dependency (every 1-2 weeks). Due to the patient's CKD, Cyclophosphamide (CP) p.o. with a dose of 50mg daily was started with careful monitoring of complete blood count to avoid myelotoxicity and prednisone therapy was continued. Erythropoietin injections were stopped. In addition, the patient received intravenous iron chelation therapy due to high ferritin levels (> 3800 μg/l). Platelet count and transfusion dependency improved and the patient is still on CP treatment. Treatment duration is planned for 6-12 months.

Treatment Considerations and Discussion

As most patients with T-LGLL have an indolent course, only half of patients require systemic treatment at the time of diagnosis and overall survival at 10 years is 70%. In asymptomatic patients, a watch and wait strategy with laboratory and clinical controls every 6 months is suggested. Treatment is only indicated in case of symptomatic disease or impaired blood values as follows: Severe neutropenia ANC <0.5 G/L or neutropenia-associated infections, anemia hemoglobin <10 g/dL or need for RBC transfusion, thrombocytopenia with platelets <50 G/L, symptomatic autoimmune diseases, symptomatic splenomegaly, and severe B-symptoms. The main goal of treatment is relief of symptoms, reduction of infections and transfusion independence. Disease related deaths are primarily related to severe infections occurring in <10% of patients. However LGLL is not curable by conventional treatment (1, 22, 32).

Immunosuppressive therapy such as methotrexate (MTX), cyclophosphamide (CP), and cyclosporine (CsA) either alone or in combination with prednisone remains the backbone of the treatment for LGL leukemia (1, 22). Initial response might be quicker when adding prednisone but has no impact on eradication of LGL clones (4). As therapy responses might be delayed, patients should be treated for at least 4 months before response assessment (1, 22). Whether MTX or CP should be given as first line therapy is not clear. To clarify this situation, a phase II randomized trial comparing first-line MTX versus CP (NCT01976182) is ongoing (26).

MTX is often preferred in the setting of neutropenia and/or rheumatoid arthritis. It is used p.o. or i.v. weekly in a dose of 10 mg/m (2) and can be continued indefinitely if tolerated. Response is achieved in approximately 55% with time to response ranging from 2 to 12 weeks and a median duration of response ranging from 2 to 4 years. In case of severe neutropenia, oral prednisone (1 mg/kg per day) is administered in addition to MTX for the first month and tapered off by the end of the second month (22).

For CP, in a dose of 50-100 mg/m (2), response rates 55–66% are described. Treatment is limited to no more than 12 months

(33). Case series demonstrated response rates to CP ranging from 60-100% in LGLL-associated PRCA (34).

If primary therapy is ineffective, a switch between MTX and CP is suggested (1). Analysis of a French cohort with 229 patients of LGL showed in 11/15 cases a clinical response with CP failed treatment of MTX (4). CsA is mostly reserved for the treatment of resistant disease (24). Dose ranges from 2–10 mg/kg/day, mostly 3 mg/kg/day and it shows an ORR of 56% and maintained as long as is it reasonably tolerated (4, 22, 24).

Other second-line agents are bendamustine, purine analogs and alemtuzumab (25, 32). Alemtuzumab, an anti-CD52 monoclonal antibody, demonstrated an ORR of 74% in a small phase II trial. However, due to toxicity, its use is limited to refractory cases and prophylactic antibiotics and CMV monitoring are necessary (1, 22, 35). Purine analogs (e.g. 2-chlorodeoxyadenosine, pentostatin and fludarabine) display a high ORR of 80% with a short period of treatment (1-3 courses) and the potential of inducing durable remission. However, data is limited and based on small case series and case reports (22, 33, 36–39).

There is no consensus regarding clinical management of aggressive forms of LGLL. Clinical behavior is close to aggressive leukemia and some clinicians propose a CHOP-like based or cytosine arabinoside-containing polychemotherapy, followed by autologous or allogeneic hematopoietic cell transplantation (1, 32, 40).

Considering the pathogenesis of LGL leukemia, various specific inhibitors were evaluated in T-LGLL. Tofacitinib, a JAK3-specific inhibitor, showed in T-LGLL patients an improvement of RA symptoms and a hematological response in 6/9 (67%) cases (26, 41). BNZ-1 a multi-cytokine inhibitor that inhibits interleukin (IL)-2, IL-15 and IL-9 signaling showed promising results in reducing cytokine mediated cell survival being investigated in a phase I/II trial (42). However, results are pending. The histone deacetylase (HDAC) inhibitor Belinostat has recently demonstrated a marked activity in refractory T-LGL (43). Interestingly, anti-CD20 MoAb Rituximab showed promising response in RA-associated LGL-leukemia (44).

Our first patient had a rare subtype of T-LGL with a specific phenotype: CD3+, CD16+, CD56+ and CD5+ but CD4-/CD8-. Regarding differential diagnoses, CD4-/CD8- T-LGL displays an immunophenotype and clinical pattern overlapping with the aggressive lymphoma hepatosplenic T-cell lymphoma (HSTCL). (45) [(p4)]As HSTCL is usually CD5 and CD57 negative, it is helpful in distinguishing it from Tγδ-LGLL (46). Moreover, in contrast to described cases in literature, our patient showed an asymptomatic course without splenomegaly or autoimmune cytopenia (11, 27, 47). A *STAT3* mutation was not detected. According to Teramo et al., CD3+, CD56+ and Tγδ- LGLL seems to correlate with an indolent presentation, which is compatible with the immunophenotypic profile and indolent course of our patient (11).

Our second patient with a γδ-T cell subpopulation being CD3+, CD16-, $CD57^{mid}$, $CD56^{dim}$ γδ-T cells showed a symptomatic course with ITP and PRCA. T-LGL is seen in 15% to 20% of patients with PRCA (48). Frequent red blood cell transfusions caused iron overload. Treatment with cortisone and CP resulted in transfusion independence and further confirmed the therapeutic potential of CP for T-LGLL combined with PRCA. The precise underlying mechanism of CP in LGLL-associated PRCA is still not known. It is suspected to work by reducing cytotoxic T-lymphocytes that damage antibody-bound erythroblasts directly (49).

In conclusion, LGL is a rare disease and prospective data are scarce. Diagnosis of LGL is complex and oligosymptomatic clinical presentation can delay diagnosis. Patients with LGL cells as described above should prompt a careful workup to rule out reactive LGL expansion from clonal LGL leukemia. Differential blood count, blood smear, immunophenotyping and TCR-rearrangement analysis are mandatory. If diagnosis of LGLL is confirmed, close controls depending on severity of either symptoms or lab findings are necessary for patients requiring therapy. However, the majority of cases are indolent and close monitoring is necessary.

AUTHOR CONTRIBUTIONS

JS wrote the manuscript. JS, AP, CK, PS, HK, and GH contributed to the manuscript preparation and have read and approved all drafts. GH reviewed and approved the final version of the manuscript. All authors contributed to the article and approved the submitted version.

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Advances in Cellular Therapy for T-Cell Prolymphocytic Leukemia

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T-cell prolymphocytic leukemia (T-PLL) is a rare, aggressive hematologic malignancy with a poor prognosis. Alemtuzumab (Campath) remains the cornerstone for treatment, with an 80% complete response (CR). Hematopoietic stem cell transplant (HSCT) is considered the standard of care as consolidative therapy in eligible patients. However, allogeneic stem cell transplant is also complicated by increased rates of infections from chemotherapy, acute graft-versus-host disease (GVHD), and chronic GVHD. This review aims to report the available literature on the efficacy and complications of consolidative HSCT. It also discusses the importance of patient selection and pre- and post-transplant complications including atypical infections and GVHD.

Keywords: T-PLL, CART cell, allogeneic stem cell transplant, CMV reactivation, autologous stem cell

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INTRODUCTION

T-cell prolymphocytic leukemia (T-PLL) is a rare aggressive malignancy originating from the mature post-thymic T cell. Although the incidence of this malignancy is only 2.0/million/year in Western countries, it is considered as one of the most common mature T-cell leukemias (1). Patients usually present with a steep increase in lymphocyte counts, organomegaly, lymphadenopathy, and occasional skin lesions (2–4). Diagnosis is most often established by the presence of characteristic mature post-thymic T-cell immunophenotype on flow cytometry, that is, TdT−, CD1a−, CD2+, CD5+, and CD7+ positive (2). High expression of CD52 provides an effective therapeutic approach for these patients with Campath (alemtuzumab), an anti-CD52 monoclonal antibody that has robust activity in newly diagnosed and recurrent T-PLL (5, 6). Despite achieving impressive response rates of up to 80%, the median overall survival (OS) is only 10–16 months, as most patients relapse at 12 months. Very few options are available for salvage therapy after relapse (7, 8).

Single-gene sequencing has provided deep insight into the pathophysiology of this disease, thereby creating several potential therapeutic targets. Recent studies have discovered that the loss of ataxia telangiectasia mutated gene (ATM) and activation of T-cell leukemia/lymphoma gene play a pivotal role in oncogenesis (9). Targeted therapy with inhibition of HiDAC (Histone Deacetylase), BCL2 (B-Cell Lymphoma-2), and JAK-STAT (Janus Kinases, Signal Transducer and Activator of Transcription proteins) have shown to be very promising in Phase I and preclinical studies (9, 10). Despite multiple therapeutic options that are currently being studied, the current standard of care is a consolidative allogeneic stem cell transplant following induction therapy with Campath in transplant-eligible patients (11–13). Further collaborative studies combining these therapeutic modalities are needed to improve prognosis and OS.

ROLE OF INDUCTION AGENTS IN T-CELL PROLYMPHOCYTIC LEUKEMIA

Alemtuzumab remains the cornerstone agent for active T-PLL. It is a fully humanized anti-CD52 antibody that induces antibody-dependent cell lysis, apoptosis, and complement activation (14). Campath has shown overall responses (ORs) of up to 90% or higher when compared with traditional chemotherapy-based combinations (6, 7). Complete response (CR) rate at induction was not improved when used in combination with other conventional agents (15). In a pivotal study by Dearden et al., intravenous Campath resulted in an OR rate (ORR) of 91% and CR of 81%. These outcomes were superior to those of subcutaneous Campath, which showed a 33% CR, establishing intravenous Campath as the standard induction regimen (7, 8). Despite a high ORR, the duration of remission is short-lived, with most patients relapsing within 12 months, necessitating further consolidative therapy. Alemtuzumab can have a lasting impact, as its clearance decreases with repeated dosing, due to progressive loss of CD52 receptors from the destruction of malignant and normal T cells. This results in a 7-fold increase in concentration after 12 weeks of therapy (16). CD52 is a glycoprotein that is expressed on the cell surface of various hematopoietic cells. It is primarily expressed on the cell surface of mature lymphocytes, natural killer cells, eosinophils, neutrophils, monocytes macrophages, and dendritic cells (17). Hence, Campath treatment can have a lasting impact on the function of host and donor T cells, thereby influencing outcomes of consolidative transplant.

ROLE OF HEMATOPOIETIC STEM CELL TRANSPLANT

Hematopoietic stem cell transplant (HSCT) is an effective form of consolidation for T-PLL. Both autologous (Auto-HSCT) and allogeneic stem cell transplants (Allo-HSCT) prolong OS and progression-free survival (PFS) when compared with no consolidation therapy after induction Campath (11). Allogeneic stem cell transplantation is currently the only available potential curative option for T-PLL. Recommendation for consolidative stem cell transplant is primarily made from case reports and retrospective studies (11–13, 18–21).

CONSOLIDATIVE TRANSPLANT VERSUS OBSERVATION

Krishnan et al. performed a multicenter retrospective analysis of 28 patients treated between 1996 and 2008 with either a consolidative autologous stem cell transplant (N = 15) or an allogeneic SCT (N = 13). OS and PFS were compared with those of 23 patients who were treated with Campath alone as first-line or second-line therapy. The patients in the non-transplant arm had achieved CR and survived for at least 6 months after the last dose of Campath. Among 15 patients who underwent autologous transplant, 11 patients were in CR1, 2 in CR2, and 2 in PR at the time of transplant.

All patients in this arm achieved a CR following an autologous transplant. Nine of these patients relapsed at a median of 15 months (5–56 months). There was 1 case of treatment-related mortality (TRM) secondary to pneumonitis. The median survival of patients receiving an autograft was 52 months. Among patients receiving allogeneic transplants, 9 were in CR1 and 4 in partial response (PR).

The allogeneic arm had 30% TRM that was attributed to fungal infection, refractory graft-versus-host disease (GVHD), pseudomonal sepsis, and Epstein–Barr virus (EBV)-associated post-transplant lymphoproliferative disorder (PTLD). Median OS was 33 months. The study showed a median OS of 48 months in patients receiving consolidative stem cell transplants (either auto or allo), which was more than the median survival in the non-transplant arm (20 months). The patients in the non-transplant arm were well-matched in patient characteristics to the transplant arm. This study showed that consolidation with HSCT after induction Campath was more beneficial than induction Campath alone. Even though patients had a median OS of 52 months with an Auto-HSCT and 33 months with an Allo-HSCT, the survival was not statistically different between these groups ($p = 0.2$). Patients undergoing allogeneic transplants had a high TRM of 30.7%, but survivors had long-term CR at a median follow-up of 6 years. The autologous arm, unfortunately, had a 60% relapse rate (RR), and all patients who relapsed died of progressive disease. This TRM may be reduced in the modern era with the introduction of reduced-intensity conditioning.

ROLE OF ALLOGENEIC STEM CELL TRANSPLANT

Currently available recommendations are based on retrospective studies from international and national research organizations. There are a few prospective studies; however, no interventional study has been reported. Given the incidence of this disease, it would be very arduous to design such a study.

A retrospective study from the Center for International Blood and Marrow Transplant Research (CIBMTR) reported 47 patients who underwent an Allo-HSCT for PLL from 1995 through 2005; 77% of the patients received matched unrelated donors. Twelve patients in this group received partially matched or single allele mismatch. Median PFS at 1 year was 33% (95% CI of 20%–47%), and 1-year OS was 48% (95% CI of 33–62 months) with a median OS of 11.2 months. In this study, 46% of the patients had refractory PLL when they had an allogeneic stem cell transplant. Of the patients, 52% (95% CI 38–66) developed grade 2–4 GVHD, and the 1-year incidence of chronic GVHD was 42% (95% CI 28–57). Factors such as age, conditioning intensity, T- or B-PLL, CR after single or multiple lines of therapy (CR1 *vs.* CR2), and presence of acute or chronic GVHD were not shown to influence OS. Due to the size of the

study and the heterogeneity in the patient population, the authors were unable to identify factors influencing outcomes with Allo-HSCT (12).

The European Society for Blood and Marrow Transplantation (EBMT) database has reported outcomes of 41 patients with T-PLL who underwent an allogeneic stem cell transplant from 1995 to 2006. Patients had received allografts from either a matched sibling donor (51%) or a matched unrelated donor. At a median follow-up of 36 months, this study reported a 3-year relapse-free survival of 19% and an OS of 21%. Three-year non-relapse mortality (NRM) and relapse incidence were 41%. Multivariate analysis showed that conditioning regimens containing total body irradiation (TBI) and a shorter interval between diagnosis and HSCT were associated with favorable relapse-free survival. No other recipient or donor-related factors had an impact on OS or PFS (13). Hence, this study further indicated that early referral to HSCT is associated with favorable outcomes.

The French registry reported a 36% (95% CI −17 to 54) 3-year OS and 26% PFS (95% CI 14–45) in 27 patients. Ten patients received HLA identical sibling allograft and 18 matched unrelated donors (one patient received a second Allo-SCT). Notably, this study only had 11% of patients who had refractory disease; the other patients were in complete remission or at least in a PR. With a median follow-up of 33 months, the estimated 3-year OS was 36% (95% CI −17 to 54%), and PFS was 26% (95% CI 14–45%). There were no factors associated with OS in the univariate analysis, and a trend for improved OS was seen in patients who received TBI in the conditioning regimen (21).

Most recently, EBMT has reported a prospective observational study of patients receiving an allogeneic stem cell transplantation for T-PLL from 2007 to 2012. A total of 54 patients were screened for this study. The study excluded patients with non-confirmed T-PLL diagnosis by a central laboratory, age ≥ 65 years, refractory disease at Allo-HSCT, cord, and mismatched unrelated donor transplants.

Thirty-seven patients were evaluable for the study endpoints; 44% of the patients received a transplant in CR1.

Most patients in the study had been treated with Campath before stem cell transplant, and the median time interval between the last dose of Campath and Allo-HSCT was 75 days; 30% of these patients received TBI doses of 6 Gy or higher. This study had a median follow-up of 50 months (12–78 months), the 4-year OS was 42% (25%–59%), and PFS was 30% (14%–46%). The median OS was 27.8 months, and PFS was 19.2 months. No factors were noted to have an impact on the outcome in multivariate analysis (22).

Single-center retrospective studies have reported a 4-year OS of 56%, NRM of 34%, a 4-year RR of 21%, a median PFS of 15 months (95% CI 12–99), and OS of 56 months [95% CI 15–56; (23)]. Sellner et al., in their case series of 10 patients, studied the utility of T-cell receptor (TCR)-based minimal residual disease (MRD) quantification for monitoring disease status in T-PLL. They reported a cumulative OS and PFS of 20%, an RR of 50%, and an NRM of 30% in the median follow-up period of 58 months (3–92 months). This interesting study aimed to correlate quantitative MRD monitoring by clone-specific real-time PCR of TCR rearrangements and the TCR repertoire diversity by next-generation sequencing (NGS). Patients who achieved MRD negativity with immunological interventions had a corresponding increase in the poly-clonality of their T cells (24).

Table 1 summarizes the abovementioned studies and highlights important data including nature of transplant, disease status prior to transplant, OS, and TRM.

Newly diagnosed T-PLL patients who need to be treated should be induced with intravenous Campath, preferably in experienced centers. All patients must be referred promptly to the Bone Marrow Transplant Team during induction. Based on the above-published retrospective studies, the National Comprehensive Cancer Network (NCCN) recommends that patients who obtain a CR or PR after initial therapy should be considered for a consolidative allogeneic stem cell transplant.

However, Allo-HSCT is associated with significant treatment-related mortality and morbidity. Patient's performance status, donor availability, disease status at the time of HSCT, presence of atypical infections occurring secondary to Campath, and other general medical comorbidities play a crucial role in determining the risk versus benefit of proceeding with an allogeneic stem cell transplantation. The hematopoietic cell transplantation (HCT)-specific comorbidity index (HCT CI) published and validated by Sorror et al. includes a comprehensive pre-transplant assessment of preexisting comorbidities. A score of 3 or more in this assessment predicts 41% 2-year NRM (25). Autologous stem cell transplant as consolidative therapy can be considered in patients whose risk of undergoing an allogeneic stem cell transplant outweighs the potential benefit of cure. Although autologous stem cell transplant does not have the potential of cure, Krishna et al. reported an OS of 52 months in the Auto-SCT arm *vs.* 20 months in the non-transplant arm. Consolidative HSCT is preferred over observation after obtaining an optimal response to alemtuzumab. Prospective randomized trials with novel induction agents are crucially needed to improve outcomes; however, the rarity of this disease poses a significant challenge to the feasibility of such a study.

NON-RELAPSE MORTALITY FOR ALLOGENEIC HEMATOPOIETIC STEM CELL TRANSPLANTATION

Most published data have reported 30%–40% treatment-related mortality; however, Allo-HSCT offers a potential long-term survival benefit for some patients. The main contributors to NRM are GVHD and infections. A retrospective analysis from the CIBMTR and EBMT did not show any association between age and mortality (12, 13). Recent advances in reduced-intensity conditioning regimens have reduced TRM in other diseases needing consolidative HSCT (26). Hence, it is hoped that the introduction of reduced-intensity conditioning regimens for T-PLL would result in improved TRM with longer follow-up.

In a single-center experience of treating more than 80 PLL patients, almost half of those achieving remission have proceeded

TABLE 1 | Studies of Stem cell transplant for PLL.

Study	Auto- *vs.* Allo-SCT	Status at transplant	Conditioning regimen	Donor status	OS (median), months	Relapse rate	Acute GVHD grade 2–4	Chronic GVHD —1 year	Treatment-related mortality
Krishan et al.	Auto	CR1 and CR2, PR	84% TBI based		52	60% at 1 year	–	–	6.6%
	Allo	CR1, PR	MAC—33% All TBI based RIC—67% Flu/Mel	MUD 58% MRD—42%	33	30.7% at 1 year	23%	–	30.7%
Kalaycio et al.	Allo	CR, PR 46% refractory disease	MAC—40% >500 cGy or >9 mg/kg Bu RIC—30% <500 cGy or <9 mg/kg Bu Neither—30%	MRD—23% MUD—49% MMUD—25% Ukn—2%	11.2	39% at 1 year	52%	42%	28%
Wiktor-Jedrzejczak et al.	Allo	CR, PR 50% refractory disease	TBI based 54% Chemo based—32% Unknown—14%	MRD—51% MUD—49%	12	41% at 3 years	39%	44%	41% at 3 years
Guillaume et al.	Allo	CR, PR, 11% refractory disease	MAC—41% RIC—59% TBI based—56% Chemo—44%	MRD—37% MUD—63%	26	47% at 3 years	51%	40%	31% at 3 years
Wiktor-Jedrzejczak et al., 2019	Allo	CR, PR	MAC—35% (>6 Gy) RIC—65% (<6 Gy) Only TBI based	MRD—43% MUD—57%	27.8	38% at 4 years	19%	43%	32% at 4 years
Dholaria et al.	Allo	CR, PR	MAC—73% Flu/Bu Pen/Bu RIC—27% Flu, Cy TBI Flu, Mel	MRD—46% MUD—27% MMUD—18% Cord—9%	56	23% at 4 years	28%	54%	32% at 4 years
Sellner et al.	Allo	CR, PR	Flu Cy—40% Flu/TBI—60%	MRD—40% MUD—40% MMUD—10% Haplo-10%	10	50% at 58 months	–	–	30% at 58 months

Allo, allogeneic; Auto, autologous; CR1, complete response 1; CR2, complete response 2; PR, partial response; TBI, total body irradiation; MAC, myeloablative conditioning; RIC, reduced-intensity conditioning; Flu, fludarabine; Mel, melphalan; Bu, busulfan; Pen, pentostatin; Cy, cyclophosphamide; MRD, matched related donor; MUD, matched unrelated donor; MMUD, mismatched unrelated donor; Haplo, haplo-identical; Cord, cord blood; Ukn, Unknown.

to either an autologous or an allogeneic stem cell transplant. Most centers provide a "washout period" of 6 weeks to 3 months from completion of induction Campath to allogeneic stem cell transplant (Insert). This is thought to reduce the risk of failure of engraftment and reduce the risk of ongoing infection. In a case series reported by Shumilov et al., they noted that 5/10 patients succumbed to NRM. This was primarily attributed to post-transplant infectious complications. Cytomegalovirus (CMV) reactivation was observed in 60% of patients with 1 lethal infection. It is to be noted that no letermovir prophylaxis was given to these patients, and hence, the rates of reactivation may be lower in the letermovir era (27, 28).

Routine monitoring for CMV reactivation, anti-infective prophylaxis for herpes virus, and *Pneumocystis jiroveci* pneumonia are recommended for all patients even during induction with alemtuzumab-based regimens and must be continued during and post Allo-HSCT. These patients should be considered for letermovir prophylaxis if they have undetectable CMV DNA prior to transplant (28). It is advisable to screen these patients for fungal colonization with imaging and to consider further workup and treatment prior to stem cell therapy (29). Infectious screening for *Strongyloides* should be performed especially in patients originating from endemic regions, with the help of Serological testing and stool

specimens. These patients can be treated with Ivermectin before transplant.

Screening for latent tuberculosis using QuantiFERON or Tuberculin skin test must be performed in these patients before stem cell transplant, and patients should be treated for latent tuberculosis infection (LTBI) concomitantly pre- and post-transplant (30).

Retrospective and prospective studies report an incidence of grade 2–4 acute GVHD ranging from 19% to 52%, with a 40%–55% incidence of chronic GVHD. The graft versus leukemia activity in T-PLL has been shown by correlating minimal residual kinetics (by TCR-based MRD quantification) with the TCR diversity alterations in patients receiving immunomodulation such as immunosuppression or donor lymphocyte infusions after an allogeneic transplant (24). Despite a washout period of 6 weeks from Campath, robust donor T-cell graft versus leukemia activity was noted in the study. Hence, early recognition and aggressive management of grade 2–4 GVHD play a pivotal role in improving treatment-related mortality. Therapeutic advancements and investigative trials in acute and chronic GVHD have led to the Food and Drug Administration (FDA) approval of agents like ruxolitinib, ibrutinib, and belumosudil (31–33). These recent advances should indeed contribute to decreasing treatment-related mortality in the upcoming years.

RECENT ADVANCES IN CELLULAR THERAPY FOR T-CELL PROLYMPHOCYTIC LEUKEMIA

A recent case report has suggested acceptable toxicity to intrathecal (IT) Campath for refractory leptomeningeal prolymphocytic leukemia. IT Campath was also successful in the eradication of the leptomeningeal disease, which is resistant to triple IT chemotherapy and total brain irradiation (34). There are no published data on the efficacy of a consolidative allogeneic transplant in reducing the risk of central nervous system (CNS) relapse in T-PLL. CD30 is one of the cell surface proteins that is expressed on T cells, becoming an apt target against which chimeric antigen receptor-T (CAR-T) cells can be manufactured. However, targeting pan T-cell antigens not only would lead to severe T-cell immunosuppression but also would lead to autologous CAR-T destruction (35, 36).

CAR T-cell therapy has also been based on the TCR beta chain constant (TRBC) locus clonality; this technique may be more applicable in T-cell malignancies. Normal T-cell populations have a mixture of both TRBC 1- and TRBC 2-positive cells, while malignant T cells express only one beta chain. Hence, CAR T cells targeting the TRBC of the malignant clone would specifically target the malignant T-PLL cells and spare the normal T cells (37). The complementarity determining region 3 (CDR-3) is a hypervariable region of the TCR, which is responsible for binding the antigen. This would also be a potentially interesting target against which CAR T-cells can be manufactured (38).

There is a paucity of clinical trials for this uncommon disease. Several agents that have been implicated in the biology of T-PLL are currently being studied in phase 1 and preclinical studies. These include HiDAC, JAK-STAT, and BCL2 inhibitors (39–41). A combination of these novel agents with stem cell transplantation is also currently being studied in the form of post-transplant maintenance to reduce RRs (NCT02512497).

CONCLUSION

1. Anti-CD52 antibody, Campath, as a single agent given intravenously remains the standard of care for induction therapy in T-PLL. Despite high ORRs, the CR is short-lived; and stem cell consolidation therapy is essential to provide an opportunity for cure (6).
2. Early referral to stem cell transplantation for patients receiving induction Campath is crucial for improving OS (13). All patients younger than 75 years should be referred for consideration of consolidative HSCT.
3. Allogeneic transplant is considered for patients who are younger than <75 years, with Eastern Cooperative Oncology Group (ECOG) <2, and with minimal comorbidities, as assessed by the HCT CI.
4. Response to Campath, availability of suitable donors, patient compliance, and adequate social support are some of the other important factors taken into consideration for patient's suitability for Allo-HSCT.
5. Autologous HSCT can be considered in patients for whom the risk of an allogeneic transplant can outweigh the benefit, or in patients lacking suitable donors.
6. Adequate washout period of at least 6–12 weeks from Campath induction is preferred before proceeding with an allogeneic or autologous transplant.
7. Thorough investigation and treatment of underlying infections pre- and post-transplant play an important role in the reduction of mortality.
8. Reduced-intensity conditioning regimens, prophylactic antiviral agents such as letermovir, and the recent increase in the availability of multiple FDA-approved agents for acute and chronic GVHD are hoped to reduce TRM (26, 33).

This is an extremely exciting era for T-PLL, as deep insight into the intracellular mechanisms has led to the application of various agents to achieve an improved response.

The combination of these agents with cellular immunotherapy will elicit deep responses and improve RRs, thereby improving OS in this rare but fatal disease.

AUTHOR CONTRIBUTIONS

IV performed the literature search and data for the article. KB provided the concept and framework and edited and revised the article. All authors listed have made a substantial, direct, and intellectual contribution to the work and approved it for publication.

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Prognostic Significance of Comprehensive Gene Mutations and Clinical Characteristics in Adult T-Cell Acute Lymphoblastic Leukemia Based on Next-Generation Sequencing

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Background: Adult T-cell acute lymphoblastic leukemia (T-ALL) is a heterogeneous malignant tumor with poor prognosis. However, accurate prognostic stratification factors are still unclear.

Methods: Data from 90 adult T-cell acute lymphoblastic leukemia/lymphoma (T-ALL/LBL) patients were collected. The association of gene mutations detected by next-generation sequencing and clinical characteristics with the outcomes of T-ALL/LBL patients were retrospectively analyzed to build three novel risk stratification models through Cox proportional hazards model.

Results: Forty-seven mutated genes were identified. Here, 73.3% of patients had at least one mutation, and 36.7% had ≥3 mutations. The genes with higher mutation frequency were *NOTCH1*, *FBXW7*, and *DNMT3A*. The most frequently altered signaling pathways were NOTCH pathway, transcriptional regulation pathway, and DNA methylation pathway. Age (45 years old), platelet (PLT) (50 G/L), actate dehydrogenase (LDH) (600 U/L), response in D19-BMR detection, TP53 and cell cycle signaling pathway alterations, and hematopoietic stem cell transplantation (HSCT) were integrated into a risk stratification model of event-free survival (EFS). Age (45 years old), white blood cell (WBC) count (30 G/L), response in D19-BMR detection, TP53 and cell cycle signaling pathway alterations, and HSCT were integrated into a risk stratification model of overall survival (OS). According to our risk stratification models, the 1-year EFS and OS rates in the low-risk group were significantly higher than those in the high-risk group.

Conclusions: Our risk stratification models exhibited good prognostic roles in adult T-ALL/LBL patients and might guide individualized treatment and ultimately improve their outcomes.

Keywords: T-cell acute lymphoblastic leukemia/lymphoma, next-generation sequencing, mutations, clinical characteristics, risk stratification

INTRODUCTION

T-cell acute lymphoblastic leukemia (T-ALL) in adults is an aggressive and heterogeneous hematopoietic malignancy caused by the clonal proliferation and abnormal differentiation of T lymphoid progenitor cells. Nowadays, due to the standard frontline intensive chemotherapy, 85% of T-ALL patients have achieved complete remission (CR) (1, 2). However, there is still up to 40% of adults who relapse after intensive chemotherapy, with 5-year overall survival (OS) less than 7% (3). Therefore, finding new therapeutic targets and using precisely targeted drugs are of great significance to improve the therapeutic efficacy of T-ALL.

Currently, the intensity of T-ALL treatment is based on the risk stratification using a combination of age, white blood cell (WBC) count, and extramedullary infiltration, cytogenetic, and early response to induction chemotherapy. However, it is still difficult to accurately predict the prognosis of adult T-ALL patients according to present risk stratification models. With the rapid development of next-generation sequencing (NGS) in recent years, the genomic analyses of T-ALL have been extensively explored and various genetic markers associated with T-ALL pathogenesis were identified (4–7). It has been indicated that genomic analyses could systematically identify genetic risk loci for T-ALL susceptibility (8) and support prenatal origin (9, 10). A latest study demonstrated that the mutated gene profile of adult T-ALL patients differed from that of pediatric patients and indicated an association with age in T-ALL patients (11). Furthermore, genomic analysis is conducive to comprehend the genetic basis of clonal evolution and relapse in T-ALL (12–14). A recent study also revealed that the genomic analyses can early predict the relapse of adult T-ALL driven by mutated genes and may guide clinical decisions (15). In addition, gene mutations and signaling pathway alterations based on genomic analyses are important predictors of clinical outcome in adult ALL (16). Up-to-date risk stratification of T-ALL patients based on the genome analyses showed that gene mutations had impacts on prognosis and were conducive to subdivide cases into different risk groups (17). Therefore, integration of gene mutations into current risk stratification criteria may be beneficial to improve prognosis identification and therapeutic efficacy. However, relative data are mostly lacking in adult T-ALL.

In this study, we simultaneously collected gene mutation profiles by NGS and clinical characteristics in 90 adult T-cell acute lymphoblastic leukemia/lymphoma (T-ALL/LBL) patients. Statistical analysis identified that some gene mutations were significantly correlated with clinical prognostic indicators including CR, minimal residual disease (MRD), event-free survival (EFS), relapse-free survival (RFS), and OS. Based on these prognosis-related gene mutations and clinical characteristics, we established three T-ALL risk stratification models to predict long-term prognosis and guide individualized regimens.

PATIENTS AND METHODS

Patients and Treatment Protocol

A retrospective analysis had been conducted on 90 T-ALL/LBL patients hospitalized in Wuhan Union Hospital from June 2016 to June 2021. All patients, who were diagnosed as T-ALL/LBL according to the 2016 World Health Organization (WHO) diagnostic criteria, underwent bone marrow (BM) examinations such as cell morphology, immunophenotype, fluorescence *in situ* hybridization (FISH), fusion gene, cytogenetics, and molecular genetics (namely, NGS).

According to the Chinese guidelines (2021 version), all patients in our study received induction and intensive chemotherapy [daunorubicin, vincristine, cyclophosphamide, l-asparaginase, and prednisone (DVCLP), daunorubicin, vincristine, l-asparaginase, and prednisone (DVLP), hyper-fractionated cyclophosphamide, vincristine, doxorubicin, and dexamethasone/methotrexate, cytarabine (Hyper-CVAD/MA)]. Some T-ALL/LBL patients with suitable transplantation donors accepted hematopoietic stem cell transplantation (HSCT) after remission (if age ≤55 years old). This study has been approved by the Ethics Committee of Tongji Medical College of Huazhong University of Science and Technology and followed the principles of the Declaration of Helsinki.

Flow Cytometry

In accordance with WHO's guidelines, all 90 cases were diagnosed as T-ALL/LBL by particular immunophenotypic markers (usually TdT positive, usually expressing cCD3 and CD7, variably expressing CD1a, CD2, CD3, CD4, CD5, CD7, and CD8). T-ALL/LBL was further classified into pro-T-ALL, pre-T-ALL, cortical T-ALL, and medullary T-ALL according to the European Group for the Immunological Characterization of Leukemias (EGIL) classification standard (2, 18).

Cytogenetic Analysis

Clonal karyotypes in mitotic phases were detected by G-banding chromosome analysis under microscope and were described according to the International System for Human Cytogenetic Nomenclature (ISCN, 2013).

Next-Generation Sequencing

The mononuclear cells isolated from the newly diagnosed patients' BM were later used for whole genome DNA (gDNA) extraction, and then NGS technology was applied to determine the type, location, and frequency of each gene mutation using a predesigned hematopoietic tumor-related hotspot gene panel (Further details of gene panels are available in the **Supplementary Appendix**). Detailed methodology was described below. The gDNA concentration was required to be ≥10 ng/μl, OD260/OD280 = 1.7–1.9, and the total mass ≥1,000 ng. The Illumina standard library (Illumina, Inc.) was then constructed and Agilent 2100 (Agilent, Inc.) was used to assess the spectrum of DNA fragments in the library, and the main peak size of the library was about 350 bp. The Roche NimbleGen liquid phase hybridization capture chip was used to target capture 214 genes with 445k in size (Roche, Inc.). QPCR quantification was carried out to measure the library concentration; the concentration of each library should be ≥10 nmol/L. PE75 sequencing was performed on Illumina Nextseq 550AR (Illumina, Inc.) after completion of the library control. Sequencing data were analyzed using the following methods: the in-house developed quality control tools were firstly used to initiate the preprocessing and quality control analysis of the raw sequencing data, followed by using the Burrows-Wheeler Alignment (BWA) algorithm to compare the processed

sequencing data with the reference human genome (version: GRCh37/hg19). Picard was chosen for PCR duplication labeling, and GATK's BaseRecalibrator was used for quality value correction of sequence alignment results. Based on the cosmic database, we used a self-built Panel of Normals (PON) with a large sample to exclude germline mutations and common single nucleotide polymorphisms (SNPs) and filter output of the variants manually. Based on the paired samples, the MuTect2 software was used for single-nucleotide variation (SNV) and Insertion/Deletion (INDEL) mutation detection, and the self-built method was used for internal tandem duplication (ITD) and protein transduction domain (PTD) mutation detection. Detection limit of NGS was set to 0.5%. Variants were annotated using Annovar software for all tests, and to ensure data quality, the average effective depth of each sample captured in the target area was required to be ≥1,000x, and it was required that all reads that support mutant types have a quality and base quality higher than 30.

Statistical Methods

The follow-up was carried out until June 2021. OS was calculated from the date of diagnosis of T-ALL/LBL to the date of death for patients who died or the last follow-up date for those who were alive at the time of the analysis. EFS was calculated from the beginning of treatment until the date of induction failure, first relapse, or death. Response in BM was evaluated on the 19th day (D19-BMR) during induction treatment and was categorized as M1 (lymphoblasts <5%), M2 (5%–25%), and M3 (≥25%). Univariate and multivariate analyses were performed to identify potential prognostic factors. The chi-square (X^2) test and Fisher's exact test were applied to identify pairwise relationships between genetic alterations. The variables with $P < 0.1$ in univariate analysis were incorporated into the Cox proportional hazards model for multivariate analysis. CR, MRD, EFS, RFS, and OS were calculated by the Kaplan–Meier method, and then differences between groups were compared by the log-rank test.

The candidate risk factors were included into the Cox proportional hazards model and filtered by least absolute shrinkage and selection operator (LASSO) regularization. The models were checked by variance inflation factor (VIF) and C-index. All analyses were performed by R statistical software 4.0.1. A two-sided $P < 0.05$ indicated that the difference was statistically significant.

RESULTS

Gene Mutational Analysis Based on Next-Generation Sequencing

Gene Mutation Profiles

Among the 90 newly diagnosed T-ALL/LBL patients, 66 cases (73.3%) had at least 1 mutation and 33 cases (36.7%) had more than 3 mutations. There were even 2 cases with 6 mutations. The gene with the highest mutational frequency was *NOTCH1* 30.0% (27/90), followed by *FBXW7* 16.7% (15/90), *DNMT3A* 14.4% (13/90), *PHF6* 12.2% (11/90), *RUNX1* 11.1% (10/90), *JAK3* 10.0% (9/90), and *IDH2* 7.8% (7/90) (**Table S1** and **Supplementary Figure S1**). Pairwise correlations of these gene mutations in our dataset were visually depicted by Circos plots (**Figures 1A**–**H**).

Mutated genes are grouped by signaling pathways. The mutational landscapes of 90 adult T-ALL/LBL patients were described in **Figure 1I**. Signaling pathway analyses were further performed, and the most frequently altered pathway was the NOTCH pathway (34.4%, 31/90), followed by the transcriptional regulation pathway (24.4%, 22/90), DNA methylation pathway (18.9%, 17/90), Janus kinase/signal transducer and activator of transcription (JAK/STAT) pathway (18.9%, 17/90), lymphoid differentiation and development pathway (15.6%, 14/90), histone methylation pathway (14.4%, 13/90), RAS signal pathway (11.1%, 10/90), TP53 and cell cycle pathway (6.7%, 6/90), phosphatidylinositol 3-kinase/protein kinase-B/mammalian target of rapamycin (PI3K/AKT/mTOR) pathway (6.7%, 6/90), and Wnt/β-catenin pathway (2.2%, 2/90) (**Table S1**, **Supplementary Figure S1**). The frequency of other mutated genes and altered signaling pathways were shown in **Supplementary Table S1** and **Figure S1**.

The Pairwise Relationship Between Genetic Alterations

The pairwise analysis of all mutated genes and signal pathways were shown in **Tables S2, S3**. By integrated mutational analysis, we found significant co-occurrence of *NOTCH1* mutations and *FBXW7* mutations, *NOTCH1* mutations and *IL7R* mutations, *FBXW7* mutations and *IL7R* mutations, *PHF6* mutations and *NRAS* mutations, and *DNMT3A* mutations and *IDH2* mutations ($P < 0.05$ for all comparisons) (**Table S4**). Results also disclosed some frequently co-occurring signal pathways, including histone methylation signaling pathway and lymphoid differentiation and development signaling pathway, RAS signaling pathway and lymphoid differentiation and development signaling pathway, RAS signaling pathway and transcriptional regulation signaling pathway, lymphoid differentiation and development signaling pathway and transcriptional regulation signaling pathway, and JAK/STAT signaling pathway and NOTCH signaling pathway ($P < 0.05$ for all comparisons) (**Table S4**). No mutated genes or altered signal pathways were found mutually exclusive in our study.

Prognostic Value of Gene Mutations

We further analyzed the prognostic value of gene mutations (**Table S5**) and found that *FBXW7* mutations and *PTEN* mutations were related to increased CR rate ($P < 0.001$ and $P < 0.05$, respectively), while *DNMT3A* mutations were related to decreased CR rate ($P < 0.05$). However, *NOTCH*, *PHF6*, *JAK3*, and *IL7R* mutations had no significant effect on CR. Patients with *FBXW7* mutations had a significantly increased MRD negative rate ($P = 0.006$). However, no gene mutations had remarkable effects on EFS in our study. Patients with *WT1* mutations had significantly decreased RFS ($P < 0.001$). The OS of patients with *TP53* or *FLT3* mutations was significantly shortened (both $P < 0.05$), while *NOTCH1*, *FBXW7*, *IL7R*, *IDH2*, and *DNMT3A* mutations had no remarkable effects on OS.

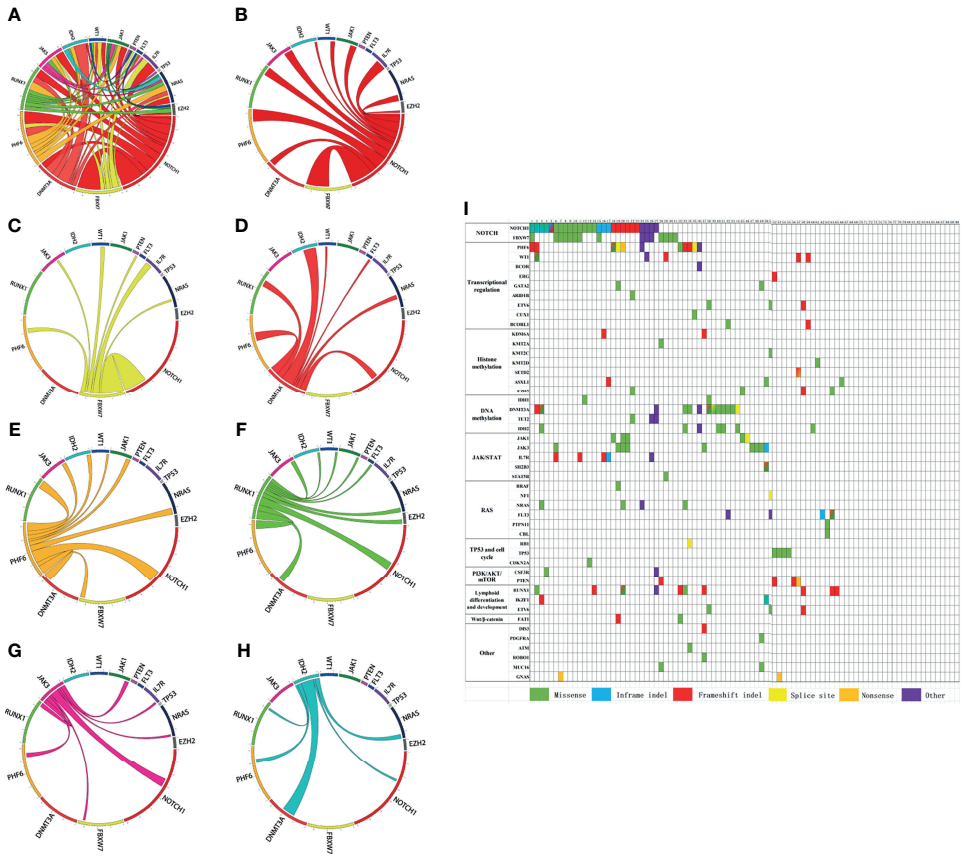

FIGURE 1 | **(A–H)** Circos plots visually depict the pairwise correlation of gene mutations in our dataset. **(I)** Mutated genes are grouped by signaling pathways. The figure shows the mutational landscapes of 90 adult T-cell acute lymphoblastic leukemia/lymphoma (T-ALL/LBL) patients. Each column represents a patient, and each row represents a gene. Each color indicates a type of mutation. Blended color square denotes more than two mutation types, which are represented by the corresponding colors.

Univariate analysis of signaling pathways (**Table S6**) showed that DNA methylation pathway alterations, TP53 and cell cycle pathway alterations, and lymphoid differentiation and development pathway alterations were related to decreased CR rate (all $P < 0.05$). DNA methylation signaling pathway alterations and lymphoid differentiation and development signaling pathway alterations were related to increased MRD positive rate ($P < 0.05$, respectively). Patients with TP53 and cell cycle signaling pathway alterations had significantly decreased EFS ($P < 0.001$), while patients with JAK/STAT pathway alterations had significantly increased EFS ($P < 0.05$). However, no signaling pathways had effects on RFS in our study. Results also indicated that the OS of patients with TP53 and cell cycle signaling pathway alterations was significantly shortened ($P = 0.001$), while the OS of patients with JAK/STAT signaling pathway alterations was significantly extended ($P < 0.05$).

Clinical Characteristics Analysis

Clinical Characteristics of Patients

Besides gene mutational analysis, we also summarized the primary clinical characteristics of these 90 newly diagnosed T-ALL/LBL patients (**Table S7**). The median age was 27 years (range from 14 to 70 years old). The median follow-up time was 6 months. Here, 85.6% of patients (77/90) were diagnosed as T-ALL, while the other 13 patients were T-LBL. Additionally, according to the immunophenotype of patients, 32 of them were categorized as pro-T (35.6%), 36 as pre-T (40%), and 22 as cortical T subtype (24.4%). Furthermore, up to 46.7% cases in our study (42/90) met criteria for early T-cell precursor (ETP)-ALL according to the 2016 WHO (19). Moreover, 33 common leukemia fusion genes in our study were detected by RT-PCR (Further details of the 33 fusion genes are available in the Supplementary Appendix.).

Univariate Analysis of Clinical Characteristics

The clinical characteristics associated with prognostic markers including CR, MRD, EFS, RFS, and OS were screened out by univariate analysis. As shown in **Table S7**, age, immunophenotype, WT1 expression, day 8 prednisone response, and day 19 lymphoblast percentage are predictors of reaching CR rate and MRD negative rate. These and other clinical characteristics were predictors of EFS, RFS, and OS as summarized in **Table S7**. The number of cases who had a

certain gene fusion in our study was slightly less (14/90), and univariate analysis showed that the fusion genes were not associated with the prognosis of adult T-ALL/LBL patients, so that fusion genes were not included in risk stratification.

Multivariate Analysis of Gene Mutations and Clinical Characteristics

The statistically significant risk factors in gene mutations and clinical characteristics from univariate analysis above were chosen for further multivariate analysis. It revealed that Hb >100 g/L and M1 in D19-BMR detection were independent favorable prognostic factors for CR, while DNA methylation signaling pathway alterations and ETP were independent negative prognostic factors for CR. Cortical T and M1 in D19-BMR detection were independent favorable prognostic factors for MRD, while DNA methylation signaling pathway alterations were independent negative prognostic factors for MRD. Age ≤45 years old, PLT >50 G/L, LDH ≤600 U/L, HSCT, and M1+M2 in D19-BMR detection were independent favorable prognostic factors for EFS, while TP53 and cell cycle signaling pathway alterations were independent negative prognostic factors for EFS. Age ≤45 years old, WBC count ≤30 G/L, HSCT, and M1+M2 in D19-BMR detection were independent favorable prognostic factors for OS, while TP53 and cell cycle signaling pathway alterations were independent negative prognostic factors for OS. However, risk factors for RFS by univariate analysis were too few to carry out further multivariate analysis.

Risk Stratification Models of Overall Survival in 90 Adult T-ALL/LBL Patients

Univariate and multivariate analyses showed that age (45 years old), WBC count (30 G/L), response in D19-BMR detection, TP53 and cell cycle signaling pathway alterations, and HSCT were independent predictors for OS (**Table 1**). Then, the above five independent predictors of OS were integrated into an OS rate estimation nomogram (**Figure 2A**). The C-index of the nomogram was 0.844 (**Figures 2B–D**). The calibration plots showed good agreement between predictions and actual observations in our study (**Figures 2E–G**). In order to well evaluate the prognosis of patients, the receiver operating characteristic (ROC) analysis was conducted and the area under receiver operating characteristic curves (AUC) was calculated. The Youden Index was used to determine the optimal cutoff point that has the highest combination of sensitivity and specificity to discriminate between low-risk and high-risk patients. With the threshold score of 140 for OS nomogram, 54 patients with total points ≥140 (AUC ≥86.4) were defined as low-risk group and 36 patients <140 (AUC <86.4) as high-risk group. The 1-year OS rate of T-ALL/LBL patients in the low-risk group was significantly higher than that in the high-risk group [all patients: 70.4% vs. 30.6%, $P < 0.0001$; hazard ratio (HR): 7.956, 95% CI: 3.915–16.17] (**Figure 2H**).

Of these 90 adult T-ALL/LBL patients, 39 patients received HSCT after chemotherapy. The median follow-up time after HSCT was 153 days (range from 23 to 1,200 days). Among them, 13 patients relapsed after HSCT. The cumulative incidence rate (CIR) was 33.3% (13/39), and the non-relapse mortality (NRM) was 3.8% (1/26) (**Supplementary Figure S2**). The median follow-up time of leukemia-free survival was 233 days (range from 23 to 1,200 days).

In order to remove the impact of HSCT on the prognosis for patients, we adopted "censored data" to process the transplantation data and then built a new risk stratification model for OS in 90 adult patients. Univariate and multivariate analyses showed that age (45 years old), LDH (600 U/L), response in D19-BMR detection, and TP53 and cell cycle signaling pathway alterations were independent predictors for OS (**Table 2**). The new risk stratification model of OS was also built into a nomogram (**Figure 3A**). The C-index of the nomogram was 0.792 (**Figures 3B–D**). The calibration plots

TABLE 1 | Univariate and multivariate analysis for OS in 90 adult T-ALL patients.

Variable	Univariate		Multivariate				
	HR (95% CI)	*P*	HR (95% CI)	*P*	c-index	vif	nomo score
Age at diagnosis (45y)	4.868 (2.438-9.721)	9.55742E-07	3.1854 (1.41962-7.1476)	0.00496	0.844	1.266289	0/63
WBC (30G/L)	1.88 (1.016-3.478)	0.04123618	2.9731 (1.50880-5.8585)	0.00164		1.168878	0/40
TP53 and cell cycle	4.28 (1.639-11.18)	0.001376429	3.0074 (1.12213-8.0603)	0.02859		1.017995	0/35
Response in D19-BMR detection (M1+M2/M3)	3.407 (1.823-6.367)	4.74972E-05	2.1497 (1.10235-4.1923)	0.02471		1.093628	0/37
HSCT	0.1537 (0.07346-0.3218)	4.99E-08	0.1764 (0.07721-0.4029)	3.84E-05		1.134547	0/100

TABLE 2 | Univariate and multivariate analysis for OS in 90 patients removing the impact of HSCT.

Variable	Univariate		Multivariate				
	HR (95% CI)	*P*	HR (95% CI)	*P*	c-index	vif	nomo score
Age at diagnosis (45y)	7.087 (3.332-15.07)	3.66E-07	8.018 (3.272-19.649)	5.32E-06	0.792	1.237466	0/100
TP53 and cell cycle	4.464 (1.69-11.79)	2.53E-03	4.294 (1.558-11.834)	0.00484		1.015417	0/51
LDH (600U/L)	1.803 (0.8758-3.711)	0.11000	3.630 (1.599-8.237)	0.00205		1.248115	0/42
Response in D19-BMR detection (M1+M2/M3)	3.78 (1.814-7.877)	3.85E-04	3.185 (1.440-7.045)	0.00422		1.090931	0/48

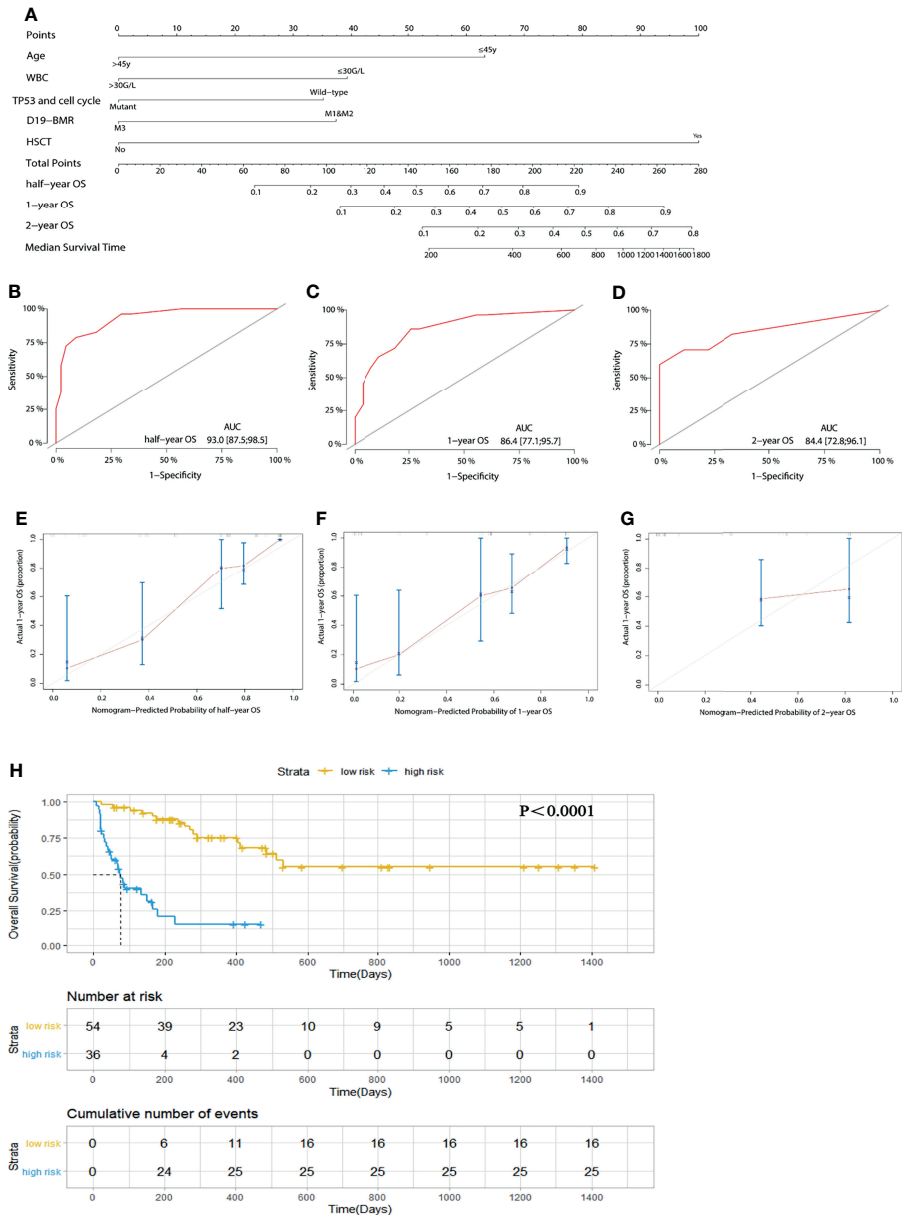

FIGURE 2 | **(A)** A nomogram predicts the half-year, 1-year, and 2-year overall survival (OS) of 90 adult T-cell acute lymphoblastic leukemia/lymphoma (T-ALL/LBL) patients. **(B–D)** The AUC of nomogram for the half-year, 1-year, and 2-year OS. **(E–G)** Calibration curves for predicting half-year, 1-year, and 2-year OS. **(H)** Kaplan–Meier survival curves of OS. The diagonal gray lines could help to judge the agreement between predictions and actual observations in the AUC and calibration curves. The dotted lines drawn on the Kaplan–Meier curves were used to reveal the median survival time of patients when 50% of patients had the event. The data in the tables showed the number at risk and cumulative number of events at specific time points.

also showed good agreement between predictions and actual observations in our study (**Figure 3E**). With the threshold score of 170 for OS nomogram, 27 patients with total points ≥170 (AUC ≥78.5) was defined as low-risk groups and 63 patients <170 (AUC <78.5) as high-risk groups. The 1-year OS rate of T-ALL/LBL patients in the low-risk group was significantly better than that in the high-risk group (69.6% vs. 21.7%, $P < 0.00019$; HR: 3.8, 95% CI: 1.803–8.01) (**Figure 3F**).

Risk Stratification Model of Event-Free Survival in 90 Adult T-ALL/LBL Patients

Univariate and multivariate analyses showed that age (45 years old), PLT (50 G/L), LDH (600 U/L), response in D19-BMR detection, TP53 and cell cycle signaling pathway alterations, and HSCT were independent predictors for EFS (**Table S8**). Then, the above six independent predictors of EFS were integrated into the nomogram of estimating EFS rate with the

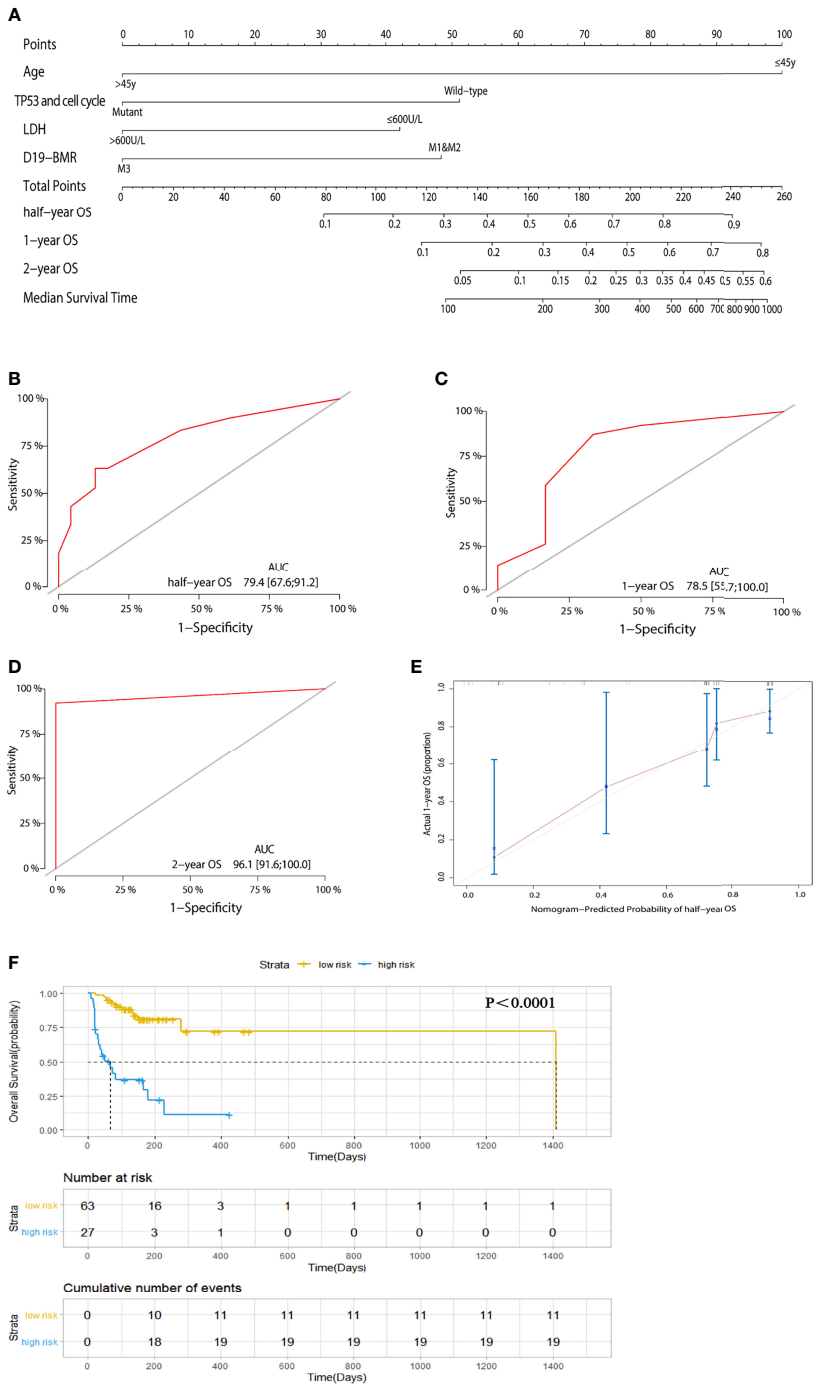

FIGURE 3 | **(A)** A nomogram predicts the half-year, 1-year, and 2-year overall survival (OS) of 90 adult T-cell acute lymphoblastic leukemia/lymphoma (T-ALL/LBL) patients while removing the impact of hematopoietic stem cell transplantation (HSCT). **(B–D)** The AUC of nomogram for the half-year, 1-year, and 2-year OS. **(E)** Calibration curves for predicting 1-year OS. **(F)** Kaplan–Meier survival curves of OS.

C-index 0.844 (**Supplementary Figures S3A–D**). The calibration plots also showed good consistency between predictions and actual data (**Supplementary Figures S3E–G**). With the threshold score of 150, 58 patients with total points ≥150 (AUC ≥85.4) belonged to the low-risk group and 32 patients <150 (AUC <85.4) belonged to the high-risk group. The 1-year EFS rate of T-ALL/LBL patients in the low-risk group was significantly higher than that in the high-risk group

(allpatients: 67.2% vs. 25.0%, $P < 0.0001$; HR: 7.002, 95% CI: 3.642–13.46) (**Supplementary Figure S3H**).

DISCUSSION

To date, there is still a lack of universally accepted criteria combining gene mutations with clinical characteristics for T-ALL risk stratification. Therefore, in this study, we established three novel risk stratification models by the combination of gene mutations and clinical characteristics with EFS and OS to predict therapeutic efficacy and prognosis in adult T-ALL/LBL patients, which displayed favorable predictive efficacy. One latest study involving genomic analyses of ALL by copy number alteration (CNA) profiling indicated that 8 genes (*IKZF1*, *CDKN2A/2B*, *PAR1*, *BTG1*, *EBF1*, *PAX5*, *ETV6*, and *RB1*) had potential to serve as risk stratification markers (20), which partly overlapped with our results about gene mutations, indicating the applicability of our study.

TP53 is a typical tumor suppressor gene. *TP53* mutation is involved in the pathogenesis of various tumors, including T-ALL. The frequency of *TP53* mutations in newly diagnosed T-ALL in our study was slightly higher than previously reported (4.4% vs. 2%–3%) (21). In Pediatric Oncology Group protocol POG8862, *TP53* mutations usually occurred in relapsed T-ALL children, who had a worse survival than children without *TP53* mutations (22). In addition, *TP53* mutations were found associated with worse 5-year EFS and OS (23), which was consistent with our results. In our study, TP53 pathway alteration is an independent unfavorable risk factor for EFS and OS. Besides, the OS in the patients with *TP53* mutations was significantly shortened, whose median survival time was 53 days.

DNMT3A mutations frequently occur in myeloid tumors but are less common in lymphoid malignancies that are mainly found in T-cell lineage diseases (24, 25). Besides, the mutation frequency of *DNMT3A* increased with age and was extremely rare in children and adolescents with T-ALL (25). Mutation frequency of *DNMT3A* in our study was 14.4%, which was higher than previously reported, 9.1% (25), but lower than previously reported, 17.8% (26). Previous studies demonstrated that *DNMT3A* mutations were significantly associated with shorter EFS and OS, which were independent prognostic factors for EFS but not OS (25). Another study from MRC UKALL XII/ECOG E2993 reported that *DNMT3A* was an independent prognostic marker in adult T-ALL that might be useful for risk stratification of high-risk early immature adult T-ALL (27). In our study, the median times of reaching both CR and MRD in patients with *DNMT3A* mutations are much longer than those of patients without *DNMT3A* mutations. Furthermore, DNMT3A pathway alteration is an independent unfavorable risk factor for CR and MRD, which suggested that the patients with *DNMT3A* mutations and DNA methylation signaling pathway alterations have worse early response to chemotherapy. Decitabine, a DNA hypomethylating agent, was reported to be a promising therapeutic agent for relapsed ALL after HSCT (28). Besides, a patient with relapsed T-ALL after HSCT achieved an effective response to the combined treatment of decitabine and venetoclax (29). So, hypomethylating agent combined with chemotherapy might be recommended for T-ALL patients with *DNMT3A* mutations and DNMT3A pathway alterations to increase the CR rate.

NOTCH1 was a class I transmembrane glycoprotein that functions as a ligand-activated transcription factor, directly transducing extracellular signals on the cell membrane and triggering the expression of specific target genes in the nucleus (30). Activation of NOTCH signaling pathway by *NOTCH1* and/or *FBXW7* mutations was a prominent oncogenic event in the hematopoietic system, also critical for the development of T cells and the regulation of many important cellular processes. In our study, the mutation frequency of *NOTCH1* (30%) was lower than the previously reported 45.8%–66% (16, 31–33). The mutation frequency of *FBXW7* was between the reported data 18% (31) and 9.4% (32). The mutation frequency of *NOTCH* signaling pathway was lower than reported data 59%–73.3% (27, 34–36). The role of *NOTCH1* mutations in T-ALL is still controversial. Our study showed that *NOTCH1* mutations have no significant impact on CR, MRD, EFS, RFS, and OS, which was completely consistent with results of some studies (31, 33, 35, 37). However, some researchers reported that T-ALL patients with *NOTCH1*/*FBXW7* mutations had better OS when compared with wild-type cases (5, 27, 37), and *NOTCH1* mutations predicted a faster early treatment response (38). Apart from the favorable role, Zhu et al. (39) reported that *NOTCH1* mutations were relevant to shorter OS in T-ALL patients. Therefore, a larger sample size is needed for the confirmation of the role of *NOTCH1* mutations.

In this study, we also identified the pairwise relationship between genetic alterations and found significant co-occurrence of *NOTCH1* mutations and *FBXW7* mutations, *NOTCH1* mutations and *IL7R* mutations, *FBXW7* mutations and *IL7R* mutations, *PHF6* mutations and *NRAS* mutations, and *DNMT3A* mutations and *IDH2* mutations. Of note, T-ALL is a genomically heterogeneous malignancy as discussed, and co-occurrence of specific mutations could contribute to leukemogenesis (13). Preclinical studies suggest that co-occurring mutations may impact treatment responsiveness, since the treatment response to docetaxel monotherapy in lung tumors was markedly impaired when *KRAS* mutants co-occurred with *TP53* mutations (40). Furthermore, *KRAS* mutations co-occurring with *TP53* mutations are associated with increased intratumoral T-cell infiltration, programmed cell death protein (*PD-1*) expression, and prolonged clinical benefit from anti-PD-1 immunotherapy in non-small cell lung cancer (NSCLC) (41). Although *IDH1* and *IDH2* both regulate DNA methylation, mutations to *IDH1* and *IDH2* are mutually exclusive (42), which was also observed in our study. Furthermore, it has been reported that *IDH1* and *IDH2* mutations are frequently co-occurring with *DNMT3A* mutations in AML. In particular, the prognosis was significantly worse for the co-occurrence of *DNMT3A* mutations with *IDH2* mutations (43). In addition, it has been reported that *DNMT3A*, *IDH1*, and *IDH2* mutations were

uniquely present in the early immature adult T-ALL and conferred worse prognosis in adult T-ALL (27), which is consistent with our study. Some previous studies revealed that *NOTCH1/FBXW7* mutations co-occurred (44) and were significant favorable prognostic predictors for OS in adult T-ALL patients in the absence of *K/NRAS* mutation or *PTEN* mutations (45). Moreover, it has been demonstrated that JAK/STAT signaling pathway alterations were co-occurring with alterations of NOTCH signaling pathway (46, 47) and *PHF6* mutations but not with *K/NRAS*, and this population may not benefit from HSCT (46). It has been demonstrated experimentally that *PHF6* loss can enhance the oncogenic activity of *NOTCH1* mutations; therefore, *PHF6* and *NOTCH1* co-mutation are more tightly linked to T-ALL pathogenesis and leukemia-associated mortality (48, 49). Several studies demonstrated that *IL7R* mutations may be oncogenic drivers in ETP-ALL (50, 51) and positively correlated with *PHF6* mutations in the development of T-ALL (52). Interestingly, it has been observed that *PTPN2* deletions were co-occurring with alterations of IL7R/JAK-STAT signaling pathway and inclined to associate with improved OS in children, but not in adults in a large cohort of 430 adult T-ALL patients (53). Hence, co-occurring mutations may account for the limited activity of single targeted agent. Rational combination therapies are of great promise to provide precise and effective long-term disease control or remission.

The incidence of ETP-ALL gradually increased with age, which was 5.5%–13% in children (54, 55) and 30%–50% in adults (56–58). The incidence of adult ETP-ALL in our data was 46.7%. These differences may attribute to ethnic variations and demographic structure. The average age of ETP-ALL patients in this study was 37.5 years old, higher than 32 as previously reported (59). ETP-ALL has been found related to unfavorable prognosis because of poor response to chemotherapy and high relapse rate (54, 55, 60, 61). The 10-year OS for ETP-ALL was only 19% (54). However, a recent research found that not all patients with ETP-ALL had worse prognosis (62). It has been also reported that patients with ETP-ALL seemed to have an intermediate risk outcome and might have a similar prognosis compared with typical T-ALL patients if receiving intense treatment (63). In this study, ETP-ALL was an independent poor prognostic factor for CR and MRD but did not impact long-term outcomes such as EFS, RFS, and OS, which indicated that ETP-ALL was not the strictly independent factor for all prognostic markers.

Some current pediatric risk stratification models include MRD status of patients (64). In adult T-ALL, MRD $\geq 10^{-4}$ is associated with higher recurrence rate and decreased OS, which has been included in criteria for high-risk patients (16). In our study, T-ALL/LBL patients with detectable MRD had worse EFS and OS. But we found that MRD is not an independent risk factor for EFS and OS. Actually, adult ALL patients show greater heterogeneity than pediatric patients. Moreover, PCR- and flow cytometry-based MRD assessment has limited sensitivity. Standardization of methodologies and harmonization of terminology are still lacking for MRD diagnostics. These are probably the reason why MRD status has not been implemented in the risk stratification of adult T-ALL/LBL. Hence, improved detection methods and larger sample size are necessary for further validation.

It is increasingly important to accurately stratify patients who benefit from HSCT. A meta-analysis including 2,962 patients have shown a survival benefit for HSCT for patients <35 years old but not for those >35 years (65). In addition, 1,646 adults diagnosed with standard-risk or high-risk ALL in the Medical Research Council (MRC) UKALL XII/ECOG 2993 have shown superiority of HSCT on the prognosis (66). The consensus from the Chinese Society of Hematology has also recommended that HSCT is the standard of care for adult ALL patients at either standard risk or high risk who receive adult chemotherapy regimens (67). In our study, the HSCT was an independent favorable predictor for EFS and OS.

The independent risk factors we included in our risk stratification models are different from all previous models mainly because we emphasized gene mutations detected by NGS. The integration of gene mutations and clinical characteristics of adult T-ALL/LBL patients improved our understanding of their clinicobiological features, optimized the current prognostic-related risk stratification models, and provided a foundation for formulating treatment regimens. However, its limitations also deserve commentary. This was a non-randomized retrospective analysis with some potential biases. In addition, the number of cases in this study was slightly less, so that comprehensiveness of the results is limited. Therefore, it is necessary to recruit more patients and prolong follow-up time in the subsequent project to confirm the validity of our risk stratification models on adult T-ALL treatment decisions and prognosis.

DATA AVAILABILITY STATEMENT

The NGS data have been deposited in public, community supported repository. The name of the repository and accession number can be found below: Genome Sequence Archive in National Genomics Data Center and accession number HRA001815 that are publicly accessible at https://bigd.big.ac.cn/gsa-human/browse/HRA001815. Other related data are available on personal request through the corresponding author (QW) and will be made available after approval of HY, MH, and JD, who created and maintain the database.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by the Ethics Committee of Tongji Medical College of Huazhong University of Science and Technology. Written informed consent to participate in this study was provided by the participants' legal guardian/next of kin.

AUTHOR CONTRIBUTIONS

QW conceived and designed the study. HY, MH, JD, LY, CQ, YT, and TL collected and analyzed data. HY, MH, and JD wrote the paper. These three authors have contributed equally to this work and share first authorship. QW reviewed and edited the article. All authors read and approved the article.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fonc.2022.811151/full#supplementary-material

Supplementary Figure S1 | (A) Frequency of mutated genes in our dataset. **(B)** Frequency of altered signaling pathways in our dataset.

Supplementary Figure S2 | (A) The cumulative incidence rate (CIR) of the 39 patients received HSCT after chemotherapy. **(B)** The non-relapse mortality (NRM) of the 39 patients received HSCT after chemotherapy.

Supplementary Figure S3 | (A) A nomogram predicts the half-year, 1-year and 2-year EFS of 90 adult T-ALL/LBL patients. **(B–D)** The AUC of nomogram for the half-year, 1-year and 2-year EFS. **(E–G)** Calibration curves for predicting half-year, 1-year and 2-year EFS. **(H)** Kaplan-Meier survival curves of EFS.

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Mature T-Cell leukemias: Challenges in Diagnosis

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T-cell clones can frequently be identified in peripheral blood. It can be difficult to appreciate whether these are benign and transient or whether they signify a clonal disorder. We review factors that aid in understanding the relevance of T-cell clones. Conversely, obvious pathological T-cell clones can be detected in blood, but there is uncertainty in how to categorize this clonal T cell population, thus, we adopt a multidisciplinary review of the clinical features, diagnostic material and radiology before making the diagnosis. In this review we shall discuss some of these challenges faced when diagnosing mature T-cell leukemias.

Keywords: diagnostics, mature T and NK-cell neoplasms, T-PLL, T-cell prolymphocytic leukemia, large granular lymphocyte (LGL) leukemia, adult T-cell leukemia/lymphoma (ATL), T cell lymphoma

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INTRODUCTION

Mature T-cell neoplasms with leukemic involvement are rare and while many can present with archetypal features that allow for easy diagnostic categorization, other cases can be more difficult to sub-classify. Accurate and precise diagnosis requires integration of all the clinical findings along with morphological assessment, immunophenotyping, cytogenetic and molecular analysis of the peripheral blood, bone marrow and lymph node and radiology (1). A multi-disciplinary review of these cases is paramount to avoid incorrect diagnosis, for example, a histopathologist reviewing a lymph node biopsy may suggest a patient has nodal peripheral T-cell lymphoma in the absence of information regarding the white cell count and clinical picture (2).

In this review, we shall discuss some of the features that aid in subclassifying the mature T-cell leukemias and differentiating them from nodal peripheral T-cell lymphoma with leukemic involvement. We shall highlight rare examples of these diseases in order to avoid potential diagnostic pitfalls.

WHAT IS THE DIFFERENTIAL FOR A CLONAL T-CELL POPULATION IDENTIFIED IN PERIPHERAL BLOOD?

Lymphocytosis due to an increase in T-lymphocytes can easily be distinguished from clonal B-cell populations by basic flow cytometry methods. Once it has been established that the increase in lymphocytes are T cells, and further characterizing the T-lymphocyte population by morphology, immunophenotyping, cytogenetic and molecular analysis, an attempt to establish clonality is

recommended, particularly in cases where the white cell count is low. This can be performed by several methods, namely, PCR-based methods, next generation sequencing (NGS) and flow cytometry of the TCR-V_β repertoire (albeit limited use in everyday practice) or more recently TRBC1 (3–7).

Persistent T-cell lymphocytosis and expansion of T-cell populations can be seen in many cases of chronic infection, for example HIV and CMV and indeed reactive to other malignancies (8–10). Similarly immune dysregulation due to primary immunodeficiencies or autoimmune conditions such as autoimmune lymphoproliferative disorders (ALPS) can lead to significant lymphoid proliferation and peripheral blood involvement. Thus, even in the absence of clonal T-cell expansion a persistent T-cell lymphocytosis may indicate significant pathology that requires multi-disciplinary team input both for both diagnosis and management. This discussion is beyond the scope of this paper. One important point to note is that many of these conditions in themselves increase the risk of lymphoma.

REACTIVE VERSUS T-CELL NEOPLASM?

The identification of a T-cell clone is not synonymous with a neoplasm. T-cell clones can be detected due to reactive causes, infection and senescence and can be persistent in these cases (11, 12). There is a spectrum of disorders both infective and autoimmune that can be associated with polyclonal expansion of T cells through to monoclonal expansion through to neoplastic proliferations of T cells. This is especially the case with large granular lymphocytic proliferations which can be seen in autoimmune conditions, and Felty's syndrome, but similarly it is known that there is a strong link between Rheumatoid arthritis and LGLL. Furthermore, the pathogenesis of lymphoproliferative disorders, such as LGLL has been linked to chronic T cell activation with viruses such as HTLV or EBV implicated (13). This boundary and how we define these clones can be complex and can change with time (14). Furthermore, with improvements in diagnostics and also availability, there will be an increase in individuals identified with persistent T-cell clones with normal or even low lymphocyte counts. Interestingly, there is an association between clonal hematopoiesis (CHIP), MDS and clonal T-cell disorders. Not only do they share many of the same recurrent mutations seen predominantly in epigenetic regulators such as *DNMT3A* and *TET2*, suggestive that this may be early mutations in common progenitors, but they also often co-exist and there are numerous reports of co-existing MDS with LGLL or angioimmunoblastic T-cell lymphoma (15, 16).

T-cell clones of uncertain significance may be detected by molecular analysis solely, or there may be a small T-cell population identified by flow cytometry often with a large granular lymphocyte phenotype (as described below) (17, 18). While there is no equivalent to monoclonal B-lymphocytosis, many of these incidental clones can be considered as 'T-cell clones of uncertain significance' if the criteria for diagnosis of large granular lymphocytic leukemia (LGLL) or other mature T-cell leukemia are not met. However, the significance of the T-cell clones in the context of cytopenias and therefore how to manage them is not clear (19). It should also be noted that large granular lymphocytic proliferations can be seen with other hematological and non-hematological conditions, namely, myelodysplasia, plasma cell dyscrasias, aplastic anaemia, post-stem cell transplant, HIV infection and treatment with dasatinib (20–24). The presence of mutations in *STAT3* and *STAT5b* does not immediately define a diagnosis of LGLL as these mutations are not specific to this disease (14). Thus, if patients do not have sufficient evidence for a positive diagnosis of a defined T-cell leukemia, then we prefer to consider them as having T-cell clones of uncertain significance. Akin to MGUS and monoclonal B cell lymphocytosis of uncertain significance, these should however be followed up as these clones may acquire secondary events that drive progression and develop into malignancy (25). Our preference depending on clinical situation is to monitor patients every 6 months in the first instance and then annually if no progression occurs.

ACUTE VERSUS MATURE T-CELL NEOPLASM?

While distinguishing between T-lymphoblastic leukemia (T-ALL) and mature T-cell neoplasms is usually very straightforward with the presence of immature markers such as CD1a, CD34, and TdT in the former, there have been unusual cases of T-prolymphocytic leukemia, T-PLL seen with lack of surface CD3 and CD45 that can make the diagnosis more difficult (26, 27). Similarly, there have been reports of mature T-cell neoplasms aberrantly expressing immature markers, such as CD1a (28, 29).

MATURE T CELL LEUKEMIA WITH NODAL/CUTANEOUS INVOLVEMENT VERSUS NODAL/CUTANEOUS T CELL LYMPHOMA WITH LEUKEMIC INVOLVEMENT?

The mature T-cell leukemias are sufficiently diverse from one another that they are usually readily discernible; however distinguishing them from nodal or cutaneous lymphomas with leukemic involvement can be challenging and thus requires integration of all results before reaching a diagnosis, occasionally this can require multiple biopsies and can take time before a conclusion can be made (**Table 1**).

LARGE GRANULAR LYMPHOCYTIC LEUKEMIA

Large granular lymphocytic leukemia (LGLL) typically presents with cytopenias, most commonly neutropenia. Median age at

TABLE 1 | Summary of the defining features of the mature T cell leukemias.

	T-PLL	T-LGLL	ATLL	SS
Classic Clinical Features	**Rapidly progressive** High white cell count Lymphadenopathy, splenomegaly, skin involvement **effusions**	**Indolent** Often clone is modest size <20 × 10^9/L Associated cytopenias Associated autoimmune history	**Presence of HTLV1** Variable involvement by skin, nodes, blood, marrow and extranodal disease **Hypercalcemia**	**Erythroderma** Generalized lymphadenopathy Pruritus
Morphology	**Basophilic prolymphocytes with cytoplasmic blebbing** Small cell (20%) and Sezary (5%) variants	**Large granular lymphocytes**	Variable **"Flower cells"**	**Cerebriform cells**
Typical Immunophenotyping (rare variations do exist for all diagnoses)	$CD2^+$, $CD3^+$, $CD5^+$, **$CD7^{++}$** CD4/8 variable $CD1a^-$, TdT^-, $CD25^{-/+}$ **$TCL1^+$**	$CD2^+$ $CD3^+$ $CD5^+$ **$CD8^+$** **$CD56^+$ $CD57^+$** (NK and $CD4^+$ and δγ cases also seen)	$CD2^+$ $CD3^+$ $CD5^+$ **$CD4^+$ $CD25^+$** **CD7–**	$CD2^+$ $CD3^+$ $CD5^+$ $CD4^+$ **CD7– CD26–**
Specific molecular or cytogenetic aberration	**t(14,14); inversion 14; t(X,14);** iso8q; complex cytogenetics	***STAT3* and *STAT5b* mutations**	High frequency of mutations	Non-specific and heterogeneous pattern of translocations and mutations

Those listed in bold can be helpful in differentiating from each other as are quite specific to that disease category.

presentation is 66 years (30–32). There is a strong association with autoimmune conditions, and approximately 15% of patients with LGLL will also have rheumatoid arthritis.

Morphology

Typically there are no dysplastic features on the peripheral blood unless there are co-existing conditions and the cytopenias are evident. The large granular lymphocytes tend to be infrequent but are characteristic large lymphocytes with abundant cytoplasm and azurophilic granules. The distribution of the lymphocytes is intrasinusoidal in the bone marrow trephine that is otherwise normo- or hypercellular.

Immunophenotyping and Molecular Analysis

The characteristic immunophenotypic profile of LGLL is that of mature cytotoxic T cells most commonly αβTCR, CD2, CD3, CD8, CD56, and CD57 positive. Less commonly, LGLL can be comprised of CD4 positive T-cells, NK cells (classified as chronic lymphoproliferative disorder of NK cells in the most recent WHO classification), or have a γδTCR (33). While these can all be readily differentiated from T-LGLL by flow cytometry, it is important to consider their differential diagnoses such as aggressive NK cell leukemia or hepatosplenic T cell lymphoma, especially if patents have a more aggressive clinical picture.

Clonality may be assessed by flow cytometry using TRBC1 or more commonly by assessing for TCR gene rearrangements. Molecular analysis of *STAT3* and *STAT5b* can be helpful as recurrent mutations in these genes have been identified in LGLL, but are not specific.

Making the Diagnosis

The WHO diagnostic criteria for LGLL are defined as a persistent (>6 months) increase in the number of peripheral blood large granular lymphocytes, usually 2–20 × 10^9/L without a clearly defined cause (33). However, it is stated that LGL counts of less than 2 × 10^9/L that otherwise meet the diagnosis are still consistent.

Hence, this diagnosis can only be made once persistence of the clone has been demonstrated. Often the authors are asked to review cases where patients have been investigated for cytopenias and while there are persistent T-cell rearrangements identified by molecular analysis, there is no associated lymphocyte population with LGLL phenotype identified by flow cytometry or infiltrate seen on bone marrow trephine. In these cases we would suggest continued infrequent monitoring and reassessment if the clinical situation changes but that this does not meet the diagnostic criteria for LGLL (34).

We have seen cases with very high white count with lymphocytes >100 × 10^9/L and so while low level clones are more common, they are not a defining feature.

Similarly, rare patients have presented with a predominantly nodal distribution of disease and this must not be assumed to be PTCL NOS based on distribution alone.

Cases of LGLL with more unusual immunophenotypic profiles such as γδTCR can lead to other differential diagnoses such as gamma delta hepatosplenic T-cell lymphoma (35, 36). However, by combining the clinical features such as generalized symptoms, rapidity of onset of symptoms, presence of hepatosplenomegaly, and bone marrow sinusoidal expansion by lymphoma cells the two can be readily distinguished, emphasizing that the pathologist cannot make the diagnosis in isolation, without knowing the clinical picture.

T-PROLYMPHOCYTIC LEUKEMIA

T-prolymphocytic leukemia (T-PLL) characteristically presents at a median age of 65 years. Patients with ataxia telangiectasia have an increased risk of T-PLL and in these cases, the presentation can be in their 20s. Often the illness presents

rapidly, with a rapidly rising white cell count, with generalized symptoms and also effusions, ascites, edema, and peri-orbital edema and skin infiltration. However, T-PLL can have an indolent pre-phase that is detected incidentally, when patients will not have these symptoms and have smaller more stable clones but with the characteristic phenotype as described below.

Morphology

The morphology can be variable with three characteristic appearances described. These include the more typical prolymphocytes with blebbing of the cytoplasm and single nucleolus; small cell variant with cells displaying condensed chromatin and nucleoli invisible by light microscopy; and cerebriform variant with an irregular nuclear outline similar to the lymphocytes seen in Sézary syndrome.

Immunophenotyping

The lymphocytes are post-thymic and express mature markers positive for CD2, CD3, CD5 and CD7 and CD52. $CD4^+$ $CD8^-$ is most commonly seen, with rarer cases expressing only CD8 or double positive. The latter is quite specific to T-PLL compared to other mature T cell leukemias, and so can be helpful for making the diagnosis. Similarly, TCL1 expression can be assessed by flow cytometry and is specific to T-PLL.

Cytogenetics and Molecular Analysis

Changes involving chromosome 14 are the most common genetic alteration, seen in over 90% of cases. Inv(14)(q11q32) and t(14;14)(q11;q32) causes juxtaposition of TCRα and TCL1 or TCL1B leading to activation (37). This rearrangement can be identified by FISH (karyotype has a lower sensitivity), and the aberrant TCL1 protein expression can also be detected by flow cytometry or immunohistochemistry (38). The translocation t (X;14) is present in approximately 10–20% cases and involves the rearrangement of the TCRα locus with the proto-oncogene MTCP1 (39–41).

Other cytogenetic abnormalities are also commonly found, namely, abnormalities of chromosome 8 which often results in increased expression of MYC, deletions in 11q23, 12p, 22q, and 17 or abnormalities in chromosome 6 have also been identified with the majority of patients exhibiting a complex karyotype (37, 40, 42–47). While molecular analysis is also performed, the recurrent mutations in genes such as *ATM*, *JAK3*, and *STAT5b* are not specific (40).

Making the Diagnosis

As well as assessing for TCL1 expression, in our center we will also perform FISH to look for the characteristic inversion 14 (q11q32) or t(14;14)(q11q32), but importantly also perform cytogenetics to look for other aberrations that are frequently seen in T-PLL.

International consensus criteria have been recently published to guide the diagnosis (48). When specific cytogenetic aberrations or protein expression are detected, the diagnosis is certain; however, there is a small subset of cases which have been diagnosed elsewhere to have T-PLL on the basis of a leukemic clonal T-cell population "compatible" with T-PLL by flow cytometry and with involvement by a "T-PLL specific site" that on further investigation with a wider T-cell panel, has been reclassified as PTCL-NOS due to lack of cytogenetic aberrations, and features that would be more unusual for T-PLL such as weak CD7.

Identification of $CD4^+$ $CD8^+$ double positive T-cell populations, can be very suggestive of the diagnosis of T-PLL. While ATLL is characteristically $CD4^+$ $CD25^+$, CD25 expression can also be seen in T-PLL and so does not differentiate between the two, HTLV analysis aids in differentiation of these cases. Typically Sézary cells do not express CD7, which can help diagnostically. A recent case that had been referred to our center as possible T-PLL was in a patient with marked erythroderma and a relatively modest lymphocytosis, in this case the weak CD7 positivity pushed the referring center to this diagnosis, however, the clinical history of the erythroderma being significant for many years and the discrepancy of the extent of cutaneous involvement and progression with lack of progression of the leukemic component made the diagnosis of T-PLL very unlikely. Skin biopsy showed evidence of a CD4 positive T-cell lymphoma infiltrate with small to intermediate sized T-cell infiltrate with focal epidermotropism. This in conjunction with the lack of any specific cytogenetic aberration by FISH analysis for TCRAD break-apart allowed the regional skin lymphoma unit and our center to conclude that this was most in keeping with Sézary syndrome. This highlights the importance of multidisciplinary involvement in difficult cases such as these.

Similarly, cases can be seen where nodal lymphomas are incorrectly diagnosed as T-PLL due to the leukemic involvement or the converse when initially the patient presents with lymphadenopathy but the lymphocytosis is not such a feature or perhaps in a more indolent phase (49, 50).

ADULT T-CELL LEUKEMIA/ LYMPHOMA (ATLL)

Despite the marked heterogeneity of this disease, given the knowledge of the etiological infectious agent, HTLV1, differentiating this from other mature T cell neoplasms is generally easier than other mature T cell leukemias. However, HTLV1 serology should be undertaken in any case of cutaneous, nodal or leukemic mature T-cell neoplasm in order not to miss this diagnosis (51).

Morphology

Several morphological variants have been described with the archetypal variant being medium to large sized "flower cells" with nuclear indentations (33, 52).

Immunophenotype

Mature T-cell markers are expressed, namely, CD2, CD3, and CD5, but usually lack CD7. The majority are CD4 positive and CD8 negative but CD8 positive and double positive cases have been described (33, 53). CD25 is strongly expressed in nearly all cases. CD30 expression is variable.

Cytogenetics and Molecular Analysis

The genomic landscape of ATLL is complex with a high frequency of mutations with regional variations and variations dependent on subtype of ATLL (54–56). Most frequently mutated genes are *PLCG1*, *PRKCB*, *VAV1*, *IRF4*, *FYN*, *CARD11*, and *STAT3*.

Sézary Syndrome

Sézary syndrome usually presents in patients in the older (60 years plus) age group. Symptoms most frequently include erythroderma and generalized lymphadenopathy. The classical triad of erythrodermic pruritic rash covering >80% of the body surface area, lymphadenopathy and circulating Sézary cells can aid in diagnosis (33, 57). The history is usually quite short, due to the rapid progression, however a secondary Sézary syndrome occurring following a more prolonged history with documented preceding mycosis fungoides has also been defined (57).

Morphology

Sézary cells in the peripheral blood typically show cerebreiform nuclei. Skin changes histologically are similar to mycosis fungoides with less epidermotropism. Effacement of the lymph nodes with dense monotonous infiltrates can be seen (33).

Immunophenotype

The immunophenotype of the cells usually are CD3 and CD4 positive and lack CD7 and CD26. Rarer phenotypes have been seen such as loss of other T cell antigens, CD4 negative CD8 positive disease or double positive (57)

Cytogenetic and Molecular Analysis

The cytogenetic rearrangement seen are non-specific with complex cytogenetics and numerous mutations identified in studies exploring the molecular landscape (58, 59).

Making the Diagnosis

It is important to analyze all compartments (skin, peripheral blood, lymph nodes) for possible involvement. Histological changes in the skin can be non-specific and thus numerous skin biopsies are frequently taken before a diagnosis is made. Furthermore, many inflammatory skin conditions may lead to reactive T cell clones in the peripheral blood which can complicate diagnosing the skin condition. This emphasizes the importance of peripheral blood analysis which can help confirm the diagnosis. Skin biopsy and peripheral blood show the same TR gene rearrangements. The total Sezary count is greater than 1 $\times 10^{\circ}$/L with an expanded CD4 positive population resulting in CD4:CD8 ratio of greater than 10 with loss of CD7 being quite characteristic (2, 33).

Nodal Lymphoma With Leukemic Involvement

All nodal lymphomas have been reported to have leukemic involvement in rare cases (60–62). The diagnosis of these would not be performed on peripheral blood alone and would need to be correlated with the bone marrow, lymph node histology and any clinical information. We have had a number of cases referred for second opinion on diagnosis with quite marked T-lymphocytosis, who are ultimately classified as PTCL NOS due to lack of defining markers to suggest an alternative diagnosis.

CONCLUSIONS

Not all patients who present with mature T-cell leukamias have easily classifiable disease, and in these cases, if they do not fulfil the currently recognized diagnostic categories, by definition they need to be considered as peripheral T cell lymphoma, not otherwise specified. As our use of next generation sequencing, and gene expression and methylation profiling increases, how we define these neoplasms is likely to change and improve. In the meantime, the integration of clinical, morphological, genetic and histopathological features is paramount to ensure that optimal management is employed to avoid under- or over-treatment of the patient.

AUTHOR CONTRIBUTIONS

DE, AA, and CD devised concept of article. DE wrote the first draft. All authors listed have made a substantial, direct, and intellectual contribution to the work and approved it for publication.

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REVIEW
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Cytokines in the Pathogenesis of Large Granular Lymphocytic Leukemia

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Large granular lymphocytic leukemia (LGLL) is a lymphoproliferative disorder of older adults characterized by the clonal expansion of cytotoxic T/natural killer cells due to constitutive pro-survival signaling. In recent years, it has become clear that cytokines and their receptors are aberrantly expressed in LGLL cells. The exact initiation process of LGLL is unknown, although several cytokine-driven mechanisms have emerged. Elevated levels of several cytokines, including interleukin-15 (IL-15) and platelet-derived growth factor (PDGF), have been described in LGLL patients. Evidence from humans and animal models has shown that cytokines may also contribute to the co-occurrence of a wide range of autoimmune diseases seen in patients with LGLL. The goal of this review is to provide a comprehensive analysis of the link between cytokines and pro-survival signaling in LGLL and to discuss the various strategies and research approaches that are being utilized to study this link. This review will also highlight the importance of cytokine-targeted therapeutics in the treatment of LGLL.

Keywords: interleukins, growth factors, cytokines, LGLL, therapy

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INTRODUCTION

Large granular lymphocytic leukemia (LGLL) is a lymphoproliferative disorder of older adults characterized by the clonal expansion of effector cytotoxic T cells or natural killer (NK) cells. The WHO classifies LGLL into T-cell LGLL (~85% of all cases) and chronic NK-cell lymphoproliferative disorder (NK-CLPD also known as NK-LGLL) (~10% of all cases) (1). Although sometimes included in the LGLL family, aggressive NK-cell leukemia (ANKL) is a distinct neoplasm of NK cells that is nearly always associated with Epstein–Barr virus (EBV) infection and has a very poor prognosis (2). While T-LGLL and NK-LGLL are classified as separate disorders, their pathogenesis is essentially identical and therefore will be considered together in this review.

The exact cause of LGLL is unknown. To date, studies examining the biology of LGLL have identified several altered growth factors signaling pathways in these leukemic cells, which induce molecular aberrancies believed to play a role in the development of LGLL and in its clinical and laboratory manifestations. This review aims to provide an overview of the role of cytokines in the development of LGLL.

OVERVIEW OF LARGE GRANULAR LYMPHOCYTIC LEUKEMIA DEVELOPMENT

The importance of cytokine dysregulation in LGLL pathogenesis has been well established (3). LGLL represents an expansion of activated cytotoxic lymphocytes that persist after antigenic stimulation. LGLL is initiated by an unknown pathogenic trigger or triggers to activate the initial immune cell reaction and increase the production of pro-inflammatory cytokines by LGL cells (4–7). This causes polyclonal reactive cell expansion. However, unlike the normal T-cell LGL expansions in response to antigen, which are controlled and resolved through T-cell apoptosis or differentiation into the memory T-cell pool, LGLL cells begin to clonally proliferate (6, 8). This dysregulated clonal expansion is currently attributed to alterations of multiple pro-survival and anti-apoptotic signaling pathways, especially constitutively active cytokine signaling (9). The major cytokine factors and their interactions with oncogenic signaling pathways in LGLL will be reviewed here.

ABERRANTLY EXPRESSED CYTOKINES IN LARGE GRANULAR LYMPHOCYTIC LEUKEMIA

During disease development, LGLL cells may acquire the ability to sustain proliferative signaling by producing growth factors and their cognate receptors themselves, resulting in chronic autocrine proliferative stimulations (10, 11). LGLL cells can also respond to soluble growth factors present in the pro-inflammatory microenvironment (12). The cytokines that have emerged as major players in LGLL pathogenesis are presented below.

Interleukin-15

Interleukin-15 (IL-15) is a 15-kDa, four-helix bundle cytokine that plays a crucial role in the development of innate immunity (13). It is central to NK cell and NK-T cell development and activation. IL-15 was discovered in 1994 as a T-cell proliferation factor that shared the interleukin-2 (IL-2) receptor βc and γ_C subunits (14). Signaling occurs through the IL-15Rαβγ heterotrimeric receptor complex that includes the shared βc and γ_C chains, as well as a private α receptor (15). The IL-15 gene consists of 9 exons spanning approximately 34 kb on chromosome 4q31 in humans and chromosome 8 in mice, with 73% conservation between species (13, 16). Both mice and humans have an alternatively spliced isoform of IL-15 that also encodes the mature IL-15 protein with potentially different secretion capacity (17). IL-15 has wide tissue distribution and is typically expressed by stromal cells, epithelial cells, and monocytes. However, it is not typically expressed by T cells. Expression of IL-15 by LGLL cells is abnormal and promotes LGLL cell survival (10). The role of IL-15 in the pathogenesis of LGLL has been well documented (3, 10, 18–20). IL-15 normally regulates T- and NK-cell activation, proliferation, and cytotoxicity. Zambello et al. (20) established that LGLL isolated from patients constitutively express all three of the IL-15 receptor components: IL-15Rα, βc, and γ_c. The proliferation of LGLL cells constitutively expressing IL-15 receptors is enhanced by the addition of exogenous IL-15 *in vitro* and showed enhanced cytotoxic activity (20). LGLL cells have increased membrane-bound IL-15 on their surface as compared to healthy controls (21). Typically, IL-15 is presented in *trans*- to NK and T cells that express IL-2/15Rβγ. It is therefore interesting that Chen et al. (18) demonstrated increased levels of soluble IL-15Rα (sIL-15Rα) in the serum of patients with LGLL as well as upregulated levels of IL-15Rα mRNA in patient peripheral blood mononuclear cells (PBMCs). They speculate that this increased sIL-15Rα in LGLL patient serum could be a product of increased enzymatic cleavage from cell surfaces or due to alternative splicing resulting in the soluble isoform. Chen et al. (18) also showed increased IFNγ mRNA in PBMCs from T-LGLL patients, which is known to induce expression of IL-15Rα in monocytes. IL-15 signaling contributes to LGLL pathogenesis through several mechanisms including hypermethylating DNA, altering microRNA expression, and activating several oncogenic pathways such as Jak/STAT, Ras, PI3K, and NF-kB (10). Through these mechanisms, as further detailed in subsequent sections of this review, IL-15 promotes pro-survival and anti-apoptosis signaling in LGLL as a key player in the immunopathogenesis of this disease.

Platelet-Derived Growth Factors

Platelet-derived growth factors (PDGFs) are produced by many different cell types, such as fibroblasts, endothelial cells, and macrophages. Overproduction of these factors is a known contributor to many types of cancer and disease (22, 23). The PDGFs are dimeric growth factors ranging in size from approximately 27 to 30 kDa. They activate two related transmembrane tyrosine kinase receptors, PDGF-α and PDGF-β, leading to downstream effects (22, 23). The five PDGF isoforms are PDGF-AA, PDGF-BB, PDGF-AB, PDGF-CC, and PDGF-DD. All ligands except PDGF-DD activate PDGF-α receptor dimerization in the cell. Similarly, all ligands except the PDGF-AA can activate the α and β receptors in cells (22).

Network modeling of LGLL survival pathways by Zhang et al. (3) identified PDGF as a central contributing driver of LGLL pathogenesis in addition to IL-15 (3). This network analysis indicated that after T-cell activation, constitutive IL-15 and intermittent PDGF signaling were sufficient to reproduce known dysregulations in T-LGLL. Supporting these findings, Zhang et al. (3) found patients with T-LGLL had increased circulating levels of PDGF-BB. With the use of immunohistochemical staining, PDGF-BB protein was confirmed to be located on LGLL cells. Yang et al. (11) showed that LGLL cells have increased levels of PDGF-β receptor mRNA as compared to healthy donor cells. Treating LGLL cell lines with exogenous PDGF or serum from LGLL patients led to increased LGLL cell proliferation, which was abrogated by PI3K inhibitor (11). The authors also demonstrate that downstream targets of PDGF signaling, PI3K and Akt/ERK, are constitutively active in LGLL (11). Pharmacologic disruption of this pathway in an

LGLL cell line (NKL) and primary patient samples with anti-PDGF-BB antibody led to decreases in downstream targets and increased LGLL cell apoptosis (3, 11). These findings establish PDGF as part of an autocrine loop in LGLL allowing tumor cell survival.

Interleukin-2

Interleukin-2 (IL-2) is a 16-kDa four alpha-helix bundle cytokine in the same family as IL-15 (24). Mainly produced by activated T cells, IL-2 drives T-cell growth and differentiation *via* interaction with its heterotrimeric receptor consisting of three subunits α, β, and γ_C (25). IL-2R has been shown to be increased in LGLL cells (26). Yang et al. (27) investigated the link between antigen activation, IL-2, and Fas-driven death pathways in T-LGLL. Normally, IL-2 helps to initially activate T cells but then drives the cell toward apoptosis *via* activation-induced cell death (AICD). While it has been established that despite high Fas-FasL expression LGLL cells are resistant to Fas-mediated apoptosis, the connection to IL-2 signaling is not completely understood (27, 28). LGLL cells treated with exogenous IL-2 *in vitro* had restored Fas-signaling, but there was no change in c-FLIP, a protein that inhibits the formation of the death-inducing signaling complex (DISC) machinery, compared with LGLL cells untreated with IL-2. This suggests intact functioning of this pathway and, instead, a possible disruption in regulation (27). c-FLIP has been found to be overexpressed in LGLL patients, which may contribute to the cells' resistance to Fas-induced apoptosis (27). Additionally, IL-2 signaling can activate NF-kB, Jak/STAT, and MAPK pathways, all of which can drive cell proliferation and survival (29).

Interleukin-6

Interleukin-6 (IL-6) is a well-known pro-inflammatory, four alpha-helical, cytokine secreted by many cell types including monocytes and T cells (30). IL-6 induces Jak/STAT and Ras/Erk signaling through interactions with a unique IL-6R and membrane-bound gp130 subunits of its receptor (31). Similar to IL-15, IL-6R can be both *cis*- and *trans*-presented to the gp130 receptor subunits, which dimerize to trigger intracellular downstream signaling (30). Analyses by Teramo et al. (12) revealed that the non-leukemic cell population in patients with LGLL is more prone to producing IL-6 than the healthy counterpart. It was also shown that the high levels of IL-6 that were observed in patients with LGLL were associated with the persistent stimulation of STAT3. Inhibiting this signaling with anti-IL-6 or anti-IL-6Rα antibodies led to decreased phosphorylated STAT3 and reduced LGL survival (12). Recently, Kim et al. (32) investigated IL-6 in the plasma of T-LGLL patients (n = 9) by *STAT3* mutational status as compared to healthy donors (n = 8). They demonstrated widely upregulated cytokine profiles in the LGLL patients, specifically greatly increased IL-6 and IL-15RA, regardless of *STAT3* mutation (32).

Miscellaneous Others

Interleukin-12

Early studies showed that interleukin-12 (IL-12) can act as a co-stimulatory cytokine in concert with the activation of CD3 to increase the proliferation of LGL cells *via* Jak/STAT signaling (33).

Interleukin-17 and Interleukin-23

Interleukin-17 (IL-17) production defines helper T cells (T_H) and is a central pro-inflammatory driver in the immune response (34). IL-17 signaling leads to increases in granulocyte-macrophage colony-stimulating factor (GM-CSF), IL-6, monocyte chemoattractant protein-1 (MCP-1), macrophage inflammatory protein (MIP-2), and other inflammatory cytokines (34). Outlined by Zawit et al. (35), there may be potential for immunotherapeutic targeting of the IL-17/-23 signaling axis as a treatment strategy in LGLL. Interleukin-23 (IL-23) signaling through Jak/STAT receptors in T_H17 cells can drive these cells to produce IL-17 and further perpetuate the production of pro-inflammatory cytokines (36).

sIL-2R, Interleukin-6, TNF-alpha, Interleukin-8, and Interleukin-10

sIL-2R, Interleukin-6, TNF-alpha, Interleukin-8, and Interleukin-10 were increased in the supernatant of LGLL primary sample cultures compared to controls (26). These cytokines can inhibit hematopoiesis, and IL-8 has been shown to lead to neutrophil extravasation. This may contribute to the neutropenia that these patients experience in addition to other autoimmune diseases (37).

RANTES, Interleukin-8, MIP-1 Alpha and Beta, Interleukin-10, Interleukin-18, IFNɣ, and IL1Ra

RANTES, Interleukin-8, MIP-1 alpha and beta, Interleukin-10, Interleukin-18, IFNɣ, and IL1Ra all have elevated mRNA transcripts in the PBMCs of LGL patients (38). The sera of LGLL patients demonstrated elevated levels of RANTES (Regulated upon Activation, Normal T-cell Expressed and presumably Secreted), MIP-1b, and IL-18, all of which can activate the PI3K pathway (38). Further elucidation of the mechanisms that trigger the transition from the reactive lymphoproliferation to the extreme monoclonal process and subsequent leukemogenesis revealed various phenotypic differences between the healthy and leukemic T-LGL cells. These differences include the up-modulation of various genes (IL-8, IL-18, and IFNɣ) and the presence of chemokines (MCP-1 and IP-10/CXXL10) (39). The overexpression of these chemokines and receptors (including CXCL2, hepatitis A virus cellular receptor 1, IL-18, and CCR2) in T-LGL cells are associated with viral infections. These findings support the concept that viral infections can lead to the development of T-LGL cells. Interestingly, upregulated cytokines are those typically produced by CD8+ T cells in response to viral infection, lending evidence to the idea that a virus may be triggering or perpetuating insult contributing to LGLL cell pathogenesis.

Epidermal Growth Factor, IP-10/CXCL10, Granulocyte Colony-Stimulating Factor

Recent serum analysis of LGLL patients by Olson et al. (40) found reduced epidermal growth factor (EGF) and increased levels of interferon gamma-induced protein 10 (IP-10) and

granulocyte colony-stimulating factor (G-CSF) in LGLL serum compared to that of healthy donor controls. The authors also compared cytokine profiles between T-LGLL and NK-LGLL, which they found to be largely similar between the subtypes. They state that the reason for lowered EGF in LGLL patients is unknown but conclude increased IP-10 and G-CSF, which recruit lymphocytes and stimulate the bone marrow respectively, both fit with the clinical neutropenic context of the disease.

CYTOKINE-DRIVEN ONCOGENIC PATHWAYS IN LARGE GRANULAR LYMPHOCYTIC LEUKEMIA

The interactions of cytokines and the downstream oncogenic signaling drivers active in LGLL are summarized below and in **Figure 1**.

Jak/STAT

There is abundant evidence for dysregulated STAT signaling in LGLL. First described in 2001 by Epling-Burnette et al., constitutive STAT3 activation is one of the defining features of the pathogenesis of LGLL (41). Approximately 40% of T-LGLL patients have gain-of-function *STAT3* mutations and *STAT5b* variants have also been identified in LGLL subtypes (42, 43).

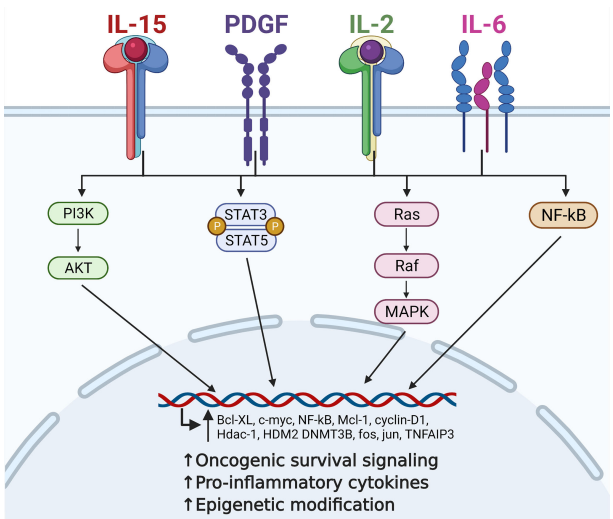

FIGURE 1 | Contribution of critical cytokine signaling to large granular lymphocytic leukemia (LGLL) immunopathogenesis. Interleukin (IL)-15, platelet-derived growth factor (PDGF), IL-2, and IL-6 are all central players in the immunopathogenesis of LGLL. Dysregulation of these cytokines leads to constitutive activation of their downstream signaling pathways such as PI3K, JAK/STAT, Ras/MAPK, and NF-kB. This leads to increased transcription of oncogenic driver genes such as *c-MYC*, *cyclin D1*, and *BCL-xL*, ultimately leading to increased malignant cell proliferation and survival. Figure made with BioRender.com.

Y640F and D661Y are the most common *STAT3* alterations, accounting for roughly 60% of cases (44). These mutations are typically found in the Src homology 2 (SH2) dimerization and activation domains of *STAT3* gene (43). The gain-of-function mutations result in stabilized dimerization, enhanced transcriptional activity, and eventually increased production of pro-survival proteins (43). In Y640F, the hydrophobic alteration to the sequence allows independent homodimerization of the protein (40). When activated, the pSTAT3 complex can then translocate to the nucleus and enhance the transcription of oncogenic driver genes such as *c-MYC*, *BCL-xL*, and *MCL1* (44).

Jerez et al. (45) linked *STAT3* mutation status to patient outcomes and clinical features showing that patients with somatic *STAT3* mutations were significantly more likely to manifest symptoms at the time of diagnosis ($p < 0.001$). These patients also typically required more treatments over the course of their disease and had a shorter "time-to-treatment-failure" than those who did not harbor *STAT3* mutations (45). The prevalence of autoimmune conditions, such as rheumatoid arthritis (RA) and autoimmune hemolytic anemia, was also higher in the *STAT3* mutated cohort. Recently, Barilà et al. (46) provided the first evidence that the presence of a *STAT3* mutation can negatively affect the survival rate of patients with LGLL (46).

To further define these clinical differences, Olson et al. (40) investigated variations in red blood cell parameters in LGLL patients grouped by *STAT3* mutation type. They found that males with D661Y *STAT3* mutations had significantly higher mean corpuscular volumes (MCVs) and lower hemoglobin levels as compared to either the Y604F group or healthy donor controls (40). This has potential implications for *STAT3* mutational status screening of LGLL patients who may present with macrocytic anemia (40). *STAT5b* mutations, N642H and Y665F, have also been found to be gain-of-function mutations in the SH2 domain and were initially discovered in a small percentage of clinically aggressive CD8+ T-LGLL (47). However, *STAT5b* mutations have subsequently also been identified in CD4+ T-LGLL patients, with incidence ranging from 15.2% to 55% reported (42, 46, 48). Clinically, these CD4+ T-LGLL patients are most often asymptomatic, without any impact on survival outcomes (46, 48). Interestingly, a recent investigation into somatic mutations of 57 NK-LGLL patients specifically showed that few (9%) had *STAT3* mutations and no *STAT5b* mutations were found (49). However, in patients negative for *STAT3* mutations, the authors observed mutations in many other genes related to cancer pathogenesis, including those related to Ras/MAPK and PI3K/Akt signaling, as well as *TET2*, which plays a role in epigenetic modification (49). *STAT3* mutations have also been identified in ~43% of patients with Felty syndrome (FS; a rare disease that shares many clinical similarities with LGLL), as well as significant increases in ten cytokines common to both LGLL and FS (50). IL-15Ra, IL-6, MIP-1a, CXCL10, and CSF-1, as well as oncostatin-M, TNFRSF9, PD-L1, CDCP1, and HGF, were those notably upregulated in both FS and LGLL, further emphasizing the link between cytokine and STAT3 dysregulation and disease pathogenesis (50). These differences in mutational

landscape delineated by the immunophenotype of the malignant cells are interesting to consider and may have future applications with regard to disease screening or treatment strategy in the age of precision medicine.

Regardless of mutational status, all LGLL patients have constitutively upregulated STAT3 activity, in large part due to pro-inflammatory cytokine drivers. As previously discussed, IL-15 and IL-6 are both increased in LGLL patients and are known activators of Jak/STAT signaling. There is evidence for IL-15 as a central pathogenic driver in LGLL initiation and progression through Jak/STAT signaling (3, 10). Physiologically, it is important to note that while short-term exposure to IL-15 increases proliferation, survival, and cytotoxic activities of LGL cells, long-term chronic activation of STAT by IL-15 has been shown to be leukemogenic (10). As described in Fehninger et al. (51), mice that were engineered to overexpress IL-15 develop spontaneous fatal LGLL. However, it is interesting to note that *STAT3* mutations alone are not sufficient to induce LGLL in a mouse model, suggesting that cytokine signaling and other pathway dysregulations are critical for oncogenesis (52).

Ras-Raf-1-MEK1-ERK/MAPK

IL-2, IL-6, IL-15, and PDGF can all activate the Ras-Raf-1-MEK1-ERK/MAPK signaling pathway. Ras and ERK have been found to be constitutively active in NK-LGLL. The aggressive LGLL cell line, PLT-2, has a G12A *KRAS* mutation (53, 54). Mizutani et al. (54) postulated that it is the *KRAS* mutation that allows the PLT-2 cell line to grow independently from any exogenous IL-2 stimulation, unlike MOTN-1, a chronic T-LGLL line, which requires IL-2 and IL-15 cytokine stimulation for survival. Inhibiting Ras in LGLL cells with a farnesyltransferase inhibitor, FTI2153, caused ERK inhibition and induced apoptosis *via* Fas signaling and independently of Fas (53). Inhibition of MEK1 also reduced the survival of NK-LGLL cells (53). All of this suggests that dysregulation of this pathway may have both pro-growth and anti-apoptotic influences on LGLL cell pathogenesis. The exact mechanisms by which MEK/ERK signaling are driving LGLL cell survival are not yet fully defined. However, it has been established that activated MAPK is capable of regulating anti-apoptotic proteins. For example, Bcl-2, BAD, and p-ERK can phosphorylate proto-oncogenic transcription factors in the nucleus such as Fos and Jun (53, 55). The Ras cascade also has the ability to crosstalk with PI3K/Akt signaling, further affecting downstream signaling in LGLL pathogenesis (56).

PI3K/Akt

Activated by Ras signaling, and PDGF, as well as IL-18, RANTES, and MIP-1, the PI3K/Akt signaling pathway is a major driver of pro-survival signaling in LGLL (3, 38). Compared to healthy donors, T-LGLL cells have increased PI3K/Akt activity, as indicated by higher levels of p-Akt, which contributes to downstream resistance to apoptosis (56). p-Akt can activate mTOR, a major driver of cell growth and proliferation (57). Schade et al. (58) show that Src family kinases can lead to constitutive activation of the PI3K pathway in LGLL, eventually leading to anti-apoptotic signaling *via* disruption of DISC formation. This effect was abrogated using a PI3K inhibitor, LY294002, which restored apoptosis and showed a reduction in ERK expression, reinforcing the concept of crosstalk between these two pathways (58).

Akt can also interfere with the regulation of transcription factor NF-kB by blocking its inhibition. This leads to increased NF-kB activity and enhanced transcription of oncogenic genes (59). Administration of the PI3K inhibitor LY294002 also resulted in significantly decreased NF-kB activity in T-LGLL cells as well as cell apoptosis, and one of two LGLL patients treated on a phase I study of the dual PI3K δ/γ inhibitor duvelisib had a prolonged partial response (3, 60).

NF-kB

NF-kB is a transcription factor that regulates the survival of immune cells and can be activated by IL-15, Ras, and Akt/PI3K. It can translocate to the nucleus, activating the transcription of pro-survival and anti-apoptotic genes, such as *cyclin D1*, *c-MYC*, *BCL-2*, and *MCL-1*, and can induce the production of IL-2 (41, 61–63). Zhang et al. (3) compared nuclear extracts of T-LGLL cells to nuclear extracts of healthy donor PBMCs and found that c-Rel, an NF-kB family protein, is increased and constitutively active in T-LGLL. When NF-kB was inhibited, the T-LGLL cells had significantly induced apoptosis that was not observed in normal healthy donor PBMCs ($p < 0.009$) (3). Interestingly, the authors also showed that the Mcl-1-driven pathogenic effect of NF-kB in T-LGLL can occur independently of STAT3 signaling, adding another facet of possible signal compensation to this complicated disease picture. Recently, Olson et al. (64) identified missense mutations in *TNFAIP3*, a negative regulator and target of NF-kB, in 8% of a cohort of 39 LGLL patients (64, 65). *TNFAIP3* expression has been previously shown to be upregulated in LGLL samples, further emphasizing the importance of NF-kB signaling in LGLL pathogenesis (65, 66). Yang et al. (67) established a link between the cytokine TRAIL (Tumor Necrosis Factor-Related Apoptosis-Inducing Ligand) and NF-kB in LGLL by demonstrating increased TRAIL mRNA and protein in LGLL cells as well as increased soluble TRAIL in sera from LGLL patients compared to healthy controls (67). TRAIL can bind death receptors to induce apoptosis in tumor cells as well as activate the NF-kB pathway. The LGLL cells express the TRAIL receptor DcR2, and activation of this receptor by TRAIL leads to increases in NF-kB signaling. Through this mechanism, NF-kB's pro-survival and anti-apoptotic activities are further driven by cytokine signaling in LGLL.

INTERACTION OF ONCOGENIC DRIVERS AND CYTOKINE SIGNALING PATHWAYS

The frequent co-existence of dysregulated cytokine signaling and oncogenic mutations has been described in LGLL. The *TNFAIP3* missense mutations in NF-kB signaling observed by Johansson

et al. (66) were significantly associated with *STAT3* mutations in LGLL patient samples, a combination also seen in other lymphomas (66). Coppe et al. (68) identified *CD40LG* as a mutated receptor in LGLL patient samples. CD40LG is involved in STAT3 signaling, as well as MAPK-Ras-Erk, and the IL-15 pathways. Interestingly, *CD40LG* mutations were also seen as functionally related to *TNFAIP3* in the analysis, meaning that there is also a potential link to NF-kB signaling dysfunction. In addition to *CD40LG* lesions, the authors identified activating mutations in *FLT3* receptor tyrosine kinase, which has implications for Ras, Jak/STAT, and PI3K/Akt signaling (68).

It has been established that increased IL-15 can affect the expression of Bcl-2 family genes. However, Hodge et al. (19) further elucidated a mechanism by which IL-15 may be driving anti-apoptotic signaling in LGLL pathogenesis. The authors demonstrate that IL-15 causes upregulation of HDM2, a p53-E3 ligase, which can drive proteasomal degradation of Bid, a protein that is essential for cell apoptosis (19). Through this mechanism, IL-15 can reduce Bid in T-LGLL and NK-LGLL samples. Inhibiting IL-15 or the proteasome degradation pathway in these samples restored Bid levels and showed increased cell death (19).

Previous work from Mishra et al. (10) demonstrates how chronic IL-15 exposure can initiate LGLL through NF-kB signaling and Myc induction in tumor cells. In normal wild-type mouse LGL cells treated with IL-15, this cytokine induces Myc expression *via* the NF-kB pathway. Myc was then shown to mediate increases in aurora kinases A and B. Elevation of *AURKA*, *AURKB*, and *MYC* was confirmed in primary LGLL patient samples, and Myc knockdown in mouse LGL cells showed reduced *AurkA* and *AurkB*. The increased aurora kinases led to centrosome aberrations and result in chromosomal aneuploidy, which is a consistent finding in patient LGLL cells. This chromosomal instability helps drive leukemic oncogenesis. Concurrent to aurora kinase upregulation, the IL-15-driven induction of Myc, NF-kB, and Hdac-1 results in the reduction of *miR-29b* when these repressor proteins bind to its promotor. Indeed, *miR-29b* levels were demonstrated to be significantly decreased in LGLL patients ($p < 0.0009$) as well as healthy donor LGL cells exposed to IL-15 ($p < 0.003$). *Mir-29b*, in turn, typically negatively regulates Dnmt3b, a DNA methyltransferase, with the expression of *DNMT3B* found to be elevated in primary LGLL patient cells. Thus, the increased Dnmt3b in LGLL results in DNA hypermethylation, leading to further chromosomal instability as well as possible silencing of tumor suppressor genes (10). Mishra et al. (10) further demonstrated increased global DNA methylation in primary samples from LGLL patients, as well as healthy LGL cells treated with IL-15 *in vitro* to support this. Through these mechanisms, it is clear that IL-15 has a critical role in the pathogenesis of LGLL.

In addition to *miR-29b*, another miRNA has recently been implicated in the pathology of LGLL. Mariotti et al. (69) identified reduced expression of *miR-146b* in CD8+ T-LGLL due to *miR-146b* promotor hypermethylation. This observed repression of *miR-146b* expression was dependent on STAT3 activation, likely *via* the action of DNMT1, and could be experimentally reversed in CD8+ T-LGLL cells by inhibiting STAT3 (69). Interestingly, the authors also demonstrate how miR-146b may contribute to the development of neutropenia in LGLL *via* interaction with Fas-ligand signaling. Absolute levels of neutrophils in LGLL patients correlated with miR-146b levels and are inversely correlated with the amount of soluble Fas-ligand (FasL) (69). The authors posit that miR-146b-target protein HuR is increased in CD8+ T-LGLL, which serves to stabilize the translation of FasL, ultimately leading to increased levels of FasL in this disease and a mechanism for the resultant neutropenia. In this way, cytokine drivers of STAT3 activation can further alter miRNA levels to drive LGLL pathogenesis.

The loss of suppressor of cytokine signaling-3 (SOCS3) may also be contributing to the pathogenic potential of IL-6 signaling in LGLL. SOCS3 is typically induced by IL-6 *via* p-STAT3. However, despite the upregulated levels of IL-6 and STAT3 observed in LGLL, Teramo et al. (12) found a decreased amount of SOCS3 mRNA and protein in LGLL patient samples compared to healthy donors. Typically, SOCS3 is responsible for negatively regulating Jak/STAT signaling. The authors demonstrated that SOCS3 does not respond appropriately to p-STAT3/IL-6 messaging in the LGLL cells, which may further drive dysregulated STAT signaling. However, after treating the LGLL cells with decitabine, a demethylating compound, appropriate IL-6-driven increases of SOCS3 mRNA and protein were observed (12). This treatment also correlated with decreased p-STAT3, decreased Mcl-1, and increased LGLL apoptosis. Decitabine's effective mechanism of action, demethylation, lends support to the conclusion that epigenetic changes may be silencing normal SOCS3 responses in LGLL. However, abnormal methylation changes to the SOCS3 promoter were not seen, leading the authors to conclude that epigenetic modification occurs elsewhere (12). In this way, IL-6 and loss of the SOCS3 regulator work together to further drive Jak/STAT signaling and LGLL pathogenesis.

Olson et al. (64) recently investigated epigenetic changes in NK-LGLL patient samples. Methylation of *TET2* promoter sequences as well as hypermethylation of negative regulators of *STAT3*, *PTPRD*, and *PTPRN* was observed. TET2 typically contributes to DNA demethylation. This study also identified loss-of-function mutations in this gene in 28% of their observed NK-LGLL patients (n = 58). These patients had significantly increased global methylation compared to healthy controls (64). Thus, in addition to driving increased STAT activation, epigenetic modification may also be facilitating further enhanced methylation of the genome in LGLL. Another study analyzed the *TET2* mutational hierarchy in NK-CLPD by performing whole-exome sequencing of different hematopoietic cells (70). It revealed that the *TET2* alteration was shared by NK-LGLL and cells of the myeloid compartments. This study concluded that the multi-hit model could explain the emergence of *TET2* mutations during the early stages of hematopoietic progenitors (70). *TET2* mutations were also associated with the $CD16^{low}$ phenotype in NK-LGLL (70).

Kim et al. (32) recently demonstrated how cytokine and epigenetic changes in LGLL can be regulated by STAT3 activity. This study demonstrated that IL-15 mRNA expression levels are significantly higher in STAT3 mutated LGLL. Additionally, T-LGLL patient samples with *STAT3* mutations had high STAT3 levels and increased pSTAT3 compared to healthy controls. Additionally, increased DNMT1, DNMT3, EZH2, and MYC protein were seen in T-LGLL compared to controls. DNMT1, DNMT3, and EZH2 are methyltransferase enzymes that can affect epigenetic modifications. These findings were recapitulated in KAI3 NK cells with $STAT3^{Y640F}$ or $STAT3^{G618R}$ mutations, and increased p65, a subunit of NF-kB, highlighting the crosstalk potential between these signaling pathways. Treatment of healthy donor CD8+ T cells with IL-6, IL-15, and MCP-1 cytokines led to enhanced phosphorylation of STAT3 and increased DNMT1, DNMT3B, and EZH2 protein. This further defines a mechanistic link between cytokine signaling and regulators of epigenetic modification. This study also observed direct binding of mutated STAT3 to DNMT1 and EZH2 protein, further defining the mechanism of action of this pathway. Treating *STAT3*-mutated LGLL cells with hypomethylating agent 5-azacytidine led to reduced cell viability, STAT3 phosphorylation, and DNMT1 (32, 71). Through these results, the authors define how cytokine signaling and STAT3 mutations in LGLL can directly drive epigenetic changes in this disease, clarifying new targets for further investigation and potential therapeutic intervention.

MECHANISMS OF CYTOKINE DYSREGULATION IN LARGE GRANULAR LYMPHOCYTIC LEUKEMIA

While it is established that cytokine signaling is involved in LGLL initiation and maintenance, how the cytokines involved become upregulated is not well characterized. The working theory for the initiation of LGLL involves an antigenic insult that triggers an inflammatory state and immune cell reactivity that gets inappropriately perpetuated through a variety of signaling and genetic mechanisms (9). It is likely that to some degree, the hyperactivation of signaling pathways such as Jak/STAT, Ras-Raf-Mek-Erk, PI3K, and NF-kB further drives cytokine production, release, and response in a feed-forward loop. However, exact details have not been thoroughly elucidated. IL-6 signaling, for example, induces STAT3, which has the ability to promote *IL-6* gene expression in an autocrine feed-forward loop, but this has yet to be demonstrated conclusively in LGLL (72, 73).

In addition to signaling deficiencies, mutations and epigenetic changes may also contribute to cytokine dysregulation in LGLL. Previous work has shown some evidence for hypermethylation of the IL-15 promotor in LGLL patient samples compared to healthy donor cells (71). Mishra et al. (74) have previously shown increased IL-15 promoter methylation in cutaneous T-cell lymphoma (CTCL), another T-cell malignancy largely driven by IL-15 pathogenesis. In the case of CTCL, the hypermethylation prevents repressor protein binding and results in aberrantly increased IL-15 expression. In LGLL cell line samples, treatment with 5-azacytidine (a hypomethylating agent) resulted in decreased IL-15 gene expression and decreased cell viability, lending evidence to epigenetic changes contributing to IL-15 overexpression in LGLL (71).

PDGF and *PDGFR* genetic and epigenetic alterations have been described previously in other hematologic malignancies but have yet to be characterized in LGLL (75, 76). Changes to PDGF receptor proteins may allow for ligand-independent activation and escape from inhibitory mechanisms or degradation pathways. Possible changes to this pathway need further investigation in the setting of LGLL, given the central role of PDGF signaling in disease pathogenesis (3).

There is clear evidence that overproduction of cytokines can lead to the development of LGLL and various types of cytopenia in patients with LGLL. The challenge in treating patients with a heterogeneous disease like LGLL is to identify patients who may benefit most from blocking the activity of cytokines. The use of targeted approaches for the neutralization of oncogenic or immunosuppressive cytokines could provide new opportunities to develop effective therapeutic strategies for LGLL patients.

CYTOKINE-DRIVEN ANIMAL MODELS OF LARGE GRANULAR LYMPHOCYTIC LEUKEMIA

The use of animal models of LGLL has greatly enriched our understanding of the pathogenesis of LGLL and provided the opportunity to test novel therapeutics in the disease context. Fehniger et al. (51) developed a transgenic mouse that overexpressed IL-15 by removing posttranscriptional checkpoint inhibitors, allowing for more efficient translation and secretion. These mice developed fatal lymphocytic leukemia between 12 and 30 weeks of age with an NK-T signature of CD3+TCRB+DX5+ markers (51). Phenotypically, the mice developed alopecia, hepatosplenomegaly, weight loss, and extreme clonal lymphocyte expansion in blood, spleen, and bone marrow. The authors described a "blast morphology" of these lymphocytes, which infiltrated many organ systems (77). This model best recapitulates the aggressive T and NK variants of LGLL. This chronic upregulation of IL-15 can induce oncogenic signaling pathways to drive the development of LGLL (10).

Klein et al. (78) described a mouse model that expresses the human $STAT5B^{N642H}$ mutation, which goes on to develop CD8+ T-cell leukemia (78, 79). This stands in contrast to a study by Dutta et al. (52), which demonstrated that activating STAT3 mutations in mice was not sufficient to induce LGLL. The $STAT5B^{N642H}$ lesion is a gain-of-function mutation in the SH2 domain. Similar to the IL-15 transgenic mice, both models have leukemic immunophenotypes positive for CD122, NKp46, and DX5, mirroring CD3+NK1.1+ T-LGL cells (77, 78). The authors also showed that these $STAT5B^{N642H}$ mutation mice could be successfully treated with ruxolitinib, a JAK inhibitor, further

emphasizing the central role of dysregulated STAT signaling in LGLL pathogenesis (78).

THERAPEUTIC BLOCKING OF CYTOKINE SIGNALING IN LARGE GRANULAR LYMPHOCYTIC LEUKEMIA TREATMENT

Currently, LGLL is not a curable disease, and the mainstay of treatment remains general immunosuppressive therapy. Frontline agents include methotrexate, cyclophosphamide, and cyclosporine, whose efficacy is typically limited to partial remissions (60). However, with new insights into LGLL pathogenesis, researchers have brought novel targets of clinical interest into pharmaceutical development. Of particular interest is those targeting cytokine signaling, which are outlined in **Table 1** and summarized in **Figure 2**.

Cytokine-directed therapeutic agents that have been tested against LGLL *in vivo* include Hu-Mikβ1, BNZ-1, and 5-azacytidine. Hu-Mikβ1 is a monoclonal antibody against CD122, the shared β-chain receptor for IL-2 and IL-15 (80, 88). In a phase I clinical trial of Hu-Mikβ1 in LGLL patients, Waldmann et al. (80) observed that Hu-Mikβ1 blocked the *trans* presentation of IL-15 to T cells but did not affect *cis* signaling. The authors demonstrated the safe use of Hu-Mikβ1 but did not find any clinical efficacy in LGLL patients (80).

BNZ-1 is a peptide that binds the common gamma-chain receptor CD132 and prevents IL-2, IL-9, and IL-15 signaling (81). Wang et al. (81) treated LGLL cell lines and primary patient samples with BNZ-1 and showed that in both cases tumor cell viability decreased and apoptosis increased. Additionally, BNZ-1 blockage of IL-2 and IL-15 signaling led to reductions in downstream mediators of these cytokine pathways such as p-STAT, p-Akt, and p-ERK targets (81). *In vivo*, inhibition of IL-15 using BNZ-1, as part of a phase-I/II clinical trial (NCT03239393), resulted in apoptosis of LGLL cells in nearly all patients within 24 h of administration and clinical responses in 20% of patients, clearly demonstrating the crucial role of this cytokine to LGLL pathogenesis and potential clinical value of this therapy (82, 83).

5-Azacytidine is a hypomethylating agent that decreased IL-15 expression and reduced cell viability in the MOTN-1 LGLL cell line (71). This evidence further implicates hypermethylation as a central driver of IL-15 and LGLL pathogenesis. The oral formulation of this potential treatment is currently being investigated in phase I/II clinical trial (NCT05141682) in LGLL patients (71).

Potential therapeutics that have yet to be tested in humans but have shown efficacy *in vitro* are siltuximab and tocilizumab. Siltuximab and tocilizumab are monoclonal antibodies against IL-6 and IL-6R, respectively. Currently approved for the treatment of RA, they inhibit JAK pathway signaling. Treating LGLL patients' PBMCs with anti-IL-6 antibodies led to malignant cell apoptosis (12). The co-occurrence of some LGLL patients with RA or RA-like

TABLE 1 | Therapies targeting cytokine signaling in Large Granular Lymphocytic Leukemia.

Therapeutic agent	Mechanism/findings	Reference
Agents tested against LGLL *in vivo*		
Hu-Mikβ1	Anti-CD122 (shared IL-2 and IL-15 receptor β-chain) monoclonal antibody. Blocks *trans* presentation of IL-15 to T cells. In a phase I study in LGLL, the drug was safe but showed no clinical efficacy.	(80)
BNZ-1	Multi-cytokine inhibitor that prevents IL-2, IL-9, and IL-15 from interacting with the gamma receptor subunit CD132. Wang et al. (81) demonstrated that treating T-LGLL cell lines and primary patient samples with BNZ-1 led to reduced tumor cell viability, decreased downstream signaling, and increased apoptosis. Additionally, Brammer et al. (83) showed apoptosis of LGLL cells in patients treated with BNZ-1 within 24 h of treatment. A phase I/II clinical trial (NCT03239392) showed a 90% decline in T and NK cells by day 15 of treatment (82).	(81–83)
5-azacytidine	Hypomethylating agent: treatment of the LGLL cell line MOTN-1 cells with 5-azacytidine resulted in decreased IL-15 expression; implicating IL-15 promoter hypermethylation as a key driver of IL-15 induced LGLL. Decreasing IL-15 production by demethylating the promoter is being explored in a phase I clinical trial (NCT05141682) evaluating an oral 5-azacytidine formulation (CC-486) in patients with LGLL.	(71)
Agents tested against LGLL *in vitro*		
Siltuximab and tocilizumab	Anti-IL-6 and anti-IL-6R, monoclonal antibodies currently approved for treatment of rheumatoid arthritis by inhibiting JAK pathway signaling. *In vitro* anti-IL-6 antibody treatment of LGLL patients' PBMCs led to malignant cell apoptosis (12).	(12, 84)
Agents of interest in LGLL		
Imatinib mesylate (STI-571)	A receptor tyrosine kinase inhibitor that can target PDGF receptors.	(85)
Secukinumab and ixekizumab	Anti-IL-17 monoclonal antibodies that prevent IL-17 receptor binding and downstream JAK/STAT and NFkB signaling. Currently, FDA-approved for ankylosing spondylitis and psoriatic arthritis treatment.	(86)
Risankizumab	Anti-IL-23 humanized monoclonal antibody binds the p19 subunit of IL-23 to block signaling. Currently, FDA-approved for plaque psoriasis treatment.	(87)

LGLL, large granular lymphocytic leukemia; IL, interleukin; PBMCs, peripheral blood mononuclear cells; CML, chronic myelogenous leukemia; FDA, Food and Drug Administration.

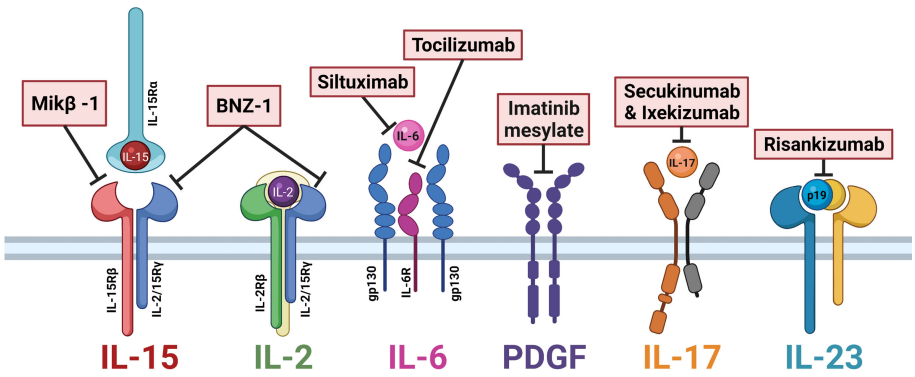

FIGURE 2 | Cytokine-directed therapies of interest in large granular lymphocytic leukemia (LGLL). Mik-β1, a CD122 monoclonal antibody, prevents the *trans*-presentation of interleukin (IL)-15. BNZ-1 binds the common gamma chain CD132, blocking IL-15 and IL-2 signaling. Siltuximab and tocilizumab block IL-6 signaling. Imatinib mesylate is a receptor tyrosine kinase inhibitor that prevents platelet-derived growth factor (PDGF) signaling. Secukinumab and ixekizumab block IL-17 signaling. Risankizumab binds the p19 subunit of IL-23 to block signaling. Figure made with BioRender.com.

symptoms may especially make this line of treatment inquiry worthwhile for further investigation.

Agents of interest in LGLL that have yet to be tested in this disease but align with known LGLL pathogenic mechanisms are imatinib mesylate (STI-571), secukinumab and ixekizumab, and risankizumab. Imatinib mesylate (STI-571) is a receptor tyrosine kinase inhibitor that can target the PDGF receptor to inhibit signaling (85). While typically used in chronic myelogenous leukemia (CML), the known pathogenic role of PDGF signaling in LGLL warrants further investigation into the usefulness of targeting this cytokine pathway (3). Secukinumab and ixekizumab are both monoclonal antibodies that prevent IL-17 receptor binding and limit downstream JAK/STAT and NFkB signaling (86). Given the role of IL-17 as a pro-inflammatory chemoattractant implicated in the pathology of various autoimmune conditions (which afflict a subset of LGLL patients), blocking IL-17 signaling is a strategy worth exploring in the setting of LGLL. Similarly, IL-23 can signal through Jak/STAT receptors in T_H17 cells to drive these cells to produce IL-17, thereby further perpetuating the inflammatory milieu (36). Risankizumab is a monoclonal antibody against IL-23 that binds the p19 subunit of IL-23 to block signaling (87). The IL-17/IL-23 signaling axis constitutes an intriguing target of therapeutic intervention for LGLL based on its known role in driving inflammation and autoimmune conditions. In summary, there are several novel cytokine-related treatment strategies worth further investigation in LGLL.

CONCLUSION

Aberrant cytokine expression and signaling are important components of LGLL pathogenesis. It is not yet clear how effective interventions that target inflammation will be in preventing the onset and/or progression of LGLL. Understanding these cytokine signaling pathways and their various components will help develop novel therapeutic agents and treatment strategies. The re-establishment of cytokine homeostasis in LGLL could benefit patients who suffer from this disease, especially those refractory to current therapeutic options.

AUTHOR CONTRIBUTIONS

CI, JB, and AM planned and conceptualized the review. CI wrote the initial draft. CI, AM, JB, AB, NC, and PP contributed to writing, review, and revision. All authors listed have made a substantial, direct, and intellectual contribution to the work and approved it for publication.

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Incidence, Treatment, and Survival of Patients With T-Cell Lymphoma, T-Cell Large Granular Leukemia, and Concomitant Plasma Cell Dyscrasias

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T-Cell malignancies are a group of heterogeneous disorders composed of primary cutaneous T-cell lymphomas (CTCLs), peripheral T-cell lymphomas (PTCLs), and T-cell leukemias, including T-cell large granular lymphocytic leukemia (T-LGLL). Cases of patients with combined T-cell malignancies and plasma cell dyscrasias (PCD) are reported in the literature, but these are mostly limited to case reports or small case series with <10 patients. Here, we described the clinical course of 26 patients and report baseline characteristics and clinical outcomes including overall survival (OS), progression-free survival (PFS), and objective response rates (ORRs) in this unique population. There was no survival difference in patients with CTCL or T-LGLL and concomitant PCD when treated with standard therapy directed at the T-cell malignancy when compared to historical controls. However, patients with PTCL and concomitant PCD had significantly inferior outcomes with rapid progression and worse OS and PFS at 1.7 years (p=0.006) and 4.8 months (p=0.08), respectively, when compared to historical controls for patients with PTCL, although the limited number of patients included in this analysis precludes drawing definitive conclusions. Treatment directed at the T-cell malignancy resulted in the eradication of the PCD clone in multiple patients (15.4%) including one with multiple myeloma (MM) who experienced a complete response after starting therapy directed at the T-cell malignancy. For patients with T-cell malignancies and concomitant PCD,

treatment with standard T-cell-directed therapies is recommended based on this analysis with continued follow-up and monitoring of the concomitant PCD. Further studies are needed to definitively elucidate the increased risk of relapse in patients with PTCL and concomitant PCD, and larger, multi-center cohorts are needed to validate these findings across T-cell malignancies and PCDs.

Keywords: T cell, CTCL, T-LGL, PTCL, MGUS, multiple myeloma, plasma cell dyscrasia, survival

INTRODUCTION

T-Cell malignancies are a group of heterogeneous disorders, including cutaneous T-cell lymphomas (CTCLs), peripheral T-cell lymphomas (PTCLs), and T-cell leukemias, such as T-cell large granular lymphocytic leukemia (T-LGLL). T-LGLL is an incurable mature T-cell leukemia characterized by the abnormal clonal proliferation of CD3+/CD5/DimCD8+/CD57+ T cells (cytotoxic T-lymphocytes, CTLs) which can result in severe neutropenia, transfusion-dependent anemia, and marrow failure. Patients require frequent therapy, with recurrent relapses and overall response rates (ORRs) approximately 40% (1), although overall survival is >10 years in most patients (2–4). PTCL, of which the primary subtypes include anaplastic large cell lymphoma (ALCL) (25%), angioimmunoblastic T-cell lymphoma (33%), and PTCL-NOS (40%), are aggressive lymphomas with poor long-term survival of 35% at 5 years outside of ALK+ ALCL (5–7). CTCL, of which the most common variety is mycosis fungoides (MF), is a chronic dermatological condition that often requires frequent, sequential therapies (8). A deeper understanding of these disorders and associated prognostic and contributing factors is essential to improve outcomes in these rare diseases.

Sporadic cases of patients with combined T-cell malignancies and plasma cell dyscrasias (PCD) have been reported in the literature. These include small series and case reports of patients with T-cell lymphomas or T-LGLL with concomitant multiple myeloma (MM), monoclonal gammopathy of undetermined significance (MGUS), and other PCDs (9–12). While the most commonly observed association is with T-LGLL, there are case reports of other T-cell malignancies including AITL and PTCL-NOS with MM. Due to the rarity of these diseases, little is known about the pathophysiology, or clinical significance of these findings, and whether clinical or disease-related outcomes are impacted. Most commonly, T-LGLL with concomitant PCD or MM has been described. These include a few singular case studies of patients that have concomitant T-LGLL and PCD, including MM and even amyloidosis (9–15). There is only one case series with >10 patients, which is mainly descriptive in nature (16), while another study with six patients is also descriptive but does start to explore the potential link between the two diseases (17). The exact mechanism of interrelation between these disorders is not well known, but there are some postulations about how they link together, particularly in the newly describe T-follicular helper-type (TFH) lymphomas, as TFH cells regulate B cells, and there is a clear association with B-cell activation in these lymphomas, including plasma cells (18, 19). Furthermore, the clinical significance, including response and survival outcomes, of these coincident disorders remains unknown.

The purpose of this study was to explore the prognostic factors and outcomes of patients who have concomitant TCL or T-LGLL and PCD. Specifically, we investigated survival outcomes in patients with concomitant T-cell malignancies and PCD and evaluate the prognostic impact on treatment response and survival in this unique population.

PATIENTS AND METHODS

Patients

This study is a retrospective review of all patients diagnosed at the OSU James Cancer Center (OSUCCC) with a concomitant T-cell malignancy and PCD between January 1, 2011 and October 1, 2021. Patients were identified from The Ohio State University (OSU) lymphoma database, OSU MM database, and OSU T-LGLL registry. This study was approved by the Institutional Review Board at OSU.

Diagnosis of T-Cell Malignancies

All diagnoses for T-cell malignancies were made based on the 2016 World Health Organization (WHO) criteria. Given the difficulty in diagnosing T-LGLL, we included specific criteria for the diagnosis of T-LGLL, adapted from the 2016 WHO criteria, recently utilized in the ECOG5998 trial and recent studies (4, 20, 21). T-LGLL diagnosis required the presence of a monoclonal T-cell receptor (TCR) and a CD3+CD8+ population on flow cytometry ≥500 cells/mm (3). A monoclonal T-cell receptor was positive if detected by TCR polymerase chain reaction (PCR) or by restriction of TCR Vbeta noted on flow cytometry. For patients diagnosed with a clonal TCR by flow cytometry, a panel of 30 TCR Vbeta rearrangements was used with positivity considered if one or more clone was detected in 10% of events or greater as previously described (22).

Diagnosis of Plasma Cell Dyscrasias

The diagnoses for PCD were made based on the 2016 WHO criteria or the revised International Myeloma Working Group (IMWG) criteria. The diagnosis of MGUS was made if a patient had the presence of a monoclonal protein, <10% clonal plasma cells on bone marrow biopsy, and no other features of MM, such as anemia, renal dysfunction, or bone disease (23). The diagnosis of MM was made in patients with the presence of a monoclonal protein and an abnormal free light chain ratio, and clinical

features of MM including anemia, renal dysfunction, and/or bone disease or a myeloma defining event such as ≥60% clonal plasma cells on bone marrow examination, more than one focal lesion on MRI ≥ 5mm, or serum-free light chain ration ≥100 (24, 25).

Follow-up and Response Assessment

All patients with T-LGLL/TCL were followed from 1998 to 2018 in the T-cell malignancy clinic at the OSUCCC, staffed by a dedicated T-cell physician. The workflow, diagnostic, and treatment approach were thus standardized over time. On treatment, patients were typically seen in the clinic every 2–3 months. Patients off treatment, or on observation, were typically followed every 6 months to 1 year. Treatment regimens varied by patient based upon the clinical scenario. Patients were also seen by a dedicated plasma cell physician in the Plasma Cell Clinic at the OSUCCC. Patients with no high-risk features were typically seen annually for MGUS. Patients with smoldering disease were seen every 3–4 months depending on clinical characteristics, and patients with active myeloma are seen monthly or sooner as needed. Treatment regimens were varied based on the clinical scenario. For patients with nodal PTCL, responses were determined *via* Lugano criteria (26). For patients with T-LGLL, responses were based off of the modified ECOG5998 criteria, as reported in a recent study (4) and a recent prospective trial in T-LGLL (27), and were assessed by the investigators. At least 4 months of treatment were needed to assess for response (**Supplementary Table S1**). For patients with CTCL, response was determined based on the criteria for consensus statement of Olsen et al. (28) For patients with MM, response criteria were determined by the International Myeloma Working Group Uniform Response Criteria for CR, namely, very good partial response (VGPR), PR, stable disease (SD), and no response (NR) (25, 29).

Statistical Analysis

Baseline demographics and clinical characteristics were reported using summary statistics for the overall sample and by the type of malignancy. Overall survival (OS) was assessed as time from T-cell malignancy diagnosis until death or censoring. Progression-free survival (PFS) was assessed as the time from T-cell malignancy diagnosis until progression, death, or censoring. Patients without OS or PFS events were censored at last follow-up. Median OS and median PFS, along with the 95% confidence intervals, were calculated using Kaplan–Meier methods for the overall sample and by malignancy type. Survival curves were compared among the type of monoclonal protein using the log-rank test. Response to treatment was also reported for the overall sample and by malignancy type. All analyses were performed using SAS version 9.4 (SAS Institute Inc., Cary, NC, USA).

RESULTS

Entire Cohort

A total of 26 patients with confirmed concomitant T-cell malignancy and PCD were included in this analysis. Full patient baseline characteristics are seen in **Table 1**. The median age at T-cell malignancy diagnosis was 63 (range, 39–82; SD, 10.9) years, and the median age at PCD diagnosis was 64 (30–82, 12.3) years; 65% (n = 17) of patients were male, and 96% (n = 25) were Caucasian. Ten (39%) of the patients presented with their T-cell malignancy first, and 10 (39%) presented with their PCD first, while 19% (n = 5) had a concurrent diagnosis, and for one patient (4%), this was unknown. The most common concurrent T-cell malignancy was T-LGLL (n = 14, 54%), followed by CTCL (n = 6, 23%) and PTCL (n = 6, 23%). The most common PCD was MGUS (n = 13, 50%), followed by MM (n = 8, 31%) and plasmacytosis (n = 2, 8%). Plasmacytoma, lymphoplasmacytic lymphoma (LPL), and a kappa light chain-predominant plasma cell proliferation were seen in one patient (4%) each. The plasmacytosis diagnosis and kappa light chain-predominant plasma cell proliferation diagnosis was given to the patients by their treating physician and included as such in this study. On review, based on IMWG criteria, these patients would likely meet diagnostic criteria for MGUS. Overall, 16/26 (62%) patients were treated for their T-cell malignancy frontline, while 9/26 (35%) were treated for their PCD frontline, and one patient (4%) did not receive treatment for either disease.

T-LGLL Patients and Treatment Response

Fourteen patients had T-LGLL with the median age at T-LGLL diagnosis of 63 (39–82; SD, 10.1) years, and the median age at PCD diagnosis was 64 (48–82; SD, 9.3) years. Nine patients (64%) were male, and 13 (93%) were Caucasian. Baseline characteristics for these patients are in **Table 2**. Among the T-LGLL patients, eight (57%) had MGUS as their PCD, while four (29%) (n = 4) and two (14%) had MM and plasmacytosis, respectively. At the time of T-LGLL diagnosis, seven patients (50%) presented with anemia [hemoglobin (Hgb) < 12 g/dl], one (7%) presented with neutropenia [absolute neutrophil count (ANC) < 1,500/mm^3], three (21%) presented with both anemia and neutropenia (two having ANC <500 and one with ANC <1,500), and three (21%) were unknown. Of the four total patients that had neutropenia at presentation, three had severe neutropenia with an ANC <500/mm^3. Nine patients (64%) were found to have a concomitant autoimmune disease including five (36%) with rheumatoid arthritis and one each (7%) with immune thrombocytopenic purpura, anti-MAG neuropathy, ANCA-associated vasculitis, and cryoglobulinemia. For patients in the T-LGLL cohort, at the time of PCD diagnosis, nine patients (64%) had anemia (Hgb <12 g/dl), and two patients (14%) had bone disease. Six patients (43%) had a serum creatinine (Cr) <1 mg/dl, while six (43%) had a Cr between 1 and 2 mg/dl, one (7%) had a Cr >3 mg/dl, and one (7%) was unknown. No clear preponderance of any particular monoclonal protein-light chain was observed (**Table 2**). Among patients with T-LGLL, 10 (71%) were treated for T-LGLL frontline, while 3 (21%) were treated for their PCD frontline. The most common frontline therapy for T-LGLL was methotrexate n=5 (36%), followed by cyclosporine (CsA) n=3 (21%). One patient (7.1%) received cyclophosphamide (Cy) and one received Cy, Doxorubicin, Vincristine, and Prednisone (CHOP). For patients that had initial treatment for their PCD (n=3), two (14%) received Bortezomib/Lenalidomide/

TABLE 1 | Baseline characteristics for all patients.

Variable	Total (%) (n=26)
Age at T-cell diagnosis, mean (SD)	63.2 (10.9)
Age at PCD diagnosis, mean (SD)	63.7 (12.3)
Sex	
Male	17 (65.4)
Female	9 (34.6)
Race	
Caucasian	25 (96.2)
African American	1 (3.8)
Primary Presenting Malignancy	
T-Cell Malignancy	10 (38.5)
PCD	10 (38.5)
Concurrent Diagnosis	5 (19.2)
Unknown	1 (3.8)
T-Cell Malignancy	
T-LGLL	14 (53.8)
PTCL	6 (23.1)
-PTCL-NOS	4 (15.4)
-AITL	2 (7.7)
CTCL	6 (23.1)
Plasma Cell Dyscrasia	
MGUS	13 (50.0)
MM	8 (30.1)
Plasmacytosis	2 (7.7)
Plasmacytoma	1 (3.8)
LPL	1 (3.8)
kappa light chain-predominant plasma cell proliferation	1 (3.8)
Monoclonal Protein-Light Chain	
IgA-L	1 (3.8)
IgA-Unk	3 (11.5)
IgG-K	8 (30.8)
IgG-L	3 (11.5)
IgM-K	2 (7.7)
IgM-L	2 (7.7)
N/A-K	2 (7.7)
N/A-L	2 (7.7)
None Detected	2 (7.7)
Unknown	1 (3.8)
Percent bone marrow plasma cells at PCD diagnosis, median (SD; range)	5 (23.0; 0.5–80.0)
M-protein quantity at diagnosis (mg/dl), median (SD; range)	533 (1,564; 15.0–6,042.0)
Serum free light chain ratio at PCD diagnosis, median (SD; range)	7.1 (38.2; 1.1–130.7)
ISS Staging For PCD	
1	4 (15.4)
2	2 (7.7)
3	3 (11.5)
N/A	17 (65.4)
First-Line T-Cell Malignancy Therapy	16/26* (61.5)
Methotrexate	5 (31.3)
Cyclophosphamide	1 (6.3)
Cyclosporine	3 (18.8)
CHOP	3 (18.8)
EPOCH	2 (12.5)
Skin Directed Therapy	2 (12.5)
First-Line PCD Therapy	9/26* (34.6)
Bortezomib/Lenalidomide/Dexamethasone	4 (44.4)
Bortezomib/Dexamethasone	1 (11.1)
Cyclophosphamide/Bortezomib/Dexamethasone	1 (11.1)
Doxorubicin/Vincristine/Dexamethasone	1 (11.1)
Daratumumab/Lenalidomide	1 (11.1)
IFRT	1 (11.1)

One patient has not received treatment for either disease.

AITL, angioimmunoblastic T-cell lymphoma; Alk Phos, alkaline phosphatase; CHOEP, Cyclophosphamide, Doxorubicin, Vincristine, Etoposide, Prednisone; CHOP, Cyclophosphamide, Doxorubicin, Vincristine, Prednisone; CTCL, cutaneous T-cell lymphoma; EPOCH, Etoposide, Prednisone, Vincristine, Cyclophosphamide, Doxorubicin; IFRT, involved field radiation therapy; LDH, lactate dehydrogenase; LPL, lymphoplasmacytic lymphoma; MGUS, monoclonal gammopathy of undetermined significance; MM, multiple myeloma; PCD, plasma cell dyscrasia; PTCL-NOS, peripheral T-cell lymphoma-not otherwise specified; R-CHOP, Rituximab–Cyclophosphamide, Doxorubicin, Vincristine, Prednisone; R-CVP, Rituximab–Cyclophosphamide, Vincristine, Prednisone; T-LGLL, T-cell large granular lymphocytic leukemia.

Dexamethasone, and one (7.1%) received Cy/Bortezomib/Dexamethasone (CyBorD). Using strict E5998 criteria for response, the frontline ORR among T-LGLL patients was 2/12 (16.7%), with 8.3% (1/12) with PR and 8.3% (1/12) achieving a CR (**Figure 3**). The median time to response was 2.5 months with a median duration of response of 8.5 months. Five additional patients would go on to have a response (4 PR, 1 CR) with further lines of therapy for an overall response rate of 58% (7/12) for any line of therapy. There were no patients who had clearance of their T-LGLL clone with treatment of their concomitant PCD.

T-Cell Lymphoma Patients and Treatment Response

Twelve patients had TCL with a median age at TCL diagnosis of 64 (range, 41–80; SD, 11.9) years. Eight (67%) of the patients were male, and all of these were Caucasian. Baseline characteristics for these patients are in **Table 3**. Six patients (50%) had PTCL, and six patients (50%) had CTCL. Of the PTCL patients, four had PTCL-NOS and two had AITL. Four (33%) of the patients had MGUS as their PCD, while five (42%) had MM, and one patient (8.3%) had each of plasmacytosis, plasmacytoma, and Kappa light chain-predominant plasma cell proliferation. For patients with PTCL, using Ann Arbor staging, one (16.7%) patient had stage I disease, one (16.7%) had stage II disease, two (33%) had stage III disease, and two (33%) had stage IV disease. For patients with CTCL, four (66.7%) had stage I disease, and one (16.7) patient had stage IV disease, while for one patient, this was unknown. Five patients (42%) had CD30+ disease. At the time of PCD diagnosis, eight patients (67%) had anemia (Hgb <12), and six patients (50%) had bone disease. The most common monoclonal protein-light chain that was seen was immunoglobulin G (IgG)-kappa, seen in six patients (50%). Among patients receiving frontline treatment for their PTCL, the therapies were CHOP (n = 2, 16.7%) and Etoposide, Prednisone, Vincristine, Cyclophosphamide, Doxorubicin (EPOCH) (n = 2, 16.7%). Using Lugano criteria, the ORR to frontline treatment for PTCL was 3/6 (50%), with two (33%) CR and one PR (17%), while three (50%) had progressive disease (**Figure 3**). The median time to response was 4.5 months. For two (16.7%) patients, the initial treatment was for CTCL with skin-directed therapy including one patient receiving topical steroids and one patient receiving bexarotene/extracorporeal photopheresis. Of the six total patients that had CTCL, four received treatment, with an ORR of 75% with 3/4 having a response (2 CR and 1 PR). Two patients were on observation only for their CTCL.

TABLE 2 | Baseline characteristics for patients with T-LGLL.

Variable	Total (%) (n=14)
Age at T-LGLL, mean (SD)	62.8 (10.1)
Age at PCD diagnosis, mean (SD)	63.6 (9.3)
Sex	
Male	9 (64.3)
Female	5 (35.7)
Race	
Caucasian	13 (92.9)
African American	1 (7.1)
Plasma Cell Dyscrasia	
MGUS	8 (57.1)
MM	4 (28.6)
Plasmacytosis	2 (14.3)
Presenting Cytopenia at T-LGLL Diagnosis	
Neutropenia (ANC <1500)	1 (7.1)
Anemia (Hgb <12)	7 (50.0)
Both	3 (21.4)
Unknown	3 (21.4)
TCR V-Beta Positive at T-LGLL Diagnosis	
Yes	8 (57.1)
No	4 (28.6)
Unknown	2 (14.3)
LGL Count (CD3CD8+) at Diagnosis	
<1,500	6 (42.9)
≥1,500	5 (35.7)
Unknown	3 (21.4)
LDH at T-LGLL Diagnosis	
≤190	10 (71.4)
>190	3 (21.4)
Unknown	1 (7.1)
Splenomegaly	
Yes	4 (28.6)
No	10 (71.4)
Associated Autoimmune Disease	
Rheumatoid arthritis	5 (35.7)
ITP	1 (7.1)
Anti-MAG neuropathy	1 (7.1)
ANCA-associated vasculitis	1 (7.1)
Cryoglobulinemia	1 (7.1)
Anemia (Hgb <12) at PCD Diagnosis	
Yes	9 (64.3)
No	4 (28.6)
Unknown	1 (7.1)
Bone Disease at PCD Diagnosis	
Yes	2 (14.3)
No	6 (42.9)
Unknown	6 (42.9)
Creatinine at PCD Diagnosis	
<1.0	6 (42.9)
1.0–1.5	4 (28.6)
1.5–2.0	2 (14.3)
2.0–2.5	0 (0.0)
2.5–3.0	0 (0.0)
>3.0	1 (7.1)
Unknown	1 (7.1)
Monoclonal Protein-Light Chain	
IgA-Unk	1 (7.1)
IgG-K	2 (14.3)
IgG-L	3 (21.4)
IgM-K	2 (14.3)
IgM-L	1 (7.1)
N/A-K	1 (7.1)
N/A-L	2 (14.3)
None detected	2 (14.3)
ISS Staging For PCD	
1	2 (14.3)
2	1 (7.1)
3	1 (7.1)
N/A	10 (71.4)
First-Line LGL Therapy	10/14* (71.4)
Methotrexate	5 (35.7)
Cyclophosphamide	1 (7.1)
Cyclosporine	3 (21.4)
CHOP	1 (7.1)
First-Line PCD Therapy*	3/14* (21.4)
Bortezomib/Lenalidomide/Dexamethasone	2 (14.3)
Cyclophosphamide/Dexamethasone/Bortezomib	1 (7.1)

One patient has not received treatment for either disease.

Patients Presenting with PCD Frontline

Nine (35%) patients were treated initially for their PCD. Three (33%) patients had T-LGLL, two (22%) had PTCL, and four (44%) had CTCL. Seven (78%) patients had MM, one (11%) patient had MGUS [decision was made to treat this patient with CyBorD due to the patient being in acute renal failure for suspected monoclonal gammopathy of renal significance (MGRS) and when the patient stabilized, and it if was determined that the patient had MGUS, then treatment was stopped), and one (11%) patient had a solitary plasmacytoma. Four (44%) patients were treated with Bortezomib/Lenalidomide/Dexamethasone, and one (11%) patient each was treated with Bortezomib/Dexamethasone, CyBorD, Doxorubicin/Vincristine/Dexamethasone, and Daratumumab/Lenalidomide, and involved field radiation therapy (IFRT) of 50 Gy. Nine patients received frontline treatment for their PCD, with two (22%) achieving VGPR, three (33%) achieving PR, three (33%) achieving SD, and one (11%) with unknown response to frontline therapy. Six patients would go on to receive treatment for their T-cell malignancy, with four (66%) achieving CR, one (17%) achieving PR, and one (17%) with NR. Two patients had high-dose Melphalan with autologous stem cell transplant (HDM-ASCT) after their first line of treatment, and one patient had HDM-ASCT after their second line treatment. Three of the nine (33%) patients in this group would achieve clearance of their PCD clone with T-cell directed therapy, but no patients in the group would achieve clearance of their T-cell clone at any point.

Clearance of Concomitant PCD Clone in Patients Treated for T-Cell Malignancies

We next evaluated whether patient's concomitant neoplasm responded to treatment of the primary disease. At our institution, the eradication of the clone is evaluated by bone biopsy with aspirate and protein electrophoresis/free light chain assay in the serum or the urine of the patients. This is in accordance with IMWG criteria. None of our patients had MRD assessment, which was performed by ClonoSEQ assay (Adaptive Biotechnologies Corporation, Seattle, USA), and none were evaluated with high-sensitivity flow cytometry. Within the

TABLE 3 | Baseline characteristics for patients with T-cell lymphoma (TCL).

Variable	Total (%) (n=12)
Age at TCL diagnosis, mean (SD)	63.8 (11.9)
Age at PCD diagnosis, mean (SD)	63.9 (15.1)
Sex	
Male	8 (66.7)
Female	4 (33.3)
Race	
Caucasian	12 (100.0)
African American	0 (0.0)
T-Cell Lymphoma	
PTCL	6 (50.0)
-PTCL-NOS	4 (33.3)
-AITL	2 (16.7)
CTCL	6 (50.0)
Plasma Cell Dyscrasia	
MGUS	4 (33.3)
MM	5 (41.7)
Plasmacytosis	1 (8.3)
Plasmacytoma	1 (8.3)
kappa light chain-predominant plasma cell proliferation	1 (8.3)
Presenting Cytopenia at TCL Diagnosis	
Neutropenia (ANC <1500)	1 (8.3)
Anemia (Hgb <12)	5 (41.7)
Neither	3 (25.0)
Unknown	3 (25.0)
Stage at PTCL Diagnosis	N=6
I	1 (16.7)
II	1 (16.7)
III	2 (33.3)
IV	2 (33.3)
Stage at CTCL Diagnosis	N=6
I	1 (16.7)
II	0 (0.0)
III	0 (0.0)
IV	4 (66.7)
Unknown	1 (16.7)
LDH at TCL Diagnosis	
≤190	2 (16.7)
>190	6 (50.0)
Unknown	4 (33.3)
CD30+ at TCL Diagnosis	
Yes	5 (41.7)
No	3 (25.0)
Unknown	4 (33.3)
HIV Positive at TCL Diagnosis	
Yes	0 (0.0)
No	8 (66.7)
Unknown	4 (33.3)
HTLV-1 Positive at TCL Diagnosis	
Yes	1 (8.3)
No	3 (25.0)
Unknown	8 (66.7)
Splenomegaly	
Yes	1 (8.3)
No	9 (75.0)
Unknown	2 (16.7)
Associated Autoimmune Disease	
Autoimmune Hemolytic Anemia	2 (16.7)
None	10 (83.3)
Anemia (Hgb <12) at PCD Diagnosis	
Yes	8 (66.7)
No	3 (25.0)
Unknown	1 (8.3)
Bone Disease at PCD Diagnosis	
Yes	6 (50.0)
No	4 (33.3)
Unknown	2 (16.7)
Creatinine at PCD Diagnosis	
<1.0	6 (50.0)
1.0-1.5	4 (33.3)
1.5-2.0	1 (8.3)
2.0-2.5	0 (0.0)
2.5-3.0	0 (0.0)
>3.0	0 (0.0)
Unknown	1 (8.3)
Monoclonal Protein-Light Chain	
IgA-L	1 (8.3)
IgA-Unk	2 (16.7)
IgG-K	6 (50.0)
IgM-L	1 (8.3)
N/A-K	1 (8.3)
Unknown	1 (8.3)
ISS Staging For PCD	
1	2 (16.7)
2	1 (8.3)
3	2 (16.7)
N/A	7 (58.3)
First-Line TCL Therapy	6/12 (50)
CHOP	2 (16.7)
EPOCH	2 (16.7)
Skin Directed Therapy	2 (16.7)
First-Line PCD Therapy	6/12 (50)
Bortezomib/Lenalidomide/Dexamethasone	2 (16.7)
Bortezomib/Dexamethasone	1 (8.3)
Daratumumab/Lenalidomide	1 (8.3)
Docetaxel/Vincristine/Dexamethasone	1 (8.3)
IFRT	1 (8.3)

entire cohort (n=26), 8/26 had clearance of their PCD clone. Of these patients, four were treated for both diseases, three were treated for only their T-cell malignancy, and one was treated for only their PCD. Full breakdown can be seen in **Table 5**. Of the patients who received treatment for their T-cell malignancy frontline, 31.3% (5/16) patients had clearance of their PCD clone.

Four (50%) of the patients had their clone clear after starting treatment for their T-cell malignancy, including two (25%) who never received PCD-directed therapy. The treatments included Cy (one patient), Bexarotene (one patient), and MTX (two patients; one with prednisone and one without prednisone). An additional patient has an unknown initial T-LGLL treatment date, but they were on CsA (for kidney transplant), a known T-LGLL treatment, at the time of the resolution of their PCD clone. Of the patients who received initial frontline treatment for their PCD, 33.3% (3/9) had clearance of their PCD clone. This included two patients with clearance after treatment for MM and one after treatment for a plasmacytoma. The treatments leading to resolution included Azacitidine/Bortezomib/Dexamethasone for MM (and MDS); Bortezomib, Lenalidomide/Dexamethasone for MM; and Bortezomib/Dexamethasone for plasmacytoma.

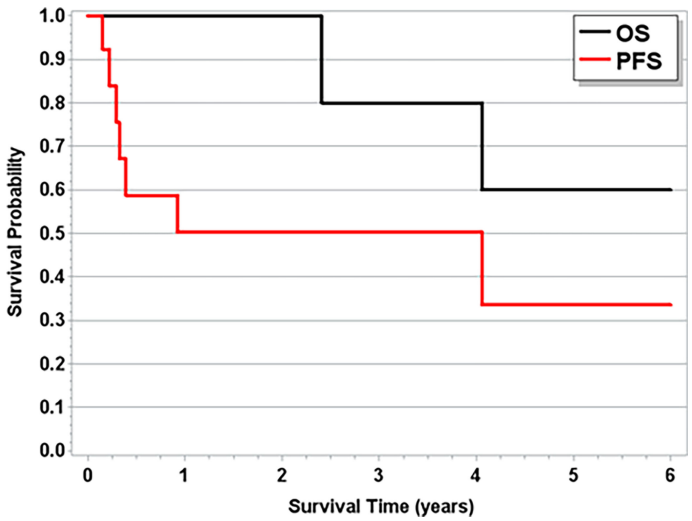

FIGURE 1 | Overall Survival and Progression Free Survival for Entire Cohort.

Of patients who received treatment for their T-LGLL, 41.7% (5/12) had clearance of their PCD clone, and neither of the two patients that were on observation for their T-LGLL had clearance of their PCD clone. No patient had clearance of their T-cell clone due to treatment of their PCD.

Survival Outcomes

With a median follow-up time of 1.8 years (range, 3 weeks–12.8 years), the median OS across all patients was 4.1 years (**Figure 1**). The median follow-up time for patients with T-LGLL was 1.9 years (range, 7 weeks–12.7 years), and for patients with TCL, it was 1.21 years (3 weeks–12.4 years). For full progression and survival outcomes, see **Tables 4A, B**. The median OS for patients with T-LGLL was not reached (**Figure 2**), while the median OS for patients with TCL was 3.4 years (**Supplementary Figure S1**). When TCL is broken down by disease, the median OS for PTCL was 1.7 years, and the median OS for CTCL was 12.4 years. In total, 42.3% of patients had progression of their T-cell malignancy. Six of the 12 (50%) patients with T-LGLL and 4/6 (67%) of patients with PTCL had refractory disease, while 0% with CTCL had progression (on frontline treatment). Median overall PFS was 3.21 years. For patients with T-LGLL, the median leukemia-free survival was 11 months (**Figure 2**), and for patients with TCL, the median

TABLE 4A | Progression and survival outcomes.

Outcome	All	T-Cell Lymphoma	T-LGLL
Progression	11/26 (42.3%)	4/12 (33.3%)	7/14 (50.0%)
Death	7/26 (26.9%)	5/12 (41.7%)	2/14 (14.3%)
Progression or death	15/26 (57.7%)	7/12 (58.3%)	8/14 (57.1%)
Median OS years (95% CI)*	4.06 (2.41-NR)	3.43 (0.65-NR)	NR (2.41-NR)
Median PFS years (95% CI)*	3.21 (0.38-9.28)	3.21 (0.28-NR)	0.92 (0.22-NR)

One T-cell lymphoma patient was excluded from time-to-event statistics due to unknown diagnosis date.

TABLE 4B | Progression and survival outcomes.

Outcome	All	PTCL	CTCL	T-LGLL
Progression	11/26 (42.3%)	4/6 (66.7%)	0/6 (0%)	7/14 (50.0%)
Death	7/26 (26.9%)	3/6 (50.0%)	2/6 (33.3%)	2/14 (14.3%)
Progression or death	15/26 (57.7%)	5/6 (83.3%)	2/6 (33.3%)	8/14 (57.1%)
Median OS years (95% CI)*	4.06 (2.41-NR)	1.66 (0.65-NR)	12.37 (3.21-NR)	NR (2.41-NR)
Median PFS years (95% CI)*	3.21 (0.38-9.28)	0.40 (0.28-NR)	12.37 (3.21-NR)	0.92 (0.22-NR)

One CTCL patient excluded from time-to-event statistics due to unknown diagnosis date.

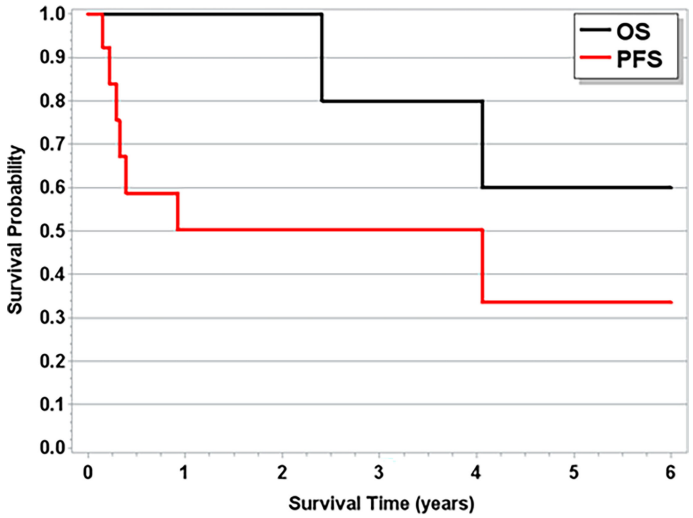

FIGURE 2 | Overall Survival and Progression Free Survival for Patients with T-LGLL.

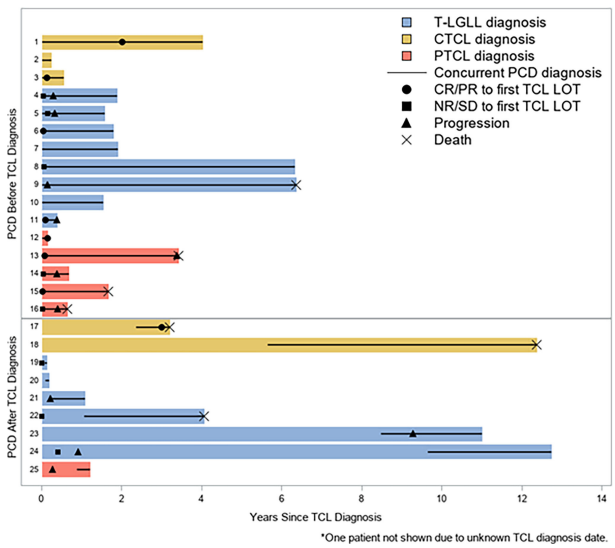

FIGURE 3 | Swimmer's Plot for Entire Cohort. Swimmer's Plot showing all patients in relation of time of diagnosis of T-Cell Lymphoma (TCL). Patients are split by whether they were diagnosed with T-cell Malignancy or PCD first. Lines on the solid color bars represent concurrent diagnosis. Legend describes when patients had progression or death.

PFS was 3.21 years (**Supplementary Figure S1**). When broken down by type of TCL (PTCL or CTCL, the median PFS among CTCL patients was 12.37 years, and the median PFS for PTCL patients was only 4.8 months. Full progression and response per patient are seen in **Figure 3** with treatment regimens in **Supplementary Table S2**. Of the patients who received treatment for their T-cell malignancy, 40% (8/20) had a response (3 PR and 5 CR). Of the patients who received treatment for their PCD, 60% (6/10) had a response (3 PR and 3 VGPR). For patients who had MM (n=8), the median PFS was 3.4 years, and the OS was 7.9 years. Full response rates by disease are seen in **Supplementary Table S1**.

DISCUSSION

In the present study, we present the largest cohort of patients with concomitant T-cell malignancies and PCD to date, with a focus on survival and treatment outcomes. For the first time, we present treatment response and survival outcomes and demonstrate that treatment of the underlying T-cell malignancy can also eradicate the concomitant PCD clone, which has implications into the pathogenesis of these diseases.

It is important to compare the results observed in this study with the established long-term survival literature for each individual disease. While an imperfect comparison, this helps to provide important, initial insights into the prognostic impact of concomitant PCD with T-cell malignancies. In the patients with T-LGLL in our cohort, the median PFS was 11 months, and OS was not reached (**Figure 2**). The OS is consistent with the established literature, as patients with T-LGLL are known to have a prolonged OS, with the ECOG 5998 study also having an OS not reached and Braunstein et al. showed a 5-year OS of 72% (4, 20, 30). Among CTCL patients, the observed median PFS of 12.4 years is similar to expected survival rates previously published for CTCL (31). Based upon our results, for patients with CTCL and T-LGLL, the survival outcomes are as expected per published literature for the respective disease types, suggesting that these patients should be treated for the first diagnosed, underlying disorder. The six patients with PTCL had a median OS of 1.7 years and a median PFS of 4.8 months. All of these patients were newly diagnosed patients with IPI scores ranging from 0 to 4. In the paper by Vose et al., median OS was nearly 2.5 years for PTCL-NOS, and AITL showed a median OS

TABLE 5 | Patients with clearance of PCD clone.

Patient Number	T-Cell Malignancy	T-cell treatment or PCD treatment first?*	First Line T-Cell Treatment	T-Cell Progression?	PCD	First Line PCD Treatment	PCD Progression After First Line Treatment?	PCD Clearance after T-Cell Treatment?
3	T-LGLL	Only T-cell	MTX	Yes	Plasmacytosis	None	No	Undetermined*
4	PTCL	PCD	CHOEP	No	Plasmacytoma	IFRT	Yes	No
5	T-LGLL	T-cell	Cyclosporine	No	MM	Bortezomib/ Lenalidomide/ Dexamethasone	Yes	Yes
11	T-LGLL	T-cell	Methotrexate	No	MM	Cyclophosphamide/ Dexamethasone	Yes	Yes
13	T-LGLL	PCD	Cyclophosphamide	No	MM	Bortezomib/ Lenalidomide/ Dexamethasone	No	No
15	T-LGLL	Only T-cell	Cyclophosphamide	Yes	MGUS	None	No	Yes
18	CTCL	Only PCD	None	No	MM	Daratumumab/ Lenalidomide	Yes	No
24	CTCL	Only T-cell	Bexarotene/ Extracorporeal Photopheresis	No	MGUS	None	No	Yes

Frontline treatment information for patients that had clearance of their PCD cline and whether they received initial treatment for their T-Cell disease or PCD and whether they had progression to front line treatments.

**Exact start date for T-cell malignancy is unknown, but the patient was on Cyclosporine (Known T-LGLL treatment) at the time of PCD clone clearance.*

of approximately 2.2 years. The results in our series among PTCL patients are worse than expected/known outcomes for these lymphomas, suggesting that patients with a concomitant PCD may have more aggressive or chemo-resistant disease (**Figure 3**). The exact reason why these patients may be experiencing worse outcomes is not known. Of the six patients with PTCL, two had AITL, and four were PTCL-NOS. AITL is a lymphoma of T-follicular helper (TFH)-derived T-lymphocytes, and over the past 10 years, some patients with previously unclassified PTCL (PTCL-NOS) have been reclassified as TFH under 2016 WHO guidelines (32). These patients often present with inflammatory symptoms (skin rash, edema, and arthralgias) c/w the B-cell regulatory function of these cells. Furthermore, it is likely that patients who have lymphomas derived from TFH cells are more likely to have concomitant PCD, as they are inherent malignancies of regulatory T-cells, and in these cases, the T-cell process likely drives the PCD (33). Frequently, these patients have complex pathological characteristics, and with the concomitant PCD, diagnosis is often protracted and delayed, which may delay treatment initiation. This highlights the importance of considering T-cell malignancies in the differential for patients with atypical PCD. While our population of PTCL patients is small (n=6), this concerning trend will need to be evaluated in a larger population of patients with additional studies for confirmation and suggests that aggressive treatment is needed for this population. Finally, we also observed patients who had resolution of their PCD clone after being treated with only T-cell-directed therapy (two patients with T-LGLL and one patient with CTCL). Furthermore, two patients had resolution of their PCD clone only after starting treatment for their T-cell malignancy (one patient with a CR for MM and one patient with resolution of their plasmacytosis; both had T-LGLL) (**Table 5**). This is an important finding, as it shows that the T-cell malignancy may be driving the monoclonal plasma cell spike and suggests that the underlying pathophysiology may be driven by the T-cell process. There is support that T-regulatory cells may maintain plasma cells, but the exact mechanism is unknown (34).

T-LGLL patients represented the largest type of T-cell malignancy in our series with 14/26 (54%) of patients with T-LGLL. Only 50% of patients with T-LGLL had progression of their disease, and only 14% died. The median OS was not reached in this group, suggesting that there is no deleterious effect of the concomitant PCD process in these patients. Interestingly, 36% of T-LGLL patients in this population had eradication of their plasma cell clone with T-LGLL directed treatment, including three patients with MM whose PCD clone was not fully eradicated with frontline myeloma-directed therapy but resolved after T-LGLL-directed treatment. This provides further evidence that the T-cell process may be driving the PCD, and treatment of the underlying T-cell malignancy, especially T-LGLL, can potentiate the eradication of the PCD clone. It has been suggested that treating the PCD clone may suppress the T-LGLL clone (16), but in our cohort, 38% of the patients who had eventual eradication of their PCD clone had treatment only for their T-cell malignancy. This does make rational sense, as patients who received T-cell-directed therapies often receive therapeutics that are known to be effective against PCD, such as cyclophosphamide. Sidiqui et al. described patients with concurrent T-LGLL and PCD, and in their study, a majority (82%) of patients developed T-LGLL after their PCD or concurrently, whereas in our study, a majority (58%) were diagnosed with their T-cell malignancy first or both malignancies at the same time (16). The variability between these two studies could simply be due to the limited sample size in both studies or earlier detection of the T-LGLL in the present series. Whatever the explanation, further studies are needed to verify the relationship between these two diseases.

It has been hypothesized that B-cell expansion can potentially result due to B-cell dysfunction in the setting of T-LGLL (35),

and this relationship has been seen with AITL and plasma cell proliferation as well (36). We show for the first time that treating the patient's T-cell malignancy may eradicate the PCD clone, especially if the patient has T-LGLL. We even see eradication of the plasma cell clone in 50% of patients with MM in this cohort. The T-LGLL may be driving the expansion of B cells as described above, leading to the development of a plasma cell clone. When the T-LGLL is treated, this clonal expansion resolves. It remains unknown whether the PCD drives the T-cell disorder or *vice versa*. To date, the exact pathophysiological mechanisms of concurrent PCD and T-cell malignancy are unknown. In MM, about one-third of patients can develop TCR-β rearrangements that share a similar immunophenotype to T-LGLL (37). Furthermore, given that T-LGLL is a disorder of terminal effector T-lymphocytes, it is possible that this induces the development of a reactive clonal expansion due to the underlying PCD or monoclonal gammopathy (38). This could be from an enhanced clonal expansion due to the chronic immune response that was initially due to the PCD (39).

This study has limitations that are inherent to all retrospective, single-center studies. The study encompassed a long period of time, during which treatment strategies changed and new agents became available. Additionally, analysis of clinical outcomes to treatment must be interpreted with caution, given low patient numbers, and only analyzing for initial progression or death. Furthermore, due to the multiple different diseases, the first-line treatment for the patients in this cohort varied extensively. It is difficult to correlate clearance of the PCD clone with survival, as only a small portion of patients had their clone resolve; it was nearly evenly split between patients who received treatment for both their T-cell malignancy and their PCD, or just treatment for the T-cell malignancy. Despite these limitations inherent to retrospective analyses, this study provides the largest dataset of patients with concomitant T-cell malignancies and PCD to date, providing a robust insight into this likely underdiagnosed population. A large multicenter retrospective review is needed to further characterize this population and definitively identify the clinical significance of these concomitant disorders. We show that treating the patient's T-cell malignancy has similar OS and PFS as compared to established baselines for T-LGLL and CTCL and may even have the potential to eradicate the PCD clone. However, for patients with PTCL (PTCL-NOS and AITL), outcomes appear worse, with similar ORR, but worse PFS, suggesting that the presence of a concomitant PCD may increase the overall risk in these patients.

CONCLUSION

We present the largest study to date on patients who have concomitant T-cell malignancies and plasma cell dyscrasias. In our analysis, we found that there was no survival difference in patients that have concomitant CTCL and T-LGLL and PCD when treated with standard T-cell-directed therapy. However, patients with concomitant PCD and PTCL had significantly inferior outcomes, with rapid progression, and worse OS and PFS highlighting the need to further evaluate these patients in a large, multi-center setting. For patients with T-cell malignancies as the primary diagnosis with concomitant PCD, treatment with standard T-cell-directed therapies is recommended with continued follow-up and monitoring of the concomitant PCD. There is the potential that treating a patient's T-cell malignancy may lead to resolution of their PCD clone, even without therapy directed at the PCD. Larger, multi-center studies are needed to validate these findings, and definitively describe the effect of concomitant T-cell malignancies and PCD.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/**Supplementary Material**. Further inquiries can be directed to the corresponding author.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by the Ohio State University Wexner Medical Center IRB. Written informed consent for participation was not required for this study in accordance with the national legislation and the institutional requirements.

AUTHOR CONTRIBUTIONS

ZB and MR collected the data. ZB, MR, AER, and JEB analyzed the data and wrote the manuscript. EM and LW performed the statistical analysis. ZB, AMi, AER, and JEB designed the study. ZB, MR, NB, DB, SD, MC, AK, FC, WH, RB, CC, DA, NC, AMe, WJ, PP, JCR, AER, and JEB cared for the patients. All authors contributed to the article and approved the submitted version.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fonc.2022.858426/full#supplementary-material

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REVIEW
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Intersection Between Large Granular Lymphocyte Leukemia and Rheumatoid Arthritis

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Large granular lymphocyte (LGL) leukemia, a rare hematologic malignancy, has long been associated with rheumatoid arthritis (RA), and the diseases share numerous common features. This review aims to outline the parallels and comparisons between the diseases as well as discuss the potential mechanisms for the relationship between LGL leukemia and RA. RA alone and in conjunction with LGL leukemia exhibits cytotoxic T-cell (CTL) expansions, HLA-DR4 enrichment, RA-associated autoantibodies, female bias, and unknown antigen specificity of associated T-cell expansions. Three possible mechanistic links between the pathogenesis of LGL leukemia and RA have been proposed, including LGL leukemia a) as a result of longstanding RA, b) as a consequence of RA treatment, or c) as a driver of RA. Several lines of evidence point towards LGL as a driver of RA. CTL involvement in RA pathogenesis is evidenced by citrullination and granzyme B cleavage that modifies the repertoire of self-protein antigens in target cells, particularly neutrophils, killed by the CTLs. Further investigations of the relationship between LGL leukemia and RA are warranted to better understand causal pathways and target antigens in order to improve the mechanistic understanding and to devise targeted therapeutic approaches for both disorders.

Keywords: rheumatoid arthritis, cytotoxic T lymphocyte (CTL), citrullination, neutropenia, STAT3 (signal transducer and activator of transcription 3), Felty syndrome

LGL LEUKEMIA CLINICAL PRESENTATION AND EPIDEMIOLOGY

Large granular lymphocyte (LGL) leukemia, is a rare hematologic malignancy accounting for 2-5% of lymphoproliferative disorders in North America and Europe (1). Recent population-based studies place the incidence of LGL leukemia between 0.2-0.72 per million people (2, 3). There are three major subtypes of disease that exhibit T-cell or natural killer (NK) cell phenotypic markers;

85% of cases are categorized as T-LGL, 10-15% as a chronic lymphoproliferative disorder of natural killer cells (CLPD-NK), and rare cases are described as aggressive NK cell leukemia (4). The median age of diagnosis is roughly 65 years (2–4).

Approximately 45-60% of patients with LGL leukemia require treatment upon presentation, with neutropenia and anemia as the main indications for treatment. Single agent immunosuppressive agents that are utilized include methotrexate, cyclophosphamide, and cyclosporine (1, 3). A "watch-and-wait" approach is appropriate in many indolent LGL leukemia patients. Unfortunately, most patients will eventually require treatment, and despite initial response, many will relapse or need life-long therapy, thus highlighting a need for continued research and new therapeutics. Reports vary in terms of survival with one of the largest population-based studies suggesting a median 9-year overall survival (3) and others indicating that overall survival is similar to control populations (2, 5). In patients requiring treatment, survival differed between symptom type, with those affected by anemia showing a median overall survival of 5.75 years and those with neutropenia exhibiting a median overall survival not yet reached 13 years after initiation of the study (6). Together, these reports demonstrate the heterogeneity of the patient population and the relatively indolent nature of the disease.

T-LGL leukemia pathogenesis is likely initiated by antigenic stimulation of cytotoxic T-cell expansion followed by somatic mutational events that activate survival pathways, subvert activation induced cell death, and drive clonal expansion (summarized in **Figure 1**). An abundance of reported genetic modifications and signaling changes point to a reliance on inflammatory and JAK/STAT signaling in LGL leukemia. In fact, nearly all patients show an increase in STAT3 activation (7–9), suggesting a stimulatory role for cytokine signaling pathways. The JAK/STAT signaling cascade is first initiated by cytokines such as IL-6, IL-2, and IL-15 and following activation, leads to transcription of STAT responsive genes that impact survival, proliferation, and immune activation (10).

Furthermore, STAT3 somatic activating mutations are the hallmark genetic lesion of LGL leukemia. Mutations were initially reported in roughly 30-40% of patients (9, 11). The

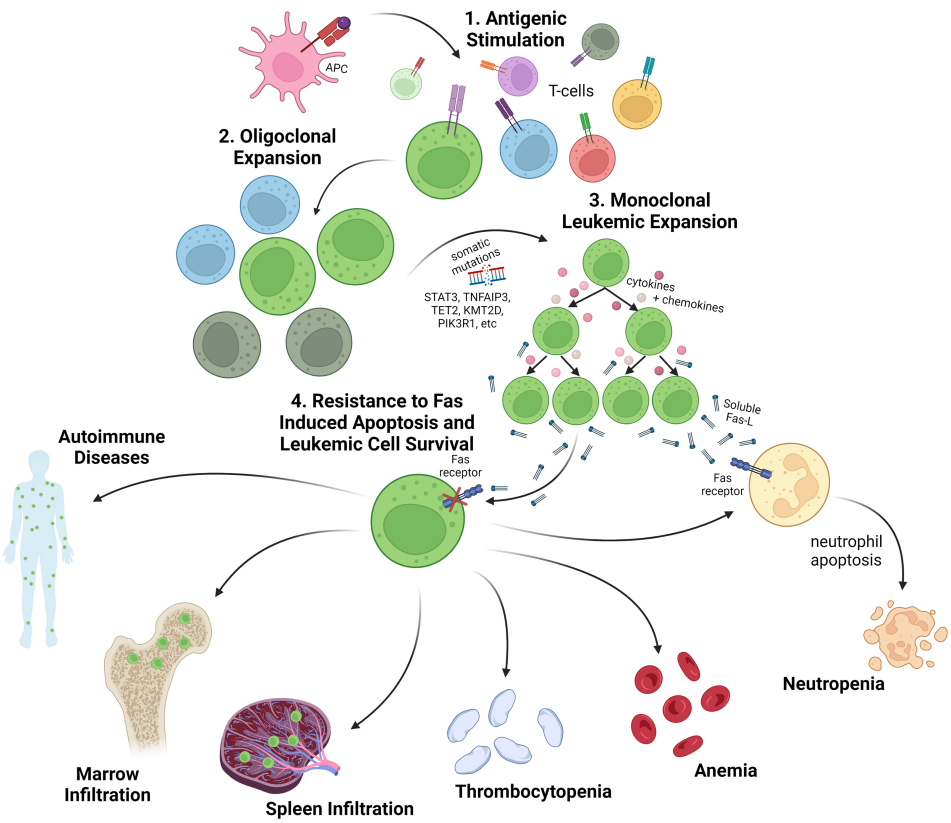

FIGURE 1 | Overview of LGL leukemia pathogenesis and clinical presentation. 1. T-cell LGL leukemia is presumed to arise following antigenic stimulation of normal T-cells. 2. Upon oligoclonal expansion of antigen reactive T-cells, somatic mutations are acquired in genes regulating key T-cell survival pathways as well as epigenetic regulators. 3. The leukemic expansion is characterized by clonal T-cell receptor rearrangements, somatic variants (especially somatic activating mutations in the *STAT3* gene), and an activated cytotoxic T-cell phenotype with secretion of inflammatory cytokines and chemokines, such as sFasL. 4. Leukemic LGLs are resistant to Fas-induced apoptosis and are characterized by activated cell survival pathways. Cytopenias, especially neutropenia and anemia, are a common disease feature and the main indicators for treatment. Leukemic LGLs also invade spleen, marrow and other organs where they contribute to cytopenias and autoimmune diseases. Created with BioRender.com.

majority of mutations occur in the SH2 domain, the region that mediates dimerization and activation of the STAT3 protein. However, recent publications report mutations in additional regions of the protein, such as the coiled-coil domain, some of which exhibit an activating phenotype. Their inclusion yields an overall *STAT3* somatic mutation rate of >50% in LGL leukemia (12–14).

Cytopenias (neutropenia, anemia, and more rarely thrombocytopenia), splenomegaly, and concomitant autoimmune diseases are the most common clinical manifestations. One of the most common symptoms of LGL leukemia is neutropenia. It is a major health concern, putting patients at risk for infection, pneumonia, or sepsis (11), especially in those with severe neutropenia (<0.5 $\times 10^9$/L) (15). Numbers vary between cohorts, but as high as 80% of symptomatic patients suffer from a neutrophil count lower than 1.5 $\times$ 10^9/L (16). Immune phenotype also correlates with neutropenia, which is found almost exclusively in CD8+ LGL leukemia (5). In one report, T-LGL leukemia patients with a CD8+, CD3+, CD16+, CD56- phenotype were the most likely to suffer from neutropenia (17). There have been several mechanisms proposed to explain LGL leukemia symptomology including: 1) LGL-secreted humoral factors, 2) LGL bone marrow infiltration, and 3) LGL-mediated cytotoxicity (17). Mechanistic drivers of neutropenia are discussed in more detail in later sections.

RHEUMATOID ARTHRITIS (RA) ASSOCIATION WITH LGL LEUKEMIA

LGL leukemia is often associated with autoimmune disorders including pure red cell aplasia, celiac disease, and others, but is most commonly associated with rheumatoid arthritis (RA) (18–20). LGL leukemia was first identified as a clonal disorder in 1985 (21). There were several descriptions of a few patients having RA with LGL leukemia around this time; indeed one of the patients in the original description of LGL leukemia was thought to have Felty syndrome, which is characterized by RA, neutropenia, and splenomegaly (22–24). RA is a systemic autoimmune disease characterized by chronic inflammation of the synovial joints, leading to pain, swelling, and destruction of the bone and cartilage (25). RA most commonly becomes symptomatic around 45–60 years of age, and women are two- to threefold more likely to develop RA than men (26). As a standalone clinical entity, RA occurs in ~1% of the world-wide population. However, reports place the incidence of RA in LGL leukemia patients as high as 36% (4, 18, 27). Of note, it is much more commonly observed in patients with T-LGL leukemia compared to those with NK-LGL leukemia (18). In the majority of patients who manifest both T-LGL leukemia and RA, the RA is diagnosed first. In a study of 56 patients with concurrent T-LGL leukemia and RA from a single clinical center, the median time that patients had RA prior to T-LGL leukemia diagnosis was six years, with a range of 0-36 years (28). LGL leukemia is rare in juvenile idiopathic arthritis (JIA) (29), likely because JIA and RA are different pathogenic entities, and has not be reported to have a relationship with late onset RA.

Importantly, once a patient with RA is found to have LGL leukemia, the patient is no longer classified as having RA. Instead, the diagnosis and treatment are centered around the LGL leukemia and the most serious complications associated with the disease (i.e. neutropenia and anemia). In this situation, the RA is considered associated with the LGL leukemia, rather than a separate disease entity. There are no case series comparing arthritis severity in canonical RA and LGL leukemia-associated RA. However, based on case reports, the severity of the arthritis in LGL leukemia appears to be similar to that occurring in canonical RA. The joint damage in both diseases is heterogeneous, with some individuals experiencing mild symptoms, while others have severe erosive joint disease.

Systematic evaluation of the clinical, genetic, and immunologic parallels between LGL leukemia and RA may reveal common mechanisms responsible for the co-occurrence of these two disorders.

PARALLELS AND COMPARISONS BETWEEN T-LGL LEUKEMIA AND RA

Despite the striking association between T-LGL leukemia and RA, the underlying mechanisms connecting the two disorders remains unknown. There are numerous points of similarity between the RA that develops in the presence and absence of LGL leukemia including common genetic, serologic, and cellular features. These features are discussed below and summarized in **Figure 2**.

Cytotoxic T-Cells (CTLs) in LGL Leukemia and RA

LGLs themselves are characterized by their large size, azurophilic cytoplasmic granules, low nuclear to cytoplasmic ratio, and round nucleus. In healthy populations, LGLs make up about 10-15% of peripheral blood mononuclear cells (PBMCs), but patients with LGL leukemia can have levels as high as 2- to 40-fold greater than their baseline (27). Diagnosis is supported by increased cell counts of > 2×10^9/L or lower counts (0.4 – 2×10^9/L) when the cells are clonal and the disease is paired with the appropriate clinical features such as RA and hematological parameters like cytopenias. Clonality assessment based upon T-cell receptor (TCR) rearrangement in $\alpha\beta$ and $\gamma\delta$ TCR genes is used to confirm diagnosis if the appropriate cell expansions are observed. Histologically, bone marrow (BM) samples show interstitial infiltrations of linear arrays of cytotoxic cells expressing CD8, cytotoxic granules containing perforin and granzyme B, and/or TIA-1 (30).

The T-LGL leukemia phenotype is typically CD3+, TCR$\alpha\beta$+, CD8+, CD16+, CD45RA+, and CD57+, and cells are CD4–, CD5dim, CD27–, CD28–, CD45RO–. Leukemic CD3+, CD8+ LGLs frequently exhibit relatively equal proportions of CD57- and CD57+ cells, which are proposed to represent progenitor and mature populations, respectively (31, 32). At the phenotypic and transcriptional level, these cells resemble chronically stimulated terminally differentiated cytotoxic T lymphocytes (CTLs), such as

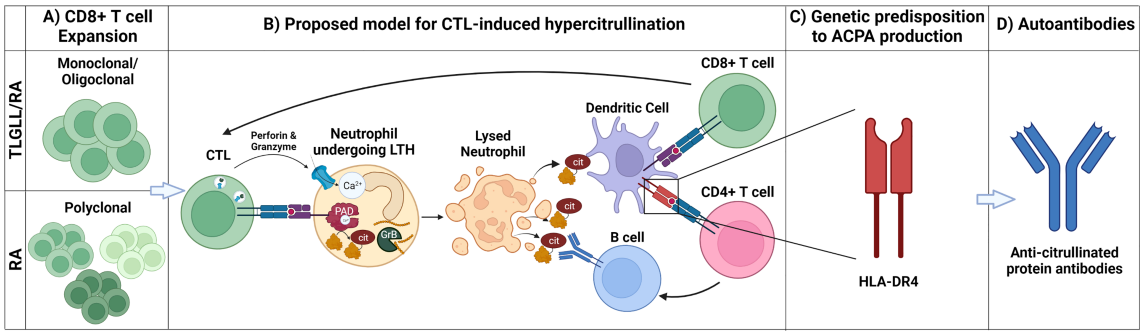

FIGURE 2 | Mechanistic parallels between T-LGL leukemia/RA and canonical RA. **(A)** CD8+ T cell expansion: T-LGL leukemia-associated RA (T-LGLL/RA) and canonical RA (RA) are characterized by the expansion of CD8+ T cells. The CD8+ T cell expansion is oligoclonal/monoclonal in T-LGLL/RA, whereas it is polyclonal in canonical RA. **(B)** Proposed model for CTL-induced hypercitrullination: In this model, clonally expanded CD8+ T cells (CTLs) targeting neutrophils release cytotoxic granules containing perforin and granzymes, inducing leukotoxic hypercitrullination (LTH). Perforin forms pores in the neutrophil membrane, allowing for calcium (Ca^{2+}) influx and activation of intracellular PAD enzymes, inducing neutrophil hypercitrullination. In parallel, granzyme B (GrB) cleavage of neutrophil antigens creates neoepitopes. As a result of the disrupted cell membrane, the neutrophils lyse, releasing autoantigens, including citrullinated and GrB-cleaved proteins. Dendritic cells (DCs) engulf these antigens and present them both to CD8+ and CD4+ T cells. The stimulated CD8+ T cells clonally expand and drive a feedforward cycle of neutrophil damage. Stimulated CD4+ T cells provide B cell help, giving rise to antibody-secreting cells producing anti-citrullinated protein antibodies (ACPAs). **(C)** Genetic predisposition to ACPA production: ACPA production is facilitated by the presentation of citrullinated antigens *via* HLA-DRs (e.g., HLA-DR4) encoded by RA-associated HLA-DRB1 susceptibility alleles. The requirement of specific RA-associated HLA-DRs for ACPA production likely explains why, despite having CTL expansion and neutrophil lysis, only a subset of patients with LGL leukemia develop RA. **(D)** Autoantibodies: Circulating APCAs are found in patients with T-LGLL/RA and canonical RA providing a serological record of the breach of immunologic tolerance to citrullinated antigens in both diseases. Created with BioRender.com.

those found in the setting of viral infection (33). Additionally, granzymes A, B, H, and K have been shown to be upregulated in LGL leukemia (34). The re-expression of CD45RA, as is observed on T-LGLs, is a feature of a sub population of effector CD8s referred to as "T effector memory cells re-expressing CD45RA" (TEMRA) cells (35). While this suggests that leukemic T-LGLs may derive from TEMRA cells (36), further comparisons using single cell approaches are needed to precisely define this relationship.

Clonal CD8+ T cell expansions have also been observed in the blood of RA patients, in the absence of known T-LGL leukemia, more frequently than in healthy controls (45% vs. 25%, respectively) (37), suggesting that antigen-driven expansion of clonal CTL populations is occurring in RA. In fact, examination of a large cohort of over 500 RA patients revealed clonal expansions in 3.6% of patients. Only 42% of patients with clonal expansions had counts above the threshold of 500 cells/μL typically considered for initial diagnosis of LGL leukemia (38). However, most patients with these clonal T-cell populations had previously been exposed to antirheumatic immunosuppressive treatments (also common treatments for LGL leukemia), which may blunt the progression along a potential continuum between RA and LGL leukemia. Given that over a million people in the US suffer from RA, these findings suggest that clonal T-cell populations are more common than the currently documented incidence of T-LGL leukemia.

As in T-LGL leukemia, the CTLs found in the synovium of RA patients are classified as effector memory or TEMRA cells (39). These cells are clonally expanded and express CD80, CD86, PD-1, and Ki67, indicating an activated and chronically stimulated phenotype (39, 40). They can persist in the joint for years, and CD3+ CD57+ cells accumulate with disease duration (41, 42). Moreover, similar to T-LGL leukemia, synovial CTLs in RA express perforin and granzymes (43). Indeed, an active role of degranulating CTLs in RA pathogenesis is supported by the findings that granzymes A, B and M are elevated in RA synovial fluid (44, 45), and serum levels of granzyme B correlate with disease activity and joint erosion (46). The accumulation of antigen-experienced clonally expanded CTLs in the RA synovium and evidence of active degranulation, implicates these cells in the pathogenesis of RA, but their precise role remains undefined.

Somatic Mutations in T-LGL Leukemia and RA

STAT3 mutations are the predominant somatic variants in T-LGL leukemia and have been associated with a variety of clinical markers of disease pathogenesis and outcome. A 2019 retrospective study of one of the largest LGL leukemia cohorts to date revealed that STAT3 mutations were associated with low hemoglobin and lower overall survival, as well as severe neutropenia (47). Another recent study confirmed higher rates of neutropenia, severe neutropenia, and cases requiring treatment in STAT3 mutated samples (48). STAT3 mutations are generally found almost exclusively in CD8+ rather than CD4+ patients (5), and more specifically, CD8+ CD16+ CD56- T-LGL leukemia patients exhibit more STAT3 mutations (49).

Numerous studies have associated STAT3 mutations with moderate and severe neutropenia in LGL leukemia (5, 9, 14, 48, 50, 51). STAT3 is a driver of soluble Fas ligand (sFasL) expression in LGLs (52), and sFasL is present at high levels in LGL leukemia patient serum (53). LGLs are resistant to FasL-induced apoptosis due to widespread activation of a network of survival signals (54). However, patient serum is sufficient to

activate cell death in normal neutrophils *in vitro* (**Figure 1**). A blocking anti-Fas monoclonal antibody rescued neutrophils from this fate (53). In addition, LGL patients with neutropenia have higher sFasL levels when compared to either healthy donor serum or serum from LGL leukemia patients with normal neutrophil counts. Furthermore, successful treatment has been associated with lower levels of sFasL (17), with methotrexate specifically inducing lower sFasL, and relapsed patients exhibiting increased sFasL (53). Thus, several lines of evidence implicate sFasL as a humoral mediator of neutropenia in LGL leukemia. Further discussion of direct LGL cytotoxic effects on neutrophils is presented below.

Interestingly, T-LGL leukemia patients with STAT3 mutations are more likely to have RA than those without (9, 50, 55–58). Whole exome sequencing in a large T-LGL leukemia cohort identified additional genes with recurrent somatic variants as well as frequent co-mutations of chromatin modifying genes in *STAT3*-mutant T-LGLs (14). Further studies are needed to define additional molecular events that correlate with RA co-occurrence in LGL leukemia.

Recent efforts identified 30 somatic mutations in clonally expanded CTLs of a small cohort of RA patients who did not have a diagnosis of T-LGL leukemia (40). Using a combination of gene targeted and exome sequencing approaches, mutations were identified in immune-related genes, proliferation-associated genes, as well as in other genes (40). Notably, these mutations were all found in clonally expanded CD8+ effector memory T cell populations, suggesting that CD8+ T cells that acquire these somatic mutations may clonally expand and play a pathogenic role in RA. However, it is important to note that somatic mutations were only found in 5/25 patients studied, and most mutations were only found in a single patient. While these data are intriguing, further studies on larger cohorts are needed to identify whether CTL mutations in RA are causal or an effect of the disease and to draw any meaningful parallels between the mutational CTL landscapes in RA and T-LGL leukemia.

Sex Bias

Although LGL leukemia generally occurs equally in males and females, with some studies showing a slightly increased incidence in males (2), the development of RA in patients with T-LGL leukemia is highly skewed toward females. One study of 56 patients with T-LGL leukemia and RA found that 73% were female (28). This parallels what has been observed in canonical RA for decades, a 3:1 female:male ratio (59, 60). While much more needs to be learned about the mechanism behind this sex bias, the increased risk of RA development in females with T-LGL leukemia suggests parallel mechanisms with canonical RA.

Immunogenetic Associations

RA is associated with a specific group of *HLA-DRB1* alleles termed the "shared epitope" alleles, so named due to the presence of a common amino acid motif (QKRAA) in the peptide binding groove of the encoded protein (61). The *HLA-DRB1* gene encodes the HLA-DRβ chain of the MHC class II molecule, HLA-DR, which serve as scaffolds for antigen presenting cells to display exogenously derived peptide antigens to CD4+ T helper cells. The HLA-DRB1 locus is highly polymorphic in humans and confers the highest genetic risk for RA development (62). While the risk for RA was initially attributed to HLA-DRB1*04 allelic variants (63), it was later appreciated that a larger group of alleles encoding for the "shared epitope" are collectively associated with RA (61). The most common RA-associated shared epitope alleles include HLA-DRB1*01:01, 01:02, 04:01, 04:04, 04:05, 10:01, and 14:02 (64).

Patients with concurrent T-LGL leukemia and RA are also enriched in HLA-DRB1*04 alleles associated with RA (65, 66). One study showed that 9/10 patients (90%) with T-LGL leukemia and RA expressed HLA-DRB1*04, whereas only 4/12 (33%) of patients with T-LGL leukemia alone expressed HLA-DRB1*04 (66). Two important caveats of these studies are that only HLA-DRB1*04 was evaluated, not other shared epitope alleles, and that individual allelic variants of HLA-DRB1*04 were not considered. This is important since some HLA-DRB1*04 variants are associated with RA (i.e. HLA-DRB1*04:01, 04:04, and 04:05), while others have been found to be protective against RA development and severity (i.e. HLA-DRB1*04:02). Although additional studies are needed to precisely compare the immunogenetic similarities between T-LGL leukemia and RA, the enrichment of RA-associated HLA-DRB1*04 alleles in patients with T-LGL leukemia who develop RA suggests the presence of a shared immunogenetic scaffold.

Antigen Specificity

Despite the observed clonal expansion and antigen-experienced phenotype, the antigen-specificity of the clonally expanded TEMRA cells in T-LGL leukemia and canonical RA remains largely unknown. One study observed close contact between LGL cells and dendritic cells (DCs) in bone marrow biopsies from patients with LGL leukemia (67). In *ex vivo* experiments, LGLs could be stimulated to proliferate when cultured with autologous bone marrow-derived, but not peripheral blood-derived, DCs, suggesting that these cells are actively responding to an antigen present in the bone marrow microenvironment. More recently, seroreactivity to human T-cell leukemia virus (HTLV-1/2) and human immunodeficiency virus (HIV-1) retroviral epitopes was identified in a subset of LGL leukemia as well as the clinically normal family members of reactive patients (68). There was no evidence of retroviral infection in reactive patients. While this viral seroreactivity has been identified in a subset of LGL leukemia, no unifying antigenic driver has been identified, and this represents a key knowledge gap in the disease.

In RA, one study has shown that RA patients have a population of CTLs that are autoreactive against epitopes from apoptotic cells that are cross-presented by dendritic cells, termed "apoptotic epitopes." These epitopes include those from vimentin and actin (69). This is interesting given that citrullinated vimentin and actin are both known targets of anti-citrullinated protein antibodies (ACPAs) in patients with RA (70, 71). In RA patients that do not respond to anti-TNF therapy, these CTLs display a TEMRA phenotype and are able to kill Tregs *in vitro* after stimulation with apoptotic epitopes, *via* a NKG2D-dependent mechanism. In addition, immunofluorescence imaging of the synovium of these patients has shown that CTLs

interact with Tregs, some of which express cleaved caspase-3, suggesting that these CTLs can kill Tregs *in vivo* (72). Much is still unknown about the epitopes recognized by CTLs in T-LGL leukemia and canonical RA. The definition of the target cells and antigens in these diseases is critical for understanding disease pathogenesis.

Serologic Profile

A hallmark feature of canonical RA is the formation of high titer autoantibodies targeting a defined set of self-proteins, making them powerful diagnostic biomarkers (73). There are two main autoantibodies that are analyzed clinically: 1) autoantibodies recognizing the Fc-portion of IgG, termed rheumatoid factor (RF); and 2) autoantibodies targeting proteins containing the post translational modification citrulline, termed anti-citrullinated protein antibodies (ACPAs). Each antibody specificity is present in approximately 70% of patients with RA and can co-occur in the same patient as well as exist separately (74). While both RF and ACPAs have high sensitivity for a diagnosis of RA, ACPAs are more specific, suggesting dysregulated protein citrullination and a breach of tolerance to these antigens as key processes in RA. ACPAs are a collection of antibodies targeting a diverse set of proteins in which arginine residues have been post-translationally deiminated by the peptidylarginine deiminase (PAD) enzymes, generating the non-classical amino acid citrulline (75). These antibodies are detected clinically using synthetic cyclic-citrullinated peptides (CCP). In addition, the development of ACPAs is associated with HLA-DRB1 shared epitope alleles (76), implicating this common genetic scaffold in the development of immune responses to citrullinated proteins.

Interestingly, RA-associated autoantibodies are also detected at high levels in individuals with T-LGL leukemia. In a study of 27 patients with T-LGL, 15 (55.6%) were positive for RF, four of whom did not have a diagnosis of RA (77). In a study of 56 T-LGL leukemia and RA cases, 82% were RF positive and 88% were positive for anti-CCP antibodies (28). In a small study comparing ACPA positivity in T-LGL leukemia patients with and without RA, 95% (18/19) of T-LGL leukemia patients with RA had ACPAs, compared to none (0/15) of the patients without RA (78). Importantly, while the data suggest that seropositivity for classic RA autoantibodies may be higher in T-LGL leukemia patients with RA compared to the general RA population, further head-to-head studies are needed to define the serologic overlap between the two disease entities. Together, these data highlight the serological similarity between patients with RA in the presence and absence of T-LGL leukemia, and support the hypothesis that dysregulated protein citrullination is a key pathogenic process both in RA and T-LGL leukemia/RA.

Treatment

Most patients with LGL leukemia eventually need treatment because of severe or symptomatic neutropenia, anemia, or associated autoimmune conditions. Because LGL leukemia is such a rare disease, most clinical evidence for drug selection is derived from retrospective studies that indicate the efficacy of three main immunosuppressive treatments: methotrexate (MTX), cyclophosphamide, and cyclosporine A (27). Interestingly, these therapies have significant parallels with treatments for canonical RA. MTX is a first-line therapy for RA, and oral cyclophosphamide and cyclosporine A are also useful to control RA (79, 80), although the use of cyclophosphamide is limited because of toxicity and cyclosporine A is reserved for refractory RA. Therefore, LGL leukemia with or without RA is usually treated as a single entity without the need for using additional therapies to treat the concomitant RA, unless joint symptoms persist. Importantly, considering that LGL leukemia is the potential driver of RA in this group of patients, in principle, any treatment controlling the leukemia should be effective in controlling RA.

Similarly, therapies introduced to treat the RA in patients with LGL leukemia have shown benefit in improving hematological parameters associated with the leukemia, including cytopenias and LGL expansion. In particular, rituximab, a monoclonal antibody therapy targeting CD20, has been shown to induce a remarkable 100% hematological response rate (either complete or partial leukemia remission) in small case series and case reports of refractory LGL leukemia with RA (81–84), and in one case of refractory LGL leukemia without RA (85). The JAK3 inhibitor tofacitinib has also been shown to induce hematological improvement in some patients with refractory LGL leukemia and RA (86). The finding that similar therapies are useful in treating both canonical RA and LGL leukemia supports the notion that these diseases share common pathogenic pathways.

Interrelationship Amongst T-LGL Leukemia, RA and Felty Syndrome

Felty Syndrome (FS) is a rare disorder occurring in 1-3% of RA patients and is defined by the presence of splenomegaly and neutropenia (87). Given its symptomatic overlap with LGL leukemia, there is considerable debate about whether FS and LGL leukemia are distinct or related entities. FS has long been associated with LGL leukemia (88, 89), and LGL leukemia may co-occur in as high as 40% of FS patients (18). Past reports have also observed a high prevalence of HLA-DRB1*04 alleles in both diseases (86.7% in FS; 82.8% in LGL leukemia/RA patients; 31.4% in LGL leukemia patients, which is similar to control population rates) (66) as well as response to methotrexate therapy in both diseases (90). Moreover, FS, LGL leukemia and RA share elevated levels of the cytokines IL-6, HGF, CDCP1 and CXCL10, and the latter correlates with more severe disease activity in RA (91, 92).

Recent studies have applied advanced molecular analyses to further define the relationship between the two diseases. A 2018 analysis of 14 FS patients found that 43% had *STAT3* mutations in the SH2 domain as detected by deep amplicon sequencing. Regardless of mutational status, a majority of bone marrow samples exhibited elevated phospho-STAT3 levels. Many of these patients had a high percentage of lymphocytes, but this did not necessarily equate to overall lymphocytosis. On average, these FS patients had smaller clone sizes than the average T-LGL leukemia patient (91). In 2021, Gorodetskiy et al. stratified FS

patients by presence or absence of clonal T cell expansion, classifying those patients with expansions as LGL leukemia/RA (n=56) and the remainder as FS alone (n=25). Interestingly, in contrast to patients with FS, LGL leukemia/RA patients exhibited increased LGL counts >2 x 10e9/L (21% vs. 0% in FS) and *STAT3* mutations (39% vs. 0% in FS) (28). This *STAT3* mutation prevalence in the LGL leukemia/RA group is similar to the frequency in previously published studies in LGL leukemia (9, 93). These data suggest that the extent of clonal T-cell expansion may distinguish LGL leukemia/RA from FS. It remains to be determined if FS patients classified in this manner will later acquire somatic activating mutation in *STAT3* and/or progress to LGL leukemia/RA. LGL leukemia/RA and FS both exhibited CD3+CD8+ T-cells with CD57, CD16 and $CD5^{-/dim}$ expression (28). Notably, T-cell clonality and *STAT3* mutations were detected more frequently in spleen samples than peripheral blood or bone marrow from ten atypical LGL leukemia/RA patients with lymphopenia, severe neutropenia, and marked splenomegaly, emphasizing the potential for LGL leukemia misdiagnosis as FS (94).

Further studies are needed to refine the diagnostic criteria to distinguish between LGL leukemia and FS, if they are indeed distinct diseases. However, substantial challenges remain to the routine application of sensitive molecular methods to uncommon specimens such as bone marrow and spleen material. Increased utilization of T-cell clonality and *STAT3* mutational profiling may lead to increased diagnosis of LGL leukemia within RA and FS patient populations, yet these events are likely detectable in all three diseases with ultrasensitive detection methods.

In summary, canonical RA and the subset of patients with LGL leukemia and RA exhibit an abundance of shared and overlapping demographic, immunologic, serologic, and genetic features. These parallels are unlikely to be fortuitous but evoke a common mechanism for RA development. The following section provides some considerations to explain the connection between these two diseases.

PROPOSED MECHANISMS FOR THE RELATIONSHIP BETWEEN T-LGL LEUKEMIA AND RA

Different models have been proposed for the co-occurrence of T-LGL leukemia and RA. Since RA is generally documented several years before LGL leukemia is diagnosed, it has been questioned whether T-LGL leukemia is a consequence of long-standing RA, whether the leukemia develops as a consequence of RA treatment (38), or whether the clonal expansion of pathogenic CTLs is indeed the driver of RA in these patients. Evidence for these three options will be discussed in detail below, and it is important to note that there may be no single model that can explain all cases of RA occurring in the setting of T-LGL leukemia. Understanding the mechanistic relationship between RA and T-LGL leukemia is critical for understanding disease pathogenesis and identifying effective preventive and treatment strategies for both disorders.

LGL Leukemia as a Consequence of RA

Clonal CD8+ T cell expansions have been observed in RA, which is not surprising given the chronic autoantigen driven nature of this disease. One possibility for the co-occurrence of RA and T-LGL leukemia is that the clonal expansion of CD8+ T cells in RA may result in the acquisition of *STAT3* and other somatic mutations, T cell transformation, and the development of leukemia. While more frequent clonal CD8+ T cell expansions have been observed in RA compared to healthy controls (45% vs. 25%, respectively), the same study found that the two groups had a similar degree of clonality, and some individuals in both the RA and healthy control groups exhibited expansions comprising ~40% of their CD8+ T cell pool (37). This suggests that although CD8+ T cell expansions are common in RA, they alone cannot explain the concomitant development of RA and LGL leukemia. In addition, T-LGL leukemia can occur in the absence of RA, demonstrating that RA is not a prerequisite for the development of leukemic T-LGLs. Thus, while it may be tempting to speculate that RA is the driver of T-LGL leukemia based on the frequent diagnosis of RA before T-LGL leukemia, it is equally likely that occult low frequency LGL clones initiate the breach of immune tolerance to self-antigens prior to the development of neutropenia and clinical discovery of T-LGL leukemia (see "Pathogenic CTLs as the driver of RA" section).

LGL Leukemia as a Consequence of RA Treatment

Another possible explanation for the co-occurrence of LGL leukemia and RA is that LGL leukemia develops as a result of the immunomodulating therapies used to treat RA, namely treatment with tumor necrosis factor (TNF) inhibitors. In one study, clonal expansions of LGL cells expressing CD3, CD56, and γδ TCRs were observed in 3.6% (19/529) of RA patients and were found to positively correlate with exposure time to TNF blocking agents (38). However, it is important to note that this phenomenon is not unique to RA. Similar clonal expansions of LGL cells with γδ TCRs have been observed in association with TNF inhibitor use in patients with ankylosing spondylitis (SpA) and psoriatic arthritis (PsA) (95). In addition, a relationship between anti-TNF use for the treatment of irritable bowel disease and the development of hepatosplenic T-cell lymphoma (HSTCL) (96), has been suggested by a literature review study that found 11% (22/200) of HSTCL cases reported in the literature were associated with IBD treatment (97). It remains to be determined if such LGL cell clonal expansions are associated with progression to LGL leukemia in any of the individuals in whom they were detected, and whether treatment may drive or expand an existing pathogenic LGL pool present in these patients. Regardless of the mechanism for their development, the lack of specificity of these clonally expanded LGL cells for RA or LGL leukemia suggests that

anti-TNF inhibitor therapy is not likely to be the mechanistic link between RA and T-LGL leukemia.

LGL Leukemia as the Driver of RA

While not all factors contributing to RA development are known, accumulating evidence suggests a central role for CTLs in RA pathogenesis, both as effectors perpetuating tissue damage and as generators of RA autoantigens (**Figure 2**). This latter role may be the key to linking T-LGL leukemia to RA development. We postulate that, in people with T-LGL leukemia and concomitant RA, the resulting autoimmunity represents a paraneoplastic syndrome caused by the expanded T-LGL clones. Moreover, parallel CTL-driven mechanisms may contribute to the development of RA in people without T-LGL leukemia.

This hypothesis is supported by the finding that a subset of RA patients have evidence of killer cell pathway activation in their joints in association with a form of lytic neutrophil cell death, termed leukotoxic hypercitrullination (LTH) (98, 99). LTH has been found to be unique among cell death and activation stimuli tested to date in its ability to hyperactivate the intracellular calcium-dependent peptidyl arginine deiminase (PAD) enzymes, leading to widespread protein citrullination in a pattern similar to that found in cells of the RA joint. LTH can be triggered by both host and pathogen-derived pore forming proteins, which allow the influx of extracellular calcium into the cell and hyperactivation of the intracellular PAD enzymes (98–100). In the subset of RA patients with LTH-associated hypercitrullination in the joint, the pore forming protein perforin was identified as the causative factor in the ability of killer cells to induce hypercitrullination in target neutrophils (98). The physiologic role of perforin is to form pores in the membrane of target cells to facilitate the delivery of granzymes, which subsequently cleave intracellular proteins, including caspases, to induce apoptosis *via* the extrinsic pathway. The observation that hypercitrullination was found in synovial fluid cells from a subset of patients with activation of the extrinsic apoptosis pathway, implicates CTL killing of neutrophils in the generation of citrullinated autoantigens in a subset of individuals (98).

A recent study on target cells engineered to express PAD2 or PAD4, two key citrullinating enzymes strongly implicated in RA pathogenesis and highly expressed by neutrophils, demonstrated a combinatorial effect of perforin and granzymes on the creation of autoantigens recognized by sera from RA patients (101). It has been hypothesized that a potential consequence of granzyme-mediated cleavage of self-proteins during the induction of target cell apoptosis is the generation of neoepitopes that may lead to the breach of immunologic tolerance and development of autoimmunity (102). The serine protease granzyme B has been most heavily studied in this regard after it was shown that the majority of autoantigens targeted across the spectrum of systemic autoimmune diseases are substrates for this protease. It was observed that a different pattern of protein fragments was generated when these antigens were cleaved by granzyme B compared to the effector caspase, caspase 8, which has a similar preference for cleaving substrates after aspartic acid residues (103). Together, these studies suggest that CTLs have the potential to modify the autoantigen pool in target cells, both by inducing hypercitrullination in PAD-expressing cells and by granzyme B-mediated cleavage of target cell proteins.

A review of granzyme B-cleaved autoantigens in systemic autoimmunity further revealed that granzyme B cleavage sites and autoreactive B and/or T cell epitopes tend to co-cluster within proteins, suggesting a causal relationship (104). This was demonstrated experimentally for PAD4, which is both a citrullinating enzyme and a target autoantigen in a subset of RA patients with the most destructive joint disease (105–108). In this study, cleavage of PAD4 by granzyme B was found to induce discrete changes in the PAD4 protein structure in regions adjacent to and remote from the granzyme B cleavage site (109). These structural changes were associated with increased presentation of peptide epitopes derived from these regions by an RA-associated HLA-DR allele. Furthermore, the granzyme B-enhanced epitopes were able to stimulate CD4+ T cell responses in patients with RA, suggesting that this process may occur *in vivo*. The findings that citrullination and granzyme B cleavage have the capacity to modify the repertoire of self-proteins present in target cells killed by CTLs coupled with the longstanding observation that RA is present in a subset of patients with T-LGL leukemia, supports the model that T-LGLs are drivers of RA development in individuals with concurrent leukemia and RA.

UNANSWERED QUESTIONS AND FUTURE RESEARCH DIRECTIONS

As detailed above, there are numerous clinical, genetic, and therapeutic overlaps between LGL leukemia and RA (**Figure 2**). It remains to be determined if the clonal CTL expansions detected in a subset of RA patients represent the early stages of a continuum between RA and LGL leukemia. If so, they may represent a biomarker of leukemic risk that warrants increased testing and monitoring. In addition, the cause of the classically observed neutropenia that is prominent in T-LGL leukemia remains unknown, but one hypothesis is the active killing of neutrophils by pathogenic CTL clones. It will be important to determine if direct CTL killing of neutrophils is a uniting feature of both disorders, as it could be responsible for the neutropenia observed in LGL leukemia and be a potent inducer of citrullinated and granzyme B-cleaved autoantigens in both diseases. Future study on the mechanistic parallels between T-LGL leukemia and RA will be critical to elucidate causal pathways and target antigens, in order to develop novel mechanism-guided treatments for these related disorders.

AUTHOR CONTRIBUTIONS

KM and KA: writing, figure generation. FA and TL: concept development, critical review. DF and ED: writing, concept development, critical review. All authors contributed to the article and approved the submitted version.

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REVIEW
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Pathogenesis and Treatment of T-Large Granular Lymphocytic Leukemia (T-LGLL) in the Setting of Rheumatic Disease

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A complex relationship exists between rheumatic diseases and cancer. This delicate balance between chronic inflammation and malignant cell transformation in hematologic neoplasms has been observed, but is not well defined. Large Granular Lymphocyte (LGL) leukemia is at the intersection of a clonal lymphoproliferative disease, chronic inflammation, and autoimmunity. The association between rheumatoid arthritis (RA) and the spectrum of Felty's Syndrome is well-known. Other rheumatic disorders have been reported including systemic lupus erythematosus (SLE), Sjogren's Syndrome (SS), vasculitis, Behcet's Disease (BD) and systemic sclerosis. The association between T-LGLL and rheumatic disease pathogenesis has been hypothesized, but has not yet been fully understood. Components of a shared pathogenesis includes chronic antigen stimulation, JAK-STAT pathway activation and overlap of various cytokines. We will summarize current knowledge on the molecular understanding between T-LGLL and rheumatic disease. There are many potential areas of research to help meet this need and lead to development of targeted therapeutic options.

Keywords: LGL, rheumatology, pathogenesis, T-LGLL, SLE (or Lupus), Behcet disease, Scleroderma (or systemic sclerosis), vasculitic, Sjogren's syndrome

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INTRODUCTION

A complex relationship exists between rheumatic diseases and cancer. This delicate balance between chronic inflammation and malignant cell transformation in hematologic neoplasms has been observed, but is not well defined. Large Granular Lymphocytic (LGL) leukemia is at the intersection of clonal lymphoproliferative disease, chronic inflammation, and autoimmunity (1). LGL leukemia is a rare type of mature T cell and NK cell neoplasm that was first characterized by McKenna et al. in 1977 (2). It was given its current name following discovery of lymphocyte clonality by Loughran et al. in 1985 (3). In 1989, the French–American–British cooperative group identified LGLL as a distinct entity among T cell leukemias (4). Based on the WHO classification, this clonal proliferation can be divided into three distinct conditions: T-LGLL, chronic lymphoproliferative disorder of NK-cells (CLPD-NK or NK-LGLL), and aggressive NK-cell leukemia, of which T-LGLL is the most common accounting for 85% of cases (5). T-LGLL is frequently described in patients with

rheumatologic disease (6). 15-40% of LGL leukemia patients have concomitant rheumatoid arthritis (RA) with Felty's Syndrome representing the most well-known association (7).

Other concomitant rheumatic disorders with LGLL have been reported including systemic lupus erythematosus (SLE), Sjogren's Syndrome (SS), vasculitis, Behcet's Disease (BD) and systemic sclerosis (SSc), but the true frequency is difficult to assess due to the rarity of T-LGLL. There is a link in the pathogenesis between T-LGLL and rheumatic disease though the exact pathobiology underlying this has yet to be fully elucidated. Further, concomitant T-LGLL with rheumatic disease is likely underreported, as flow cytometry and testing for the T-cell receptor (TCR) are not currently standard of care for patients with rheumatic diseases. Currently, it is thought that chronic T cell activation in the setting of an antigen trigger, dysregulation of apoptosis and hyperactivation of Janus kinase (JAK) signal transducer activator of transcription (STAT) pathway as well as other molecular survival pathways (1, 8) drives the development of T-LGLL. Typical disease features of T-LGLL include splenomegaly, and cytopenias, most commonly neutropenia with increased susceptibility to infection, and anemia, often with transfusion dependence. Large granular lymphocytes bear CD3+CD8+CD57+ surface phenotypes on T cells with clonal rearrangement of TCR genes (9). These LGLs have antibody-dependent and natural killer cell-mediated cytotoxicity and make up 5-10% of total lymphocytes in healthy patients (10). Currently, treatment is based on immunosuppressive therapies, which may produce an insufficient long-term response, and make targeted therapies an ideal next step for treatment (11). Due to the rarity of T-LGLL, a significant knowledge gap exists regarding the pathogenesis and management options of T-LGLL in the setting of rheumatic disease.

The pathogenesis of LGL leukemia is thought to be due to an unknown chronic antigen trigger that leads to increased activation of the JAK-STAT pathway and emergence of a clonal population (1). Hyperactivation of the JAK-STAT pathway can be due to *STAT3* mutations that are present in 30-40% of LGL cases and mainly in patients affected by CD8+ T-LGLL subtype (12). *STAT3* mutations have been reported in patients with T-LGLL and RA (13). In a study by Rajala et al, T-LGLL patients with one *STAT3* mutation (23%) and multiple *STAT3* mutations (43%) had higher incidence of RA compared to those without mutations (6%) (14). The JAK-STAT pathway is known to play a role in the pathogenesis of other rheumatic diseases as well as provide a target for new therapies. The development of a monoclonal cytotoxic lymphocyte population is the hallmark of T-LGLL and leads to production of inflammatory cytokines resulting in disease manifestations such as cytopenias (1). Some patients with LGL leukemia can present with clinical features of rheumatic disease before the diagnosis of leukemia. It is unclear if this manifestation is related to the autoimmune disease itself or occurring as a secondary lymphoproliferative process. This review will discuss the overlap of pathogenic mechanisms and treatment between T-LGLL and rheumatic diseases other than RA.

CHRONIC ANTIGENIC STIMULATION

LGL leukemia cells represent a population of cytotoxic effector memory T cells, suggesting chronic antigen stimulation (15). The role of Epstein Barr Virus (EBV), Human T-lymphotropic Virus (HTLV-1) and Hepatitis C Virus (HCV) have been suggested (1, 16–18). As in T-LGLL, various rheumatic diseases are thought to be the result of immune activation due to chronic antigen stimulation. Studies link EBV infection with autoimmune disease and some lymphoid malignancies (19). EBV has been studied extensively in RA and SLE. In SLE, the hypothesis of defective control of EBV infection in a genetically predisposed individual leads to EBV-reactive T cells, autoantibody production and resultant tissue damage (19). EBV has been found in salivary glands of patients with Sjogren's Syndrome and EBV infected plasma cells have been shown to produce anti-Ro52 and anti-La antibodies (20). Other viral syndromes including HTLV-1, human immunodeficiency virus (HIV) and HCV share clinical features of Sjogrens (21). Currently, there is no conclusive evidence LGLs are activated by HCV, but the hypothesis of chronic self-antigen stimulation is supported by immunohistochemical studies showing LGL clusters in contact with dendritic cells in bone marrow (22). Chronic antigen stimulation from HCV has been extensively studied in the setting of cryoglobulinemia. The hepatitis C E2 envelope glycoprotein interacts with CD81 expressed on lymphocytes (23) which has been shown to result in increased T cell proliferation (24) and chronic B cell stimulation resulting in clones that produce monoclonal IgM (23), underlying the pathogenesis of cryoglobulinemia. In type II mixed cryoglobulinemia, the evolution from polyclonal to oligoclonal B cell expansion due to chronic antigen stimulation is considered to be a transition between autoimmunity and neoplasia (25). It is possible similar pathways are involved in the development of lymphoma and cryoglobulinemia in Sjogren's Syndrome (25). LGL leukemia was associated with indolent B cell lymphoma in two patients with HCV who were successfully treated with antiviral therapy. In one case, LGL expansion correlated with viral replication and anti-viral treatment controlled LGL leukemia (26). In another example, a case of T-LGLL in a patient with concomitant hepatitis B, C and HIV was successfully treated with anti-viral therapy (27). In epidemiologic studies, HTLV-1 has increased incidence in patients with Sjogren's Syndrome and HTLV-1 transgenic mice have shown rheumatic disease manifestations (28). The role of HTLV-1 in LGL remains unclear, but initial studies revealed HTLV seroreactivity in some LGL leukemia patients (29). In other diseases such as vasculitis, myositis and scleroderma the role of potential viral trigger is less clear and other antigenic stimulation may be result of bacterial, environmental or other triggers.

INHERITED SUSCEPTIBILITY/ HLA PREDISPOSITION

In rheumatic diseases, the human class II major histocompatibility complex (MHC) human leukocyte antigen

(HLA) plays an important role in predisposing an individual to develop an autoimmune response. Most notable is the HLA-DR region in RA, SS, SLE and vasculitis including Giant Cell Arteritis (GCA) and anti-neutrophil cytoplasmic antibody (ANCA) associated vasculitis (AAV) (30–32). In LGLL, the HLA-DR4 marker has been shown to be prevalent in patients with Felty's/RA, but the frequency in patients with LGL leukemia that is not associated with RA is unknown (33, 34). In a small series of patients with T-LGLL, HLA-DR4 was observed in 32% of patients, in those with associated RA this was 90% (34). In another series, HLA-DR4 was highly predictive of responsiveness to cyclosporine in patients with T-LGLL supporting an immunologic mechanism underlying cytopenias (35).

ACTIVATION OF THE JAK-STAT PATHWAY

In T-LGLL and rheumatic disease mutations of the JAK-STAT pathway play a vital role (**Image 1**). Gain of function mutations have been associated with autoimmunity as well as hematologic malignancies (36). In T-LGLL, mutation in *STAT3* gene is described most commonly leading to enhancement in anti-apoptotic pathways (37). Inhibition of the JAK pathway has been a therapeutic target for a variety of rheumatic diseases. JAK inhibitors (JAKi) have been approved for use in RA, ankylosing spondylitis (AS) and psoriatic arthritis (PsA), but studies are still ongoing for use in other rheumatic diseases such as SLE, vasculitis and SS. In T-LGLL, the JAK inhibitors ruxolitinib and tofacitinib have been applied to patients with refractory T-LGLL and related RA with some success. In a small cohort of patients receiving tofacitinib, hematologic response was observed in 67% of patients and 89% had improvement in RA symptoms (38). This has not been evaluated in cases of T-LGLL and other associated rheumatic diseases.

Systemic Lupus Erythematosus

The role of the JAK-STAT pathway in SLE has extensively been studied with ongoing randomized controlled trials evaluating use of JAK inhibition in the treatment of SLE (39–41). (NCT03616912), (NCT03616964), (NCT03252587). It is well known the interferon (IFN) signature plays a key role in SLE pathogenesis and activation of the IFN-receptor leads to signal transduction through the JAK-STAT pathway (42). Genes including *STAT4* have been associated with high levels of IFN-alpha. This may predispose patients to SLE as overexpression of IFN-alpha genes has been found to be elevated in serum of patients with lupus (43–45). The proposed effect of STAT4 inhibition is immune suppression and inhibition of Th1 cell differentiation (42). T-LGLL is more commonly associated with *STAT3* gain of function mutation which is associated with early-onset lymphoproliferation as well as autoimmunity (46). In lupus, the role of *STAT3* has been identified in the pathogenesis of lupus nephritis. In a lupus murine model, *STAT3* knockout mice had a markedly reduced renal inflammatory infiltrate, as well as less pronounced renal IgG and C3 deposition, compared to controls (47). There has also been association of SLE development with polymorphisms in TYK2, another member of the JAK family, identified in a large Swedish and Finnish population (48). While the relationship between T-LGLL and SLE remains unclear the JAK-STAT pathway has been shown to play a role in the pathogenesis of both disease entities and may represent a potential treatment target.

Vasculitis

The JAK-STAT pathway has also been evaluated in various vasculidites, and has been reported in patients with T-LGLL. In a series of eleven patients with vasculitis, 91% of patients had small vessel involvement presenting with purpura and histologic evidence of leukocytoclastic vasculitis. Cryoglobulinemic vasculitis was most frequently observed followed by ANCA negative microscopic polyangiitis and one case of GCA. Biopsy of the temporal artery and renal biopsy showed no LGL infiltration (49). In this series, most cases of T-LGLL were diagnosed simultaneously with vasculitis. Thus, screening for LGL in patients with new diagnosis of vasculitis should be considered.

In a study of patients with Behcets Disease (BD), total *STAT3* expression was significantly higher compared to controls, suggesting this signaling pathway is also activated (50). In a Han Chinese population with BD, a significantly increased frequency of the *STAT3* polymorphism was also observed suggesting susceptibility to BD (51). In LGLL patients, *STAT3* mutations have been associated with gene alterations on *TNFAIP3* which is a gene responsible for encoding an NF-kB signaling inhibitor called A20 (52, 53). Notably, haploinsufficiency of A20 protein can also result in a BD phenotype (54). Atas et al. hypothesized that there may be a pathogenetic association between BD and T-LGLL, due to the fact that upregulation of IL-18 and STAT3 pathways, along with a reduction in A20 protein result in reduced NF-kB inhibition (55). This overlap suggests IL-18, STAT3 and TNFAIP3 may play important roles in the pathogenesis of both BD and T-LGLL.

In large and medium vessel vasculidites, cytokine signaling dependent on JAK1 and JAK3 has been shown to be critically important in chronic inflammation (56, 57). In GCA and Takayasu Arteritis (TAK), vessel wall inflammation is induced by Th1 and Th17 cells (56). The cytokines released by these cells are known to activate the JAK-STAT pathway (36). In mouse models, temporal artery biopsy samples have shown upregulation of *STAT1* and *STAT2* genes (57, 58). A cohort study of patients with TAK revealed increased expression of various genes related to the JAK-STAT pathway (59). There are case reports of use of successful JAK inhibition in treatment of refractory TAK (60, 61).

The relationship of T-LGLL and ANCA-Associated Vasculitis (AAV) is unknown. In a cohort study of patients with AAV and nephrotic syndrome, molecular profiling of tissue samples revealed shared STAT1 activation identifying these two histopathologically different diseases have a common molecular pathway (62). Currently no clear association with *STAT3* mutations has been described in AAV. There are many unknowns for other types of vasculitis including polyarteritis

nodosa (PAN) and IgA vasculitis owing to the rarity of these diseases. It is possible that advances in molecular profiling technology will increase understanding of these disease processes and identify future treatment targets.

Sjogren's Syndrome

In Sjogren's Syndrome (SS), studies of JAK-STAT profiling are limited. *STAT4* polymorphisms have been identified as a genetic risk factor for SS development (63). In a study of monocytes from patients with primary SS, increased expression of JAK3 and STAT4 was detected by polymerase chain reaction (PCR) compared to controls (64). In a cohort of patients with SS, stimulation of peripheral blood monocytes by IL-6 revealed increased activation of STAT3 (65). A phenotype of LGL that has been described in association with SS represents the T_{emRA} subset, which can be seen in the setting of chronic inflammation, but is classically associated with low cell proliferation and high cell death rate compared to LGLs which have prolonged survival due to STAT pathway activation (66). Overall, these findings highlight overlap between chronic inflammation and autoimmunity as well as the difficulty associated with determining which process is the primary etiology. Further studies are needed to better assess the role of the JAK-STAT pathway in development of concomitant T-LGLL and SS. There are ongoing clinical trials evaluating the use of JAK pathway inhibition for treatment of sicca symptoms. (NCT04496960, NCT05087589, NCT04916756, NCT03100942)

Systemic Sclerosis

Reports of T-LGLL and systemic sclerosis (SSc) are exceedingly rare. In a small cohort of patients with T-LGLL and autoimmune diseases, one patient with a diagnosis of systemic sclerosis was described (67). Cytokine analysis on T-LGLL cells was performed and showed increased levels of IL-6, IL-8, IL-10, soluble IL-12 and TNF alpha suggesting role of cytokine release related to the immune phenomena observed in LGLL (67). The JAK-STAT pathway has been shown to play a crucial role in differentiation of autoreactive cells and the extracellular matrix remodeling that occurs in SSc (68). IL-6 is thought to exert it profibrotic effect through JAK2/STAT3 signaling (69). Skin biopsies from SSc patients have also shown abnormal IL6/JAK/STAT3 and tofacitinib gene signatures (70). The role of JAK inhibition is also ongoing in clinical trials for skin and lung manifestations of SSc (NCT03274076, NCT04206644).

CYTOKINES

Many cytokines involved in the pathogenesis of rheumatic disease and hematologic malignancies utilize the JAK-STAT pathway to transduce intracellular signals. Increased levels of cytokines are known to contribute to disease activity. Many different cytokines have been evaluated in the pathogenesis of T-LGLL and autoimmune disease. Leukemic LGL survival is promoted by elevated levels of IL-6 resulting in activation of STAT3 (12). Other cytokines including IL-2, IL-12, IL-15, IL-18, EGF, IP-10, G-CSF have been identified (71, 72). IL-15 has been shown to cause chromosomal instability and DNA hypermethylation acting as a key "activation switch" for survival and expansion of LGLL in both humans and mice (73). In rheumatologic disease, many cytokines use the Type 1 and 2 cytokine receptor family which has been implicated in disease pathogenesis (74, 75). The PRECISE Systemic Autoimmune Diseases (PRECISEADS) study identified a pro-inflammatory cytokine network shared by four distinct rheumatic diseases including SLE, SS, RA and SSc. Patients were found to primarily have increases in CXCL10, IL-2, IL-6, and tumor necrosis factor (TNF). The pro-inflammatory profile was also characterized by an abnormal B cell distribution, a CD8 cytotoxic T cell signature, and more severe clinical features (76). *In vitro* study suggested upregulation of this cytokine signature associated with B cell enhancement of Th1 differentiation and proliferation of activated naive T cells (76). While there is overlap between certain cytokines involved in rheumatic diseases as well as T-LGLL, whether these cytokine profiles imply a causative role is still unknown. It may be inferred that increased levels of these various cytokines support a cellular immune mechanism in rheumatic diseases and an ongoing expansion of T cells.

ROLE OF IL-15 IN T-LGLL AND AUTOIMMUNE DISEASE

Interleukin-15 (IL-15) is a proinflammatory cytokine expressed by a broad range of tissues and contributes to chronic inflammation and autoimmunity (77). IL-15 has been implicated in the pathogenesis of several autoimmune diseases as well as LGLL. IL-15 is a member of the IL-2 family of cytokines, which use receptor complexes containing the common gamma-chain for signaling (77). IL-15 promotes activation of T cells, NK-cells, neutrophils, macrophages, and is critical to dendritic cell function (78). Importantly related to development of autoimmune disease, IL-15 enhances activation and maintenance of IL-17 producing T Cells (75). The role of IL-15 in autoimmune disease comes extensively from studies of rheumatoid arthritis. IL-15 has been evaluated in other rheumatic diseases including SLE, SS, BD and SSc, but its exact role remains obscure (See **Table 1 and Supplement**).

Clinical trials targeting IL-15 in rheumatic disease are scarce and limited to RA. In a proof-of-concept study in rheumatoid arthritis patients, the use of human IgG1 anti-IL-15 monoclonal antibody (HuMaxIL15) showed suitable drug tolerability with no significant effects on T lymphocyte subset and NK cell numbers. By week eight, 63% of patients achieved an improvement of 20% in both the number of tender and swollen joints (79). Following, a phase II trial of the anti-IL 15 human monoclonal antibody, AMG 714, for RA did not show efficacy (NCT00433875). AMG 714 has also been evaluated in other diseases with autoimmune basis including psoriasis (NCT00443326) and celiac disease, but failed to meet its primary endpoint (80).

In T-LGLL, excess IL-15 is thought to play a part in the link between inflammation and cancer. Initial clinical trials targeting IL-15 had been unsuccessful (81, 82), but recent positive clinical data from a phase 1/2 clinical study (NCT03239392) of BNZ-1, a multi-cytokine inhibitor was presented at the 62nd American Society of Hematology (ASH) Annual Meeting suggests that IL-15 inhibition can induce clinical responses in patients with T-LGLL, particularly those with transfusion dependence (83).

LINKING AUTOIMMUNITY AND CANCER: IL-15 REGULATORY PATHWAYS

A common feature of CD8+ T cells and NK cells is their dependence on IL-15 for homeostasis (84, 85). Zhou et al. describe the deubiquitinase, Otub1 which was shown to be a key regulator of IL-15R signaling. Otub1 deficiency was associated with anti-cancer immunity and loss of self-tolerance (86). This highlights the role of Otub1 as a potential novel checkpoint target for cancer therapy. Other clinical trials using IL-15 in treatment of cancer have shown increased activation of NK and CD8+ T cells, but when administered as monotherapy have been ineffective (87). This is thought to be due to the action of immunologic checkpoints and there are ongoing trials evaluating the use of IL-15 in combination with checkpoint inhibitors for patients with metastatic solid cancers (NCT03388632). Combination therapy of IL-15 with rituximab in a mouse model of lymphoma and alemtuzumab in a model of adult T cell leukemia revealed that IL-15 enhanced efficacy of both rituximab and alemtuzumab (88). This led to development of the phase 1 trial of IL-15 combined with alemtuzumab for patients with adult T cell leukemia (NCT02689453) as well as ongoing trials in chronic lymphocytic leukemia (NCT03759184, NCT03905135).

ROLE OF OTHER CYTOKINES IN T-LGLL AND RHEUMATIC DISEASE

Systemic Lupus Erythematosus

SLE has been considered a dominant Th2 cytokine disease though, increased levels of both Th1 and Th2 cytokines can be seen (89). An association between IL-18, SLE and T-LGLL has been proposed. IL-18 is a cofactor for Th1 cell development and cytotoxic T cell induction (90). Ogata et al. describe a case of SLE and T-LGLL with levels of IL-18 correlating with lupus symptoms as well as the number of T-LGLs in serum suggesting IL-18 may activate T-LGLL (91). In a study of 40 patients with SLE, plasma IL-18 and IL-12 concentrations were significantly higher in SLE patients than in controls (92). In mouse models, CD8+ cytotoxic T cells have been found to be elevated in IL-18 transgenic mice and aberrant expression of IL-18 resulted in the increased production of both Th1 and Th2 cytokines (90). The MRL/lpr mouse, used as a clinical model in SLE, has been found to have higher serum levels of IL-18 compared to wild-type mice (93). In the same study, injections of IL-18 lead to presentations of malar rash and glomerulonephritis. This highlights the important role IL-18 plays in SLE and possibly the development of T-LGLL, but also as a potential therapeutic target.

Sjogren's Syndrome

Levels of different cytokines in association with T-LGLL and SS have been evaluated in a series of 12 patients which revealed significantly increased levels of soluble interleukin-2 receptor, TNF-alpha, IL-6 and IL-8 compared with healthy controls (94). This increase was common to LGL leukemia patients with or without Sjogren's syndrome.

Vasculitis

Cytokine profiles in vasculitis vary based on the specific underlying diagnosis and the connection with T-LGLL is still not clearly characterized. In large vessel vasculitis such as GCA, key cytokines identified include IFN-gamma, IL-6, IL-12, IL-17, IL-18 and IL-21 (56, 95) which promote Th1 and Th17 cell differentiation (96). In patients with granulomatosis with polyangiitis (GPA), monocytes have been shown to release high levels of IL-12 leading to induction of Th1 cytokines including TNF-alpha and IFN-gamma (97). In Behcet's Disease, most studies have shown evidence of a Th1 predominant response, but Th2 and Th17 involvement have also been demonstrated (55). Levels of IL-2, IL-12, IL-18 and IFN-γ (Th1 proinflammatory cytokines) have been shown to be increased in BD (98) and elevated levels of IL-18 have also been linked with disease activity (99).

Systemic Sclerosis

Increased levels of IL-1, IL-2, IL-2R, IL-4, IL-8, IL-17, TNF-alpha, interferon, and antibodies to IL-6 and IL-8 have been found in sera of patients with SSc (100, 101). The role of IL-6 has been highlighted as increased levels have been linked to more severe skin and lung disease (102). The IL-6 inhibitor, tocilizumab is approved for use in SSc related interstitial lung disease. While a variety of cytokines are involved in autoimmunity and malignancy the question of whether anti-cytokine therapies may play a preventative role in T-LGLL is unknown. Chronic stimulation by proinflammatory cytokines including IL-6 is responsible for sustained LGL proliferation as well as an important *STAT3* activating factor (103). Studies have revealed increased levels of IL-6 in plasma of patients with LGLL compared to healthy controls (67, 104). IL-6 inhibitors are also used as treatment for other rheumatic conditions including GCA, RA and Castleman disease, but its role as use for prevention or treatment of T-LGLL is lacking clinical data. Based on the role of IL-6 in pathogenesis of LGLL, there has been consideration to use of tocilizumab as salvage therapy in T-LGLL (105). In addition to anti-cytokine therapies, similar questions arise for the role of JAK-STAT inhibitors, as this pathway plays a central role in LGLL pathogenesis. This class of drugs is more commonly being used to treat inflammatory arthritis, but due to lack of clinical data the role as preventative therapy for T-LGLL is lacking and it is unknown if patients with

inflammatory arthritis treated with these drugs are less likely to develop LGLL.

ROLE OF SPHINGOLIPIDS IN T-LGLL AND RHEUMATIC DISEASE

Sphingolipids have been shown to play a part in long term survival of cytotoxic lymphocytes (106). Dysregulation of the sphingolipid pathway in rheumatic diseases has rarely been described. In SLE, a cohort study revealed clinical and renal disease activity were associated with elevated levels of circulating sphingolipids (107). In another study of patients with biopsy proven lupus nephritis, serum levels of sphingolipids were higher compared to controls (108). As dysregulation of pro-apoptotic (ceramide, sphingosine) and pro-survival sphingolipids (sphingosine-1-phosphate) has been shown to play a role in T-LGLL (106, 109) it would be of interest to evaluate the value of sphingolipids in patients with rheumatic disease.

TREATMENT:

JAK Inhibitors in the Management of T-LGLL and Rheumatic Disease

The discovery of JAKs as targeted therapy led to improvements in treating many rheumatic diseases including RA, polyarticular juvenile idiopathic arthritis (JIA) and psoriatic arthritis. There are currently three JAK inhibitors (JAKi) approved for use in patients with rheumatic disease in the United States. Tofacitinib, baracitinib and updacitinib are approved for use in active RA in patients who have had inadequate response to methotrexate, traditional disease modifying anti-rheumatic drugs (DMARDs) and tumor necrosis factor inhibitors (TNFi). Tofacitinib is also approved for use in polyarticular JIA, psoriatic arthritis and ankylosing spondylitis. The pan-JAKi, Peficitinib is approved for RA in Japan, South Korea, and Taiwan (110). Filgotinib, a Jak 1 inhibitor is approved for RA in Japan and Europe (111).

It can be speculated that due to improvements in earlier RA diagnosis and initiation of treatment this may lead to an overall decrease in clonal expansion and development of T-LGLL. Many therapeutic options are available for RA, but their specific role in driving clonal expansion is unknown. In a study of 529 patients with RA, 19 (3.6%) patients exhibited T-LGL expansion. There was a significant association with the T-LGL clone and duration of TNF inhibitor use suggesting long term exposure may be associated with increased clonal T-LGL cells in RA patients (112). Similar results were demonstrated in a cross-sectional analysis of patients with psoriatic arthritis and ankylosing spondylitis (113). A variety of *in vitro* and murine studies have shown mechanisms of potential benefit for use of JAK inhibition in rheumatic diseases including SS, SLE, large vessel vasculitis, dermatomyositis and SSc though overall data is limited. Most clinical evidence comes from case reports however there are ongoing randomized trials with a variety of JAKi for other rheumatic disease indications.

In Sjogren's Syndrome, a phase II trial of filgotinib failed to meet its primary endpoint (NCT03100942) and there are ongoing trials evaluating the use of tofacitinib and baracitinib. Notably in SLE, a phase 2 trial of baricitinib was successful in patients with active skin and joint disease and phase 3 trials are ongoing (41). Evidence for use of JAKi in vasculitis is scarce. Most data from *in vitro*, murine models and clinical experience suggest a pathogenic basis that JAKi may be beneficial, but clinical trials are needed. Data has come primarily from studies involving large vessel vasculitides such as GCA and TAK (36). There are ongoing clinical trials evaluating the efficacy of JAK inhibitors in both of these diseases (NCT04299971, NCT03026504, NCT03725202, NCT04161898). In other vasculitides such as Behcet's and Polyarteritis Nodosa, JAKi has been reported in cases of refractory disease with some success (114). In a study of 13 patients with refractory BD, patients who were treated with tofacitinib showed improvement in vascular and joint symptoms (115). A pilot study of 10 patients with AAV treated with tofacitinib were found to have improvements in clinical symptoms and reduction in steroid requirements (116), but larger randomized trials are needed to confirm these findings. There are also ongoing trials of use of JAKi in SSc and dermatomyositis (NCT03274076, NCT03002649, NCT04966884, NCT04613219).

The role of JAK inhibitors as targeted therapy in T-LGLL associated with rheumatic disease is not known. In a study of nine patients with rheumatoid arthritis and refractory T-LGLL, tofacitinib led to hematologic response in six patients and improvement in synovitis in eight patients (38). This may suggest a role for earlier use of JAKi in patients with concomitant RA and T-LGLL, but larger studies are needed. JAKi use in other rheumatic conditions associated with T-LGLL have not been reported.

The use of JAKi in T-LGLL is currently being evaluated, though early promising data from a Phase I basket study suggests there may be some efficacy. Targeted therapy with Ruxolitinib, a JAK 1 and 2 inhibitor, was evaluated in five cases of refractory T-LGLL with partial response observed in two patients, and improvement in cytopenias in 4 patients (117). There is an ongoing trial of Ruxolitinib in relapsed or refractory T or NK cell lymphoma (NCT02974647) and this study is being evaluated in a multi-center phase II trial. Ruxolitinib safety, tolerability and efficacy was also evaluated in a four-week trial in patients with RA (NCT00550043), but there are no published results. Another targeted therapy, BNZ-1, is a multi-cytokine inhibitor that targets the gamma chain receptor subunits of IL-2, IL-9, and IL-15 leading to reduction of cytokine-mediated cell survival (118). First clinical data with BNZ-1 in LGL was completed in a phase I/II trial with 20% ORR (3PR, 1 CR), particularly in patients with transfusion-dependent anemia (83). In regard to other autoimmune disease, there is a phase II trial ongoing for alopecia, but no other active trials in rheumatic disease at this time (NCT03532958).

While standard therapies used in symptomatic T-LGLL include steroids, methotrexate, cyclosporine and cyclophosphamide, these are effective in only 30-40% of cases (11, 119). No clear treatment guidelines have been established due to a lack of clinical trial data.

In patients with T-LGLL and associated rheumatic disease co-management with a rheumatologist is key. Treating the underlying rheumatic process may be the best initial step to alleviate T-LGLL. While methotrexate is often a first line therapy in the setting of inflammatory arthritis and other rheumatic diseases, initial treatments used in T-LGLL including cyclophosphamide are often reserved for severe organ or life-threatening manifestations of rheumatic disease. There is a clear need to develop better therapies for the treatment of T-LGLL and T-LGLL in the setting of rheumatic disease.

SUMMARY

Chronic inflammation and immune activation are central to the bidirectional relationship between cancer and rheumatic disease. Components of a shared pathogenesis between T-LGLL and rheumatic disease includes chronic antigen stimulation, JAK-STAT pathway activation and overlap of various cytokines. Due to the rarity of T-LGLL in the setting of rheumatic disease this complex relationship remains difficult to define. It is important to evaluate the presence of T-LGLL in patients with rheumatic disorders, as T-LGLL is likely under-reported in this population. While T-LGLL and rheumatic conditions may share clinical and lab features, a complete history and examination by a rheumatologist is key for appropriate serologic evaluation and diagnosis of rheumatic disease. In the setting of cytopenia, early evaluation with peripheral blood flow cytometry and TCR testing would likely improve recognition and early detection of T-LGLL.

AUTHOR CONTRIBUTIONS

NC wrote the first draft of the manuscript. AS, JB, WJ contributed to manuscript revision, read and approved the submitted version.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fonc.2022.854499/full#supplementary-material

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