The T-Cell Leukaemias

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Introduction

The mature T-cell leukaemias are a rare and heterogeneous group of disorders derived from the mature or post-thymic T-cell. They comprise T-cell prolymphocytic leukaemia (T-PLL), T-cell large granular lymphocytic leukaemia (T-LGL) and Adult T-cell lymphoma/leukaemia (ATLL) [1]. There are also neoplastic T-cells in the peripheral blood in cases of Sézary syndrome (SS), the generalised, more aggressive variant of the mature T-cell cutaneous lymphoma mycosis fungoides. ATLL and SS are covered elsewhere in this text and this chapter will therefore focus on T-PLL and T-LGL leukaemia.

The diagnosis of the T-cell leukaemias is based on a multiparameter approach which encompasses clinical presentation, peripheral blood count and morphology, immunohistochemistry, flow cytometry, cytogenetics and molecular genetics. Thus, the recent advances in modern immunophenotypic and molecular tools have been crucial in characterising these disorders and in distinguishing them from their B-cell counterparts. Prognosis and response to conventional chemotherapy are generally poor, with the exception of T-LGL which is a more indolent disorder than the others in its class. The rarity of these conditions, their refractoriness to standard therapies, underlying immune suppression, multi-factorial aetiologies and lack of single identifiable therapeutic targets in the majority of cases all contribute to a great management challenge. An important aspect therefore in advancing treatment of these and other T-cell disorders is adopting an international approach to diagnosis, documentation and trial design and entry in order to recruit sufficient patient and clinico-pathological data to inform robust choices.

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T-Cell Prolymphocytic Leukaemia

Introduction

Prolymphocytic leukaemias (PLL) of B- and T-cell subtype are rare diseases which together account for around 2% of all mature lymphoid leukaemias. When first described in 1974 [2], the different cells of origin were not appreciated and the disease was described as a variant of chronic lymphocytic leukaemia (CLL). T-PLL was first documented in a patient presenting with clinical features similar to B-PLL, but in whom the cells had a T-cell phenotype [3]. The recent availability of modern immunophenotypic and molecular tools has allowed a better distinction of this disorder from its B-cell counterpart and other mature

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Table 9.1 Key characteristics of T-cell prolymphocytic leukaemia

	Characteristic Findings
Clinical features	Older adults
	M:F 1.2:1
	Splenomegaly, lymphadenopathy, skin rash, oedema and serous effusions
	Lymphocytosis often exceeding 100×10^9/l
Morphology	Prolymphocytes with prominent nucleoli, basophilic cytoplasm with blebs
	Small cell (20%) and Sézary variants (5%)
Immunophenotyping	CD2, CD3, CD5, CD7+, CD52+
	CD4/8 variable
	CD1a-ve, TdT-ve, CD25−/+
Cytogenetics	Inversion 14; t(14,14); t(X,14); iso8q; complex
Oncogenes	*TCL-1*, *MTCP-1*, *ATM*
Prognosis	Median survival <1 year with conventional chemotherapy
Treatment	Alemtuzumab alone or in combination with purine analogues
	Consolidation with HSCT

T-cell leukaemias. T-PLL is recognised in the WHO classification [1] as having three morphological variants—typical, small cell and cerebriform—all of which have a similar clinical course and identical molecular genetics. Recent studies have highlighted the role of specific oncogenes such as *TCL-1, MTCP-1* and *ATM*. However, despite better understanding of the underlying cell biology, prognosis for these patients remains poor with no curative therapy and short survival. The advent of monoclonal antibody therapy and the wider application of non-myeloablative allogeneic transplantation have improved the treatment options for this group of patients. Table 9.1 summarises the characteristic features of T-PLL.

Pathogenesis

T-PLL is a rare proliferation of mature or post-thymic lymphocytes. It has been described in the East and West without geographical or racial clustering and there are no reports of familial cases. There is no evidence that radiation or carcinogenic agents play a role in the pathogenesis of T-PLL and neither had it been thought that viruses such as HTLV1 are involved. However, a recent study found that the EBV genome was present in T-PLL cells by using polymerase chain reaction (PCR) analysis across multiple sites in the viral genome [4]. In addition, these T-PLL cells expressed a number of EBV latent antigens suggesting a potential link between T-PLL and EBV infection.

T-cell maturation is strictly controlled by the thymic cellular microenvironment and depends on complex interactions between various cytokines and growth factors [5]. T-cells rely on the T-cell receptor (TCR)-CD3 complex to present a specific antibody to bind to a foreign antigen, as opposed to B cells, which depend on immunoglobulin rearrangements. Mutations in the TCR subunits result in T-cell lymphoproliferative diseases derived from post-thymic immunocompetent lymphoid cells. In adults T-PLL arises sporadically. There is a close relationship between this sporadic form of T-PLL and the leukaemia that occurs in patients with the hereditary debilitating neurological disease ataxia telangiectasia (AT) [6]. Patients with AT have bi-allelic inactivation of the AT mutated gene (*ATM*) located at the 11q23 locus [7]. Approximately 10% of AT homozygotes develop cancer, mostly of the lymphoid system and in particular of the T-cell type [8]. Some of these patients develop abnormal clonal proliferation of T-cells with morphological, immunological, cytogenetic and molecular features (e.g. over-expression of the *TCL-1* oncogene) identical to T-PLL. Genetic abnormalities (mutations and deletions) of *ATM* are well documented in T-PLL [9–12]. *ATM* is therefore a candidate gene likely to be involved in the pathogenesis of both sporadic and AT-associated T-PLL, possibly through its role as a tumour suppressor.

Clinical Presentation

T-PLL is a disease affecting older adults with a male predominance. In the Royal Marsden Hospital series of 150 patients, the median age at presentation was 63 years (range 33–91 years) and

the male: female ratio 3:1 [13]. Patients are characteristically symptomatic at presentation with a peripheral blood lymphocytosis often exceeding 100×10^9/l [14], generalised lymphadenopathy and splenomegaly. Dermal lymphoid infiltrates are present in a third of patients and peri-orbital oedema can be a feature. Serous effusions, particularly pleural effusions, are seen in 15% of patients at diagnosis but are common in relapsed or refractory disease. Anaemia and thrombocytopenia are less common at presentation than in B-PLL [15], occurring in 25% and 45% of patients, respectively. Central nervous system involvement is rare. Human T-lymphotrophic viruses I and II (HTLV-I/II) are invariably negative by both serology and PCR. A small proportion of patients have a more indolent or "smouldering" form of T-PLL which may mimic stage A CLL. Although these patients are generally asymptomatic at presentation and may exhibit a long latent phase, they will inevitably progress after a median period of 33 months [16] to follow the more aggressive course typical for T-PLL.

Morphology and Histopathology

T-PLL has a broad morphological spectrum [17]. In half of cases the cells have a round to oval nucleus, while in the remainder the nuclei are irregular, often with convolutions. The degree of nuclear irregularity, however, is less pronounced than that seen in Sézary Syndrome (SS) or ATLL cells. There are three subtypes: typical T-PLL, a small cell variant and a cerebriform variant accounting for 75%, 20% and 5%, respectively. All three share the same immunophenotypic and cytogenetic features and are thus classified as a single disorder [1]. Typical cases have a peripheral blood lymphocytosis with pro-lymphocytes of medium size with condensed nuclear chromatin, a single prominent nucleolus, intensely basophilic agranular cytoplasm and cytoplasmic protrusions or "blebs" in most cases (Fig. 9.1). In the small cell variant the pro-lymphocytes are considerably smaller and the nucleolus is less prominent and can often only be visualised under electron microscopy. In the rarest variant the pro-lymphocytes have a cerebriform nucleus resembling Sézary cells.

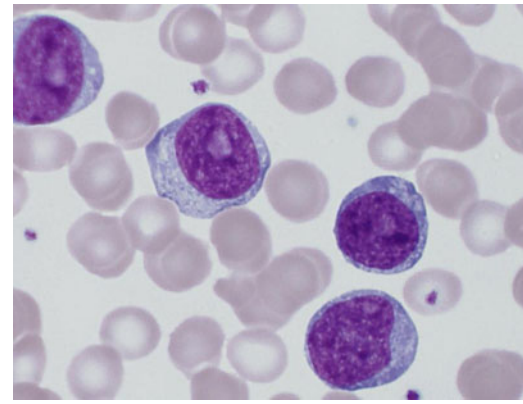

Fig. 9.1 Typical peripheral blood morphology in T-PLL showing small to medium sized lymphoid cells with a single nucleolus, basophilic cytoplasm and cytoplasmic protrusions

Although tissue histology is not essential for diagnosis, the bone marrow, lymph nodes and skin may be infiltrated. Diffuse and interstitial infiltration of the bone marrow is seen in the majority of cases and reticulin fibrosis is almost always present. Lymph nodes show paracortical expansion by T-prolymphocytes. The skin histology differs from that seen in mycosis fungoides (MF) and SS, showing dermal infiltration, preferentially around the appendages and without epidermotropism. Spleen histology shows a marked red pulp infiltration extending into the white pulp and capsule in contrast to T-cell large granular lymphocytic leukaemia which is confined to the red pulp.

Immunophenotype

T-PLL cells exhibit a mature, post-thymic phenotype and are, by definition, negative for terminal deoxynucleotidyl transferase (TdT) and the cortical thymic marker CD1a. They express the pan-T cell markers CD2, CD5 and CD7 [13]. CD7 is usually expressed with strong intensity in contrast to other mature T-cell malignancies where this marker may be weak or negative. CD3 and anti-TCR-α/β may be negative in the cell membrane but are always expressed in the cytoplasm, and the TCR-β and/or γ chain genes are rearranged in all cases. The majority (60%) have the CD4+/CD8– phenotype, but alternatively may have

CD4+/CD8+ co-expression (25%) or have a CD4–/CD8+ immunophenotype (15%) [14]. The distinctive co-expression of CD4 and CD8, the weak CD3 membrane expression and the strong CD7 expression suggest that the T-PLL cell may be at an intermediate stage of differentiation between a cortical thymocyte and a circulating mature T-cell. Cell surface antigens linked to T-cell activation such as CD25, CD38 and class II HLA-DR are variably expressed and monoclonal antibodies against natural killer cells and TIA-1 are negative. T-prolymphocytes strongly express the CD52 antigen at a high density [18], which can be targeted by the monoclonal antibody alemtuzumab. In most cases T-PLL patients express the TCR-αβ phenotype, although rare instances of TCR-γδ have been reported [19].

Molecular Features

In keeping with the aggressive clinical course of TPLL, patients usually have complex karyotypes and cytogenetic abnormalities which may occur progressively throughout the disease. The genetic hallmark of T-PLL is the inversion [inv(14)(q11;q32)] or its variant the tandem translocation [t(14;14)(q11;q32)] [20, 21]. These rearrangements of chromosome 14 are present in up to 80% of cases [22] and bring together the proto-oncogene *TCL-1* (T-cell leukaemia 1) located at 14q32.1 with the TCR α gene, located at 14q11. This results in deregulation of *TCL-1*, a gene physiologically expressed on CD4/CD8 double-negative thymocytes [23, 24]. In about 20% of patients the translocation [t(X;14)(q28;q11)] (Fig. 9.2) is reported, which results in the juxtaposition of the *MTCP-1* gene (a member of the *TCL-1* gene family) located at Xq28 with the TCR-α gene [25, 26]. Therefore, chromosomal rearrangements in T-PLL juxtapose *TCL-1* and *MTCP-1* to the TCR loci and lead to their activation. Genomic analyses of the 14q32.1 breakpoint region have revealed three additional genes; *TCL-1b, TNG1* (*TCL-1* neighbouring gene 1) and *TNG2*, which are also expressed on T-PLL cell lines and cells from patients with T-PLL but not on normal T-lymphocytes [27–29]. Activation of *TCL-1* through hypomethylation of its promoter has also been described [30]. The *TCL-1* oncoprotein is expressed in approximately 70% of T-PLL cases [31] and has been shown to associate with protein kinase B (Akt) resulting in the promotion of Akt-induced cell proliferation and survival [32].

In addition to the inversion rearrangement, which is regarded as the primary oncogenic event in T-PLL, the tumour cells usually harbour a high load of additional chromosomal aberrations. Abnormalities involving both arms of chromosome eight are frequent and over-expression of the c-myc protein is found in cases with iso8q. While the 14q abnormalities and trisomy 8q are common in western countries, they are rarely seen in Japan [33]. Although 11q23 abnormalities are seldom detected on cytogenetics, molecular analysis frequently detects mutations of the tumour suppressor gene *ATM*. Studies have demonstrated that T-PLL is associated with recurrent regions of chromosomal loss at 22q11, 13q, 6q, 9p, 12p, 11p11-p14 and 17p, as well as chromosomal gain at 8q, 14q32, 22q21 and 6p [22]. Recent single nucleotide polymorphism-based genomic mapping and global gene expression profiling has identified differential expression of a number of genes in T-PLL compared to normal CD3+T-cells [34]. These include functionally important genes involved in lymphomagenesis, cell cycle regulation, apoptosis and DNA repair, which are clustered in regions affected by known recurrent chromosomal aberrations in T-PLL. The upregulated genes were clustered on chromosome arms 6p and 8q, and the downregulated genes on 6q, 8p, 10p, 11q and 18p. This information suggests that a gene dosage effect may be involved in the pathogenesis of T-PLL and may also help to clarify the mechanisms involved in disease progression.

Differential Diagnosis

The morphology of prolymphocytes in the peripheral blood and cell markers are the vital requirements in order to make the diagnosis of T-PLL and distinguish it from other mature lymphoid leukaemias. T-PLL can be distinguished from B-PLL by immunological markers. Furthermore, skin infiltration and

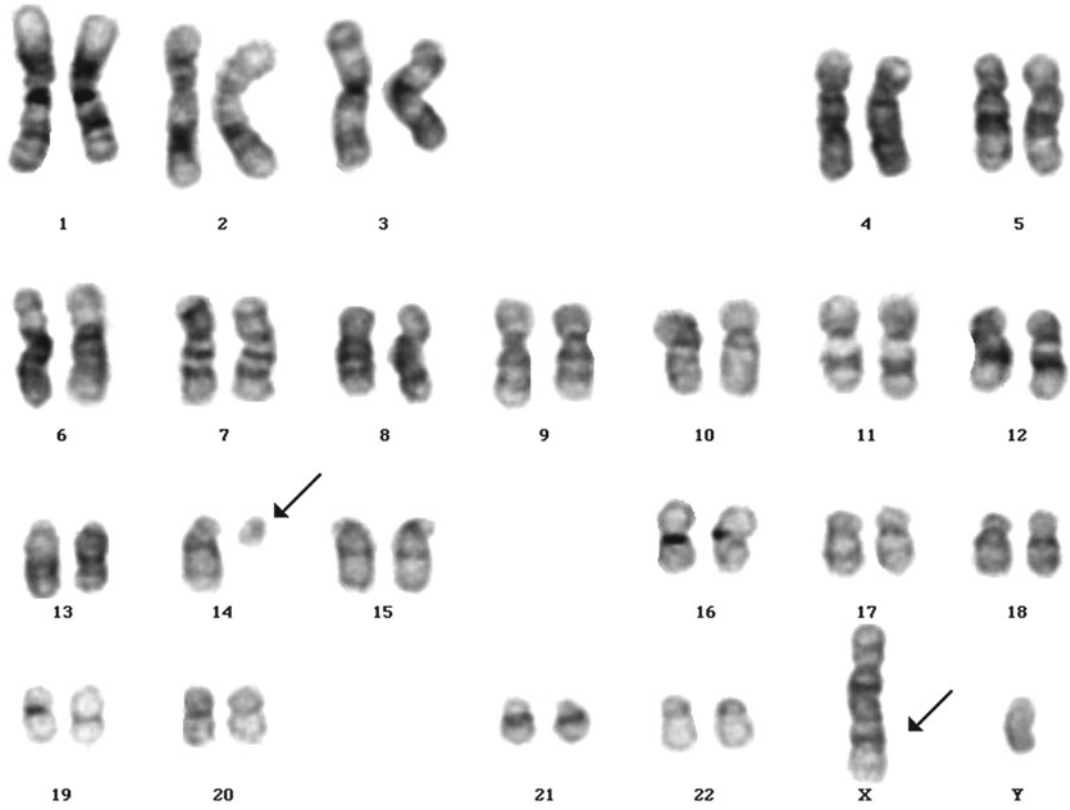

Fig. 9.2 Karyotype in T-PLL showing a t(X;14) translocation

lymphadenopathy are unusual in B-PLL, while they are present in a substantial proportion of T-PLL patients. Morphology, histology and immunological markers help to differentiate T-PLL from other mature T-cell malignancies such as T-LGL, ATLL and SS. The predominant population in T-cell LGL leukaemia is a granular lymphocyte, often with a CD8+ CD57+, CD16± phenotype, with or without expression of natural killer (NK) cell markers. The distinct geographical background, the clinical features (e.g. hypercalcemia) and positive HTLV-I serology distinguish ATLL from T-PLL. SS has distinct clinical features, skin histology and cell morphology.

Prognostic Factors

The prognosis for patients with T-PLL is poor. Data regarding specific disease variables which influence an individual's disease course and response to treatment are limited. A recent series of 84 cases reported a median overall survival of 24.7 months and a 5-year survival rate of 21% [35]. There was a highly significant correlation between poor outcome and high WBC count at presentation ($>40 \times 10^9$/l), shorter lymphocyte doubling time as well as with older age (>62 years) at presentation. Higher TCL-1 expression correlated with a higher WBC count at presentation, a rapid lymphocyte doubling time and a shorter overall survival [35]. The presence of hepatomegaly and serous effusions predict for a poor response to alemtuzumab [36]. Patients who do not respond to treatment have a median survival of only 4 months.

Treatment

T-PLL is an aggressive disease, which is often resistant to therapy. Overall prognosis is poor with a median overall survival historically of

Table 9.2 Summary of clinical trials in T-PLL

Study	Regimen	No	CR (%)	PR (%)	ORR (%)	MPFS months	MS months
Previously treated patients							
Mercieca [38]	Pentostatin[a]	55	9	36	45	6	9
Dearden [36]	Alemtuzumab	39	60	16	76	7	10
Keating [42]	Alemtuzumab[a,b]	76	38	12	50	4.5	7.5
Therapy-naive patients							
Dearden [43]	Alemtuzumab	11	100	0	100	10+	13+
Hopfinger [44]	FMC + Alemtuzumab	18	NA	NA	66 (FMC) 86 (after alemtuzumab)	10	19

[a]Retrospective analysis
[a]Compassionate-use trial
CR, Complete remission; *PR*, Partial remission; *ORR*, Overall response rate; *MPFS*, Median progression-free survival; *MS*, Median overall survival; *NA*, Not available

approximately 7 months in patients receiving conventional chemotherapy such as the alkylating agents [14]. However, survival has improved following the introduction of relatively new agents including the purine analogues and the anti-CD52 antibody (alemtuzumab; campath-1H). Pentostatin has been shown to be effective, particularly in patients who are CD25+, CD38+ and CD103+ [37]. A study of 55 patients performed in 1994 reported an overall response rate of 45% with 9% complete remissions and median response duration of 6 months [38]. This also resulted in an improvement in overall survival. Unlike standard approaches to the use of pentostatin in hairy cell leukaemia, initial doses of 4 mg/m^2 were given weekly for 4 weeks then every 2 weeks to maximum response. Interestingly, no significant difference in response rate was observed between previously treated and untreated patients rendering this agent a versatile option in treatment of T-PLL. Equally positive results have been reported with cladrabine, although study numbers are smaller [39, 40].

More recently, the anti-CD52 monoclonal antibody, alemtuzumab, has been utilised to target the CD52 antigen which is expressed at high density on the surface of T-prolymphocytes. An early study in 1997 of 14 patients reported an excellent response rate of 73% [41]. More recently, a European study of 39 patients with relapsed/refractory T-PLL, who received alemtuzumab intravenously three times a week after initial dose escalation, reported remarkable overall response rates of 76% with complete response rates of 60% [36]. Nine of the 39 patients were refractory to pentostatin. The median overall survival was 10 months and depth of response was positively associated with prolongation of survival; median overall survival was 16 months in those patients who achieved complete responses. Responses were poor in patients who had serous effusions, hepatic or CNS involvement. In the United States a retrospective analysis of 76 patients with T-PLL treated on a compassionate use with standard alemtuzumab therapy reported OR rates of 50% with 37.5% CR [42]. These patients, who had received one or more lines of treatment and had progressive and/or refractory disease, had a superior quality and duration of response to alemtuzumab compared to prior therapy. Alemtuzumab has subsequently been investigated in treatment naïve patients. In a preliminary study of 11 patients, all achieved a CR with 7 of 11 patients still alive at median follow up of 12 months (range 4–17 months) [43].

The successful use of chemo-immunotherapy in B cell malignancies has prompted similar studies in T-PLL. The German CLL study group has reported results of a prospective phase II trial of fludarabine, mitoxantrone and cyclophosphamide (FMC) followed by alemtuzumab consolidation in 18 patients [44]. The ORR was 66% following FMC, increasing to 86% after alemtuzumab, with a median progression-free survival of 10.6 months and OS of 19.2 months. Table 9.2 summarises the results of clinical trials in T-PLL.

Weidmann et al. have used a regimen consisting of fludarabine (days 1–4), cyclophosphamide (day 3), doxorubicin (day 4) together with alemtuzumab in escalating doses (days 1–4) to treat 23 patients with a range of peripheral T-cell malignancies [45]. Overall response rate in this series was 61% with a CR rate of 78% in the newly diagnosed patients. However, this cohort only included a single case of T-PLL. Anecdotal experience with the combination of alemtuzumab and pentostatin suggest that this combination may be more active than these agents used singly.

The introduction of alemtuzumab has significantly improved the outcome in T-PLL and it should be considered the treatment of choice. However, its use is not without risk. It causes prolonged T-cell depletion with significant immunosuppression and infective complications reported in approximately 13% of patients [42]. Prophylaxis for pneumocytsis and herpetic infections is required both during treatment and for a protracted period beyond completion and screening for cytomegalovirus (CMV) reactivation is advocated. Use of alemtuzumab in heavily pre-treated, often elderly patients is associated with increased risk of infectious and other treatment-related complications, although it can be well tolerated in elderly patients because of lack of organ toxicity. Tolerability is improved when alemtuzumab is used as first line therapy. Responses to alemtuzumab are still transient and further disease progression is inevitable. Furthermore, patients may down-regulate expression of CD52 at relapse making treatment with alemtuzumab ineffective. Hence, all patients who achieve a response to therapy should be considered for consolidation with a haematopoietic stem cell transplant (HSCT) to prolong disease-free and overall survival.

In a recent study 22 patients with T-PLL received a HSCT in first complete response, in second complete response or with a good partial response (PR) following alemtuzumab therapy. Thirteen were consolidated with an autologous HSCT and nine with an allogeneic HSCT (five sibling and four unrelated donor) [46]. In the patients who were autografted, 38% remain alive with median disease-free survival of 20 months (range 8–78 months). Of the nine patients who had an allograft, four had full intensity conditioning and five reduced intensity conditioning. Fifty-six percent remain alive, one patient in continued CR 7 years post-SCT. Two patients died from transplant-related mortality (TRM) and both had received full intensity conditioning. Two patients relapsed. These results demonstrate that autologous HSCT can increase disease-free survival, but two thirds of patients still relapse. While allogeneic HSCT is an attractive option, TRM with full intensity conditioning is high. There are other case reports of successful outcome with reduced intensity conditioning [47, 48], and this is a strategy which merits further study.

Treatment regimens in the future are likely to be based on intravenous alemtuzumab with the addition of purine analogues such as pentostatin, gemcitabine or nelarabine. The role of new agents like bortezomib and pralatrexate will also need to be explored. Better understanding of the molecular pathogenesis may also lead to the introduction of new therapeutic approaches targeting specific pathways such as Akt activation, ATM mutations (PARP inhibitors) and telomerase, all of which may be active in T-PLL.

T-Cell Large Granular Lymphocyte Leukaemia

Introduction

The term T-LGL was coined by Loughran in 1993 [49] to describe this clonal proliferation of mature, post-thymic T-cells. LGLs make up 10–15% of the total peripheral blood mononuclear cell count in normal adults [50]. The majority of these cells are of the CD3-natural killer (NK) lineage, with the minority of the CD3+ T-cell lineage (15%). T-cell LGLs are post-thymic, antigen-primed cytotoxic CD8+ T lymphocytes and NK-cell LGLs belong to the innate immune system with the capability of non-major histocompatibility complex (MHC)–restricted cytotoxicity [51]. LGL proliferations are clonally derived from either CD3−/CD56+ or CD3/CD8+ LGLs and are designated natural killer LGL leu-

kaemia or T-cell LGL leukaemia, respectively. The WHO classifies NK-LGL leukaemia within the spectrum of NK cell disorders, which are covered elsewhere in the text. This chapter will therefore focus on T-LGL leukaemia, which makes up approximately 85% of cases of LGL leukaemias.

In contrast to the other T-cell disorders, T-LGL leukaemia is generally an indolent condition with a median survival in excess of 10 years. The aetiology is not fully understood, but there is a strong association with autoimmune disorders, suggesting a common immunogenetic pathogenesis. The most common presenting features are cytopenias, characteristically neutropenia which may manifest as increased susceptibility to and severity of infection. There is no genetic hallmark of T-cell LGL and diagnosis relies on morphology, characteristic cell surface markers and demonstration of clonality. Treatment modalities are predominantly immunosuppressive in nature and are only indicated for symptomatic patients. Table 9.3 summarises the key features of T-LGL leukaemia.

Table 9.3 Key characteristics of T-cell large granular lymphocyte leukaemia

	Characteristic findings
Clinical features	Median age 55–60 years
	M = F
	60% symptomatic
	LGLs in peripheral blood $2–20 \times 10^9$
	Cytopenias, characteristically neutropenia. Recurrent infection.
	Strong association with autoimmune disorders (e.g. rheumatoid arthritis)
Morphology	LGLs with abundant cytoplasm
	Fine or coarse azurophilic granules
Immunophenotyping	CD3+, TCR-αβ, CD8+, CD57+,CD16+
Cytogenetics	Clonal rearrangement of TCR-β chain gene
	No characteristic cytogenetic abnormality
Oncogenes	Abnormalities of Fas/Fas-ligand pathway, dysregulated apoptosis
Prognosis	Generally an indolent disorder
	Median survival >10 years
Treatment	Only if symptomatic
	Immunosuppression—methotrexate, cyclophosphamide, cyclosporine A

Pathogenesis

The aetiology of T-LGL leukaemia is not clear. There is a strong association with autoimmune disorders suggesting a common immunogenetic pathogenesis. A polyclonal immune response with chronic activation of T-cells by an autoreactive or viral antigen is believed to be the initial stimulus to expansion of LGLs [52–54]. It is not clear whether a second molecular event is necessary to establish the neoplastic phenotype. There has been much debate in the literature as to whether T-LGL leukaemia represents a neoplastic or reactive condition; however, the demonstration of clonality supports a neoplastic proliferation. It has been suggested, however, that T-cell LGL leukaemia could represent an autoimmune disorder caused by chronic antigenic stimulation leading to extreme expansion of only one clone of CD8+ cytotoxic T-cells [54, 55]. There have been several reports of detection of HTLV-1 antibodies in cases of T-LGL leukaemia [56, 57], but in contrast to ATLL, these represent just a few cases and there is no universal association. Recent studies have demonstrated involvement of hCMV in the ontogeny of CD4+ T-LGL leukaemia, and it has been suggested that it could be the antigenic stimulus responsible for the initiation and maintenance of the disease [58]. There is no evidence that any other virus is involved in the pathogenesis of T-LGL leukaemia. Rare cases of T-LGL have been reported as a form of lymphoproliferative disorder after autologous or allogeneic transplant [59, 60], which must be distinguished from the oligoclonal T-cell populations frequently present in transplant patients at the point of lymphocyte reconstitution [61]. Clonal T-LGL populations have also been reported in association with low grade B-cell dyscrasias such as hairy cell leukaemia, CLL and monoclonal gammopathy of

unknown significance [62–64]. These do not usually progress to clinically significant disease and appear to represent a type of host response. The co-association of B-cell pathology with T-LGL leukaemia suggests that either a common antigen drives clonal B- and T-cells, or that humoral malignancy could serve as the stimulus for lymphocyte expansion representing an overactive anti-tumour surveillance [64].

Dysregulation of several intracellular signalling pathways may account for the inherent resistance to apoptosis of LGL cells in vitro [65] including Fas/Fas ligand [66, 67], phosphatidylicnositol-3-kinase [68] and mitogen-activated protein kinase/extracellular signal-regulated kinase/Ras [69]. The degree of cytopenias (most commonly neutropenia) is usually out of proportion to the level of bone marrow infiltration, supporting an immune component to the pathogenesis. Possible mechanisms for neutropenia include deregulated Fas/Fas ligand induced apoptosis of myeloid cells, immune complex or antibody mediated neutrophil destruction, hypersplenism or direct inhibition of myeloid maturation [51, 70, 71].

Clinical Features

In contrast to the other T-cell leukaemias, T-cell large granulocyte leukaemia is a relatively indolent condition, with a median survival in excess of 10 years [51]. It is a rare disorder, accounting for 2–3% of cases of small lymphocytic leukaemia [1]. The median age at diagnosis is 55–60 years, but cases are reported in all age groups [72, 73] and there is no reported gender predilection or geographical distribution. The total lymphocyte count is normal or slightly raised, usually under 15×10^9/l, and the majority are LGLs. Traditionally, an increase in peripheral blood LGLs to greater than 2×10^9/l for more than 6 months was required to make the diagnosis of LGL leukaemia. However, these criteria are not strictly necessary if T-cell clonality can be demonstrated. Sixty percent of patients are symptomatic at presentation [74] and the most common presenting feature is neutropenia, which is manifest in the form of increased frequency and severity of infection and oral ulceration. Opportunistic infections are uncommon. At diagnosis 85% of patients are neutropenic of whom 50% are within the severe range ($<0.5 \times 10^9$/l) [73]. Anaemia and thrombocytopenia are present in 50% and 20%, respectively [73]. Severe anaemia due to red cell hypoplasia has been reported in the context of T-LGL [75]. B symptoms are present in 20–40% of patients [51]. Lymphadenopathy is very rare but mild-to-moderate splenomegaly and hepatomegaly are found in 20–50% and 10–20% of patients, respectively [51].

There is a strong association with autoimmune disorders, most commonly rheumatoid arthritis, which co-exists in up to 30% of patients with T-LGL leukaemia [70]. Systemic lupus erythematosus, Hashimoto's thyroiditis, immune-mediated cytopenias and pulmonary artery hypertension have also been described in patients with T-LGL leukaemia [49, 76, 77]. A polyclonal autoimmune response may represent the initiating element and explain this association and hypergammaglobulinaemia is common. Based on the tissue infiltration in the bone marrow and spleen as well as demonstration of clonality and the occasional finding of non-recurring cytogenetic abnormalities, T-LGL leukaemia has been classified as a leukaemia rather than a reactive condition [78].

Morphology and Histopathology

There is a peripheral blood lymphocytosis due to an increased number of LGLs beyond the 10–15% of total peripheral blood mononuclear cells that LGLs account for in normal adults. Neoplastic cells are similar to normal LGLs; medium to large cells with eccentrically placed nuclei, moderately condensed chromatin and abundant, weakly basophilic cytoplasm with coarse azurophilic granules [1] (Fig. 9.3). The granules often exhibit a characteristic ultrastructural appearance described as parallel tubular arrays and contain a number of proteins such as perforin and granzyme B that play a role in the cytotoxic function of these lymphocytes [79]. Macrocytosis can sometimes be a feature but smear cells are rare.

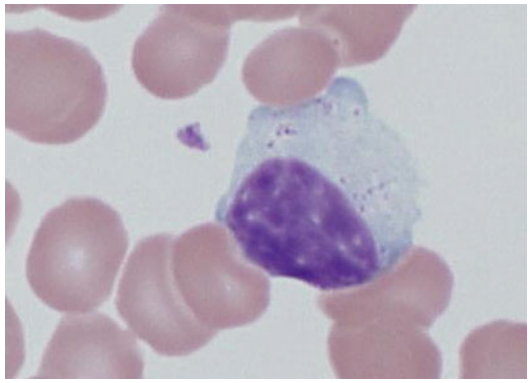

Fig. 9.3 Typical peripheral blood morphology in T-LGL Leukaemia showing large lymphoid cells with eccentric nucleus and abundant pale cytoplasm containing coarse azurophilic granules

Tissue histology is rarely essential for diagnosis. The bone marrow shows a variable, often low level, infiltrate by cells of similar morphology to those in the peripheral blood. The degree of cytopenias (most commonly neutropenia) is usually out of proportion to the level of bone marrow infiltration, supporting an immune component to the pathogenesis. In patients with severe neutropenia, there is often a picture of maturation block at the myelocyte stage, with mature granulocytic cells poorly represented. It is usual for there to be a normal number of megakaryocytes even in cases with significant thrombocytopenia. Cases with marked anaemia often show either a lack of maturing erythroblasts or megaloblastic erythropoiesis. Histologically, the pattern of infiltration may be interstitial, random focal, diffuse or nodular [80]. Nodules with non-clonal B-cell centres surrounded by CD4+ cells, with interstitial CD8+ cells, are a characteristic finding in T-LGL [81]. Care must be taken to examine the cell morphology to distinguish T-LGL from the low-grade B-cell lymphoproliferative disorders. The infiltrates are composed of small to medium lymphocytes, the nuclei of which have irregular contours, condensed nuclear chromatin and inconspicuous nucleoli [80]. The granules are not visible on the thin rim of cytoplasm. An association with trilineage dysplasia has been noted in a significant minority of patients [82]. Diffuse infiltration of red splenic pulp with preservation of sinuses and white pulp cords is characteristic of T-cell LGL leukaemia.

Immunophenotype

Neoplastic proliferations of LGLs are classified as T-cell or NK-cell according to their expression of T-cell markers such as CD3. T-LGL leukaemias have a mature T-cell phenotype and usually express surface CD3, CD8, CD16 and CD57, whereas NK-LGL leukaemias express CD2 and cytoplasmic but not surface CD3 and are variably positive for CD16, CD56 and CD57 [83]. CD57 is a 110-kDa glycoprotein found on NK cells and activated, effector CD8+ T-cells and is a characteristic marker for LGL leukaemia [51]. TIA-1 is also usually expressed. T-cell activation markers such as HLA-DR determinants and CD38 are expressed in a variable number of cases. Most cases of T-LGL leukaemia involve CD8+ cytotoxic T-cells; however, rarer incidences of CD4+ CD8−, CD4+ CD8+ and even CD4− CD8− disease are reported [50, 84]. Abnormally diminished or lost expression of CD5 and/or CD7 is common [85, 86]. The most common immunophenotype is therefore CD3+, TCR-αβ, CD8+, CD57+ and CD16+. Expression of the CD94/NKG2 and KIR families of NK-associated MHC-class I receptors can be detected in 50% or more of cases of T-LGL leukaemias [1]. All KIR positive cases expressed a single KIR isoform [86] and this finding can serve as a surrogate marker of clonality.

Molecular Features

The diagnosis of T-LGL leukaemia requires demonstration of clonality of the expanded LGL population by TCR gene rearrangement studies to distinguish the neoplastic proliferation from a reactive lymphocytosis [87]. This can be achieved using southern blot and/or PCR studies using primers specific to the variable regions of the TCR-β or -γ chain genes or by flow cytometry using monoclonal antibodies against the variable regions of the TCR-β or -γ chain. The majority of cases have TCR-β chain gene rearrangement. Only in a minority the TCR-β is in germline configuration and there is rearrangement of the TCR-γ chain gene [88]. There is no characteristic chromosomal abnormality detected in patients with T-LGL leukaemia and the majority will have

normal cytogenetics. In those patients (<10%) with chromosomal abnormalities, inversion of 12p and 14q, deletion of 5q and trisomy 3, 8 and 14 have been reported [89–91]. T-LGL leukaemia cells constitutively express Fas (CD95) and Fas-ligand, which is found at high levels in the patient's sera [92]. Activated cytotoxic T-cells are usually eliminated by Fas-mediated apoptosis. However, a defective CD95 apoptotic pathway confers resistance to the leukaemia cells [74]. The serum level of Fas-ligand has been shown to be a marker of disease activity and to fall on successful treatment [93].

Differential Diagnosis

The diagnosis is made by a combination of morphological examination and the demonstration of T-cell clonality in the context of a characteristic immunophenotype: CD3, CD8, CD16 and CD57 positivity. Morphology and membrane markers distinguish T-LGL leukaemia from other B- and T-cell disorders. Cases morphologically resembling T-LGL leukaemia but with an NK immunophenotype are classified with the NK disorders. There is no agreement on the level of lymphocytosis required for the diagnosis of T-LGL [84], but a reactive lymphocytosis often has a value of $<5 \times 10^9$ and T-LGL leukaemia $>5 \times 10^9$. However, values of LGLs greater than 2×10^9 are consistent with this diagnosis [1]. Traditionally, the LGL lymphocytosis should be present for at least 6 months in order to make the diagnosis, but this criterion takes on a lesser importance with the advent of TCR rearrangement studies and the ability to demonstrate clonality. Reactive causes of LGL lymphocytosis such as following splenectomy or viral infection can be distinguished by the germline configuration of the TCR chain genes.

Prognostic Factors

The uniformity of clinical, morphological and laboratory features contrasts with phenotypic, functional and genotypic heterogeneity and hence clinical course for an individual is difficult to predict. The overall prognosis is good with a median survival in excess of 10 years [51]. The clinical course of T-LGL leukaemia is often stable or slowly progressive and the cause of death is most frequently infection and rarely progressive disease. Spontaneous remissions have been reported [94]. Transformation of T-LGL to high-grade large T-cell lymphomas has been reported in a minority of cases [95]. Rare cases of an aggressive CD56+ variant of T-LGL leukaemia have been described [96], but are not classified separately by the WHO. The expression of CD26, a surface glycoprotein with an essential role in T-cell function, including being a marker of T-cell activation and a mediator of T-cell activating signals, has also been associated with a more aggressive disease course [97]. There is no data regarding disease features which are predictive of a response to any particular treatment modality other than HLA DR4 positivity, which is highly predictive of response to Ciclosporin A [98].

Treatment

T-LGL leukaemia is an indolent disorder and is usually managed with immunosuppressive medications at doses similar to those used in the treatment of autoimmune disorders [78]. Approximately 60–70% of patients will require treatment at some point during the course of their illness [99]. The main indication for treatment is neutropenia leading to recurrent infection, with other indications including symptomatic anaemia or thrombocytopenia, massive splenomegaly and systemic features. Symptomatic improvement may occur despite failure to normalise neutrophil counts and cytopenias may improve without eradication of the malignant clone [51, 98, 100]. Therefore the aim of treatment, in contrast to the other T-cell leukaemias, is primarily to alleviate cytopenias without necessarily eliminating the clone. A substantial proportion of patients are asymptomatic and should be monitored closely but do not require treatment.

Because of the rarity of T-cell LGL leukaemia, there have been no prospective clinical trials

reported and treatment data is derived from retrospective case series and single institution studies, with no established "gold standard" therapy. First line therapy is usually with single line immunosuppressive agents such as low dose methotrexate (10 mg/m^2 weekly), cyclophosphamide (50–100 mg daily) or cyclosporine A (5–10 mg/kg daily) with response rates in the order of 50–60% [101–103]. There are no randomised controlled trials to compare these agents. The onset of response to treatment is generally slow, with a median response time reported as 4 months in a recent study, although one patient had a delayed response at 14 months [104]. Cross resistance is absent among these agents which have all demonstrated good overall safety, efficacy and tolerability in the majority of patients. Responses can be sustained with continued treatment. However, reports of second malignancies (*Error! Bookmark not defined.*), taken in conjunction with the intrinsic potential for high grade transformation in T-cell LGL leukaemia [95], raise a note of caution. HLA-DR4 positivity has been shown to be highly predictive of responsiveness to cyclosporine A [98], but there are no other data to predict an individual's response to a particular agent. In the majority of cases there is no correlation between clinical response and reduction in the degree of bone marrow infiltration and number of circulating LGLs, in keeping with immunosuppressive or immunomodulatory rather than cytotoxic mechanism of action of these agents. Accordingly, there is no evidence that high dose therapy will benefit these patients by eradicating the clone.

The purine analogues including fludarabine, cladribine and pentostatin have also demonstrated efficacy in T-cell LGL leukaemia [38, 105, 106] and have the advantage of being administered as single discrete courses without the need for long-term maintenance therapy. As such they offer potentially attractive alternatives for younger symptomatic patients with T-cell LGL leukaemia. There are reports of the successful use of alemtuzumab in T-LGL [107, 108], which has been shown to strongly express the CD52 antigen [109]. The use of alemtuzumab would have associated increased expense and infective complications compared to other agents. Other monoclonal antibodies including anti-CD2 (Siplizumab, MedImmune, Gaitherburg, MD) and the humanised MiK-beta-1 monoclonal antibody (anti-CD122—the beta subunit of both interlukin-2 and interleukin-15 receptors) are being evaluated in phase I studies for T-LGL leukaemia and other T-cell disorders. Both interleukin-2 and interleukin-15 are thought to impact on the proliferation, survival and activity of LGLs [50, 110]. Though well tolerated, Mikβ1 monoclonal antibody treatment for T-cell LGL leukaemia did not result in amelioration of cytopenias in any of 12 patients treated with this agent, despite down-regulation of surface receptors seen in seven patients [111].

Haematopoietic growth factors are useful primarily in the early weeks to months required to see remissions with immunosuppressive therapies and as adjuvants to other treatments. Long-term steroid therapy should be avoided due to adverse effects. It is largely accepted that splenectomy, in the absence of splenic bulk and/or immune thrombocytopenia, has little to add to the management of T-cell LGL leukaemia and often induces a transient response in neutrophil count at the expense of increasing lymphocytosis [112, 113]. Haematopoietic stem cell transplantation would rarely be indicated in this indolent disorder, but both autologous and allogeneic transplants have been undertaken in younger patients who are refractory to other therapies [114]. The heavily immunosuppressive conditioning regimens and GVHD prophylaxis regimens may have contributed to the responses observed.

Conclusion

The mature T-cell leukaemias are a rare and heterogenous group of post-thymic T-cell lymphoproliferative disorders which encompass T-PLL, T-LGL and ATLL. Unfortunately, with the exception of the more indolent T-LGL, they have in common their unresponsiveness to conventional chemotherapy, lack of randomised controlled trials to guide management and poor prognosis. New understanding of these diseases

on a cellular and molecular level has paved the way for targeted therapies which may circumvent the treatment challenges for which the diseases in this category are renowned. It is crucial that a collaborative international approach to trial design and the introduction of new therapies is undertaken in order to achieve the goal of improving the outlook for patients diagnosed with these rare and aggressive disorders.

References

1. Swerdlow SH, Campo E, Harris N, Jaffe ES, Puleri SA. WHO classification of tumours of haemopoietic and lymphoid tissues. world health organisation classification of tumours. Lyon: IARC Press; 2008.
2. Galton DA, Goldman JM, Wiltshaw E, Catovsky D, Henry K, Goldenberg GJ. Prolymphocytic leukaemia. Br J Haematol. 1974;27:7–23.
3. Catovsky D, Galetto J, Okas A, Galton DA, Wiltshaw E, Stathopoulos G. Prolymphocytic leukaemia of B and T cell type. Lancet. 1973;2(7823):232–4.
4. Lan K, Murakami M, Choudhuri T, Tsai DE, Schuster SJ, Wasik MA, et al. Detection of Epstein-Barr virus in T-cell prolymphocytic leukemia cells in vitro. J Clin Virol. 2008;43(3):260–5.
5. Bommhardt U, Beyer M, Hunig T, Reichardt HM. Molecular and cellular mechanisms of T cell development. Cell Mol Life Sci. 2004;61:263–80.
6. Brito-Babapulle V, Catovsky D. Inversions and tandem translocations involving chromosome 14q11 and 14q32 in T-prolymphocytic leukemia and T-cell leukemias in patients with ataxia telangiectasia. Cancer Genet Cytogenet. 1991;55:1–9.
7. Xu Y, Ashley T, Brainerd EE, Bronson RT, Meyn MS, Baltimore D. Targeted disruption of ATM leads to growth retardation, chromosomal fragmentation during meiosis, immune defects and thymic lymphoma. Genes Dev. 1996;10:2411–22.
8. Taylor AM, Metcalfe JA, Thick M, Mak YF. Leukaemia and lymphoma in ataxia telangiectasia. Blood. 1986;87:423–38.
9. Stilgenbauer S, Schaffner C, Litterst A, Liebisch P, Gilad S, Bar-Shira A, et al. Biallelic mutations in the ATM gene in T-prolymphocytic leukemia. Nat Med. 1997;3:1155–9.
10. Stoppa-Lyonnet D, Soulier J, Lauge A, Dastot H, Garand R, Sigaux F, et al. Inactivation of the ATM gene in T-cell prolymphocytic leukemias. Blood. 1998;91:3920–6.
11. Yuille MA, Coignet LJ. The ataxia telangiectasia gene in familial and sporadic cancer. Recent Results Cancer Res. 1998;154:156–73.
12. Yamaguchi M, Yamamoto K, Miki T, Mizutani S, Miura O. T-cell prolymphocytic leukemia with del(11)t(1;11)(q21;q23) and ATM deficiency. Cancer Genet Cytogenet. 2003;146:22–6.
13. Matutes E, Brito-Babapulle V, Yullie MR, Catovsky D. Prolymphocytic leukemia of B- and T-cell types. In: Cheson BD, editor. Chronic lymphoid leukemias. New York: Marcel Dekker; 2001. p. 525–41.
14. Matutes E, Brito-Babapulle V, Swansbury J, Ellis J, Morilla R, Dearden C. Clinical and laboratory features of 78 cases of T-prolymphocytic leukemia. Blood. 1991;78:3269–74.
15. Catovsky D, Galetto J, Okos A, Galton DA, Wiltshaw E, Stathopoulos G. Prolymphocytic leukaemia of B and T cell type. Lancet. 1973;2:232–4.
16. Garand R, Goasguen J, Brizard A, Buisine J, Charpentier A, Claisse JF. Indolent course as a relatively frequent presentation in T-prolymphocytic leukaemia. Groupe Francais d'Hematologie Cellulaire. Br J Haematol. 1998;103:488–94.
17. Matutes E, Talavera GJ, O'Brien M, Catovsky D. The morphological spectrum of T-prolymphcytic leukaemia. Br J Haematol. 1986;64:111–24.
18. Ginaldi L, De Martinis M, Matutes E, Farahat N, Morilla R, Dyer MJ, et al. Levels of expression of CD52 in normal and leukemic B and T cells: correlation with in vivo therapeutic responses to Campath-1H. Leuk Res. 1998;22:185–91.
19. Sugimoto T, Imoto S, Matsuo Y, et al. T-cell receptor gammadelta T-cell leukemia with the morphology of T-cell prolymphocytic leukemia and a postthymic immunophenotype. Ann Hematol. 2001;80:749–51.
20. Croce CM. Role of chromosome translocations in human neoplasia. Cell. 1987;49:155–6.
21. Croce CM, Isobe M, Palumbo A, Puck J, Ming J, Tweardy D, et al. Gene for alpha-chain of human T-cell receptor: location on chromosome 14 region involved in T-cell neoplasms. Science. 1985;227:1044–7.
22. Soulier J, Pierron G, Vecchione D, Garand R, Brizard F, Sigaux F, et al. A complex pattern of recurrent chromosomal losses and gains in T-cell prolymphocytic leukemia. Genes Chromosomes Cancer. 2001;31:248–54.
23. Virgilio L, Narducci MG, Isobe M, Billips LG, Cooper MD, Croce CM. Identification of the TCL1 gene involved in T-cell malignancies. Proc Natl Acad Sci USA. 1994;91:12530–4.
24. Narducci MG, Stoppacciaro A, Imada K, Uchiyama T, Virgilio L, Lazzeri C, et al. TCL1 is overexpressed in patients affected by adult T-cell leukemias. Cancer Res. 1997;57:5452–6.
25. Stern MH, Soulier J, Rosenzwajg M, Nakahara K, Canki-Klain N, Aurias A, et al. MTCP-1: a novel gene on the human chromosome Xq28 translocated to the T cell receptor alpha/delta locus in mature T cell proliferations. Oncogene. 1993;8:2475–83.
26. Madani A, Choukroun V, Soulier J, Cacheux V, Claisse JF, Valensi F, et al. Expression of p13MTCP1 is restricted to mature T-cell proliferations with t(X;14) translocations. Blood. 1996;87:1923–7.

27. Hallas C, Pekarsky Y, Itoyama T, Varnum J, Bichi R, Rothstein JL, et al. Genomic analysis of human and mouse TCL1 loci reveals a complex of tightly clustered genes. Proc Natl Acad Sci USA. 1999;96: 14418–23.
28. Pekarsky Y, Hallas C, Isobe M, Russo G, Cruce CM. Abnormalities at 14q32.1 in T cell malignancies involve two oncogenes. Proc Natl Acad Sci USA. 1999;96:2949–51.
29. Sugimoto J, Hatakeyama T, Narducci MG, Russo G, Isobe M. Identification of the TCL1/MTCP1-like 1 (TML1) gene from the region next to the TCL1 locus. Cancer Res. 1999;59:2313–7.
30. Yuille MR, Condie A, Stone EM, Wilsher J, Bradshaw PS, Brooks L, et al. TCL1 is activated by chromosomal rearrangement or by hypomethylation. Genes Chromosomes Cancer. 2001;30:336–41.
31. Herling M, Khoury JD, Washington LT, Duvic M, Keating MJ, Jones D, et al. A systematic approach to diagnosis of mature T-cell leukemias reveals heterogeneity among WHO categories. Blood. 2004;104: 328–35.
32. Laine J, Kunstle G, Obata T, Sha M, Noguchi M. The protooncogene TCL1 is an Akt kinase coactivator. Mol Cell. 2000;6:395–407.
33. Kojima K, Kobayashi H, Imoto S, Matsuo Y, Kobayashi H, Yano T, et al. 14q11 abnormality and trisomy 8q are not common in Japanese T-cell prolymphocytic leukemia. Int J Hematol. 1998;68: 291–6.
34. Durig J, Bug S, Klein-Hitpass L, Boes T, Jons T, Martin-Subero JI, et al. Combined single nucleotide polymorphism-based genomic mapping and global gene expression profiling identifies novel chromosomal imbalances, mechanisms and candidate genes important in the pathogenesis of T-cell prolymphocytic leukaemia with inv (14)(q11q32). Leukaemia. 2007;21(10):2153–63.
35. Herling M, Patek KA, Teitell MA, Konopleva M, Ravandi F, Kobayashi R, et al. High TCL1 expression and intact T-cell receptor signalling define a hyperproliferative subset of T-cell prolymphocytic leukaemia. Blood. 2008;111:328–37.
36. Dearden CE, Matutes E, Cazin B, Tjønnfjord GE, Parreira A, Nomdedeu B, et al. High remission rate in T-cell prolymphocytic leukemia with Campath-1H. Blood. 2001;98:1721–6.
37. Delgado J, Bustos JG, Jimenez MC, Quevedo E, Hernandez-Navarro F. Are activation markers (CD25, CD38 and CD103) predictive of sensitivity to purine analogues in patients with T-cell prolymphocytic leukemia and other lymphoproliferative disorders? Leuk Lymphoma. 2002;43:2331–4.
38. Mercieca J, Matutes E, Dearden C, MacLennan K, Catovsky D. The role of pentostatin in the treatment of T-cell malignancies: analysis of response rate in 145 patients according to disease subtype. J Clin Oncol. 1994;12:2588–93.
39. Palomera L, Domingo JM, Agullo JA, Soledad Romero M. Complete remission in T-cell prolymphocytic leukemia with 2-chlorodeoxyadenosine. J Clin Oncol. 1995;13:1284–5.
40. Delannoy A. 2-chloro-2'-deoxyadenosine: clinical applications in hematology. Blood Rev. 1996;10: 148–66.
41. Pawson R, Dyer MJ, Barge R, Matutes E, Thornton PD, Emmett E, et al. Treatment of T-cell prolymphocytic leukemia with human CD52 antibody. J Clin Oncol. 1997;15:2667–72.
42. Keating MJ, Cazin B, Coutre S, Birhiray R, Kovacsovics T, Langer W, et al. Campath-1H treatment of T-cell prolymphocytic leukaemia in patients for whom at least one prior chemotherapy regimen has failed. J Clin Oncol. 2002;20:205–13.
43. Dearden C, Matutes E, Cazin B, Tjønnfjord GE, Parreira A, Nomdedeu B, et al. Very high response rates in previously untreated T-cell Prolymphocytic leukaemia patients receiving alemtuzumab (Campath-1H) therapy. Blood. 2003;102, Abstract 2378.
44. Hopfinger G, Kandler G., Koller E, et al. (2007) T-PLL 1 protocol of the German CLL Study Group (GCLLSG)-A Prospective phase 2 trial of fludarabine phosphate, Mitoxantrone and cyclophosphamide (FCM) followed by alemtuzumab consolidation as first line treatment in T-PLL. Blood. 2007;110, abstract 2130.
45. Weidmann E, Hess G, Krause SW et al. Combination chemoimmunotherapy using alemtuzumab, fludarabine, cyclophosphomide and doxorubicin (FCD) is an effective first line regimen in peripheral T-cell lymphoma (PTCL). Blood. 2004;104, abstract 2460.
46. Krishnan B, Cazin B, Ireland R, et al. Improved survival for patients with T-cell prolymphocytic leuk receiving alemtuzumab therapy followed by stem cell transplantation. IWCLL. Leuk Lymphoma. 2007;48 Suppl 1:S180.
47. Garderet L, Bittencourt H, Kaliski A, Daniel M, Ribaud P, Socié G, et al. Treatment of T-prolymphocytic leukaemia with nommyeloablative allogeneic stem cell transplantation. Eur J Haematol. 2001;66:137–9.
48. De Lavallade H, Faucher C, Furst S, El-Cheikh J, Vey N, Coso D, et al. Allogeneic stem cell transplantation after reduced-intensity conditioning in a patient with T-cell prolymphocytic leukaemia: graft-versus-tumor effect and long-term remission. Bone Marrow Transplant. 2006;37:709–10.
49. Loughran Jr TP. Clonal diseases of large granular lymphocytes. Blood. 1993;82:1–14.
50. Lamy T, Loughran TP. Large granular lymphocyte leukemia. Cancer Control. 1998;5:25–33.
51. Sokol L, Loughran Jr TP. Large granular lymphocyte leukemia. Oncologist. 2006;11(3):263–73.
52. Zambello R, Trentin L, Facco M, Cerutti A, Sancetta R, Milani A, et al. Analysis of the T cell receptor in the lymphoproliferative disease of granular lymphocytes: superantigen activation of clonal CD3+ granular lymphocytes. Cancer Res. 1995;55:6140–5.

53. Kanchan K, Loughran Jr TP. Antigen-driven clonal T cell expansion in disorders of hematopoiesis. Leuk Res. 2003;27:291–2.
54. O'Keefe CL, Plasilova M, Wlodarski M, Risitano AM, Rodriguez AR, Howe E, et al. Molecular analysis of TCR clonotypes in LGL: a clonal model for polyclonal responses. J Immunol. 2004;172:1960–9.
55. Wlodarski MW, O'Keefe C, Howe EC, Risitano AM, Rodriguez A, Warshawsky 1, et al. Pathologic clonal cytotoxic T-cell responses: nonrandom nature of the T-cell-receptor restriction in large granular lymphocyte leukemia. Blood. 2005;106:2769–80.
56. Loughran Jr TP, Coyle T, Sherman MP, Starkebaum G, Ehrlich GD, Ruscetti FW, et al. Detection of human T-cell leukemia/lymphoma virus, type II, in a patient with large granular lymphocyte leukemia. Blood. 1992;80:1116–9.
57. Loughran Jr TP, Hadlock KG, Perzova R, Gentile TC, Yang Q, Foung SK, et al. Epitope mapping of HTLV envelope seroreactivity in LGL leukaemia. Br J Haematol. 1998;101:318–24.
58. Rodriguez-Caballero A, Garcia-Montero A, Barcena P, Almeida J, Ruiz-Cabello F, Tabernero MD, et al. Expanded cells in monoclonal TCRβ+/CD4+/NKa+/CD8−/+dim T-LGL lymphocytosis recognize hCMV antigens. Blood. 2009;112(12):4609–17.
59. Narumi H, Kojima K, Matsuo Y, Shikata H, et al. T-cell large granular lymphocytic leukemia occurring after autologous peripheral blood stem cell transplantation. Bone Marrow Transplant. 2004; 33(1):99–101.
60. Au WY, Lam CC, Lie AK, Pang A, Kwong YL, et al. T-cell large granular lymphocyte leukemia of donor origin after allogeneic bone marrow transplantation. Am J Clin Pathol. 2003;120(4):626–30.
61. French LE, Alcindor T, Shapiro M, McGinnis KS, Margolis DJ, Porter D, et al. Identification of amplified clonal T cell populations in the blood of patients with chronic graft-versus-host disease: positive correlation with response to photopheresis. Bone Marrow Transplant. 2002;30(8):509–15.
62. Arons E, Sorbara L, Raffeld M, Stetler-Stevenson M, Steinberg SM, Liewehr DJ, et al. Characterization of T-cell repertoire in hairy cell leukemia patients before and after recombinant immunotoxin BL22 therapy. Cancer Immunol Immunother. 2006;55(9):1100–10.
63. Martinez A, Pittaluga S, Villamor N, Colomer D, Rozman M, Raffeld M, et al. Clonal T-cell populations and increased risk for cytotoxic T-cell lymphomas in B-CLL patients: clinicopathologic observations and molecular analysis. Am J Surg Pathol. 2004;28(7):849–58.
64. Viny AD, Lichtin A, Pohlman B, Loughran T, Maciejewski J. Chronic B-cell dyscrasias are an important clinical feature of T-LGL leukemia. Leuk Lymphoma. 2008;49(5):932–8.
65. Yang J, Epling-Burnette P, Painter J, Zou J, Bai F, Wei S, et al. Antigen activation and impaired Fas-induced death-inducing signaling complex formation in T-large-granular lymphocyte leukemia. Blood. 2008;111(3):1610–6.
66. Lamy T, Liu JH, Landowski TH, Dalton WS, Loughran Jr TP. Dysregulation of CD95/CD95 ligand-apoptotic pathway in CD3(+) large granular lymphocyte leukemia. Blood. 1998;92:4771–7.
67. Schade AE, Wlodarski MW, Maciejewski JP. Pathophysiology defined by altered signal transduction pathways; the role of JAK-STAT and P13K signalling in large granular lymphocytes. Cell Cycle. 2006;5:2571–4.
68. Schade AE, Powers JJ, Wlodarski MW, Maciejewski JP. Phosphatidylinositol-3-phosphate kinase pathway activation protects leukemic large granular lymphocytes from undergoing homeostatic apoptosis. Blood. 2006;107:4834–40.
69. Epling-Burnette PK, Bai F, Wei S, Chaurasia P, Painter JS, Olashaw N, et al. ERK couples chronic survival of NK cells to constitutively activated Ras in lymphoproliferative disease of granular lymphocytes (LDGL). Oncogene. 2004;23:9220–9.
70. Lamy T, Loughran Jr TP. Clinical features of large granular lymphocyte leukemia. Semin Hematol. 2003;40:185–95.
71. Liu JH, Wei S, Lamy T, Epling-Burnette PK, Starkebaum G, Djeu JY, et al. Chronic neutropenia mediated by fas ligand. Blood. 2000;95:3219–22.
72. Rose MG, Berliner N. T-cell large granular lymphocyte leukemia and related disorders. Oncologist. 2004;9:247–58.
73. Miranda EG, Loughran Jr TP. Chronic T-cell and NK-cell leukemias. In: Cheson BD, editor. Chronic lymphoid leukemias. New York: Marcel Dekker; 2001. p. 543–65.
74. Lamy T, Loughran Jr TP. Current concepts: large granular lymphocyte leukaemia. Blood Rev. 1999; 13:230–40.
75. Kwong Y, Wong K. Association of pure red cell aplasia with T large granular lymphocyte leukaemia. J Clin Pathol. 1998;51:672–5.
76. Sampalo Lainz A, Lopez-Gomez M, Jimenez-Alonso J. Proliferation of large granular lymphocytes in patients with systemic lupus erythematosus. Rev Clin Esp. 1995;195:373–9.
77. Starkebaum G. Chronic neutropenia associated with autoimmune disease. Semin Hematol. 2002;39:121–7.
78. Burks EJ, Loughran Jr TP. Pathogenesis of neutropenia in large granular lymphocyte leukemia and Felty syndrome. Blood Rev. 2006;20(5):245–66.
79. McKenna R, Parkin J, Kersey J, Gajl-Peczalsca K, Peterson L, Brunnign R. Chronic lymphoproliferative disorder with unusual clinical, morphologic, ultrastructural and surface marker characteristics. Am J Med. 1977;62:588–96.
80. Argnasson B, Loughran T, Starkebaum G, Kadin M. The pathology of large granular lymphocyte lymphoma. Hum Pathol. 1989;20:643–51.
81. Osuji N, Beiske K, Randen U, Matutes E, Tjonnfjord G, Catovsky D, et al. Characteristic appearances of

the bone marrow in T-cell large granular lymphocyte leukaemia. Histopathology. 2007;50:547–54.
82. Dhodapkar M, Li CY, Lust J, Tefferi A, Phyliky RL. Clinical spectrum of clonal proliferations of T-large granular lymphocytes: a T-cell clonopathy of undetermined significance? Blood. 1994;84(5):1620–7.
83. Chan WC, Catovsky D, Foucar K, Montserrat E. T-cell large granular lymphocyte leukemia. In: Jaffe ES, Harris NL, Stein H, Vardiman JW, editors. World Health Organization classification of tumours: tumours of haematopoietic and lymphoid tissues. Lyon: IARC Press; 2001. p. 197–8.
84. Semenzato G, Zambello R, Starkebaum G, Oshimi K, Loughran Jr TP. The lymphoproliferative disease of granular lymphocytes: updated criteria for diagnosis. Blood. 1997;89:256–60.
85. Lundell R, Hartung L, Hill S, Perkins SL, Bahler DW. T-cell large granular lymphocyte leukaemias have multiple phenotypic abnormalities involving pan-T-cell antigens and receptors for MHC molecules. Am J Clin Pathol. 2005;124(6):937–46.
86. Morice WG, Kurtin PJ, Leibson PJ, Tefferi A, Hanson CA. Demonstration of aberrant T-cell and natural killer-cell antigen expression in all cases of granular lymphocytic leukaemia. Br J Haematol. 2003;120(6):1026–36.
87. Rambaldi A, Pelicci P, Allavena P, Knowles 2nd DM, Rossini S, Bassan R. T cell receptor beta gene rearrangements in lymphoproliferative disorders of large granular lymphocytes / natural killer cells. J Exp Med. 1985;162:2156–62.
88. Vie H, Chevalier S, Garand R, Moisan JP, Praloran V, Devilder MC. Clonal expansion of lymphocytes bearing the gamma delta T-cell receptor in a patient with large granular lymphocyte disorder. Blood. 1989;74:285–90.
89. Loughran Jr TP, Kadin ME, Starkebaum G, Abkowitz JL, Clark EA, Disteche C. Leukemia of large granular lymphocytes: association with clonal chromosomal abnormalities and autoimmune neutropenia, thrombocytopenia, and hemolytic anemia. Ann Intern Med. 1985;102:169–75.
90. Oshimi K, Yamada O, Kaneko T, Nishinarita S, Iizuka Y, Urabe A. Laboratory findings and clinical courses of 33 patients with granular lymphocyte-proliferative disorders. Leukemia. 1993;7:782–8.
91. Wong KF, Chan JC, Liu HS, Man C, Kwong YL. Chromosomal abnormalities in T-cell large granular lymphocyte leukaemia: report of two cases and review of the literature. Br J Haematol. 2002;116:598–600.
92. Liu JH, Wei S, Lamy T, Li Y, Epling-Burnette PK, Djeu JY, et al. Blockade of Fas-dependent apoptosis by soluble Fas in LGL leukemia. Blood. 2002; 100(4):1449–53.
93. Saitoh T, Karasawa M, Sakuraya M, Norio N, Junko T, Shirakawa K. Improvement of extrathymic T cell type of large granular lymphocyte (LGL) leukemia by cyclosporin A: the serum level of Fas ligand is a marker of LGL leukemia activity. Eur J Haematol. 2000;65:272–5.
94. Takeuchi M, Tamaoki A, Soda R, Takahashi K. Spontaneous remission of large granular lymphocyte T cell leukemia. Leukemia. 1999;13:313–4.
95. Matutes E, Wotherspoon A, Parker N, Osuji N. Transformation of T-cell large granular lymphocyte leukaemia into a high-grade large T-cell lymphoma. Br J Haematol. 2001;115(4):801–6.
96. Gentile TC, Uner AH, Hutchison RE, Wright J, Ben-Ezra J, Russell EC. CD3+, CD56+ aggressive variant of large granular lymphocyte leukemia. Blood. 1994;84:2315–21.
97. Dang NH, Aytac U, Sato K, O'Brien S, Melenhorst J, Morimoto C. T-large granular lymphocyte lymphoproliferative disorder: expression of CD26 as a marker of clinically aggressive disease and characterization of marrow inhibition. Br J Haematol. 2003;121:857–65.
98. Osuji N, Matutes E, Tjonnfjord G, Grech H, Del Giudice I, Wotherspoon A. T-cell large granular lymphocyte leukemia: a report on the treatment of 29 patients and a review of the literature. Cancer. 2006;107:570–8.
99. Pandolfi F, Loughran Jr TP, Starkebaum G, Chisesi T, Barbui T, Chan WC, et al. Clinical course and prognosis of the lymphoproliferative disease of granular lymphocytes. A multicenter study. Cancer. 1990; 65:341–8.
100. Sood R, Stewart CC, Aplan PD, Murai H, Ward P, Barcos M. Neutropenia associated with T-cell large granular lymphocyte leukemia: long-term response to cyclosporine therapy despite persistence of abnormal cells. Blood. 1998;91:3372–8.
101. Battiwalla M, Melenhorst J, Sauntharajah Y, Nakamura R, Molldrem J, Young NS. HLA-DR4 predicts haematological response to cyclosporine in T-large granular lymphocyte lymphoproliferative disorders. Br J Haematol. 2003;123:449–53.
102. Loughran Jr TP, Kidd PG, Starkebaum G. Treatment of large granular lymphocyte leukemia with oral low-dose methotrexate. Blood. 1994;84:2164–70.
103. Matrai Z, Lelkes G, Milosevits J, Páldiné HP, Pecze K. T-cell large granular lymphocytic leukemia associated with pure red cell aplasia, successfully treated with cyclophosphamide. Orv Hetil. 1997;138: 2075–80.
104. Aribi A, Huh Y, Keating M, O'brien S, Ferrajoli A, Faderl S. T-cell large granular lymphocytic (T-LGL) leukemia: experience in a single institution over 8 years. Leuk Res. 2007;31(7):939–45.
105. Witzig TE, Weitz JJ, Lundberg JH, Tefferi A. Treatment of refractory T-cell chronic lymphocytic leukemia with purine nucleoside analogues. Leuk Lymphoma. 1994;14:137–9.
106. Dincol G, Diz-Kuçukkaya R, Bicakci E. T-cell large granular lymphocytic leukaemia: successful response to 2-deoxycoformycin. Neth J Med. 2008;66(2):85–7.
107. Rosenblum MD, LaBelle JL, Chang CC, Margolis DA, Schauer DW, Vesole DH. Efficacy of alemtuzumab treatment for refractory T-cell large granular lymphocytic leukemia. Blood. 2004;103:1969–71.

108. Ru X, Liebman HA. Successful treatment of refractory pure red cell aplasia associated with lymphoproliferative disorders with the anti-CD52 monoclonal antibody alemtuzumab (Campath-1H). Br J Haematol. 2003;123:278–81.
109. Osuji N, Del Giudice I, Matutes E, Morilla A, Owusu-Ankomah K, Morilla R. CD52 expression in T-cell large granular lymphocyte leukemia-Implications for treatment with alemtuzumab. Leuk Lymphoma. 2005;46:723–7.
110. Aprile JA, Russo M, Pepe MS, Loughran Jr TP. Activation signals leading to proliferation of normal and leukemic CD3+ large granular lymphocytes. Blood. 1991;78:1282–5.
111. Morris JC, Janik JE, White JD, Fleisher TA, Brown M, Tsudo M. Preclinical and phase I clinical trial of blockade of IL-15 using Mikbeta1 monoclonal antibody in T cell large granular lymphocyte leukemia. Proc Natl Acad Sci USA. 2006;103:401–6.
112. Loughran Jr TP, Starkebaum G, Clark E, Wallace P, Kadin ME. Evaluation of splenectomy in large granular lymphocyte leukaemia. Br J Haematol. 1987;67:135–40.
113. Gentile TC, Loughran Jr TP. Resolution of autoimmune hemolytic anemia following splenectomy in CD3+ large granular lymphocyte leukemia. Leuk Lymphoma. 1996;23:405–8.
114. Toze CL, Shepherd JD, Connors JM, Voss NJ, Gascoyne RD, Hogge DE. Allogeneic bone marrow transplantation for low-grade lymphoma and chronic lymphocytic leukemia. Bone Marrow Transplant. 2000;25:605–12.