Butlletin Nº 157

T-Cell Large Granular Lymphocytic Leukemia (T-LGLL)

The immune system is a complex network of cells, tissues, and organs that work together to keep the body free from infections and neoplastic processes. Among the participating cells, lymphocytes stand out for their ability to identify pathogens and generate both innate and adaptive immune responses.

Three main subfamilies of lymphocytes can be distinguished: B lymphocytes, responsible for antibody production, T lymphocytes, essential in the adaptive response, and Natural Killer (NK) cells, which are part of the innate immune response.

T lymphocytes belong to the adaptive immune system and originate in the bone marrow from a common lymphoid progenitor. Afterwords, migrate to the thymus, where they mature and acquire the ability to recognize antigens via the TCR surface receptor (TCR).

During their maturation, they undergo a process of recombination of the TCRα, TCRβ, TCRγ, and TCRδ genes, paralleled by phenotypic changes that range from a CD4–CD8– state to the CD4+CD8+ phenotype. Subsequently, they undergo a selection process that removes those cells with reactivity toward self-antigens.

Finally, T lymphocytes differentiate into two main subpopulations:

- **T helper lymphocytes (CD4+CD8-):** Responsible for modulating the immune response through the secretion of cytokines and the activation of other cells.
- **Cytotoxic T lymphocytes (CD4-CD8+):** Responsible for eliminating infected or tumor cells.

These mature CD4+ or CD8+ T cells then migrate to the peripheral blood and secondary lymphoid organs, where they encounter antigen, an essential process for each T cell subpopulation to develop its role in the immune response and ensure that the response is specific and effective.

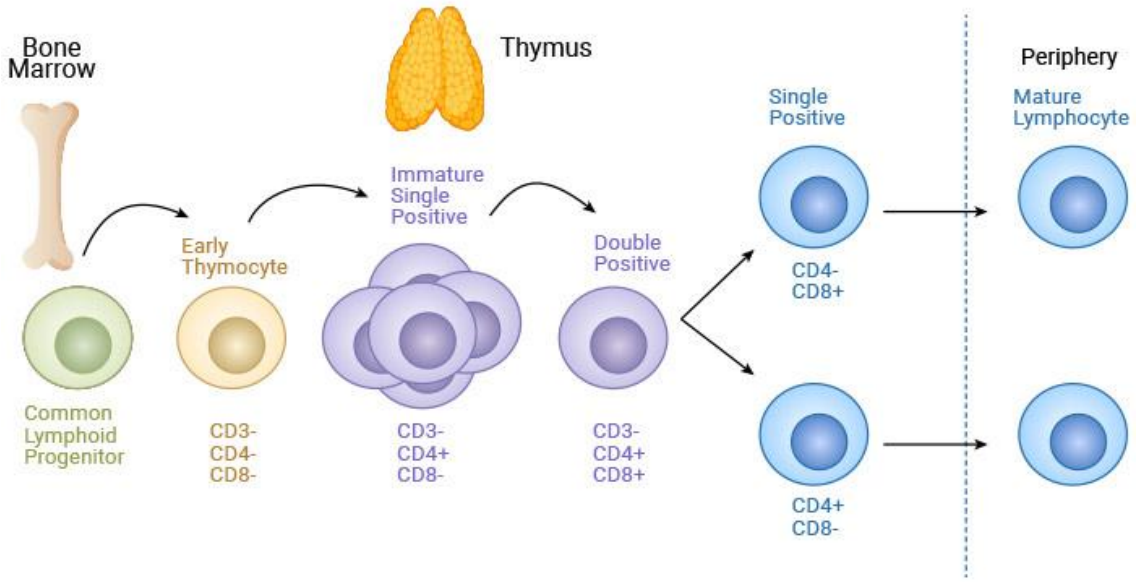

Figure 1: Schematic of T-lymphocyte maturation [Image on the internet]. CUSABIO team. T Cell Activation - The Switch for T Cell Executive Function [cited 2025 Dec 25]. Available from: *https://www.cusabio.com/c-21187.html*

MATURE T AND NK-CELL NEOPLASMS:

Neoplasms of mature T and NK cells constitute an heterogeneous group of rare lymphoid malignancies involving either T lymphocytes or NK cells, that often share similar pathophysiological mechanisms.

Their incidence ranges from 10% to 15% of mature cell lymphomas worldwide. In our laboratory, Catlab, according to the analysis of lymphoproliferative syndrome studies conducted during 2023, these neoplasms accounted for only 2% of the total, confirming their low prevalence.

According to the latest 2022 WHO classification (WHO-HAEM5), mature T and NK cell neoplasms are divided into nine families. Within the “Mature T- and NK-Cell Leukemias” family, six entities are included, characterized by presenting as a leukemic disease, and among these we find T-large granular lymphocyte leukemia (T-LGLL).

This classification framework is essential for contextualizing T-LGLL, which represents a diagnostic challenge due to its phenotypic similarity to reactive populations.

T-CELL LARGE GRANULAR LYMPHOCYTIC LEUKEMIA:

Granular large T-cell lymphocytic leukemia is a rare chronic lymphoproliferative disorder that accounts for 2–5% of mature T-cell leukemias. In fact, it is the most prevalent phenotype among chronic T- and NK-cell lymphoproliferative syndromes. This is shown in the study published in Catlab Informa No. 149, which analyzed the results obtained from Euroflow screening at Catlab, where granular

large T-cell lymphocytic leukemia accounted for 35% of the cases among the total of chronic T- and NK-cell lymphoproliferative disorders diagnosed in 2023.

This entity usually follows an indolent clinical course (85% of cases) and occurs equally in both sexes. Although it does not have a clearly defined age peak, most cases are diagnosed in patients between the ages of 45 and 75.

T-LGLL is characterized by a clonal expansion of large granular cytotoxic T lymphocytes, which manifests as a persistent increase of these cells in peripheral blood, usually with concentrations greater than 2 x 10^9 cells/L, for more than six months, and without an identified cause.

Current evidence suggests that the pathophysiological origin of T-LGLL is a chronic antigenic stimulation of cytotoxic T lymphocytes, predominantly $CD8^+$, in which alterations in the survival and apoptosis pathways favor persistence and clonal expansion.

In this context, several pathophysiological mechanisms have been proposed that could contribute to clonal expansion across different stages, among which the activation of the JAK–STAT pathway stands out. This JAK–STAT pathway is one of the most important routes for regulating proliferation, survival, differentiation, and immune response. In T-LGLL, mutations are acquired that activate STAT3, promoting the expansion of these cytotoxic lymphocytes and the increased expression of anti-apoptotic proteins such as BCL-2.

Regarding the genetic profile, molecular alterations are one of the supporting criteria for identifying the clonal nature of a LGLL proliferation according to WHO-HAEM5 (2022–2024).

Two main mutations have been described in association with LGLL:

- **STAT3 mutations:** these are particularly recurrent mutations in CD8+ T-LGLL. STAT3 activation, as discussed in the pathophysiology section, has been linked to its pathogenesis, particularly neutropenia. For this reason, it is believed that the absence of the STAT3 mutation in the CD4+ variant explains why this variant does not present with neutropenia.

- **STAT5b mutations**: These have been described in more than half of CD4+ TCRαβ+ T-LGLL cases, which are very rare in CD8+ T-LGLL.

This differential distribution may be useful to support the diagnosis and understand biological differences between variants.

Although the WHO-HAEM5 does not establish strict criteria for the diagnosis of T-cell large granular lymphocytic leukemia, several publications and clinical guidelines have attempted to define them. In this regard, Jevremovic D et al.

(2019) propose the following criteria, of which two of the three essential criteria must be met.

The essential criteria are:

- Abnormal T-lymphocyte immunophenotype with CD5 and/or CD7 downregulation and/or CD16 expression and presence of NK-associated receptors.
- T-cell monoclonality confirmed by PCR.
- Sinusoidal pattern of cytotoxic lymphocyte infiltration in the bone marrow.

Additionally, desirable criteria have also been described, which are:

- Presence of STAT3 or STAT5b mutations.
- Elevated peripheral blood cytotoxic T-lymphocyte count, usually >2×10^9/L (may be lower in some cases).

Morphology and immunophenotype of T-LGLL often overlap with those of normal terminal effector T cells, which complicates diagnosis and requires close diagnostic integration among flow cytometry, molecular biology, and clinical assessment.

T-LGLL IMMUNOPHENOTYPE:

The majority of T-LGLL cases present a **CD3+CD8+** phenotype and express a **TCRα/β+**, this is because it is a disorder of mature cytotoxic T cells and, therefore, the immunophenotype is consistent with that of a terminally differentiated effector T cell (CD8+CD57+CD28–CD45RA+). However, the LGLL-T phenotype frequently shows characteristic aberrations that allow it to be differentiated from reactive populations (loss of CD5, loss of CD7, overexpression of CD16 or CD56, and increased CD57).

CFM is the key tool to identify these aberrations and characterize the LGLL-T clone, especially in early stages or at low counts.

The expected immunophenotype is:

CD2+, CD3+, CD8+, CD4-, CD57+, TCRαβ+, CD16+, and cytotoxic markers (TIA1+, Granzyme B+, Granzyme M+, and Perforin+)

Weak expression or loss of pan-T markers CD5 and CD7 is common.

Beyond the conventional immunophenotype, T-LGLL can also present as CD4+, as well as CD4−CD8−, CD4+CD8+, and some TCRγδ+. In the CD4+ subtype, the clinical course is usually more indolent and the mutational profile also differs, since more than half of cases harbor STAT5b mutations, whereas there is an absence of STAT3 mutations.

CLONAL T-LGLL PHENOTYPES IN PATIENTS WITHOUT T-LGLL:

The presence of persistent clonal expansions of large granular lymphocytes has been described in **non-neoplastic contexts**. For this reason, it is crucial to know in which situations these clonal populations appear and to differentiate them from a true T-LGLL.

The presence of these clonal expansions has been described in the following scenarios:

- **Transplanted patients**
- **Autoimmune diseases and chronic viral infections**
- **Other hematologic neoplasms**
- **Elderly patients**

In many cases, patients may present with persistent clonal populations for more than six months, but are asymptomatic, without cytopenias and with an absolute count of large granular lymphocytes below the diagnostic threshold for LGLL (<500 cells/µL). In these cases, we speak of **monoclonal T-lymphocytosis of uncertain significance (T-CUS)**.

The publication “T-cell clones of uncertain significance “When is the rogue clone dangerous” by Gianpietro Semenzato (2024) suggests that the presence of these clonal populations may be present in approximately 1–2% of the healthy population, and is more common in older adults.

In the face of the possibility of reactive or neoplastic clonal populations, multiparametric flow cytometry is essential to establish origin, detect atypical phenotypes, and expand molecular studies as necessary.

FLOW CYTOMETRY (FCM) ANALYSIS STRATEGY:

In **Catlab**, we follow the **Euroflow** consortium's proposed algorithm for analyzing lymphocyte populations, but with some modifications tailored to the diagnostic needs of our center and the profile of the samples received.

This strategy consists of first performing a study with the LST (Lymphoid Screening Tube). This tube contains a combination of antibodies that allow us to identify the cellular lineage causing the lymphocytosis, classify the cells into the main subpopulations, detect the most common aberrant phenotypes, and assess B-lymphocyte clonality.

The following table describes the LST Tube configuration:

V450	V500	FITC	PE	PERCP-CY5.5	PE-CY7	APC	APC-H7
CD20/CD4	CD45	CD8/SmIgλ	CD56/SmIgκ	CD5	CD19/TCR γ/δ	CD3	CD38

Table 1: Configuration of the antibody panel for the characterization and quantification of the main lymphocyte populations (T, B, and NK) and the evaluation of B-cell clonality through Kappa and Lambda light chains.

The markers used to identify T cells, their subpopulations, and possible aberrations are:

- CD3: Identification of T lymphocytes.
- TCRγ/δ: T-cell receptor subtypes (most T lymphocytes will be α/β in both normal and atypical cells).
- CD4 and CD8: Major T-lymphocyte subpopulations.
- CD5 and CD56: Common immunophenotypic aberrations in T-LGLL.

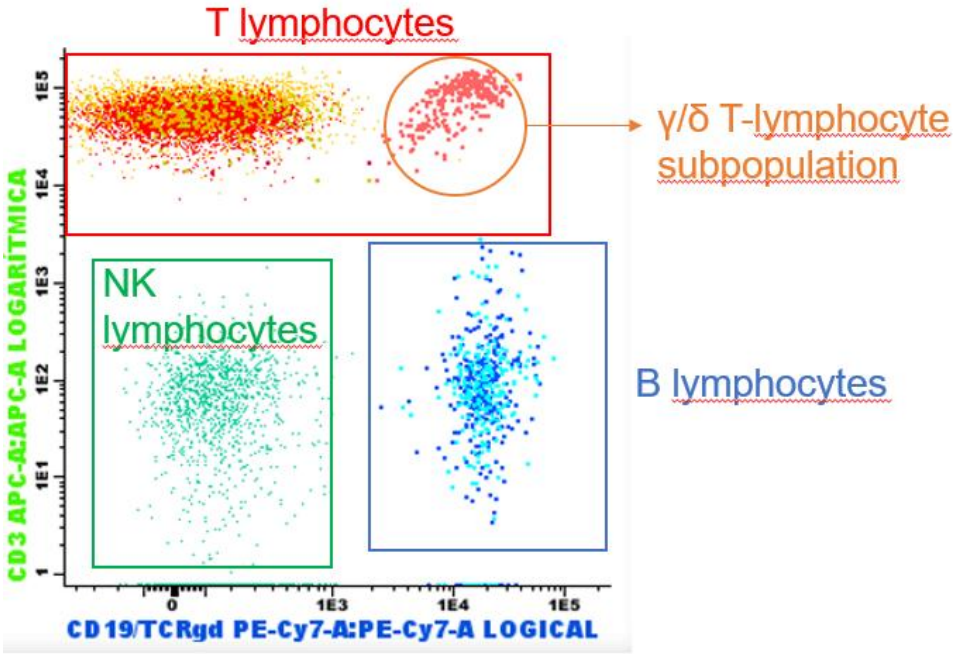

Figure 2: Dot plot showing the CD3 marker, which allows the identification of the T-lymphocyte population.

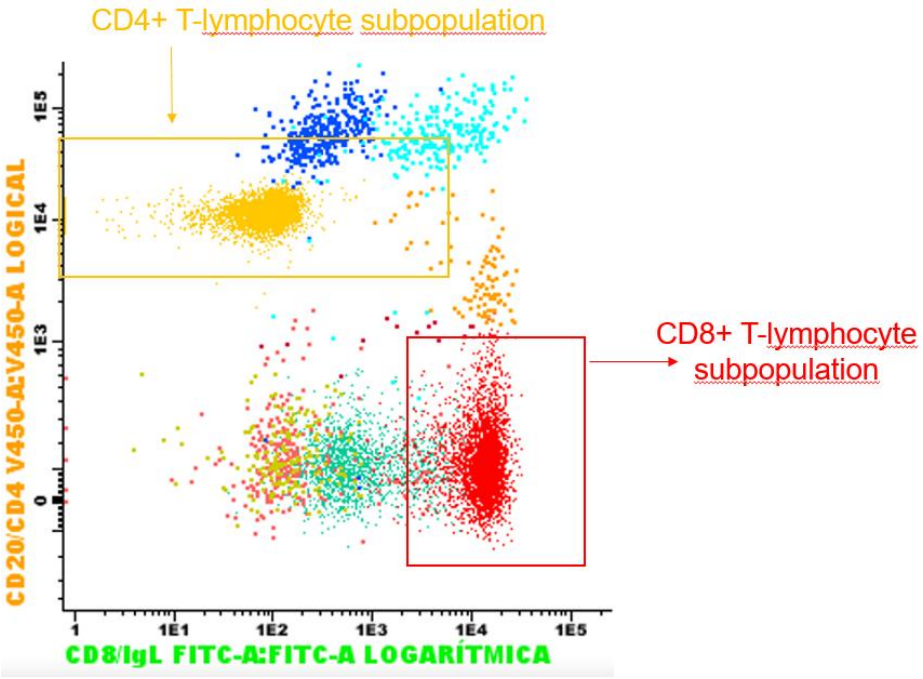

Figure 3: Dot plot showing the CD4 and CD8 markers, which allow the identification of T-lymphocyte subpopulations.

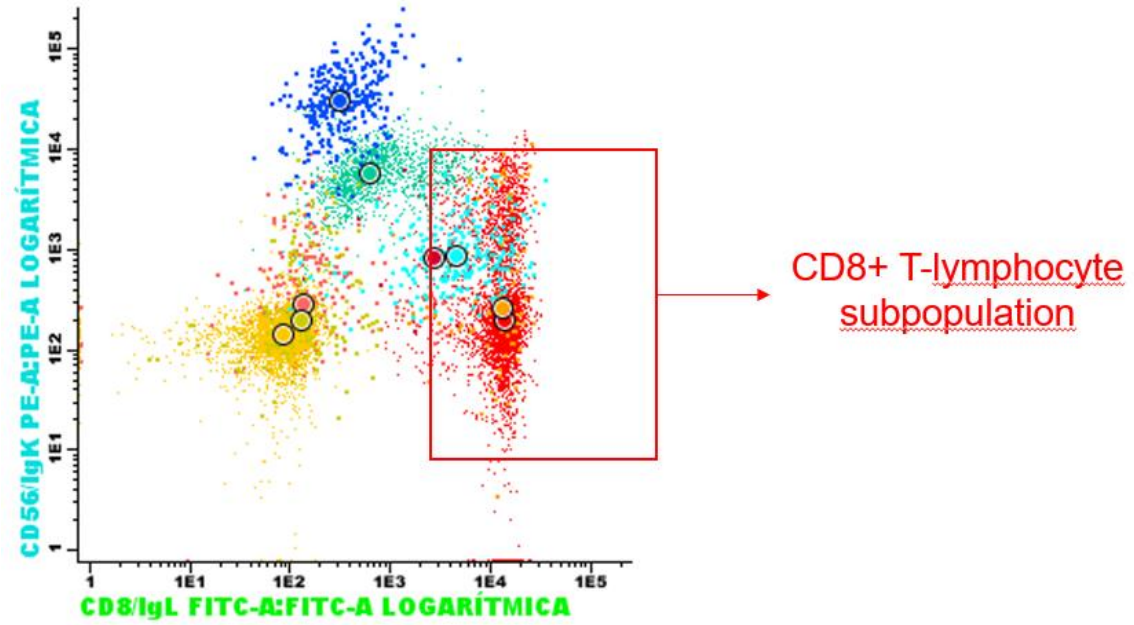

Figure 4: Dot plot showing the CD56 and CD8 markers, which allow the identification of CD8+ T-lymphocyte subpopulations that partially express CD56.

If an expanded T cell population that is aberrant or suggestive of LGLL is detected, we will expand the study to confirm its clonality and to characterize its phenotype according to cell type.

Clonality analysis will be performed through confirmatory molecular study of TCR rearrangement, which involves PCR (the “gold standard”). However, we first employ an orientative flow cytometry strategy based on the **anti-TRBC1 antibody**.

The use of the TRBC1 antibody allows for a sensitive, rapid, and simple assessment of T-cell clonality. It is based on the recognition of the expression of one of two isoforms or variants of the constant region of the TCR beta chain (TRBC1 or TRBC2), which are mutually exclusive. The result will be suggestive of clonality if TRBC1 expression is <3% or >97%, although some authors (Shi M. et al., 2019) consider a TRBC1 cutoff of <15% or >85% appropriate for assessing clonality. This technique has demonstrated **96% concordance** with molecular techniques for assessing the clonality of αβ T cells (Muñoz-García, N. et al., 2021).

Once cell clonality is demonstrated, we must perform an extensive phenotyping of the population of interest using the T-panel in order to track it.

- **T-Panel:** this panel consists of six complementary tubes that integrate differentiation, activation, cytotoxicity and TCR receptor markers, a configuration that allows for comprehensive phenotypic characterisation of the clone and the identification of potential phenotypic aberrations.

	V450	**V500**	**FITC**	**PE**	**PERCP-CY5.5**	**PE-CY7**	**APC**	**APC-H7**
TUBE-1	CD4	CD45	CD7	CD28	CD3	CD2	CD26	CD8
TUBE-2	CD4	CD45	-	CD45RO	CD3	CD45RA	CD27	CD8
TUBE-3	CD4	CD45	CD8	CD25	CD5	-	Cy-TCL1	CD3
TUBE-4	CD4	CD45	CD57	CD30	CD11c	TCR γδ	CD3	CD8
TUBE-5	CD4	CD45	Cy Perfo	Cy Granz	CD3	CD2	CD94	CD8
Perforin control	-	CD45	Cy perforin negative control	-	CD3	-	-	-
TUBE-6	CD4	CD45	CD8	CD279	HLADR	-	CD3	CD10

"Cy": cytoplasmic marker

Table 2: Configuration of the T-panel for the characterization of different T-lymphocyte subpopulations..

Finally, if an expansion of granular lymphocytes with an aberrant phenotype and confirmed monoclonality by PCR) is demonstrated, the WHO-HAEM5 criteria will be applied to classify it as T-LGLL. Additionally, testing for STAT3 and STAT5b mutations is recommended, as their presence reinforces the diagnosis and helps predict the clinical course.

In cases where a clonal population is detected but the patient is asymptomatic and the cell count is <500 cells/µL, it should be considered a **Monoclonal T-Lymphocytosis of Uncertain Significance (T-CUS)**. At Catlab, a follow-up at 6–12 months is proposed to determine whether the population remains stable or progresses towards T-LGLL, based on the following strategy:

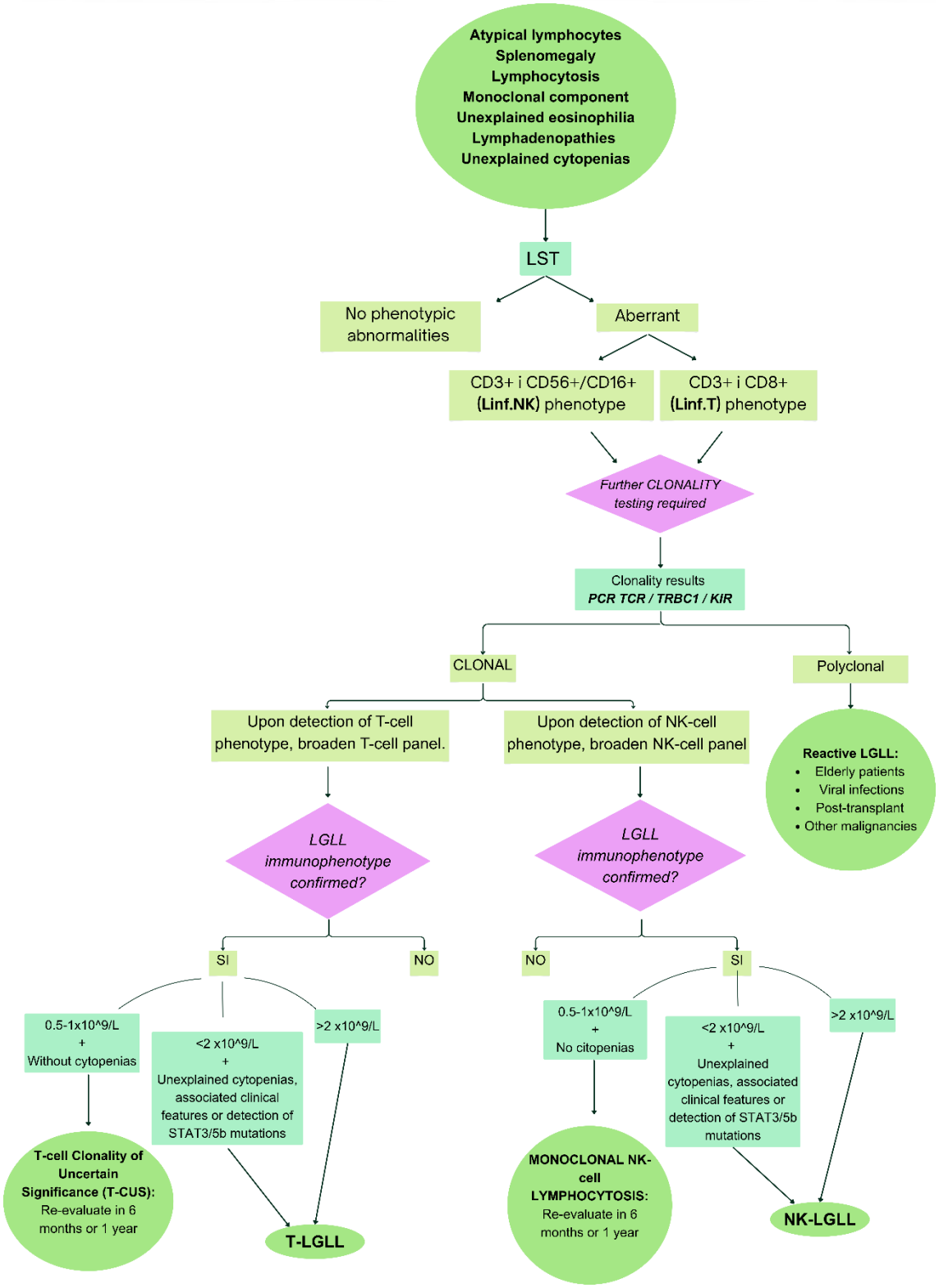

Figure 5: Schematic of the FCM analysis strategy used at Catlab for the study of T-LGLL and NK-LGLL.

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EXAMPLE OF T-LGLL DIAGNOSED AT CATLAB::

Below there's an illustrative clinical case of T-LGLL diagnosed at Catlab

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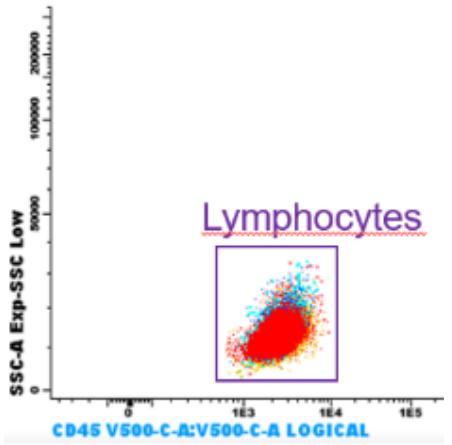

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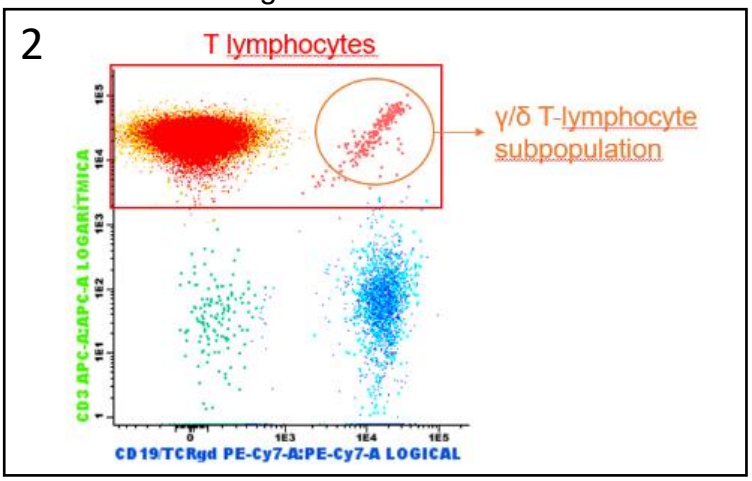

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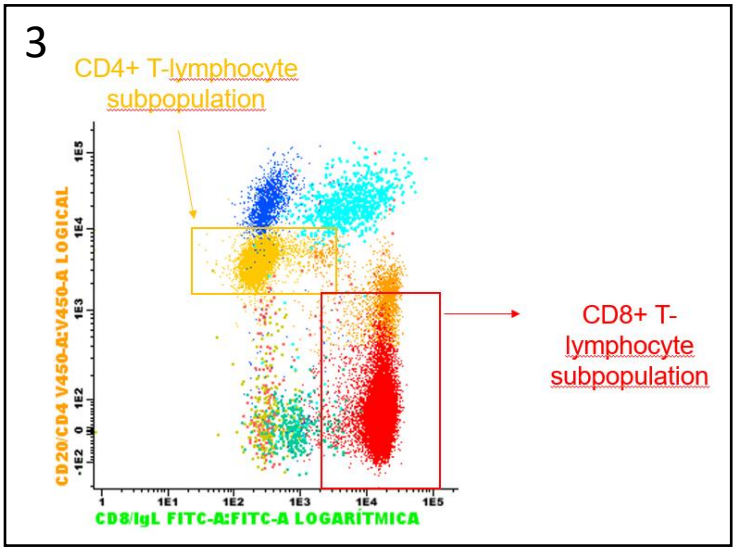

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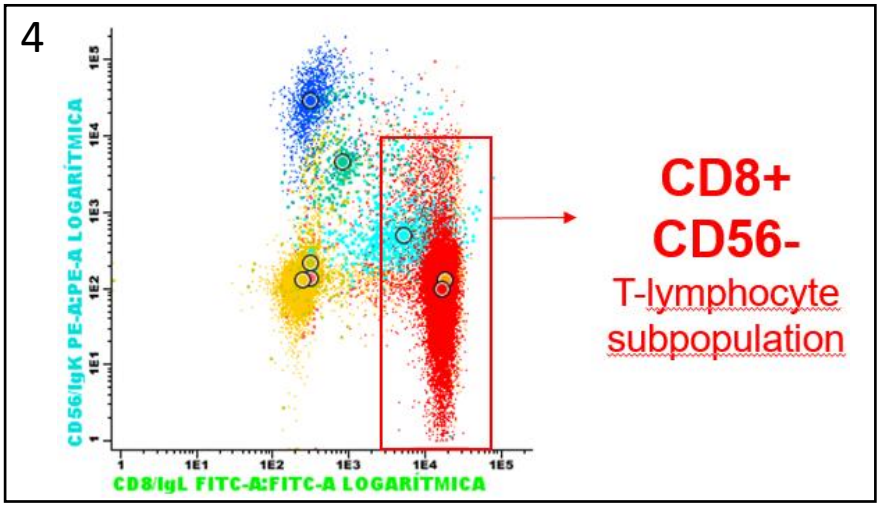

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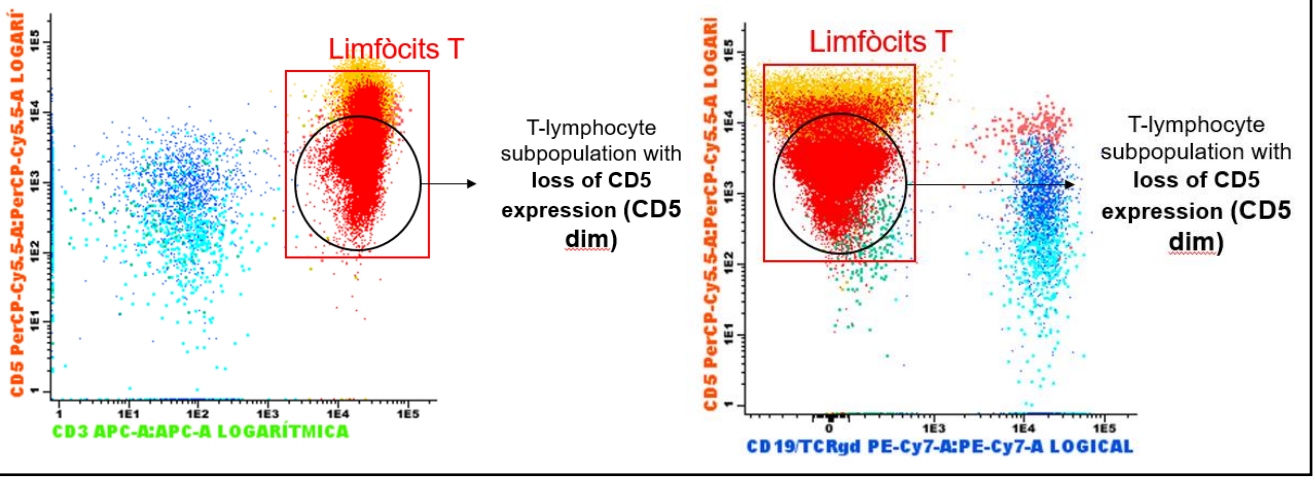

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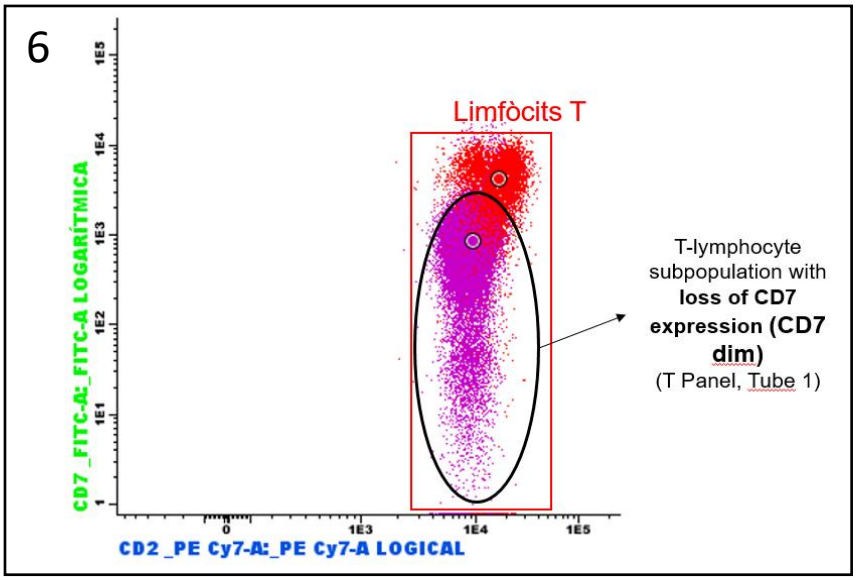

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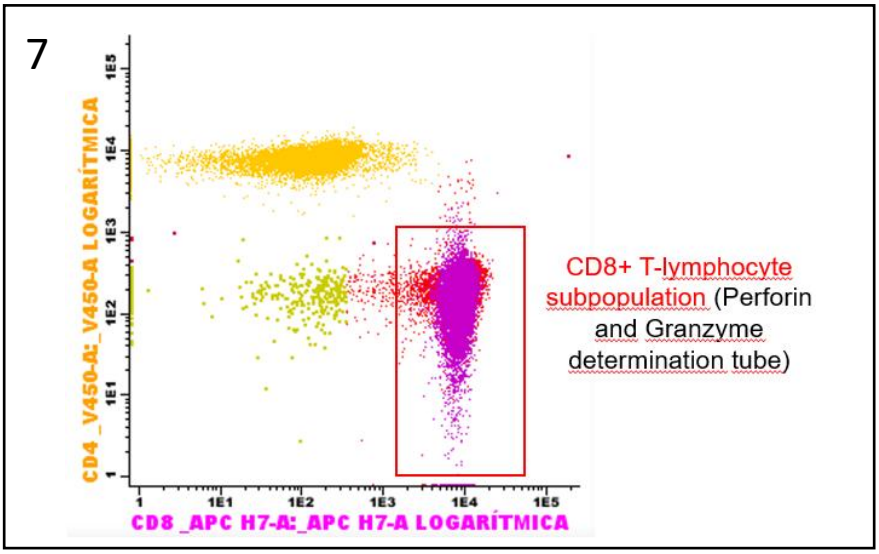

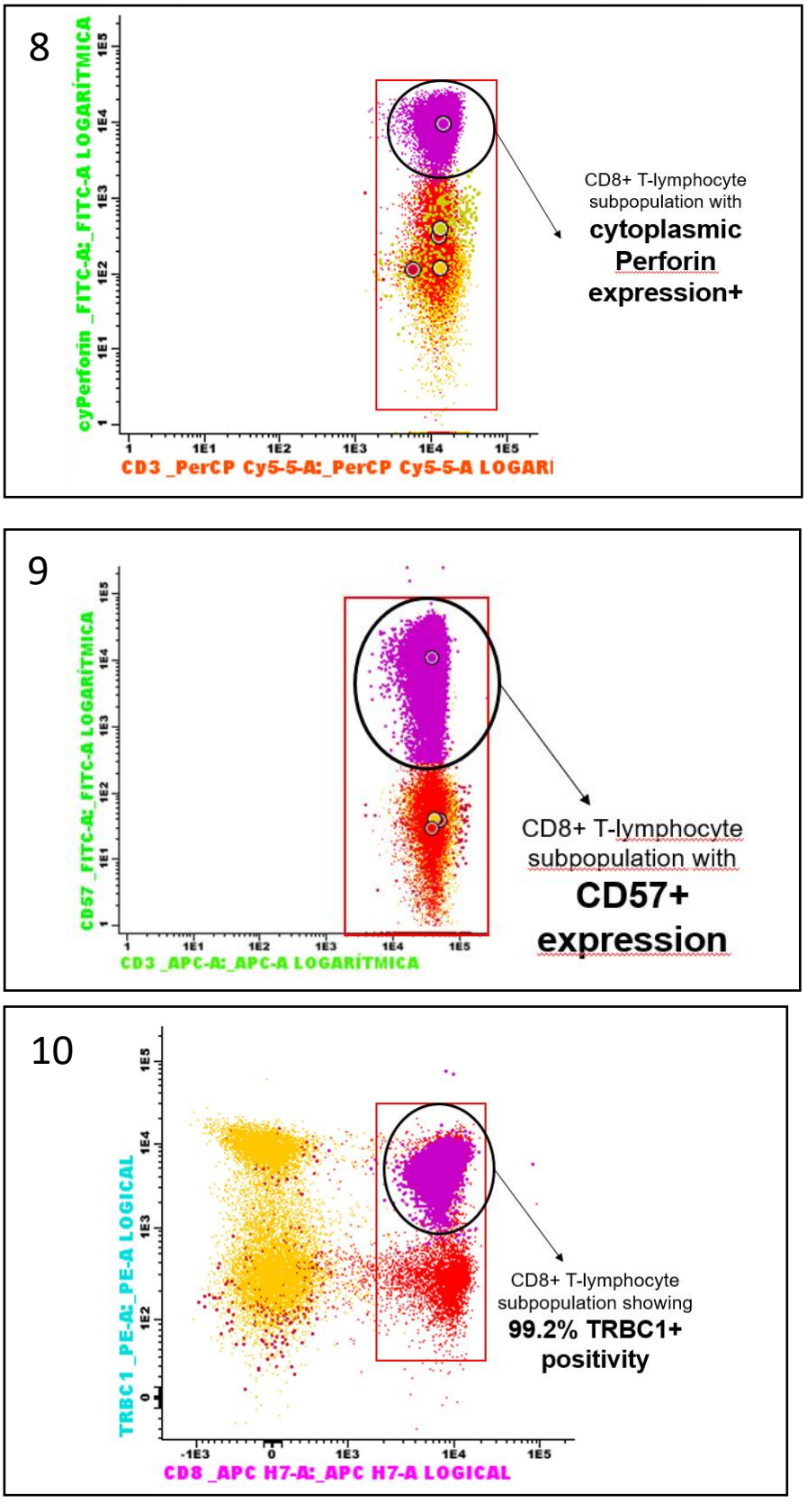

Figure 6: Detection of an aberrant T-lymphocyte population corresponding to T-LGLL via multiparametric flow cytometry at Catlab. The final phenotype obtained for the pathological population was: CD3+, CD8+, CD56-, CD5dim, CD26-, CD28-, CD2+, CD7dim, CD57+, CD11c+, CD30-, CD94+, CytPerforin+, CytGranzyme+.

In conclusion, the correct characterisation of granular-type lymphocyte proliferations (T and NK) requires integrating clinical, morphological, phenotypic and molecular data. In this context, CFM, applying the standardised Euroflow protocols, is an essential tool for the differential diagnosis among these entities (T-LGLL, NK-LGLL, T-CUS and NK-CUS). The application of these protocols ensures reproducibility between centres, and at Catlab we have implemented this harmonised methodology to provide robust and clinically relevant results, consolidating cytometry as a fundamental pillar in the evaluation of T-lymphocyte proliferations.

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