

T-cell lymphomas: recommendations for next-generation sequencing

Linfomas de células T: recomendaciones sobre el estudio de secuenciación masiva

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Abstract

Introduction: The integration of laboratory and clinical technologies and knowledge is key to an accurate diagnosis of hematologic neoplasms. Over the past decade, next-generation sequencing (NGS) has enabled the molecular characterization of these diseases, identifying mutations, copy number variations, and recurrent fusion genes. This advancement has driven the update of recommendations for the diagnosis and management of patients with hematologic neoplasms. Recent classifications by the World Health Organization (WHO), the International Consensus Classification (ICC), and the European LeukemiaNet (ELN) highlight the relevance of genetic data, incorporating them into their diagnostic and risk stratification algorithms. In this context, the implementation of targeted NGS panels is recommended for their ability to simultaneously analyze multiple genetic alterations. **Objective:** Although NGS is available in many laboratories, its use is not yet standardized, and there is no clear consensus on the methodology or the genes to be evaluated for each pathology. **Material and method:** The Molecular Biology Group in Hematology (GBMH), in collaboration with a panel of experts, developed this consensus guide. **Results:** Genes of interest were compiled based on their clinical relevance and the scientific evidence. **Conclusions:** This guide aims to assist professionals in the use of targeted NGS panels in T-cell lymphomas.

Keywords: T-cell lymphoma. Next generation sequencing (NGS). Consensus guidelines.

Resumen

Introducción: La integración de tecnologías y conocimientos del laboratorio y la clínica es clave para un diagnóstico preciso de las neoplasias hematológicas. En la última década, la secuenciación masiva (NGS) ha permitido caracterizar el perfil molecular de estas enfermedades, identificando mutaciones, variaciones en el número de copias y genes de fusión recurrentes. Este avance ha impulsado la actualización de las recomendaciones para el diagnóstico y tratamiento de los pacientes con neoplasias hematológicas. Las clasificaciones recientes de la World Health Organization (WHO), la International Consensus Classification (ICC) y la European LeukemiaNet (ELN) destacan la relevancia de los datos genéticos, incorporándolos en sus algoritmos diagnósticos y de estratificación del riesgo. En este contexto, la implementación de paneles dirigidos de NGS se recomienda por su capacidad para analizar múltiples alteraciones genéticas. **Objetivo:** Aunque

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la NGS está disponible en muchos laboratorios, su uso no está estandarizado y no hay consenso sobre la metodología ni los genes a evaluar en cada trastorno. **Material y método:** El Grupo de Biología Molecular en Hematología (GBMH), en colaboración con un panel de expertos, elaboró esta guía consensuada. **Resultados:** Se recogen los genes de interés según su relevancia clínica y evidencia científica. **Conclusiones:** Esta guía busca orientar a los profesionales sobre el uso de paneles dirigidos de NGS en los linfomas de células T.

Palabras clave: Linfoma de célula T. Secuenciación masiva (NGS). Guías de consenso.

Introduction

T-cell lymphomas (T-NHLs) comprise a broad and heterogeneous group of lymphoproliferative neoplasms originating from T lymphocytes and represent a minority of all non-Hodgkin lymphomas. The peripheral T-cell lymphoma subgroup, which encompasses most T-cell lymphomas, accounts for approximately 15% of non-Hodgkin lymphomas. Although some T-cell entities have a clinically indolent course, most T-NHLs are aggressive and generally show suboptimal responses to treatment.

Diagnosis is based on histologic and immunohistochemical examination, ideally of a lymph node biopsy. Bone marrow biopsy may be used as an alternative in cases with sufficient marrow infiltration.

Genetic studies in T-NHLs provide diagnostically useful information and, in some cases, are essential for appropriate diagnostic and prognostic classification. Among the tests to be performed, initial confirmation of T-cell clonality is recommended through detection of clonal rearrangements in the T-cell receptor (TCR) gene. Subsequently, depending on the immunohistochemical findings, fluorescence in situ hybridization (FISH) studies may be expanded, typically to assess t(2;5)(p23;q35), which results in the *ALK::NPM1* fusion found in certain anaplastic lymphomas and defines an entity with a more favorable prognosis. In cases without *ALK* expression, testing for *DUSP22* and *TP63* (3q28) rearrangements is useful.¹ In the rare cases of adult T-cell leukemia/lymphoma, detection of chromosome 14 inversion – inv(14)(q11q32) or t(14;14)(q11;q32) – is particularly useful because it supports the differential diagnosis from other T-cell lymphoproliferative disorders.² In peripheral T-cell lymphoma, subtypes with different biologic behavior have been described according to gene expression patterns, including the *TBX21* and *GATA3* variants,³ although this methodology is not currently standardized in most centers.

Regarding next-generation sequencing (NGS), given the diagnostic complexity of certain T-NHL cases, its usefulness lies in detecting molecular profiles associated with T-cell neoplasms and helping distinguish

them from other lymphoma subtypes. In addition, in cases with an unfavorable clinical course, NGS may identify molecular alterations amenable to targeted therapy, an area currently under investigation in numerous clinical trials.

Relevant methodological aspects in T-cell lymphomas

- Timing of testing: at diagnosis in selected cases and at relapse or in patients with treatment-refractory disease.
- Studies to be performed: FISH techniques can be carried out directly on paraffin-embedded samples, including lymph node or bone marrow biopsy specimens.

For NGS, library preparation may be performed using DNA extracted from paraffin-embedded tissue when a lymph node biopsy is available or from bone marrow samples when infiltration has been confirmed at that site. A particular feature of T-cell lymphomas is that they frequently harbor mutations associated with myeloid disorders. This requires either the use of a broad panel covering both lymphoid and myeloid genes or the preparation of 2 separate libraries, one using a lymphoid panel and the other a myeloid panel. In addition, panels integrating the detection of single-nucleotide variants, copy-number alterations, and fusions are recommended because they enable unified assessment of most genomic abnormalities relevant to T-cell lymphomas.

Studies to be performed using next-generation sequencing panels in T-cell lymphomas

Following a review of the literature, genes (Table 1) and genomic alterations (Table 2) of interest for assessment in patients with T-NHL were selected and agreed upon by the lymphoma group. Among these, several genes are particularly relevant in clinical practice and are described below:

- *DNMT3A*, *TET2*, and *IDH1/IDH2*. These genes, which are frequently mutated in several myeloid disorders,

Table 1. Genes with the most relevant point variants associated with T-cell lymphomas included in the next-generation sequencing (NGS) panel

Genes	Clinical value	Level of evidence
<i>ARID1A</i>	Diagnosis	Optional
<i>CARD11</i>	Diagnosis	Optional
<i>CD28</i>	Diagnosis	Optional
<i>CDKN2A</i>		Optional
<i>DDX3X</i>	Diagnosis, treatment	Recommended
<i>DNMT3A</i>	Diagnosis, treatment	Recommended
<i>EZH2</i>	Diagnosis, treatment	Recommended
<i>FYN</i>	Diagnosis	Recommended
<i>GATA3*</i>	Diagnosis, prognosis	Recommended
<i>IDH2</i>	Diagnosis, treatment	Recommended
<i>JAK1</i>	Diagnosis, treatment	Recommended
<i>JAK3</i>	Diagnosis, treatment	Recommended
<i>KMT2D</i>	Diagnosis, prognosis	Recommended
<i>RHOA</i>	Diagnosis	Recommended
<i>STAT3</i>	Diagnosis, treatment	Recommended
<i>STAT5B</i>	Diagnosis, treatment	Recommended
<i>TET2</i>	Diagnosis, treatment	Recommended
<i>TP53</i>	Diagnosis, prognosis	Recommended
<i>ARID1A</i>	Diagnosis	Optional
<i>CARD11</i>	Diagnosis	Optional
<i>CD28</i>	Diagnosis	Optional
<i>CDKN2A†</i>		Optional
<i>PLCG1</i>	Diagnosis	Optional
<i>SETD2</i>	Diagnosis	Optional
<i>TNFRSF1B</i>	Diagnosis	Optional
<i>TOX*</i>	Diagnosis	Optional
<i>VAV1</i>	Diagnosis	Optional

*Overexpression is more relevant than recurrent point variants. Assessment using an NGS panel that includes copy-number analysis or RNA sequencing is recommended.

†Deletions or loss of the locus are more relevant than recurrent point variants. Assessment by deletion or copy-number analysis is recommended.

including acute myeloid leukemia (AML), myelodysplastic syndromes (MDS), and chronic myelomonocytic leukemia (CMML), may also be affected in patients with T-NHL, particularly peripheral T-cell lymphoma and the angioimmunoblastic subtype. Detection of these

Table 2. Genes with relevant structural alterations in T-cell lymphomas included in the next-generation sequencing (NGS) panel for fusion detection. If a fusion-detection method is unavailable, assessment using FISH probes is recommended

Structural alterations	Clinical value	Level of evidence
Fusions		
<i>DUSP22</i>	Diagnosis	Required in ALK- negative cases
<i>PDGFRA</i>	Diagnosis, prognosis	Recommended
<i>ALK-NPM1</i>	Diagnosis	Recommended
<i>TP63</i>	Diagnosis, prognosis	Recommended
<i>TCL1A/B-TRA</i>	Diagnosis	Optional
<i>MTCP1-TRA</i>	Diagnosis	Optional
<i>CTLA4-CD28*</i>	Diagnosis	Optional
<i>ITK-SYK</i>	Diagnosis	Optional
<i>ICOS-CD28*</i>	Diagnosis	Optional

*These alterations are more effectively detected by fusion-targeted NGS, particularly RNA sequencing. FISH does not always reliably detect these fusions because of the close genomic proximity of the involved loci.

mutations is therefore useful for diagnosis and for the differential diagnosis from other lymphoma subtypes. It is also relevant when considering epigenetic treatment options, including hypomethylating agents and histone deacetylase inhibitors.^{4,5}

- *RHOA*. The p.G17V mutation in the *RHOA* gene is found in 68% of angioimmunoblastic T-cell lymphomas, frequently in association with *TET2* and *IDH2* mutations. It is involved in lymphomagenesis and is associated with a follicular helper T-cell phenotype. Its detection currently has diagnostic value in this entity.^{6,7}

- *STAT3* and *STAT5B*. Mutations in these genes occur in lymphoproliferative syndromes involving natural killer cells and large granular lymphocytes and are associated with an adverse prognosis in the case of *STAT5B*. Studies have suggested that activating mutations affecting these proteins may represent therapeutic targets for JAK inhibitors.^{8,9}

- *GATA3*. Mutations in this gene are found in 14% of adult T-cell leukemias/lymphomas, and overexpression may occur in 30% of peripheral T-cell lymphomas. *GATA3* has diagnostic, prognostic, and therapeutic relevance and is associated with a poorer prognosis in peripheral T-cell lymphoma.¹⁰

- *JAK1* and *JAK3*. Mutations in these genes may occur in 20–38% of ALK-negative anaplastic

lymphomas, 10–40% of T-cell prolymphocytic leukemias, and 5–18% of NK/T-cell lymphoproliferative disorders. They are particularly relevant because they may be susceptible to targeted treatment with JAK inhibitors, as is currently under investigation.¹¹⁻¹³

In conclusion, although evidence supporting the usefulness of NGS studies in T-cell lymphomas is increasing, their implementation in routine clinical practice remains very limited. Diagnosis continues to rely primarily on comprehensive histologic and immunohistochemical evaluation. Therefore, upfront NGS testing is generally reserved for highly selected cases in which the diagnosis remains inconclusive, such as gray-zone lymphomas or Hodgkin lymphoma with inconclusive histology.

During follow-up, in cases with an unfavorable clinical course, molecular testing is recommended whenever the findings may influence therapeutic management, such as when clinical trials involving targeted therapies are available, when off-label treatments may be considered according to a specific mutational profile, or when histologic transformation into another entity is suspected and molecular characterization may improve classification.

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Conflicts of interest

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Ethical considerations

Protection of humans and animals. The authors declare that no experiments involving humans or animals were conducted for this research.

Confidentiality, informed consent, and ethical approval. The study does not involve patients' personal data and does not require ethical approval. The SAGER guidelines do not apply.

Declaration on the use of artificial intelligence. The authors declare that no form of generative artificial intelligence was used in the writing of this manuscript.

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