leads to effective reconstitution of gp91^{phox} expression, and NADPH oxidase in vitro and in vivo protects X-CGD mice from experimental *Burkholderia cepacia* infection, thereby providing a preclinical proof of concept. Although in some in vitro studies gp91^{phox} reached levels higher than normal, the transgene was expressed at physiological levels across all lineages when transduced X-CGD patient cells were engrafted into immunodeficient mice.

The lentiviral vector designed by Wong et al was compared with a myeloid-specific chimeric promoter currently in clinical trial[7] but not with other regulated vectors. The overall improvement over the chimeric myeloid promoter–based vector is considerable in that the expression pattern of endogenous gp91^{phox} is well recapitulated in myeloid and B cells, managing to increase expression levels without compromising expression specificity. Emerging technologies based on gene-correction approaches by homology-directed repair into the *CYBB* locus[10] could, in principle, provide a more robust physiological regulation vs regulated lentiviral vectors but the efficiency and long-term safety of gene editing is still under investigation. Overall, the strategy developed by Wong et al holds promise as an improved gene therapy platform for X-CGD, if further testing confirms the results obtained so far.

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LYMPHOID NEOPLASIA

Comment on Barilà et al, page 1036

T-LGLL: variety is the spice of this leukemia

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In this issue of *Blood*, Barilà et al[1] characterize the clinical and biological features of Tγδ large granular lymphocyte leukemia (Tγδ LGLL). They report that Tγδ LGLL, compared with the more common Tαβ variant, displays distinctive features, is associated with a less indolent form of the disease, and has shorter overall survival (OS). Patients with Tγδ LGLL seem to benefit from therapy with noncytotoxic ciclosporin (CSA).

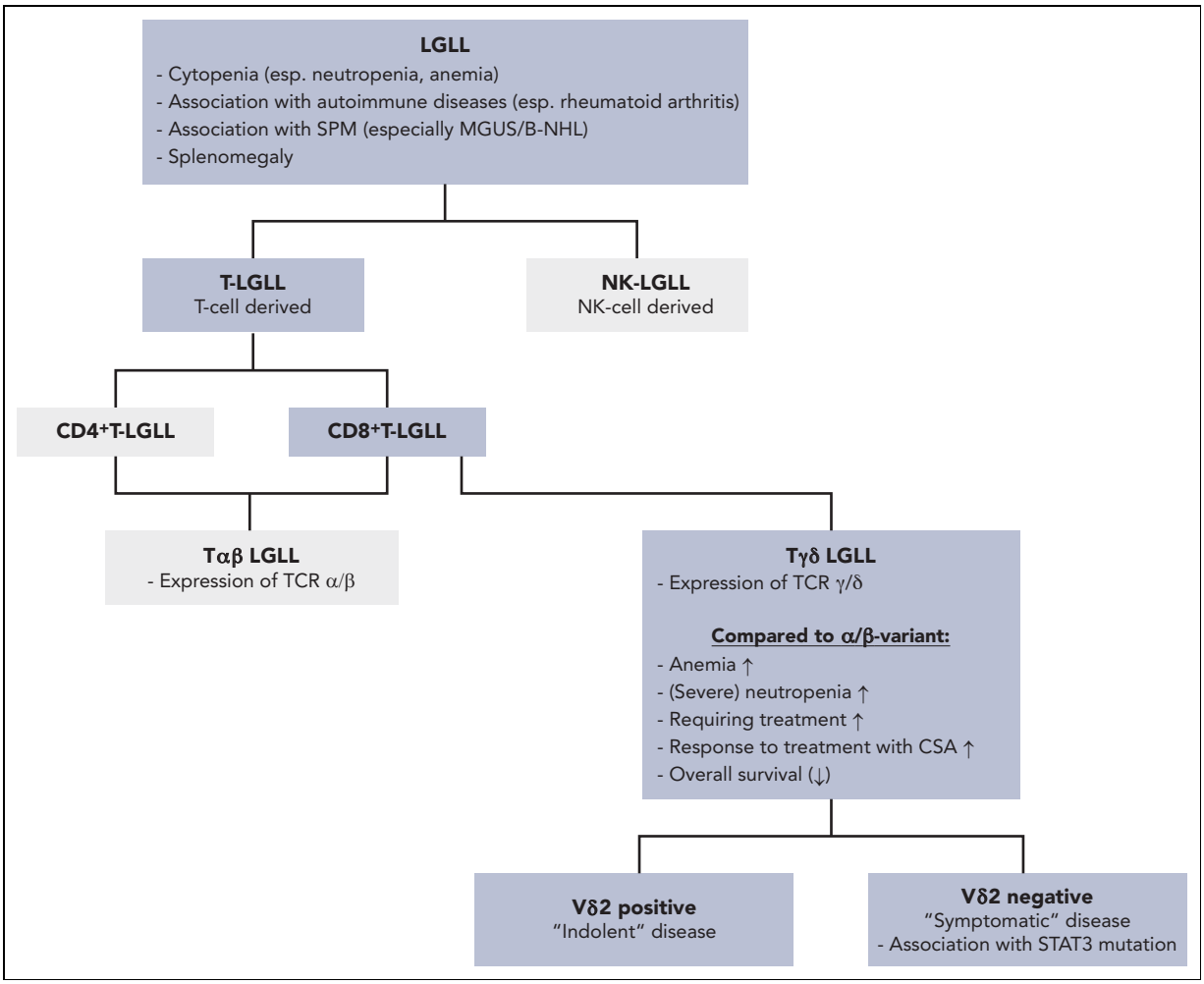
LGLL is a disease that is incompletely understood. The updated 2022 World Health Organization classification distinguishes 3 subtypes of monoclonal diseases of large granular lymphocytes (LGL): T cell–derived T-LGLL, the rarer natural killer (NK) cell–LGLL (both deemed rather indolent), as well as aggressive NK cell leukemia.

T-LGLL is commonly classified as a leukemia characterized by monoclonal cytotoxic T cells; however, it is a matter of debate whether this designation is adequate, as T-LGLL is not only a leukemia, but rather a disease characterized by (autoimmune-mediated) cytopenia, associated autoimmune disorders, and a disproportionate increase in secondary primary neoplasms, in particular, B-cell diseases. This triad is used to determine the indication for therapy.[2]

Phenotypically and clinically, a rarer variety, CD4$^+$ T-LGLL, can be distinguished from CD8$^+$ T-LGLL. Furthermore, within CD8$^+$ T-LGLL a Tαβ variant can be distinguished from a Tγδ variant based on the T-cell receptor (TCR) chains expressed (see figure).

However, clonal LGLs do not necessarily indicate a disease; they are regularly detected after stem cell and organ transplantation. Furthermore, it is unclear how several borderline conditions such as Felty syndrome or hypoplastic myelodysplastic syndrome need to be classified within the LGLL landscape.

Barilà et al provide a further important piece to this puzzle: the largest cohort on Tγδ LGLL published to date with molecular characterization, as well as information on response to treatment. The authors collected data on 137 patients with Tγδ LGLL followed at 8 international centers and found that Tγδ LGLL is a variant with distinctive clinical features. Of special interest, Tγδ LGLL seemed significantly more frequently symptomatic with reduced OS compared with Tαβ LGLL.[1] This finding contradicts the paradigm that both Tαβ LGLL and Tγδ LGLL



Dissecting LGLL. B-NHL, B-non-Hodgkin lymphoma; esp., especially; MGUS, monoclonal gammopathy of undetermined significance; SPM, second primary malignancy; STAT, signal transducer and activator of transcription.

are chronic conditions with a similar, rather indolent course of disease. However, Tγδ LGLL itself is not a homogenous entity: positivity for the Vδ2 receptor chain seems to be associated with a more indolent form of disease (see figure).

The analysis by Barilà et al further suggests that the choice of therapy also needs to be based on the subtype of T-LGLL: generally, independent of T-LGLL variant, low-dose methotrexate (MTX) or cyclophosphamide is used in first-line therapy, whereas immunosuppression with CSA is reserved for second- or third-line therapy.[2] The data presented by Barilà et al suggest that "one treatment fits all" might no longer be correct for T-LGLL patients: patients with the γδ variant do not seem to respond well to MTX, but do benefit from therapy with CSA. The clinical relevance is emphasized by the observation that response to therapy translated into prolonged progression-free survival and prolonged OS.[1]

The differences found clinically between Tαβ and Tγδ LGLL by the authors are partially reflected by differences on the biological level. Under normal circumstances, Tγδ lymphocytes comprise less than 10% of peripheral blood $CD3^+$ T cells. Unlike Tαβ lymphocytes, Tγδ lymphocytes are not dependent on the major histocompatibility complex for antigen presentation. Butyrophilin/butyrophilin-like proteins have been identified as potential specific antigen-presenting molecules in this cell subtype.[3,4]

The hypothesis that the initiating event of T-LGLL is chronic antigen stimulation is intriguing; however, tangible supporting evidence is missing so far. Indeed, in Tαβ LGLL TCR clonotypes have been demonstrated to be private to the disease (ie, absent in healthy controls) and to the patient,[5,6] although a recent study observed that over half of the T-LGLL clonotype TCRs share structural similarities with TCR from the same patients' non-leukemic repertoires.[7] In Tγδ LGLL the dominant clonotypes seem to be mainly public (ie, shared with at least 1 healthy donor).[8] At the genetic level, however, both subtypes appear to share somatic mutations as well as putative drivers.[9]

In interpreting the data presented by Barilà et al, some caution is still warranted:

- Experience shows that the diagnosis of Tγδ LGLL is more difficult than that of Tαβ LGLL, partially due to the lower median LGL cell count. It is, therefore, possible that Tγδ LGLL cases are more likely to be late-stage

diseases compared with Tαβ LGLL cases.

- T-LGLL is also known to be associated with a variety of autoimmune cytopenia such as pure red cell aplasia (PRCA). Patients with PRCA respond well to noncytotoxic immunosuppression—for example, with CSA. Most patients that had received CSA in this cohort were treated due to anemia. Undiagnosed PRCA must be ruled out in this context, as Barilà et al mention in the Discussion. Provocatively, this raises the question whether careful diagnosis of the symptom determining concomitant disease is more important than differentiation of the T-cell receptor in choosing the optimal therapy for T-LGLL patients.

T-LGLL is a disease with a wide range of manifestations, and its position at the intersection of chronic inflammation, autoimmune disease, and monoclonal hematologic disease is highly intriguing.[3] The major questions to be answered are:

- What unites these clinical cases, and what separates them?
- Is it one disease with a unifying pathophysiology or the reverse, clinically and/or molecularly distinct entities?

The exploration of the LGLL landscape has just begun. The heterogeneity of T-LGLL is not limited to the pathologic classification: even in a single patient, Tγδ, Tαβ, or NK LGLL clones can coexist. Furthermore, clonal drifts are common. The impact of genetic and epigenetic changes, not least on the interplay of the leukemic cells and the nonleukemic immune repertoire, is poorly understood. Here initial studies at the single-cell level have provided first exciting insights.[7] These and future studies should pave the way for improved targeted and personalized therapies in this orphan disease.

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TRANSPLANTATION

Comment on Yu et al, page 1070

A novel RIPK1 inhibitor attenuates GVHD

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In this issue of *Blood*, Yu et al identify the role of enterocyte RIPK1/RIPK3 in the generation of graft-versus-host disease (GVHD) and generate a potent RIPK1 inhibitor, Zharp1-211, which inhibits GVHD without impacting graft-versus-leukemia (GVL) in a murine model.[1]

Acute GVHD in the gastrointestinal (GI) tract is a critical determinant of mortality and morbidity after allogeneic stem cell transplantation.[2] Intestinal epithelial cells (IECs) have been emerging as a pivotal lineage for the initiation and amplification of acute GVHD, while also representing a major target.[3] The characteristic pathological feature of acute gut GVHD is the apoptosis of IECs. More recently, multiple nonapoptotic gene-regulated cell death pathways have been identified including necroptosis.[4] In necroptosis, receptor-interacting serine/threonine-protein kinase 1 (RIPK1) and RIPK3 recruit mixed lineage kinase domain-like (MLKL) which executes cell death by forming membrane pores. Autophagy is a pivotal pathway by which IECs are protected from necroptosis. Thus, if the autophagic protein ATG16L1 is abrogated in IECs, RIPK3 overtly promotes MLKL phosphorylation, necroptotic cell death, and GVHD.[5] However, necroptosis independent effects of the RIPK1/RIPK3 complex during GVHD have not been described.

Yu et al demonstrate that inactivation or depletion of RIPK1 or RIPK3 specifically in IECs profoundly reduces GVHD mortality and attendant visceral GVHD histopathology in autophagy-competent recipients.[1] This effect occurred in association with reduced levels of circulating cytokines and chemokines. Intriguingly, the protective effects of RIPK1 and RIPK3 inactivation were not dependent on the necroptotic or caspase-8 dependent apoptotic programmed cell death pathways. Indeed, MLKL deletion in recipient IECs delayed the onset of GVHD but did not prevent lethality. Rather, the authors found that RIPK1/RIPK3 complexes bind JAK1 to phosphorylate and activate STAT1 in IECs, resulting in enhanced chemokine (CCL9 and CCL10) secretion at multiple time points after bone marrow transplantation. Donor T-cell migration to the GI tract during GVHD is known to be dependent on multiple chemokine receptors such as CXCR3,[6] which is the receptor for CCL9 and CCL10, and CCR5.[7] RIPK1/RIPK3 complexes also