patients with classic hairy cell leukemia who have relapsed and require therapy.

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GENE THERAPY

Comment on Wong et al, page 1007

Towards improved yet regulated gene therapy for X-CGD

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In this issue of *Blood*, Wong et al[1] exploit bioinformatic tools to design and test a minimal (core) promoter region to produce sufficient physiological expression of the *CYBB* gene, which is defective in patients affected by X-linked chronic granulomatous disease (X-CGD). X-CGD is the most common form of CGD in males. CGD is an inborn error of immunity caused by a defective reduced NAD phosphate (NADPH) complex, which is a key component of innate immune defense against bacterial and fungal pathogens.[2] The gp91^{phox} protein encoded by the *CYBB* gene is required for the production of reactive oxidase species and is expressed predominantly in myeloid and B-cell lineages but not in primitive hematopoietic stem and progenitor cells (HSPC).[2]

Allogeneic transplantation is a curative treatment that may be performed in patients with X-CGD with a well-matched donor. Despite the improved outcomes achieved in the past decade,[3] allogeneic transplantation still carries a significant risk of complications. Autologous HSPC gene therapy (GT) is a promising alternative therapy. Several clinical trials have explored GT for X-CGD using integrating vectors, with more than 25 patients treated to date.[4] The first studies based on the use of γ retroviral vectors were hampered by a high incidence of insertional mutagenesis as well as gp91^{phox} inactivation due to methylation of the viral vector promoter.[2] This suggested that the proper regulation of the *CYBB* gene is critical for safety and success of HSPC GTs, fueling the development of myeloid-restricted gp91^{phox} expression[5] as well as of combined transcriptional and post-transcriptional regulation strategies designed to avoid HSPC ectopic expression.[6] More recently, clinical trials based on lentiviral vectors using a chimeric myeloid promoter showed initial evidence of restored NADPH activity and clinical efficacy.[7] However, transgene expression did not reach physiological levels, and the proportion of oxidase-positive cells was variable (1% to 63% at last follow-up).[7] These results, together with recent evidence suggesting that chronic inflammation in CGD may exert a negative effect on HSPC and their transduction, thereby increasing the risk of oncogenesis,[8] further emphasize the need to improve the efficacy and safety of the GT platforms used to treat X-CGD.

The limited cargo capacity of viral vectors also contributes to the many challenges of achieving clinically relevant yet tight physiological expression and regulation of a transgene. Transgene size has hampered the production of high-titer lentiviral vectors in diseases such as CGD and β-thalassemia, in which the endogenous locus control region is too big to be included within the viral vector, making obtaining sufficient levels of transgene expression challenging. Several groups have developed transduction enhancers that could, in principle, result in sufficient transgene copies even with low-titer vectors.[9] Here, thanks to the bioinformatics-guided design of a lentiviral vector to express CYBB gene from a minimal endogenous enhancer-promoter region, Wang et al have achieved increased transduction levels while preserving physiological expression of the corrective gene. The enhancer-promoter optimization has allowed Wang et al to significantly reduce the cargo size, thus improving vector titers and transduction efficacy that contribute, together with the improved expression profiles, to functional restoration of immune cells deriving from modified HSPC. This level of fine regulation could reduce the risk associated with non-physiological levels of gp91^{phox} expression in HSPC potentially triggering aberrant reactive oxygen species production.[2] The newly designed vector

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