the fat-to-cell ratio was lower in young subjects. Although no association between age and HSPCs were evident, myeloblasts and proerythroblasts were both more frequent and smaller by their cell size in young marrows. These findings raise a captivating follow-up question. Could myeloid precursor cells be more sensitive to aging-related alterations than the stem cells these originate from?

The authors confirmed the occurrence of periosteal and perivascular niches by demonstrating that HSPCs were positioned more proximal to bone and vasculature than would occur by chance. In addition, HSPCs were also commonly located close to megakaryocytes, which were instead located distally to bone and blood vessels. Although HSPC proximity to bone and vasculature did not vary by age, their proximity to megakaryocytes was more frequent in young marrows. Megakaryocytes in younger subjects were also larger in size, but it remains unclear whether HSPCs clustered in the vicinity of large megakaryocytes. Collectively, these results indicate that a distinct megakaryocyte niche could modulate HSPC activity in young marrows. The potential megakaryocytic niche raises intriguing questions for future studies about its role beyond normal hematopoiesis. Could megakaryocytes protect HSPCs from genotoxic insults? Could a decline in megakaryocyte niches be pharmacologically prevented?

Although this study may not have immediate clinical implications, it underscores that at least some hematopoietic cells are not randomly positioned in the human bone marrow and that hematopoiesis is topographically remodeled with age. The diagnostic evaluation and staging of hematological neoplasms are conventionally based on cytomorphology, histopathology, flow cytometry, karyotyping, and genomics. Spatial information such as the proximity of 2 distinct cells or histological structures could reveal important intercellular signaling activity and be translated to clinically relevant prognostic information or treatment biomarkers. Looking ahead, future studies are also necessary to explore remodeling patterns found in different hematological diseases and patient subgroups.

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## REFERENCES

1. Sarachakov A, Varlamova A, Svelolkin V, et al. Spatial mapping of human hematopoiesis at single-cell resolution reveals aging-associated topographic remodeling. *Blood*. 2023;142(26):2282-2295.
2. Boulais PE, Frenette PS. Making sense of hematopoietic stem cell niches. *Blood*. 2015;125(17):2621-2629.
3. Acar M, Kocherlakota KS, Murphy MM, et al. Deep imaging of bone marrow shows non-dividing stem cells are mainly perisinusoidal. *Nature*. 2015;526(7571):126-130.
4. Méndez-Ferrer S, Michurina T V, Ferraro F, et al. Mesenchymal and haematopoietic stem cells form a unique bone marrow niche. *Nature*. 2010;466(7308):829-834.
5. Xie Y, Yin T, Wiegraebe W, et al. Detection of functional haematopoietic stem cell niche using real-time imaging. *Nature*. 2009;457(7225):97-101.
6. Ciregan D, Meier U, Schmidhuber J. Multi-column deep neural networks for image classification. *2012 IEEE Conf. Comput. Vis. pattern Recognit*. 2012:3642-3649.
7. Coudray N, Ocampo PS, Sakellaropoulos T, et al. Classification and mutation prediction from non–small cell lung cancer histopathology images using deep learning. *Nat Med*. 2018;24(10):1559-1567.
8. Dembrower K, Crippa A, Colón E, Eklund M, Strand F; ScreenTrustCAD Trial Consortium. Artificial intelligence for breast cancer detection in screening mammography in Sweden: a prospective, population-based, paired-reader, non-inferiority study. *Lancet Digit Health*. 2023;5(10):e703-e711.
9. Walter W, Pohlkamp C, Meggendorfer M, et al. Artificial intelligence in hematological diagnostics: game changer or gadget? *Blood Rev*. 2023;58:101019.

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**LYMPHOID NEOPLASIA**

*Comment on Haebe et al, page 2296*

# Energy overpowers sweet tooth in FL

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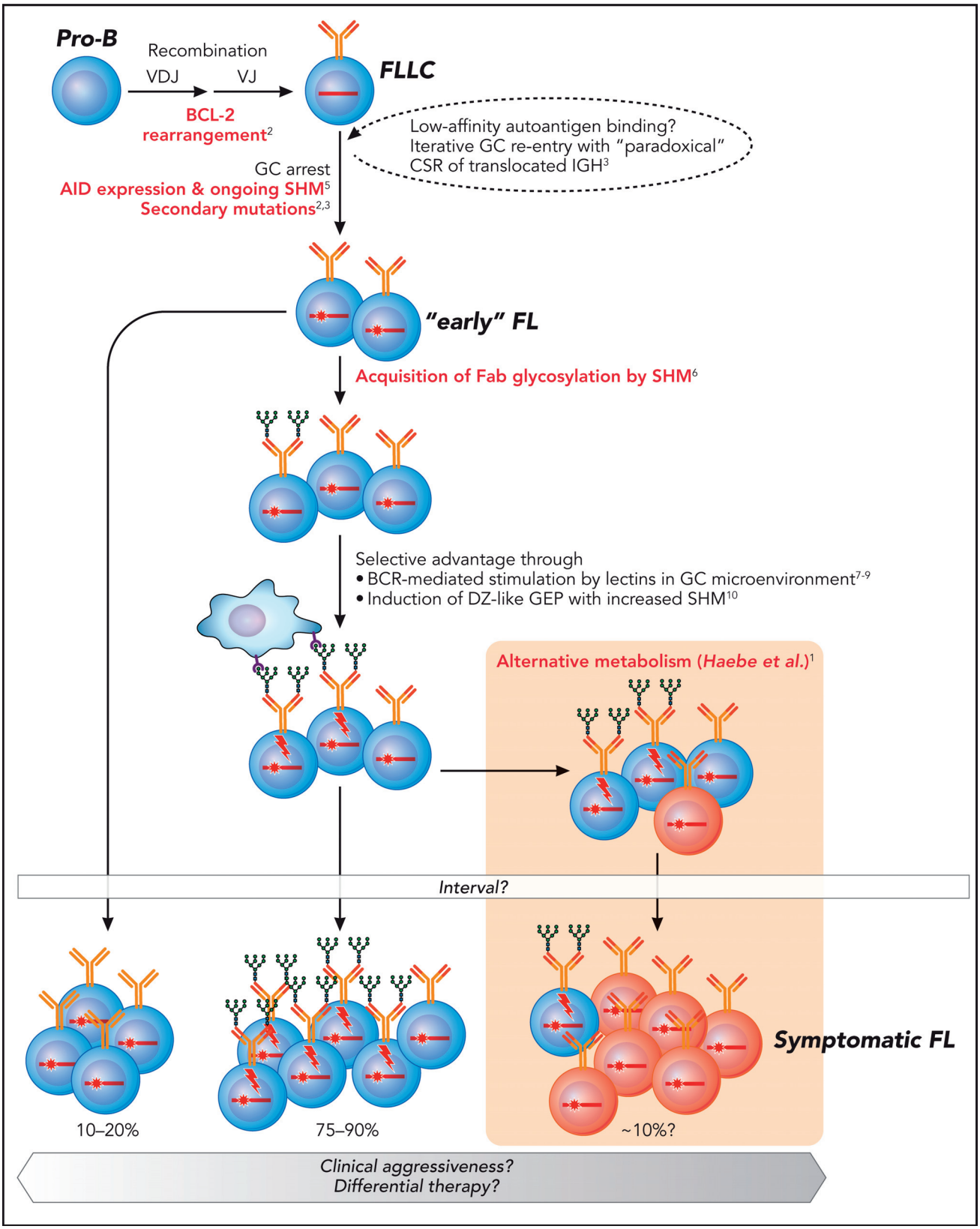
**In this issue of *Blood*, Haebe et al[1] demonstrate by single-cell sequencing that an accepted dominant driver of follicular lymphoma (FL) (ie, stimulation by its clonotypic B-cell receptor) can apparently be outcompeted by upregulation of generic metabolic pathways.**

As a malignancy of the adaptive immune system, FL is a paradigmatic and fascinating disease for exploring lymphomagenesis: FL frequently does not behave like a "true" malignancy with seeming dormancy for years, and FL histopathology and evolution remain dependent on the germinal center microenvironment. Therefore, a comprehensive description of its peculiar pathophysiology, which evidently still follows many rules of the normal adaptive immune system, requires integration of functional genetics of oncogenic mutations with mechanistic understanding of physiological and aberrant immune signaling in the germinal center. In other words, cancer geneticists and immunologists have to integrate their knowledge and skills to fully understand FL. The work by Haebe et al provides an intriguing example of such an integrated approach.

Functional genetics has successfully identified and characterized recurrent mutations in FL, ranging from the seminal B-cell lymphoma 2 (BCL2) gene translocation occurring during VDJ recombination as the common founding event, followed by the chromatin-modifying variants that prolong or increase the sojourn of the cells in the germinal center, to numerous activating mutations in signaling pathways (see figure).[2,3] Although we have just begun to decipher the complex interactions between FL cells and the cellular germinal center microenvironment at the molecular level,[4] we consider FL as neoplastic B cells that are arrested at the germinal center stage with continuous activity




Model of FL pathogenesis with integration of genetic and immunologic drivers. The findings of Haebe et al are highlighted by the peach-colored field. Occurrence of lymphomagenic driver events is highlighted in red. AID, activation-induced deaminase; BCL2, B-cell lymphoma 2 gene; BCR, B-cell receptor; CSR, class switch recombination; DZ, dark zone; FLLC, FL-like cell; GC, germinal center; GEP, gene expression profile; IgH, immunoglobulin heavy chain; SHM, somatic hypermutation. Professional illustration by Patrick Lane, ScEYEnce Studios.

of activation-induced deaminase, the enzyme initiating somatic hypermutation.[5]

Irrespective of oncogenic mutations, simple but meticulous analyses of the mutational patterns in the clonal B-cell receptor expressed by FL cases led to a striking discovery over 20 years ago[6]: Most FL cases express a B-cell receptor (BCR) that has acquired ≥1 N-linked glycosylation motifs in their Fab portions by somatic hypermutation. High-mannose residues attached at these N motifs render FL cells susceptible to BCR-mediated stimulation by lectins presented by the FL microenvironment.[7-9] This purely immunologic mechanism exerts an apparently decisive selective advantage. With a similar approach used by Haebe et al, a recent case report demonstrated induction of a dark zone–like gene expression profile and increased somatic hypermutation by acquisition of a Fab N-motif in FL.[10]

The findings by Haebe et al, however, challenge the dominance and power of BCR-driven lymphomagenesis in FL. First, the Levy group, who wrote the current article, deserves credit for conducting a prospective trial with repetitive and extensive cytologic sampling of several anatomic FL localizations over time. Second, they leveraged single-cell RNA sequencing of fresh aspirates with highly consistent quality criteria with respect to sequencing accuracy and potential batch effects. Finally, they integrated gene expression profiling data with BCR sequencing of individual cells and sequencing of recurrent FL driver genes.

On the basis of this approach, they identified 2 of 17 patients whose lymphoma clone was composed of 2 major subclones, one of which expressed a BCR with a particular N motif and the other that lacked that motif. Acquisition of additional or alternative N motifs did not appreciatively influence the stability of the subclones. In contrast to the previous report,[10] a notable aspect of the patients described here was segregation of N-motif–discrepant subclones by anatomic location. Stability of anatomic segregation over time suggests a relatively early segregation event. Unexpectedly, the subclones lacking an N motif were able to outcompete their Fab-glycosylated counterpart, including by anatomic progression with infiltration of additional lymph nodes.

The challenging question then becomes how FL cells that apparently fail to receive continuous BCR-mediated stimulation from the germinal center microenvironment can outcompete their Fab-glycosylated kin. Targeted panel sequencing failed to yield any candidate subclonal genetic driver. However, coexistence of N-motif–discrepant subclones within the same patient provided the essential experimental leverage to uncover differential gene expression profiles between these subclones.[10] By comparative gene expression profiling, Haebe et al first confirmed that the transcriptional program of N-motif–carrying FL cells is characterized by increased expression of immune signaling pathways, including the BCR but also cytokine signaling. In marked contrast, FL cells lacking Fab glycosylation had higher expression of metabolism gene sets and energy sources, including glycolysis, oxidative phosphorylation, fatty acid metabolism, and mammalian target of rapamycin complex 1 signaling.

These findings create a new integrative model of FL pathogenesis that incorporates genetics, immune signaling, and now metabolism (see figure). First, the new model demonstrates that BCR-mediated "paraphysiological or superphysiological" signaling does not necessarily provide a decisive selective advantage over time, as hitherto assumed. Second, the results delineate coexisting FL subclones with clearly different needs for expansion that should lead to equally differentiated therapies that target subclone-specific vulnerabilities.

As predictable for experimental findings that open a new perspective, several research questions arise: Do the anatomic locations influence the gene expression pattern, or do they simply keep the subclones spatially separated? Is the increased metabolism in itself sufficient to provide competitive advantage, or is it the reaction to an as yet unknown other mechanism that drives expansion and therefore increases demands for energy consumption? Are the different drivers detectable by proteomics (eg, phosphorylation in signal transduction pathways) or metabolomic changes (eg, cellular energy consumption)?

Novel questions to be addressed with respect to clinical translation of the results of Haebe et al will include determining whether the identified gene expression profiles dictate also a higher clinical aggressiveness, as suggested by outcompetition. As mentioned by the authors, a true challenge is to develop diagnostic tests that reliably indicate the prevalent gene expression profile without the need to repetitively sample several lymphoma locations. Turning finally to the minority of FL cases that entirely lack Fab glycosylation: Are these the most innocuous cases, or are they also dominated by increased metabolism, perhaps having definitively outcompeted any N-motif–carrying emerging subclones during tumor evolution before clinical diagnosis? As mentioned, these analyses are more challenging because they would require interpatient and intercase comparisons. The new perspective opened by Haebe et al will certainly provide further revelations into the pathogenesis of FL for the foreseeable future.

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## REFERENCES


1. Haebe S, Day G, Czerwinski DK, et al. Follicular lymphoma evolves with a surmountable dependency on acquired glycosylation motifs in the B-cell receptor. *Blood*. 2023;142(26):2296-2304.
2. Kumar E, Pickard L, Okosun J. Pathogenesis of follicular lymphoma: genetics to the microenvironment to clinical translation. *Br J Haematol*. 2021;194(5):810-821.
3. Milpied P, Gandhi AK, Cartron G, et al. Follicular lymphoma dynamics. *Adv Immunol*. 2021;150:43-103.
4. Mourcin F, Verdiere L, Roulois D, et al. Follicular lymphoma triggers phenotypic and functional remodeling of the human lymphoid stromal cell landscape. *Immunity*. 2021;54(8):1788-1806.e1787.
5. Scherer F, Navarrete MA, Bertinetti-Lapatki C, Boehm J, Schmitt-Graeff A, Veelken H. Isotype-switched follicular lymphoma displays dissociation between activation-induced cytidine deaminase expression and somatic hypermutation. *Leuk Lymphoma*. 2016;57(1):151-160.
6. Zhu D, McCarthy H, Ottensmeier CH, Johnson P, Hamblin TJ, Stevenson FK. Acquisition of potential N-glycosylation sites in the immunoglobulin variable region by somatic mutation is a distinctive feature of follicular lymphoma. *Blood*. 2002;99(7):2562-2568.
7. Amin R, Mourcin F, Uhel F, et al. DC-SIGN-expressing macrophages trigger activation of mannosylated IgM B-cell receptor in follicular lymphoma. *Blood*. 2015;126(16):1911-1920.

8. Coelho V, Krysov S, Ghaemmaghami AM, et al. Glycosylation of surface Ig creates a functional bridge between human follicular lymphoma and microenvironmental lectins. *Proc Natl Acad Sci U S A*. 2010;107(43):18587-18592.

9. Schneider D, Duhren-von Minden M, Alkhatib A, et al. Lectins from opportunistic bacteria interact with acquired variable-region glycans of surface immunoglobulin in follicular lymphoma. *Blood*. 2015;125(21):3287-3296.

10. Van Bergen CAM, Kloet SL, Quinten E, et al. Acquisition of a glycosylated B-cell receptor drives follicular lymphoma towards a dark zone phenotype. *Blood Adv*. 2023;7(19):5812-5816.

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THROMBOSIS AND HEMOSTASIS

*Comment on Schönborn et al, page 2305*

# VITT-like disorder HITs the headlines

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**Antibodies to platelet factor 4 (PF4) are central to the pathology of heparin-induced thrombocytopenia (HIT) and vaccine-induced thrombocytopenia and thrombosis (VITT). However, in this issue of *Blood*, Schönborn and colleagues present a new scenario, VITT-like disorder.[1]**

HIT was first described in 1958[2] associated with heparin infusions; HIT is typically associated with a drop in platelet count at a median of 5 to 10 days after initiation of heparin (often unfractionated) and has a prothrombotic phenotype, mainly involving deep vein thrombosis but also arterial thrombosis.[3] Presentation is usually in hospital, and treatment requires use of an alternative nonheparin anticoagulant and avoidance of platelet transfusions despite the thrombocytopenia. Diagnosis is confirmed by assays involving PF4-heparin and immunoglobulin G (IgG) antibodies to this complex.

VITT was identified in 2021[3-5] related to adenoviral vaccines for COVID-19. VITT presents as an acute medical emergency and is associated with thrombocytopenia and thrombosis. It usually occurs 5 or more days (median 14 days) after vaccination, commonly following the first adenoviral COVID-19 vaccination. Thromboses are seen in atypical sites, including central venous sinus thrombosis (CVST) (often in multiple veins) and splanchnic thrombosis, with 20% arterial events (myocardial infarction, stroke, or arterial limb thrombosis) with associated mortality of 20%.[6] Diagnosis is based on very high D-dimer levels and positive anti-PF4 antibody enzyme-linked immunosorbent assay (ELISA). Many automated "fast HIT" assays were not positive in cases of VITT.

What of cases diagnosed as severe HIT, known as spontaneous or autoimmune HIT, that had not had previous heparin exposure? Are some cases presenting as strokes with thrombocytopenia positive for anti-PF4 antibodies, or are these antibodies prevalent in other conditions such as antiphospholipid syndrome?

In their study, Schönborn and colleagues investigated the specific laboratory findings in VITT compared with HIT to determine if a VITT-like disorder was evident before the COVID-19 pandemic. Three main anti-PF4 assays were used in this publication: first, rapid anti-PF4 antibody assays, using chemiluminescence technology (HemosIL AcuStar) (the standard assay is anti-PF4/heparin and a new rapid assay that detects anti-PF4 antibodies, as detected in VITT cases); second, anti-PF4 IgG antibodies detected by ELISA; third, platelet activation assays (only available in highly selective laboratories) demonstrating increased platelet activation with the addition of heparin (HIPA assays) as seen in HIT, compared with increased platelet activation with the addition of PF4 (PIPA assays) confirmed in VITT.

The authors reported a cohort of cases referred for HIT testing. However, their presentation was comparable to VITT, with a strongly positive anti-PF4 IgG antibody by ELISA and platelet activation positive to PF4 (PIPA) and not to heparin (HIPA), as seen in VITT. Furthermore, all samples were positive using the new rapid anti-PF4 antibody test. Clinically, there was no relation to heparin therapy, and the cases were pre-COVID-19 pandemic, therefore unrelated to either primary COVID-19 infection or COVID-19 vaccination. Thromboses were analogous to VITT with CVST and splanchnic bed thrombosis as well as arterial events, usually stroke. Associated thrombocytopenia and markedly raised D-dimer levels were evident. Although the cause of this new VITT-like disorder is not clear, there was confirmed infection in more than 50% of cases. Furthermore, 2 cases had recurrence of presentation a number of years after the first episode.

To aid confirmation of this new disorder and the distinction between the different assays for HIT and VITT-like disorders, a large cohort of normal, healthy controls were tested; these were anti-PF4 antibody negative, a similar finding for cases of stroke and thrombocytopenia and antiphospholipid syndrome. In VITT cases, 99% were positive to the new rapid anti-PF4 assay, although 15.5% were also positive to the rapid anti-PF4/heparin assay. But in platelet activation studies, a platelet stimulation was seen with PF4 but not with heparin. However, in confirmed cases of HIT, 96.9% were positive in the rapid anti-PF4/heparin assay, but 30.5% were also positive in the rapid anti-PF4 assay. Aside from increased platelet activation to heparin (HIPA), more than 50% also demonstrated a response in the presence of PF4 (PIPA).

Preceding the identification of this new condition, VITT-like disorder, which appears comparable to VITT (related to adenoviral vaccines), some HIT cases unrelated to heparin exposure now fulfill a diagnosis of VITT-like disorder.[7,8] Importantly, it is the presentation of life-threatening thrombosis that has implications for treatment. In VITT, we now know therapeutic heparin therapy is not contraindicated, but we have alternative non-heparin-based therapies that can be used. Furthermore, intravenous immunoglobulin was initiated promptly