

involved with DNA replication and cell cycle regulation. The more differentiated subsets had higher expression of cytokine signaling, chemotaxis, and interferon signaling. Analysis of transcription regulation found associations with cancer in the least differentiated group and immune-related genes in the most differentiated groups. One of the hypotheses generated from these results was that clinical manifestation of LCH depends on the mix of less and more differentiated cells within the lesion.¹⁰

Shi et al investigated the molecular alterations in the circulating mononuclear phagocytes in LCH patients by a modified version of single-cell tagged reverse transcription. Transcription profiles identified 11 clusters, including CD14⁺ classical monocytes, CD16⁺⁺ nonclassical monocytes, CD14⁺CD16⁺ intermediate monocytes, a minor cDC1 cluster, a major cDC2 cluster, plasmacytoid DCs, B cells, plasma cells, and immature progenitors. Analysis of differentially expressed genes from the cfBRAF^{V600E} mutational level groups revealed widespread activation of the MAPK pathway, with diverse aberrations in the expression levels of RAS-MAPK-ERK pathway-related genes across the circulating myeloid compartment and the expression levels of genes varying by cfBRAF^{V600E} mutational group and cell subset.

Universal activation of the MAPK pathway in all of the circulating monocyte populations of LCH patients can be due to a bone marrow precursor mutation leading to cell-intrinsic activation of this pathway across all of these lineages or it could be due to cell-extrinsic effects. By looking at a lineage that should not harbor a MAPK-activating mutation, which of these 2 options is correct should be revealed. Any differential expression of MAPK-related signaling in these cells would suggest that cell-extrinsic effects are happening and are dependent on cell type. Although hypothesized, the investigators did not provide evidence that the only cells harboring the BRAF^{V600E} mutation expressed MAPK DEGs. Simultaneous detection of DNA mutations and RNA transcripts at a same single cell could address this problem in future studies.

One caveat of their study is that the overall number of sequenced cells is small. Moreover, the control cells were not evenly distributed among the different cell types. Hence, it is not clear whether the overall

transcriptomic landscape is affected by the level of BRAF^{V600E}, especially given the intriguing observation of widespread MAPK activation across all cell types. Another confounding finding is the relatively large number of high-risk multisystem LCH patients without cfBRAF^{V600E}. It needs to be determined whether this was due to the heterogeneity of LCH and racial diversity or to presence of additional MAPK mutations that were not tested.

This study significantly advances our understanding of LCH immunopathogenesis, indicating that the oncogenic BRAF^{V600E} mutation may lead to activation of the MAPK pathway in LCH lesions, as well as in circulating mononuclear phagocytes, the presumed precursors of tissue LCH cells. However, the data leave unanswered questions, many of which will require additional functional validation using in vitro and in vivo models. How does one reconcile the data from that study with previous results indicating that multisystem and single-lesion diseases are derived from cells with different maturational status? Will patients with neurodegenerative LCH have the same composition of peripheral mononuclear phagocytes? Are the observations made in that study a common theme in histiocytic disorders, such as Erdheim-Chester disease and juvenile xanthogranuloma?

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PHAGOCYTES, GRANULOCYTES, AND MYELOPOIESIS

Comment on Tsaknakis et al, page 1249

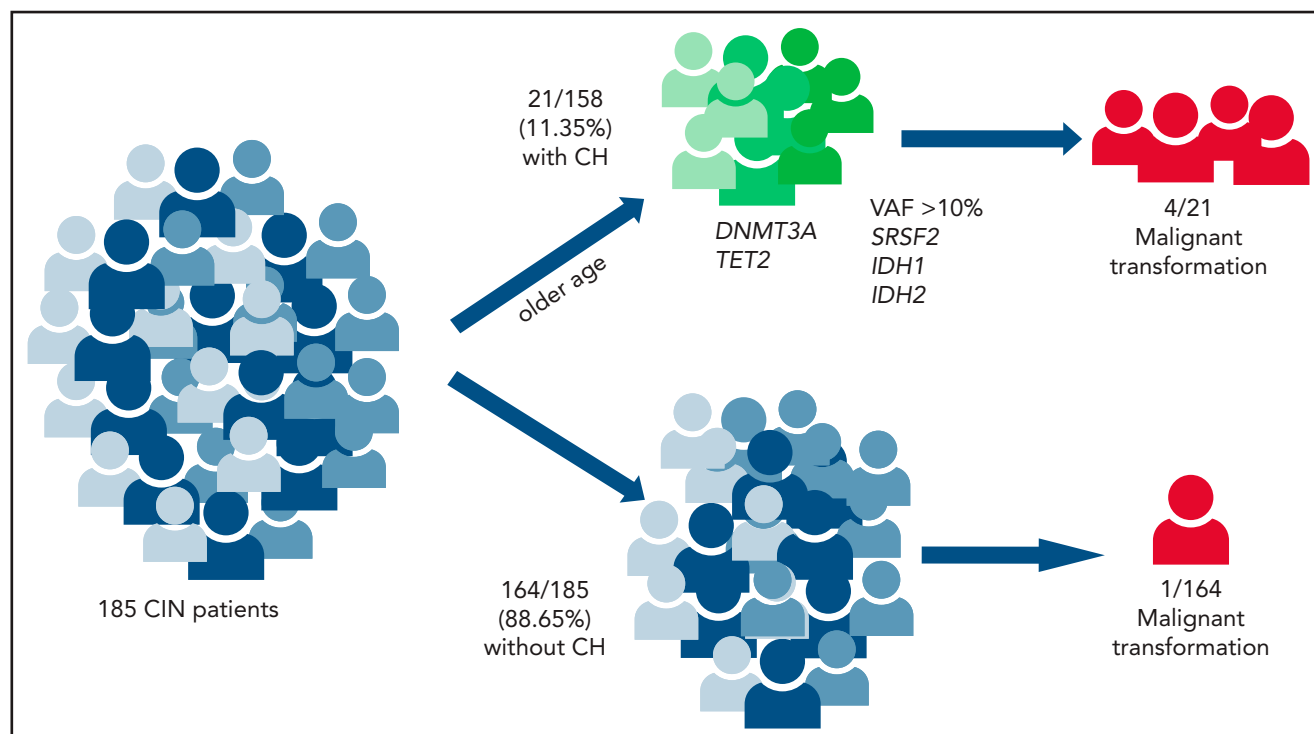
Clonal hematopoiesis in CIN

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In this issue of *Blood*, Tsaknakis and colleagues¹ used targeted next-generation sequencing (NGS) to determine the frequency and clinical significance of clonal hematopoiesis (CH) in a cohort of 185 patients with chronic idiopathic neutropenia (CIN).

CH is a common, aging-related phenomenon involving the expansion of hematopoietic stem cells with somatic mutations. The prevalence of CH varies

with the detection method and variant allele frequency (VAF) threshold for defining clonality. That said, >10% of healthy individuals will have CH (defined



CH and progression to myeloid malignancy in CIN. Patients with CIN ($n = 185$) were assessed for CH by using targeted NGS. CH was detected in 21 (11.35%) of them. Progression to myeloid malignancy was associated with VAF >10% and mutations in *SRSF2* or *IDH1/2*. Created with BioRender.com.

as a VAF of $\geq 2\%$) by the age of 80 years.^{2,3} Importantly, although the presence of CH confers a significantly increased risk of the subsequent development of a hematologic malignancy, the overall incidence of such malignancies among individuals with CH is low.^{2,3} Thus, an understanding of the factors associated with the existence and evolution of CH is crucial to the identification of individuals who may warrant more vigilant monitoring.

Chronic idiopathic neutropenia (CIN) is defined as isolated neutropenia without a known underlying congenital or acquired cause. CIN occurs in late childhood or adulthood, affects primarily women, and typically runs a benign course. CIN, also known as idiopathic cytopenia of undetermined significance-CIN (ICUS-N), is a subtype of ICUS. ICUS is characterized by persistent cytopenias of ≥ 1 blood lineages without meeting criteria for a myelodysplastic syndrome (MDS) or having other known etiologies.⁴ In previous studies, CH was detected in more than one-third of patients with ICUS (in which case it is known as clonal cytopenia of undetermined significance (CCUS) and was highly predictive of the subsequent development of a myeloid malignancy.^{5,6} The frequency of patients

with isolated neutropenia (ICUS-N/CIN) in these prior studies of ICUS/CCUS was low, however, and therefore the specific incidence and prognosis of CH in these particular patients is not clear.

Tsaknakis and colleagues examined the frequency and clinical significance of CH in 185 patients with CIN. Using targeted NGS of genes recurrently mutated in myeloid malignancies, they found 21 of 185 (11.35%) with CH (defined as VAF $\geq 2\%$) and 164 of 185 (88.65%) without. Those with CH were significantly older than non-CH patients and had lower platelet counts. Notably, the presence of CH was not associated with the severity of neutropenia. Most patients (18 of 21) with CH had only 1 mutation, whereas 2 patients had 2 mutations each and 1 patient had 3, for a total of 25 somatic mutations in the 21 patients. Consistent with studies of CH in the general population, the most frequently mutated genes were *DNMT3A* and *TET2*.

Over the course of the study (median follow-up, 132 months), 5 patients with CIN developed myeloid malignancies (4 from the CH group and 1 from the non-CH group), demonstrating a significantly elevated risk (relative risk, 31.24) of

transformation in the presence of CH. The VAFs of the mutant clones were >10% in all of the cases that transformed, and the most frequently mutated genes in those cases were *SRSF2* and *IDH1/2*. In fact, all patients with *SRSF2* and *IDH1/2* mutations developed MDS or leukemia. Finally, 9 patients with CH had serial sampling for NGS. Of those 9 patients, 3 transformed into myeloid malignancy, and transformation was preceded by expansion of the variant clone. Notably, predictors of transformation in this study were similar to those previously reported in CH, which include the presence of multiple mutations, VAF >10%, the presence of mutations in specific high-risk genes (including *TP53*, *IDH1/2*, *RUNX1*, *PHF6*, and spliceosome genes), and altered red blood cell indices.^{2,7,8} Also consistent with previous data,⁷ isolated mutations in *DNMT3A* or *TET2*, although common among the patients with CH, were not predictive of transformation over the duration of this study.

Together, the new data show that, although CH confers an increased risk of malignancy in patients with CIN, the overall frequency of CH was much lower in this specific population (11%) than in prior studies of the broader ICUS population

(>30%). The overall incidence of transformation was also lower in patients with CIN than in all patients with ICUS (2.7% vs 25%).⁵ Thus, this study highlights the unique nature of CIN as a subset of ICUS with a more favorable clinical course. Nevertheless, although much lower than the prevalence in the whole ICUS population, the relative prevalence of CH in patients with CIN who are <70 years of age was significantly higher than in age-matched individuals from the general population based on historical studies (relative prevalence, 2.56).^{2,3,9} Aside from age, clinical features, such as degree of neutropenia and other hematopoietic parameters (except for platelet counts, which were lower in the CH group but still within normal range and possibly explained by their older age), were not associated with CH. Therefore, CH should be considered in all patients with CIN. There are few specific recommendations for monitoring of CH in these patients; however, Tsaknakis et al noted that they follow up on patients with clonal CIN by using serial peripheral blood (PB) analysis and NGS and perform bone marrow analyses for concerning changes in PB counts or morphology. This approach seems reasonable, and the finding of an elevated (>10%) or increasing VAF or the presence of high-risk mutations should prompt concern for possible transformation.

The reasons for the reduced CH and transformation in CIN compared with other ICUS variants are not clear. Many gaps remain in the understanding of the pathogenesis of ICUS, as well as the development and progression of CH. Recent studies suggest that various hematopoietic stressors such as genotoxic stress, ribosome biogenesis stress, and inflammation can promote the development and progression of CH.¹⁰ Whether some of the same stressors contribute to cytopenias in ICUS remains to be determined. Further studies are clearly needed to elucidate the intrinsic and extrinsic drivers of both ICUS and CH and to identify the mechanisms by which specific mutations and environmental factors interplay to suppress normal hematopoiesis and to promote the expansion and/or transformation of mutant hematopoietic stem cells. Ultimately, better mechanistic insight will inform the identification and treatment of patients with cytopenia who are at risk for CH and malignant transformation.

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THROMBOSIS AND HEMOSTASIS

Comment on Greinacher et al, page 1269

COVID-19 and VITT: same or different?

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In this issue of *Blood*, Greinacher and colleagues¹ examine humoral responses in vaccine-induced thrombotic thrombocytopenia (VITT) and COVID-19 to determine whether these illnesses are immunologically distinct or represent a disease continuum.

At a surface level, vaccine-induced thrombotic thrombocytopenia (VITT), a recently described complication of adenoviral-based COVID-19 vaccines, and COVID-19 have much in common. Both illnesses owe their origins to severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), with COVID-19 being caused by infection with the SARS-CoV-2 virus, whereas VITT is precipitated by vaccines encoding the SARS-CoV-2 proteins. Both conditions harness the immune system to powerful effect. Severe COVID-19 is associated with a heightened inflammatory response, characterized by increased levels of proinflammatory cytokines and markedly elevated levels of IgG and IgA antibodies to the receptor binding domain and spike protein,^{2,3} whereas VITT is also a byproduct of an aberrant immune response to vaccination. Mortality in both diseases is closely

linked to hypercoagulability. High rates of venous thrombosis are reported in patients with severe COVID-19 infection⁴ and in those with VITT,^{5,6} with both illnesses accompanied by marked abnormalities in coagulation.⁵⁻⁷

However, when viewed clinically, the 2 diseases are dissimilar. COVID-19 is a viral infection, whereas VITT is not.⁸ COVID-19 disproportionately affects individuals with comorbidities that include older age, hypertension, and cardiovascular disease, whereas VITT occurs in healthy individuals with no discernible risk factors.⁸ Venous thrombosis in COVID-19 is linked to disease severity and occurs in predictable locations, such as the lower legs or pulmonary bed,⁹ unlike thrombosis in VITT, which often manifests in atypical locations, such as the cerebral and splanchnic vascular beds.^{5,6}