REGULAR ARTICLE

blood advances

Effects of IL-1β inhibition on anemia and clonal hematopoiesis in the randomized CANTOS trial

Janghee Woo,[1] Darlene Lu,[1] Andrew Lewandowski,[1] Huilei Xu,[1] Pablo Serrano,[1] Margaret Healey,[1] Denise P. Yates,[1] Michael T. Beste,[1] Peter Libby,[2] Paul M. Ridker,[2,3] and David P. Steensma[1]

[1]Novartis Institutes for BioMedical Research, Cambridge, MA; [2]Cardiovascular Division, Brigham and Women's Hospital, Harvard Medical School, Boston, MA; and [3]Center for Cardiovascular Disease Prevention, Brigham and Women's Hospital, Boston, MA

Key Points

- CH mutations and anemia enrich proteomic signatures associated with inflammation compared with only CH mutations or no CH mutations.
- Canakinumab treatment is associated with improved hemoglobin response in patients with concurrent anemia and CH mutations.

Canakinumab, a monoclonal antibody targeting proinflammatory cytokine interleukin-1β (IL-1β), improved hemoglobin levels while preventing recurrent cardiovascular events in the Canakinumab Anti-inflammatory Thrombosis Outcomes Study (CANTOS). This cardiovascular (CV) preventive effect was greater in patients with *TET2* mutations associated with clonal hematopoiesis (CH). The current proteogenomic analysis aimed to understand the clinical response to canakinumab and underlying proteomic profiles in the context of CH and anemia. The analysis included 4595 patients from the CANTOS study who received either canakinumab or placebo and evaluated multiplexed proteomics (4785 proteins) using SomaScan and targeted deep sequencing for CH mutations. Incident anemia was more common in the presence of CH mutations but reduced by canakinumab treatment. Canakinumab treatment was significantly associated with higher hemoglobin increment in patients with concurrent CH mutations and anemia than patients with CH mutations without anemia or without CH mutations. Compared with those without CH mutations, the presence of CH mutations was associated with proteomic signatures of inflammation and defense response to infection, as well as markers of high-risk CV disease which was further enhanced by the presence of anemia. Canakinumab suppressed hepcidin, proinflammatory cytokines, myeloid activation, and complement pathways, and reversed pathologically deregulated pathways to a greater extent in patients with CH mutations and anemia. These molecular findings provide evidence of the clinical use of IL-1β blockade and support further study of canakinumab for patients with concurrent anemia and CH mutations. This study was registered at www.clinicaltrials.gov as #NCT01327846.

Introduction

Anemia of inflammation (AI) is a condition of usually mild to moderately decreased hemoglobin observed in systemic inflammatory conditions, including various autoimmune diseases, obesity, diabetes mellitus, cardiovascular disease, and cancer.[1] Inflammation onset is accompanied by an increase in

Submitted 5 September 2023; accepted 29 October 2023; prepublished online on *Blood Advances* First Edition 7 November 2023; final version published online 14 December 2023. https://doi.org/10.1182/bloodadvances.2023011578.

Novartis is committed to sharing with qualified external researchers, access to patient-level data, and supporting clinical documents from eligible studies. These requests are reviewed and approved by an independent review panel based on scientific merit.

Data availability is according to the criteria and process described in the data request portal at www.clinicalstudydatarequest.com.

The full-text version of this article contains a data supplement.

inflammatory cytokines, such as interleukin-6 (IL-6) and IL-1β, which in turn causes skewed myeloid differentiation and hypoferremia via an upregulation of hepcidin, a master regulator of iron homeostasis that is produced by hepatocytes in response to cytokine signals.[1-3] Hepcidin prevents iron absorption in the small intestine and iron release from macrophages, leading to impaired erythropoiesis and enhanced erythrophagocytosis.[1,3,4]

Clonal hematopoiesis of indeterminate potential (CHIP) is a hematological condition defined by the expansion of hematopoietic clones driven by somatic mutations in hematopoietic stem and progenitor cells in people without overt bone marrow disorders.[5] CHIP is common in older adults, confers an increased risk of hematological cancer and cardiovascular disease, and is associated with increased overall mortality. Notably, a genome-wide analysis of >4000 individuals with CHIP showed that somatic mutations in *DNMT3A*, *TET2*, or *ASXL1* genes were accompanied by increased levels of circulating IL-1β and IL-6.[6] Moreover, the expression of inflammasome-related genes, including *IL-1B* and *IL-18*, increased from non-CHIP through CHIP to lower-risk myelodysplastic syndromes.[7] Patients with clonal cytopenia of undetermined significance (CCUS), a condition defined by CHIP plus anemia or another cytopenia, have inflammatory cytokine levels as high as those observed in patients with lower-risk myelodysplastic syndromes.[8] In addition, multiple preclinical studies suggest that clonal expansion in CH may increase in response to bacteria-induced systemic inflammation,[9-14] and this effect may be exacerbated by older age.[13,14]

A recent large clinical trial of canakinumab, a monoclonal antibody that directly neutralizes IL-1β, investigated the molecular effects of IL-1β blockade in patients with high cardiovascular (CV) risk. The Canakinumab Anti-inflammatory Thrombosis Outcome Study (CANTOS; NCT01327846) enrolled 10 061 patients with a history of myocardial infarction and baseline levels of high-sensitivity C-reactive protein (hsCRP) ≥ 2 mg/L.[15] Treatment with canakinumab prevented recurrent CV events,[15] the effect being more pronounced in patients with *TET2* variants treated with canakinumab.[16] A further exploratory biomarker analysis of CANTOS revealed that canakinumab treatment was associated with reduced incident anemia and improved hemoglobin levels compared with placebo, suggesting that targeting the IL-1β pathway may provide clinical benefit to patients with AI.[17] This proteogenomic analysis of CANTOS aimed to identify the effect of canakinumab on anemia in patients stratified based on the presence and type of CH mutations and to characterize the biological consequences of canakinumab treatment in these patient subgroups by integrated biomarker analyses.

Methods

Study patients and samples

The CANTOS study was conducted from April 2011 to June 2017 across 39 countries and sponsored by Novartis AG (Basel, Switzerland); patients were followed up for a median of 3.7 years.[15] The study had been approved by local institutional review boards, and all patients provided written informed consent, including a separate informed consent for patients who provided additional blood samples for genetic analyses and direct biomarker analysis. A total of 3946 DNA samples were collected from patients participating in the genomic substudy, of which a total of 3923 passed the quality control for the custom gene sequencing analysis. Patients participating in the biomarker substudy provided 10 mL blood samples at baseline, month 3, and month 12 of study enrollment. Proteomic analysis was conducted in serum samples at these time points from the 4595 patients who also agreed to the additional research.

Targeted genomic sequencing, SomaScan proteomic assay, and circulating cytokines

Genomic DNA sequencing, proteomic assay, and circulating cytokine assays were performed as previously described.[16,18-20] In brief, the presence of CH mutations at baseline was measured using 74-gene targeted genomic sequencing[16]; additionally, serum samples from pretreatment, month 3, and month 12 time points were analyzed using multiplexed proteomic assays (SomaScan, measuring 4785 unique proteins) and 8 individual cytokine enzyme-linked immunosorbent assays.[18,20] The supplemental Appendix provides additional details.

Statistical analysis

All analyses were conducted using SAS version 9.4 TS1M6 (Statistical Analysis System Institute, Cary, NC) and R version 3.6.1 (The R Foundation for Statistical Computing, Vienna, Austria).

Baseline characteristics were summarized descriptively (mean and standard deviation). Odds ratios (ORs) for associations of baseline characteristics and CH mutations were calculated using logistic regression adjusted for continuous age (years). Patients across all dose levels of canakinumab (50 mg, 150 mg, or 300 mg) were pooled for all analyses in this data set.

For patients without anemia at baseline, hazard ratios (HRs), and 95% confidence intervals (CIs) for association between incidence of anemia and CH mutations were determined using a Cox proportional hazards regression adjusted for age, baseline hemoglobin levels, and baseline hsCRP. Other baseline covariates were considered (ie, sex), but the final model was decided based on prior findings and clinical relevance. For patients with anemia at baseline, linear mixed-effects models were used to estimate mean treatment effects on hemoglobin levels over time. The models assumed an unstructured covariance matrix and included fixed effects for treatment groups, time points, baseline hemoglobin level, and interactions between treatment groups and time, in which time was included as a quadratic term. A model considering time as a categorical factor was also considered but not reported because similar results were obtained. The *P* value for the interaction between time (as a quadratic term) and treatment is reported in the corresponding figure(s). For CH-risk groups,[21] a linear model was used that included a treatment by risk-group interaction with covariates including age, baseline hemoglobin, and baseline hsCRP. The nominal *P* value tested the pairwise comparison of treatment within each risk group. Additional details regarding proteomic analysis are provided in the supplemental Appendix.

Results

Baseline patient and disease characteristics

As reported earlier,[15] patients in CANTOS randomly received canakinumab 50 mg, 150 mg, or 300 mg subcutaneously every

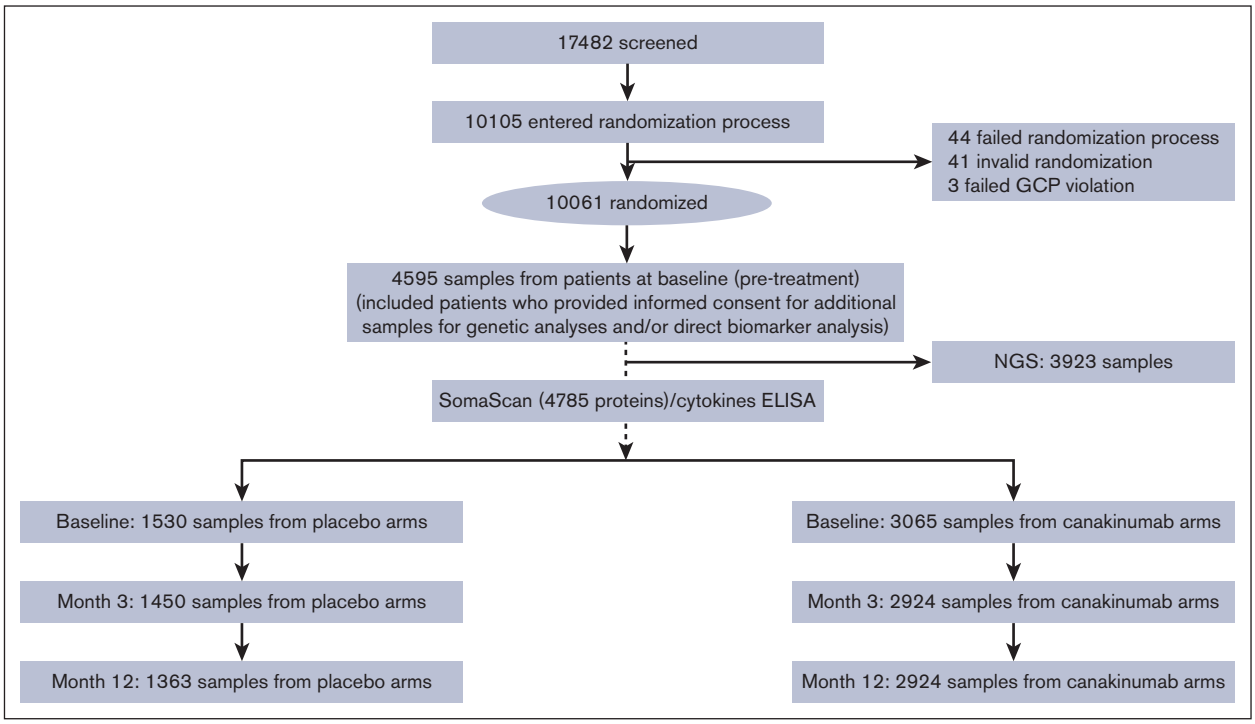

Figure 1. CONSORT flowchart of patients from the CANTOS study used for proteogenomic analysis. ELISA, enzyme-linked immunosorbent assay; GCP, Good Clinical Practice; NGS, next-generation sequencing.

3 months or a matching placebo. Among 4595 samples included in the proteogenomic analysis, 3065 patients received canakinumab and 1530 received placebo (Figure 1).

Eligible patients with samples available for the proteogenomic analysis were representative of the overall CANTOS population with similar baseline characteristics (Table 1). As expected, patients with CH mutations had a higher mean and median age compared with the overall patient population. Mean and median levels of hsCRP and IL-6 at baseline were highest in patients with baseline anemia and CH mutations. Of note, the highest rate of heart failure was observed in the patients with baseline anemia and CH mutations (19% for no CH vs 35% for CH and anemia).

Canakinumab reduces the risk of incident anemia, whereas CH mutations increase the risk of incident anemia in patients without anemia

Among patients with or without anemia at baseline, hemoglobin levels for each CH mutation group were similar compared with those without CH mutations (supplemental Figure 1A). The impact of CH mutations and canakinumab treatment was assessed in patients without baseline anemia. Canakinumab treatment, compared with placebo, resulted in less incidence of anemia in patients without CH mutations (n = 3195; HR, 0.72 [95% CI, 0.61-0.84]; $P < .0001$; supplemental Figure 1B, left). In patients with CH mutations (n = 279), a similar treatment effect due to canakinumab treatment was observed, but the difference did not reach statistical significance, likely because of the lower sample size: HR = 0.77 (95% CI, 0.49-1.19); $P = .238$ (supplemental Figure 1B, right). The presence of CH mutations was associated with a higher incidence of anemia in both placebo and canakinumab treatment groups, confirming the enhanced risk of incident anemia associated with CH mutations (supplemental Figure 1C).

Canakinumab treatment is associated with a significant increase of hemoglobin levels in patients with concurrent CH mutations and anemia

In patients with baseline anemia and without CH mutations, we observed no treatment effect by quadratic-time interaction in hemoglobin levels because of canakinumab ($P = .436$; Figure 2A, first panel). However, in patients with concurrent CH mutations and anemia, a significant treatment effect by quadratic-time interaction on hemoglobin levels was observed because of canakinumab treatment ($P < .001$; Figure 2A, second panel). Similar effects were observed for *TET2* mutations ($P < .001$; Figure 2A, third panel); the number of patients with *DNMT3A* mutations (n = 6; n = 1 treated with placebo) was insufficient to perform hypothesis testing (Figure 2A, fourth panel). In contrast, in patients with "other" mutations (CH mutations besides *TET2* and *DNMT3A*) (Figure 2A, fifth panel), the difference did not reach statistical significance until after month 20.

Patients with CH mutations, who were treated with canakinumab (n = 221), had significantly higher hemoglobin levels in those with baseline anemia, at all time points from 3 months onwards, compared with patients without baseline anemia, despite starting at a lower hemoglobin level (Figure 2B).

In addition, patients with more severe anemia (hemoglobin levels < 11 g/dL; supplemental Figure 2A, left) treated with canakinumab had a numerically higher change from baseline to month

Table 1. Baseline patient and disease characteristics

Baseline characteristic, MEAN (SD)	CANTOS (N = 10 061)	Proteomic analysis set (n = 4595)	Proteogenomic analysis set (n = 3458)	No CH (n = 3154*)	CH (n = 304*)	CH without baseline anemia (n = 250†)	CH with baseline anemia (n = 54*)
Age, y	61.1 (10.04)	61.95 (9.71)	61.98 (9.67)	61.56 (9.6)	66.34 (9.27)	65.49 (8.99)	70.26 (9.65)
Female, n (%)	2587 (25.7)	1168 (25.4)	858 (24.8)	776 (24.6)	82 (27.0)	68 (27.2)	14 (25.9)
BMI, kg/m^2	30.64 (5.95)	31.28 (6.05)	31.2 (5.88)	31.37 (5.91)	29.48 (5.3)	29.55 (4.88)	29.16 (6.97)
hsCRP, mg/L	6.62 (12.45)	6.65 (15.7)	6.54 (17.46)	6.59 (18.21)	6.02 (5.33)	5.7 (5.14)	7.55 (5.97)
Hemoglobin level, g/L	141.91 (14.98)	142.45 (14.71)	142.9 (14.34)	143.1 (14.26)	140.83 (14.97)	145.43 (11.91)	119.52 (7.29)
Type 2 diabetes mellitus, n (%)	4029 (40)	1905 (41)	1404 (41)	1286 (41)	118 (39)	98 (39)	20 (37)
Hypertension, n (%)	8008 (80)	3729 (81)	2789 (81)	2529 (80)	260 (86)	214 (86)	46 (85)
Heart failure, n (%)	2173 (22)	915 (20)	683 (20)	600 (19)	83 (27)	64 (26)	19 (35)
Anemia, n (%)†	1316 (13.1)	570 (12.4)	399 (11.5)	345 (10.9)	54 (17.8)	0 (0)	54 (100)
IL-6, ng/mL	4.07 (6.30)	4.07 (6.37)	4.02 (6.36)	4 (6.41)	4.14 (5.73)	3.64 (3.85)	6.38 (10.43)
IL-18, ng/mL	287.35 (139.46)	285.28 (138.59)	286.49 (139.64)	287.27 (141.23)	278.18 (121.41)	283.1 (120.9)	256.1 (122.38)
Hepcidin, log2RFU	N/A	13.92 (1.27)	13.92 (1.24)	13.94 (1.21)	13.72 (1.47)	13.82 (1.28)	13.26 (2.11)
Platelet count, g/L	232.44 (68.12)	229.97 (68.18)	232.42 (67.15)	232.74 (66.74)	229.18 (71.25)	231.71 (71.77)	217.48 (68.23)
IL-1 RA, ng/mL	590.58 (477.5)	591.48 (484.89)	593.52 (477.77)	600.53 (489.83)	519.74 (316.19)	531.22 (327.13)	467.58 (256.94)
TNFα, ng/mL	2.55 (6.41)	2.48 (6.19)	2.5 (6.77)	2.46 (6.79)	2.89 (6.46)	2.98 (7.12)	2.48 (1.3)
IL1β, log2RFU	N/A	11.61 (0.48)	11.6 (0.46)	11.6 (0.45)	11.61 (0.53)	11.61 (0.5)	11.6 (0.69)
Treatment, n (%)							
Placebo	3344 (33)	1530 (33)	1144 (33)	1047 (33)	97 (32)	82 (33)	15 (28)
50-mg canakinumab	2170 (22)	998 (22)	762 (22)	692 (22)	70 (23)	58 (23)	12 (22)
150-mg canakinumab	2284 (23)	1062 (23)	814 (24)	743 (24)	71 (23)	58 (23)	13 (24)
300-mg canakinumab	2263 (22)	1005 (22)	738 (21)	672 (21)	66 (22)	52 (21)	14 (26)

BMI, body mass index; Q, quartile; RA, receptor agonist; RFU, relative fluorescent units; SD, standard deviation.
*Number of patients with baseline proteomic and genomic data available. N = 3585, 338, or 59 for No CHIP, CHIP, or CHIP with baseline anemia with only genomic data, respectively.
†Anemia was defined as baseline Hb <120 g/L for females or <130 g/L for males.

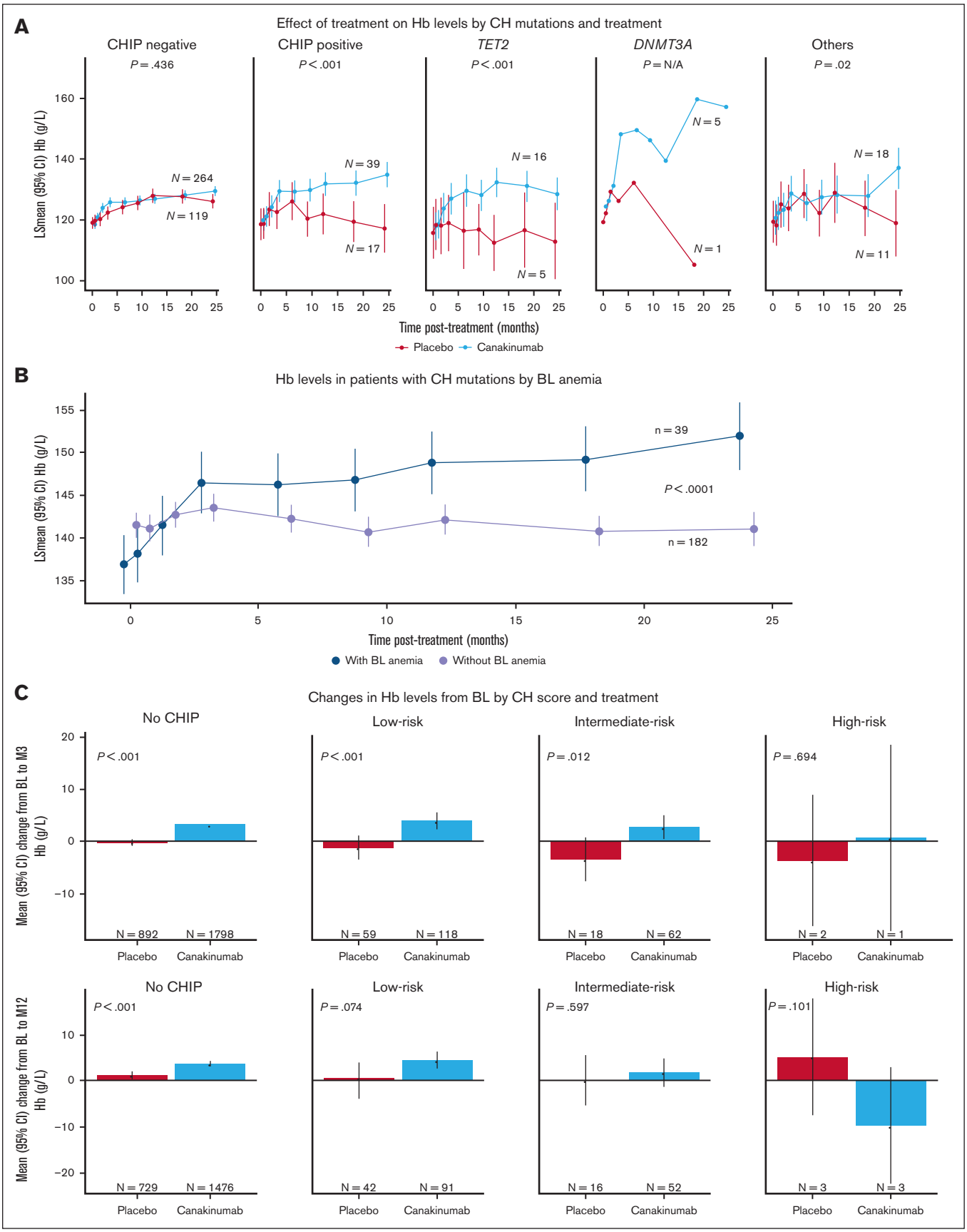

Figure 2.

3 by 2 g/dL than that in patients treated with placebo (supplemental Figure 2A, left) and patients with less severe anemia in both canakinumab and placebo arms (less severe anemia defined by hemoglobin levels ≥ 11 g/dL; supplemental Figure 2A, right). Because of the small sample size, these observations were purely descriptive and statistical testing was not performed.

We then applied a recently published clonal hematopoiesis risk score[21] to the CANTOS population to examine whether hemoglobin response to canakinumab is influenced by CH-risk group (Figure 2C). Although there were few patients in the high-risk CH group (n = 6), which precluded further analysis, canakinumab treatment consistently resulted in elevated hemoglobin levels in low- and intermediate-risk groups, whereas no changes were observed in these group of patients receiving a placebo, suggesting potential clinical benefits in the patients with CH who were at higher risk.

Next, we examined hemoglobin response in patients with high-risk CH features associated with myeloid disease progression, such as variant allele frequency (VAF) > 0.1 and mean corpuscular volume (MCV) ≥ 100.[21,22] A cut-off of 0.1 for VAF was used to include more patients in a higher VAF group, as reported previously.[22] No association between baseline VAF > 0.1 and baseline anemia was observed ($P = .89$; supplemental Figure 2B). The risk of incident anemia was not statistically different between the 2 groups with VAF > 0.1 and VAF ≤ 0.1 (supplemental Figure 2C). However, the risk of incident anemia in patients with VAF > 0.1 was highest among other groups in the placebo arm (HR, 1.83; $P = .029$), whereas the risk was reduced in the patient group with VAF > 0.1 treated with canakinumab (HR, 1.47; $P = .074$), compared with that in patients without CH mutations (supplemental Figure 2C). The hemoglobin response in patients with baseline anemia (data not shown) was not significantly different in patients with baseline VAF > 0.1 vs VAF ≤ 0.1. In addition, higher MCV (ie, MCV ≥ 100) was associated with more CH mutations at baseline (supplemental Figure 2D-E). However, the cumulative incidence of anemia was not different in relation to MCV in patients without anemia treated with either placebo or canakinumab (supplemental Figure 2F). Hemoglobin response between baseline MCV ≥ 100 and MCV < 100 did not differ significantly within any patient groups (patients without CH mutations, patients with only CH mutations but without anemia, and/or patients with anemia and CH mutations; data not shown).

Next, we evaluated the incidence of other cytopenias and infections in relation to CH mutations and canakinumab treatment, because they were associated with canakinumab treatment.[17] The risk of thrombocytopenia did not differ between those with CH mutations and no CH mutations (OR = 0.93 [95% CI, 0.63-1.35]; $P = .73$). As reported before in other canakinumab studies for non-CH selected patients,[23] in those without CH mutations, canakinumab treatment was associated with a higher risk of thrombocytopenia (supplemental Figure 3A, left). However, CH mutations offset the risk of thrombocytopenia by demonstrating a comparable incidence of thrombocytopenia in the canakinumab and placebo arms (supplemental Figure 3A, right), suggesting a potential protective effect of CH mutations against thrombocytopenia in response to canakinumab. In contrast, the cumulative incidence of infection was not significantly different between patients with and without CH mutations, regardless of treatment (supplemental Figure 3B). Concurrent CH mutations and neutropenia were rare in CANTOS, precluding additional analysis.

Increased levels of inflammation are associated with CH mutations and further enhanced in patients with concurrent CH mutations and anemia

The presence of CH mutations was associated with older age (>65 years; OR = 2.16; $P < .001$), anemia at baseline (OR = 1.34; $P = .075$), IL-6 levels above median (OR = 1.26; $P = .07$), and tumor necrosis factor α (TNF-α) levels above median (OR = 1.39; $P = .01$) at baseline (supplemental Figure 4A). In contrast, body mass index ≥ 35 was inversely associated with CH mutations (OR = 0.45; $P < .001$).

Next, we characterized proteomic profiles of patients with CH mutations that may provide mechanistic insight into the significant association of anemia with CH mutations. Patients with CH mutations exhibited significantly higher serum levels of IL-6 ($P = .03$) and TNF-α ($P = .007$) and lower levels of hepcidin ($P = .003$) than those without CH mutations at baseline (Figure 3A-C). However, hsCRP and IL-18 levels were similar between patients with and without CH mutations, regardless of the presence of anemia at baseline (data not shown).

Using SomaScan, we compared the baseline proteomic profiles of patients with and without CH mutations (Figure 3D-E; supplemental Table 1). The pathways involved in immune response toward bacteria and inflammation (gene ontology [GO] terms in defense response, response to bacterium, and regulation of immune system) were enriched in patients with CH mutations, suggesting clinical evidence for the link between inflammatory pathways involved in infection response and CH, as previously shown in animal models (Figure 3D).[9,10,14] Expressions of proteins associated with an increased risk of CV events, such as N-terminal pro B-type natriuretic peptide,[24,25] growth differentiation factor 15,[26] and insulin-like growth factor binding protein 2,[27] were also significantly increased in patients with CH mutations, consistent with the known clinical link between CH mutations and higher CV risk. Levels of inhibitory immune regulators, such as T-cell immunoglobulin and mucin-domain containing 3, CD55, and TNF receptor were also increased. In the bone morphogenetic protein (BMP) signaling pathway, BMP antagonists (neuroblastoma

Figure 2. Canakinumab treatment is associated with improved hemoglobin levels in patients with concurrent baseline anemia and CH mutations. Hemoglobin response to treatment in patients with baseline anemia (A) stratified according to CH mutations, patients with CH mutations (B) stratified according to anemia, and all patients (C) as per the CH-risk score, and adjusted for baseline hemoglobin, hsCRP, and age. (A) Hemoglobin response to canakinumab (blue) and placebo (red) in patients with concurrent baseline anemia and indicated mutations. (B) Hemoglobin response to canakinumab in patients with baseline anemia (blue) or without baseline anemia (purple) among patients with CH mutations. (C) Changes of hemoglobin levels from baseline in response to canakinumab or placebo in patients with no CH, low-risk, intermediate-risk, and high-risk score of CH. BL, baseline; Hb, hemoglobin; LS, least squares; M, month; N/A, not applicable; *P*, *P* value of the treatment by quadratic-time effect.

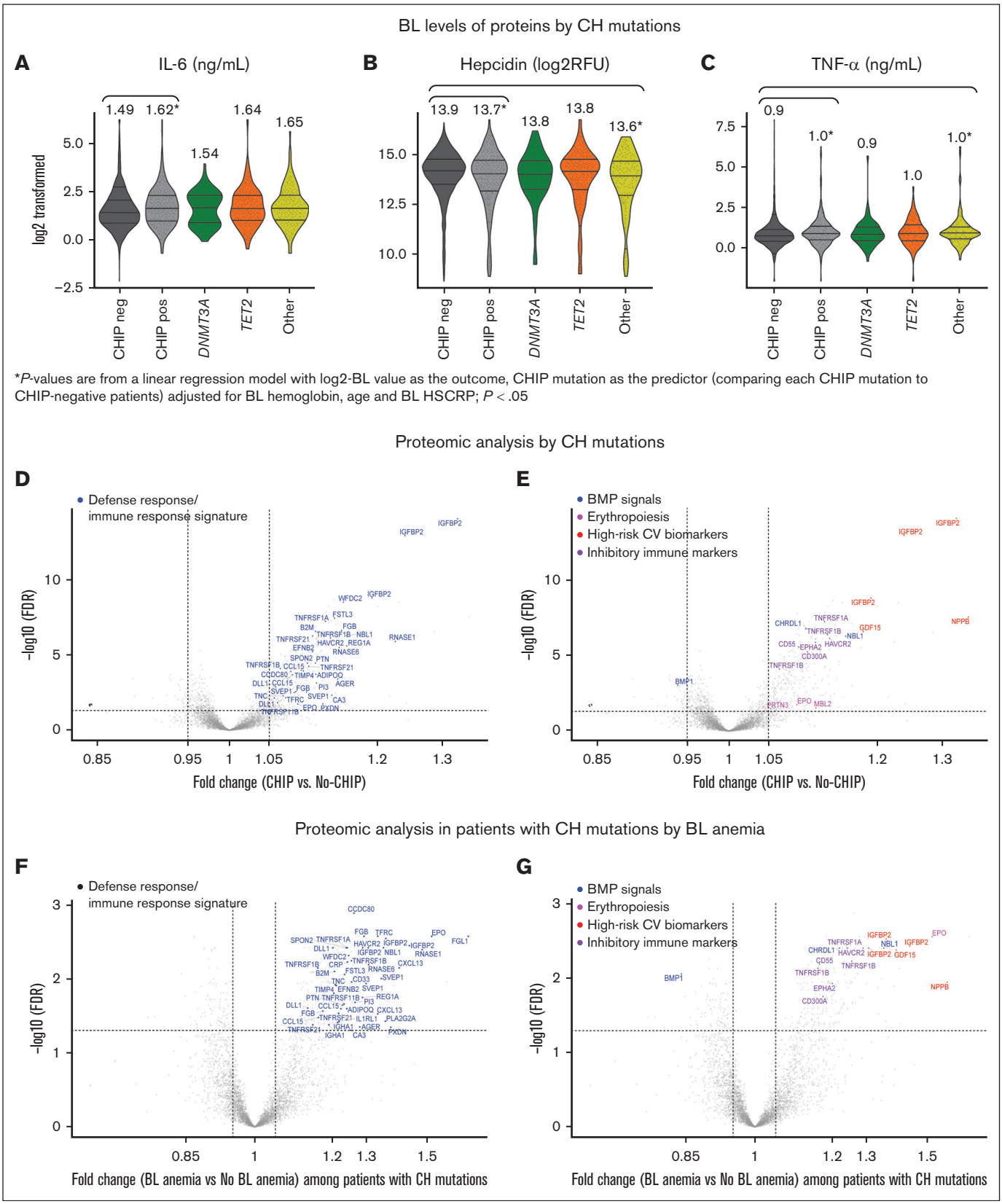

Figure 3. Proteomic signatures associated with inflammation and immune response are enriched in patients with CH mutation vs without CH mutations and increased more in patients with concurrent CH mutations and anemia. Baseline proteomic profile from SomaScan and individual cytokines ELISA characterized biological changes mediated by CH mutations and anemia. (A-C) Baseline levels of IL-6 (A), hepcidin (B), and TNF-α (C) in patients with and without CH mutations, showing mean

suppression of tumorigenicity 1 and chordin-like 1) were increased, and BMP1 level was lower in patients with CH mutations than in those with CH mutations (Figure 3E).

Next, we compared the baseline proteomic profiles of patients with and without baseline anemia among patients with CH mutations. The presence of concurrent anemia and CH mutations was associated with older age (>65 years; OR = 2.77 [95% CI, 1.48-5.43]; $P = .002$), hsCRP levels above median (OR = 2.45 [95% CI, 1.31-4.73]; $P = .006$), IL-6 levels above median (OR = 2.02 [95% CI, 1.08-3.87]; $P = .031$) at baseline (supplemental Figure 4B). The same pathways involved in the immune response to inflammation, high risks of CV events, BMP signaling, and immune modulation were significantly enriched in patients with concurrent anemia and CH mutations, compared with in patients with CH mutations and without anemia (Figure 3F-G).

Canakinumab treatment suppresses acute inflammatory response and enhances erythropoiesis

Next, we examined the biological response to canakinumab by comparing pretreatment (at baseline) and on-treatment multiplexed, proteomic data of patients who received canakinumab (Figure 4A; supplemental Table 2).

At month 3 of canakinumab treatment, we observed significant reduction in acute phase proteins or inflammatory cytokines (such as CRP, IL-15, haptoglobin, hemopexin, and cystatin F) and many complement molecules (C9, factor B, and C1s). In contrast, proteins associated with red blood cells (RBCs), for example, acetylcholinesterase (abundant on the RBC membrane) and hemoglobin, were significantly increased upon canakinumab treatment. Key pathways in GO terms that were significantly downregulated or upregulated during canakinumab treatment are shown in Figure 4B,C, respectively. Suppression of pathways associated with complement activation, acute inflammatory response, myeloid cell activation, and host response to bacteria suggests that canakinumab may inhibit complement-mediated hemolysis, erythrophagocytosis, and attenuate myeloid-biased differentiation (Figure 4B; supplemental Table 3). Collectively, these proteomic pathway analyses provided evidence that canakinumab suppresses the molecular pathways implicated in the pathology of AI.[1,28]

Next, we analyzed the changes from baseline to month 3 of the critical regulators of iron homeostasis and the resulting changes in RBC markers in patients treated with canakinumab compared with placebo (Figure 4D-E). The levels of IL-1β, IL-6, and hepcidin decreased significantly in patients receiving canakinumab ($P < .001$ for all 3 proteins) compared with those receiving placebo (Figure 4D). Acetylcholinesterase and hemoglobin significantly increased in patients receiving canakinumab ($P < .001$ for both), but not in patients receiving placebo (Figure 4E). The changes observed in the multiplex proteomic analysis (SomaScan) were confirmed by individual cytokine enzyme-linked immunosorbent assays: IL-6 levels were lower with canakinumab than with placebo, as observed in other studies,[17,19] but IL-1 receptor antagonist, IL-18, and TNF-α levels were comparable between the 2 groups (data not shown).

We hypothesized that suppression of IL-6 and hepcidin results in an improved hemoglobin response. We analyzed the association of changes in IL-6 and hemoglobin levels between baseline and month 3 in patients treated with canakinumab and placebo (Figure 4F). We observed 3.46 times higher odds of having both an increase in hemoglobin and a decrease in IL-6 levels in patients treated with canakinumab compared with placebo (Table 2). Similarly, patients treated with canakinumab had 1.88 times higher odds of having an increase in hemoglobin accompanied by a decrease in hepcidin (Figure 4G; Table 2).

Concurrent CH mutations and anemia enhance the suppression of inflammatory pathways mediated by canakinumab

We first investigated the effects of canakinumab treatment on key inflammatory markers in patients with or without CH mutations after adjusting for baseline hemoglobin, hsCRP, and age. Canakinumab treatment was not associated with a more pronounced reduction of IL-6, hepcidin, and TNF-α levels in patients with CH mutations vs those without CH mutations (Figure 5A). When the effect of canakinumab treatment was analyzed in patients stratified based on individual CH mutations, patients with *TET2* mutations showed a nonsignificant trend for a more pronounced reduction in IL-6 and TNF-α levels compared with patients without CH mutations (Figure 5A). The results obtained for hepcidin might have been confounded by the lower baseline level of hepcidin in patients with CH mutations vs those without CH mutation (Figure 3B).

The overall changes in proteomic profiles upon treatment with canakinumab for 3 months in patients with CH mutations (supplemental Figure 5A; supplemental Table 4) were consistent with changes observed in all patients (Figure 4A). Complement pathways and proteins involved in inflammation, myeloid activation, and host response to bacteria were significantly downregulated by canakinumab treatment in patients with CH mutations (supplemental Figure 5B; supplemental Table 5).

Because patients with concurrent anemia and CH mutations demonstrated more robust hemoglobin response to canakinumab than patients without CH mutations or with only CH mutations (Figure 2), we evaluated proteomic profiles in patients with anemia and CH mutations vs patients with only CH mutations without baseline anemia. IL-6 and hepcidin levels were more significantly suppressed in patients with CH mutations and anemia than in patients with only CH mutations (Figure 5B), but TNF-α levels were not suppressed by canakinumab. In addition, canakinumab

Figure 3 (continued) values at the top of each plot. (D-E) Volcano plots of multiplexed proteomic data comparing patients with CH mutations vs without CH mutations. Highlighted are (D) proteins associated with immune response and defense response to inflammation and infection (GO terms in defense response, response to bacterium and regulation of immune system), and (E) high-risk CV biomarkers (red), inhibitory immune molecules (purple), BMP-signaling ligands (blue; antagonists and agonist, BMP1), and proteins associated with erythropoiesis (pink) among significant proteins. (F-G) Volcano plots of multiplexed proteomic data in patients with CH mutations comparing patients with baseline anemia (CCUS) vs without baseline anemia (CHIP). Highlighted are (F) proteins associated with immune response and defense response to inflammation and infection, and (G) high-risk CV biomarkers (red), inhibitory immune molecules (purple), BMP-signaling ligands (blue; antagonists and agonist, BMP1), and proteins associated with erythropoiesis (pink) among significant proteins. FDR, false discovery rate; RFU, relative fluorescence unit.

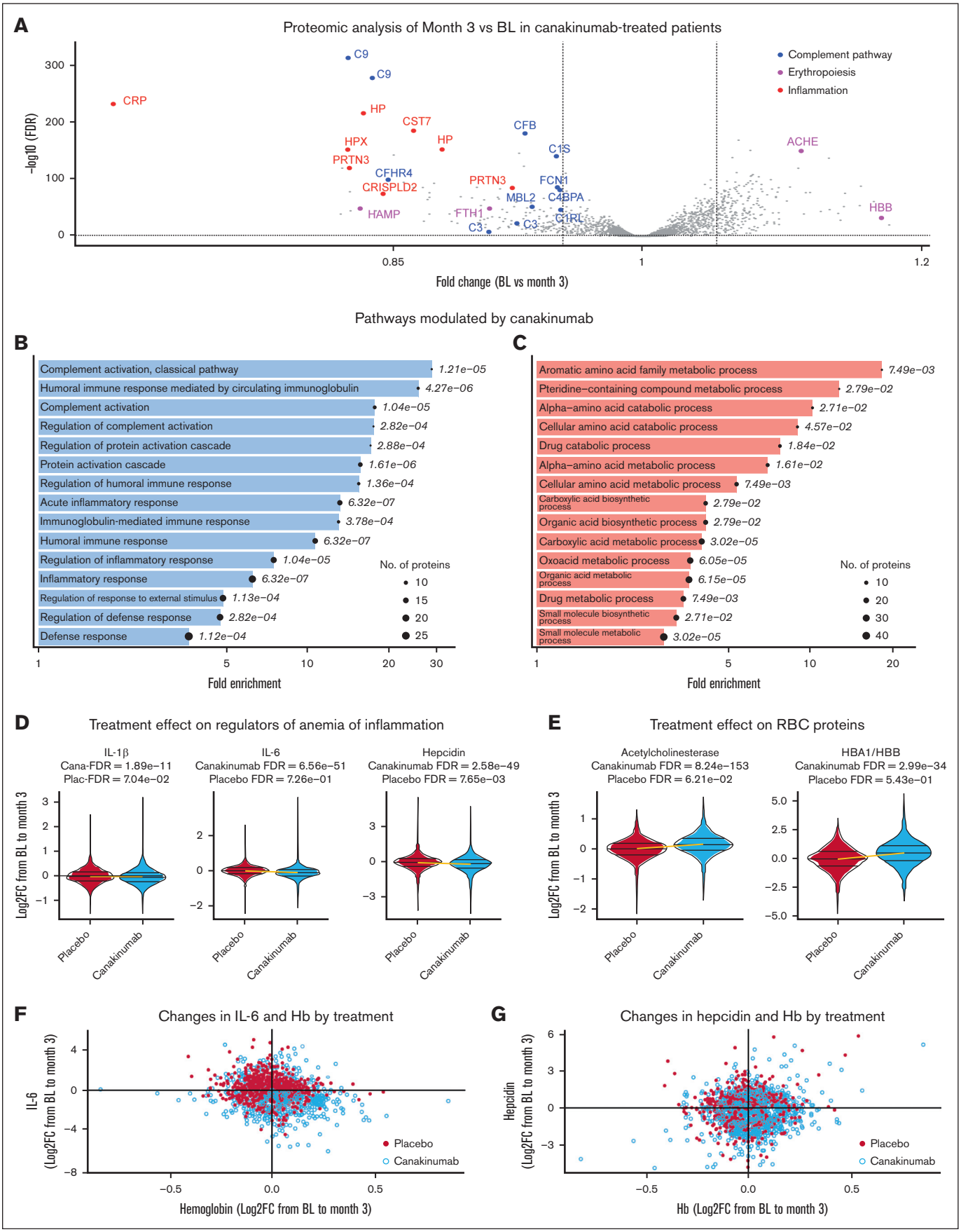

Figure 4.

Table 2. Association between Hb and IL-6 or hepcidin in patients with placebo and canakinumab treatment

	Association between hemoglobin and IL-6		Association between hemoglobin and hepcidin	
	Hemoglobin increased and IL-6 decreased	Hemoglobin decreased and IL-6 increased	Hemoglobin increased and hepcidin decreased	Hemoglobin decreased and hepcidin increased
Canakinumab	1465 (51.5%)	1380 (48.5%)	1041 (38.2%)	1684 (61.8%)
Placebo	330 (23.5%)	1075 (76.5%)	338 (24.8%)	1026 (75.2%)
OR (95% CI), *P* value	3.46 (3.00-4.00), $P < .001$		1.88 (1.62-2.17), $P < .001$	

significantly suppressed IL-6 levels in patients with *TET2* mutations and anemia more than in patients with only *TET2* mutations but without anemia. A similar trend was observed for hepcidin levels in patients with *TET2* mutations and anemia vs only *TET2* mutation (supplemental Figure 6A). In patients with *DNMT3A* or other mutations, levels of these cytokines were similar in patients with anemia vs without anemia (supplemental Figure 6B-C)

Next, we compared the pathway enrichment scores of inflammation and immune response to infection (Figure 3) between baseline and month 3 or 12 after canakinumab treatment (Figure 5C). The pathway enrichment score was significantly suppressed after canakinumab treatment in patients with concurrent anemia and CH mutations vs patients with only CH mutations and without baseline anemia. The same trend was observed in patients with *TET2* or *DNMT3A* mutations and anemia vs patients with only *TET2* or *DNMT3A* mutations (supplemental Figure 6A-C). These proteomic data, in conjunction with clinical response to canakinumab, indicate that IL-1β inhibition may reverse AI-associated pathways that are more substantially deregulated in patients with anemia and CH mutations, such as the IL-6/hepcidin axis and pathways involved in immune response to infection, than in patients without CH mutations or with only CH mutations, ultimately leading to more robust hemoglobin response in these patients with concurrent anemia and CH mutations, that is, CCUS (Figure 5D).

Discussion

As a major proinflammatory cytokine, IL-1β plays a key role in multiple physiological and pathological processes.[29] Because our integrated biomarker study included a sizable population of patients with CH mutations with or without anemia, we were able to address key biological questions of whether and how CH mutations and anemia (non-CH vs CHIP vs CCUS) can cooperate with inflammation and affect the mode of action of canakinumab in the context of erythropoiesis.

First, our proteomic data suggest that canakinumab reverses functional hypoferremia and increase iron availability by reducing IL-6 and hepcidin levels resulting in enhanced erythropoiesis and may prolong erythrocyte lifespan by inhibiting hemolysis and erythrophagocytosis. Second, patients with CH mutations and anemia in this study potentially meet the diagnostic criteria of CCUS, provided they have no underlying bone marrow abnormality.[30] Our data suggest that patients with CH and anemia (ie, CCUS) exhibited higher activation of inflammatory pathways associated with AI at the baseline than patients with CHIP or without CH mutations. Patients with CH and anemia treated with canakinumab had pronounced suppression of these pathways in association with a higher hemoglobin response. In addition, a robust hemoglobin response to canakinumab was still observed in patients with higher-risk CH (ie, intermediate-risk) per the CH-risk score classification. Furthermore, despite relatively higher hemoglobin levels for anemia criteria in this study (hemoglobin level < 13 g/dL in men or hemoglobin level < 12 g/dL in women as per the World Health Organization anemia criteria), anemia defined by these criteria, indeed, has significant clinical implications, particularly for older individuals. For example, using nationally representative data of 5329 adults aged ≥65 years (Health Survey for England),[31] the highest mortality HR was found for hemoglobin levels < 12 g/dL (HR, 2.19) for men and hemoglobin level < 11 g/dL (HR, 1.61) for women. In a separate study with 981 patients aged ≥60 years,[32] anemia, defined by the World Health Organization criteria, was significantly associated with the risk of mortality (HR, 3.33; $P = .005$). The HR of anemia was even higher than that of cancer (HR, 3.31; $P = .004$) and heart failure (HR, 2.94; $P = .008$). Thus, our results provide a scientific and clinical rationale to support early intervention or

Figure 4. Canakinumab suppresses IL-1β, IL-6, and hepcidin levels and inhibits activation of immune response, complement pathways, and myeloid differentiation. Pre- and post-treatment proteomic data from SomaScan and individual cytokine ELISA revealed key pathways that are modulated by canakinumab. (A) Volcano plot of multiplexed proteomic data in post-canakinumab patients at month 3 compared with baseline. Highlighted are significant proteins from the complement pathway (blue), erythropoiesis pathway (orange), and inflammatory pathways (pink) among those with FDR < 0.05. (B-C) Top 15 GO terms enriched in proteins downregulated (B) and upregulated (C) after canakinumab treatment showing FDR and fold enrichment. (D) Canakinumab suppresses key regulators of AI, including IL-1β, IL-6, and hepcidin. (E) Canakinumab increases RBC numbers, as evidenced by upregulated proteins in RBCs, including AChE and hemoglobin (HBA1 and HBB). (F) Changes in IL-6 and hemoglobin levels between baseline and month 3. Patients treated with canakinumab (blue) have 3.46 times higher odds of having both an increase in hemoglobin and a decrease in IL-6 than patients treated with placebo (red). (G) Changes in hepcidin and hemoglobin levels between baseline and month 3. The odds of having both an increase in hemoglobin and a decrease in hepcidin were 1.88 times higher in patients treated with canakinumab (light blue) compared with placebo (red). AChE, acetylcholinesterase; C1RL, complement component 1, r subcomponent-like; C1S, complement component 1, s subcomponent; C3, complement component 3; C4BPA, complement component 4 binding protein alpha; C9, complement component 9; CFB, complement factor B; CRIPSLD2, cysteine-rich secretory protein LCCL domain containing 2; CST7, cystatin F; FC, fold change; FCN1, ficolin 1; FTH1, ferritin; HAMP, hepcidin; HBA1, hemoglobin alpha; HBB, hemoglobin beta; HP, haptoglobin; HPX, hemopexin; MBL2, mannose-binding; lectin 2; PRTN3, proteinase 3.

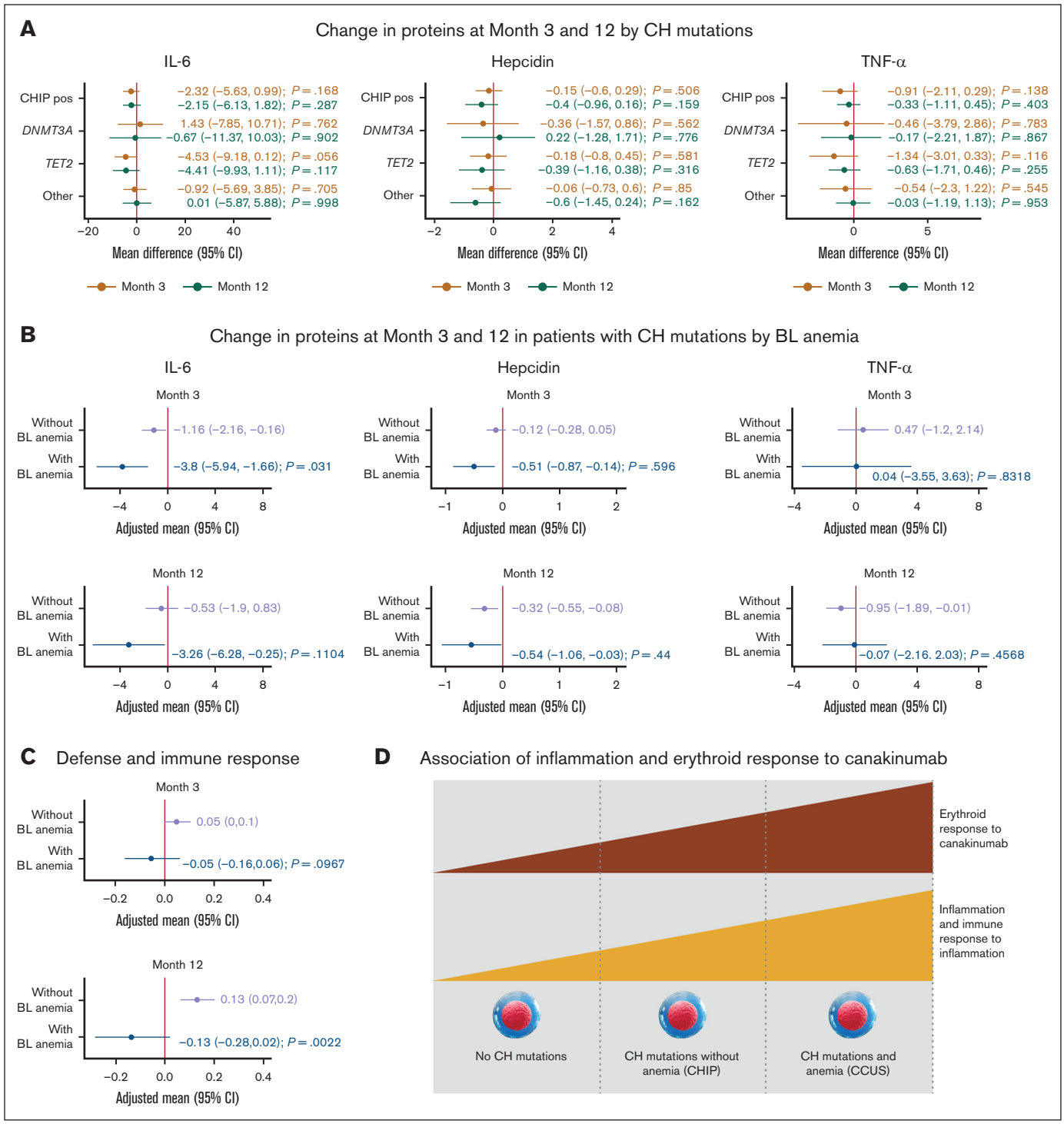

Figure 5. The concurrent CH mutations and anemia enhance the suppression of inflammatory pathways mediated by canakinumab. Pre- and post-treatment proteomic data from SomaScan and individual cytokine ELISA discovered more substantial suppression of inflammatory pathways by canakinumab in patients with CH mutations and anemia (CCUS) than patients with only CH mutations (CHIP). (A) Forest plot of mean differences in change of IL-6 (left), hepcidin (middle), and TNF-α (right) levels at months 3 and 12 in patients with CH mutations compared to those in patients without CH mutations, adjusted for baseline hemoglobin, hsCRP, and age. (B) Forest plot of mean differences in change of IL-6, hepcidin, and TNF-α levels at months 3 (top) and 12 (bottom) in patients with baseline anemia, compared to those in patients without baseline anemia among patients with CH mutations, adjusted for baseline hemoglobin, hsCRP, and age. (C) Changes of pathway enrichment scores associated with defense and immune response from baseline to months 3 (top) and 12 (bottom) of canakinumab treatment in patients stratified based on the presence of baseline anemia among patients with CH mutations, adjusted for baseline hemoglobin, hsCRP, and age. (D) Association of inflammation and erythroid response to canakinumab in patients with only CH mutations (CHIP) and concurrent CH mutations and anemia (CCUS). More elevated levels of inflammation and higher response to canakinumab were observed in patients with CCUS than in those with CHIP or no CH mutations.

prevention trials with IL-1β blockade in patients with CCUS or higher-risk CH.

Hematopoietic aging is associated with clonal hematopoiesis, myeloid-biased hematopoiesis, and anemia.[14,33] In older individuals with AI, the frequency of common CH mutations (*DNMT3A*, *TET2*, and *ASXL1*) and other rare mutations was higher than in age-matched nonanemic individuals.[14,33] In older mice, microbiome-induced IL-1 mediates myeloid-biased hematopoiesis and hematopoietic aging.[13,14] Our data demonstrated that although CH mutations contribute to the progressive development of anemia in patients without baseline anemia, canakinumab treatment increases the hemoglobin level and decreases IL-6 and hepcidin levels in patients with concurrent CH mutations and anemia. Collectively, these previous data and our current results imply that CH mutations may cooperate with IL-1 signaling in hematopoietic aging and augment anemia, which can be therapeutically targeted by canakinumab.

Characterization of proteomic profiles of patients with CH mutations compared with those of patients without CH mutations identified novel biological links that could merit further exploration in future studies. We revealed a relationship between CH mutations and high CV risk biomarkers (N-terminal pro B-type natriuretic peptide, insulin-like growth factor binding protein 2, and growth differentiation factor 15).[34] In addition, CH mutations were associated with the upregulation of immune modulatory molecules, such as T-cell immunoglobulin and mucin-domain containing 3[35] and TNF receptors,[36] indicating a potential interplay between CH and immune synapses. The observed increase in BMP antagonists and reduction of BMP1 may lead to impaired erythroid response[37] and altered hematopoiesis,[38] because the BMP-signaling pathway plays an important role in hematopoiesis[39] and stress-induced erythropoiesis.[40] Finally, the observed upregulation of proteins associated with host response to bacteria supports a biological link between CH mutations and chronic infection, which is exacerbated by aging.[9-14]

Our results suggest that the response of anemia to canakinumab depends on the specific CH mutation. The previous analysis of genomic data from CANTOS suggested that patients with *TET2* mutations derive a larger benefit from canakinumab treatment to reduce adverse CV outcomes.[16] In our analysis, canakinumab treatment showed a trend toward a higher decrease of IL-6 and hepcidin levels and was significantly associated with a higher hematological response in patients bearing *TET2* mutations. *TET2* mutations were significantly associated with higher levels of both IL-1β and IL-6 than other mutations.[6] Direct blockade of IL-1β by canakinumab may confer more potent pharmacodynamic effects in patients with *TET2* mutations, as suggested in the *TET2*-mutated autologous transplant model of macaques treated with IL-6–neutralizing antibody.[41]

This study has several limitations. CANTOS enrolled patients with a previous history of myocardial infarction and persistently high hsCRP levels, and the applicability to a population without established vascular disease or those with normal hsCRP levels is unclear. The hsCRP selection in the CANTOS population may have influenced the CH mutation and proteomic profile.[16] Multiple comorbidities, such as heart failure, chronic kidney disease, and diabetes mellitus, might have contributed to the heterogeneous etiology of anemia in this patient population. In our study, higher body mass index was inversely associated with CH mutations; however, the reverse association has been reported previously.[42] Although this analysis demonstrated a clinical hemoglobin response to canakinumab, additional studies will be required to understand clonal dynamics and mutational burden (ie, VAF) after canakinumab treatment and to establish disease-modifying potential. Moreover, because of the exploratory nature of this investigation and small sample sizes of patients with CCUS, additional studies will be needed to confirm the mechanistic links of associations that our study discovered.

In summary, this study discovered that patients with concurrent CH mutations and anemia, who may meet diagnostic criteria of CCUS,[30] exhibited more enriched proteomic signatures associated with inflammatory pathway activation, and a higher association of hematological response to canakinumab was observed in patients with concurrent CH mutations and anemia (CCUS) compared with patients without CH mutations or with only CH mutations without cytopenia (CHIP). These results are hypothesis-generating and warrant further studies. Ongoing and planned randomized intervention trials will establish the safety and efficacy of canakinumab and other anti-inflammation therapies in patients with CCUS or lower-risk myeloid disease.[43-47]

Acknowledgments

The authors thank Eric Svensson, Aviv Madar, Marc Sultan, Catarina Campbell, Yunsheng He, Katie D'Aco, Anita Fernandez, and Clariss Wache-Mainier for their contribution to the CH genomic study as well as Margaret Prescott, Lori Jennings, Lynne Krajkovich, Jaison Jacob, Pablo Serrano-Fernandez, Sergio Kaiser, and Luqing Zhang for their contribution to the SomaScan study. The authors thank Olga Ucar, of Novartis Pharmaceuticals UK; Amrita Dutta, of Novartis Healthcare Pvt Ltd, Hyderabad, India; and Vanesa Martinez Lopez, of Novartis Ireland Ltd for editorial support.

The study was funded by Novartis AG (Basel, Switzerland). P.L. receives funding support from the National Heart, Lung, and Blood Institute (1R01HL134892 and 1R01HL163099-01), the American Heart Association (18CSA34080399), the RRM Charitable Fund, and the Simard Fund.

Authorship

Contribution: J.W. and D.L. conceived the project; J.W., D.L., A.L., H.X., P.S., M.H., D.P.Y., M.T.B., P.L., P.M.R., and D.P.S. analyzed and interpreted the data; J.W. and D.L. wrote the initial draft of the manuscript; and all authors made substantial contributions to the subsequent version of the manuscript and approved the final version for submission.

Conflict-of-interest disclosure: J.W., D.L., A.L., H.X., P.S., M.H., D.P.Y., M.T.B., and D.P.S. are employees of Novartis Institute of Biomedical Research. P.L. is a consultant (nonfinancial) to or is involved in clinical trials for Amgen, AstraZeneca, Baim Institute, Beren Therapeutics, Esperion Therapeutics, Genentech, Kancera, Kowa Pharmaceuticals, Medimmune, Merck, Norvo Nordisk, Novartis, Pfizer, and Sanofi-Regeneron; is a member of the scientific advisory board for Amgen, Caristo Diagnostics, Cartesian Therapeutics, CSL Behring, DalCor Pharmaceuticals, Dewpoint Therapeutics, Eulicid Bioimaging, Kancera, Kowa Pharmaceuticals, Medimmune, Moderna, Novartis, Olatec Therapeutics, PlaqueTec, TenSixteen Bio, Soley Therapeutics, and XBiotech, Inc; has received research funding from Novartis; is a member of Board of Directors for Soley Therapeutics, TenSixteen Bio and XBiotech,

Inc; has financial interest in Soley Therapeutics, TenSixteen Bio and XBiotech, Inc; and had interests reviewed and managed by Brigham and Women's Hospital and Mass General Brigham in accordance with their conflict of interest policies. P.M.R. has received research grant support from Amarin, Esperion, Kowa, Novartis, Pfizer, and the National Heart, Lung, and Blood Institute; has served as a consultant to Agepha, ANGIOWave, AstraZeneca, Boehringer-Ingelheim, Cardiol Therapeutics CiVi Biopharma, Flame, GlaxoSmithKline, Health Outlook, Horizon Therapeutics, Janssen, Montai Health, New Amsterdam, Novartis, Novo Nordisk, Research Triangle Institute, SOCAR, Uptton, and Zomagen; and receives compensation for service on the Peter Munk Advisory Board (University of Toronto), the Leducq Foundation, Paris, France, and the Baim Institute (Boston, MA).

The current affiliation for J.W. is Winship Cancer Institute, Emory University, Atlanta, GA.

ORCID profiles: J.W., 0000-0001-9617-4301; D.P.Y., 0000-0003-1039-515X; M.T.B., 0000-0002-1343-0005; P.L., 0000-0002-1502-502X; D.P.S., 0000-0001-5130-9284.

Correspondence: Janghee Woo, Novartis Institutes for BioMedical Research, 250 Massachusetts Ave, Cambridge, MA; email: janghw1@gmail.com.

References

1. Ganz T. Anemia of inflammation. *N Engl J Med*. 2019;381(12):1148-1157.
2. Ganz T. Hepcidin in iron metabolism. *Curr Opin Hematol*. 2004;11(4):251-254.
3. Wang CY, Babitt JL. Hepcidin regulation in the anemia of inflammation. *Curr Opin Hematol*. 2016;23(3):189-197.
4. Ganz T. Hepcidin and iron regulation, 10 years later. *Blood*. 2011;117(17):4425-4433.
5. Jaiswal S. Clonal hematopoiesis and non-hematologic disorders. *Blood*. 2020;136(14):1606-1614.
6. Bick AG, Weinstock JS, Nandakumar SK, et al. Inherited causes of clonal haematopoiesis in 97,691 whole genomes. *Nature*. 2020;586(7831):763-768.
7. Schneider M, Rolfs C, Trumpp M, et al. Activation of distinct inflammatory pathways in LR-MDS is determined by genetics. *Blood*. 2022;140(suppl 1):4011-4012.
8. Nielsen AB, Hansen JW, Ørskov AD, et al. Inflammatory cytokine profiles do not differ between patients with idiopathic cytopenias of undetermined significance and myelodysplastic syndromes. *Hemasphere*. 2022;6(5):e0713.
9. Hormaechea-Agulla D, Matatall KA, Le DT, et al. Chronic infection drives Dnmt3a-loss-of-function clonal hematopoiesis via IFNγ signaling. *Cell Stem Cell*. 2021;28(8):1428-1442.e6.
10. Meisel M, Hinterleitner R, Pacis A, et al. Microbial signals drive pre-leukaemic myeloproliferation in a Tet2-deficient host. *Nature*. 2018;557(7706):580-584.
11. Cai Z, Kotzin JJ, Ramdas B, et al. Inhibition of inflammatory signaling in Tet2 mutant preleukemic cells mitigates stress-induced abnormalities and clonal hematopoiesis. *Cell Stem Cell*. 2018;23(6):833-849.e5.
12. Burns SS, Kumar R, Pasupuleti SK, So K, Zhang C, Kapur R. Il-1r1 drives leukemogenesis induced by Tet2 loss. *Leukemia*. 2022;36(10):2531-2534.
13. Caiado F, Kovtonyuk LV, Gonullu NG, Fullin J, Boettcher S, Manz MG. Aging drives Tet2+/- clonal hematopoiesis via IL-1 signaling. *Blood*. 2023;141(8):886-903.
14. Kovtonyuk LV, Caiado F, Garcia-Martin S, et al. IL-1 mediates microbiome-induced inflammaging of hematopoietic stem cells in mice. *Blood*. 2022;139(1):44-58.
15. Ridker PM, Everett BM, Thuren T, et al. Antiinflammatory therapy with canakinumab for atherosclerotic disease. *N Engl J Med*. 2017;377(12):1119-1131.
16. Svensson EC, Madar A, Campbell CD, et al. TET2-driven clonal hematopoiesis and response to canakinumab: an exploratory analysis of the CANTOS randomized clinical trial. *JAMA Cardiol*. 2022;7(5):521-528.
17. Vallurupalli M, MacFadyen JG, Glynn RJ, et al. Effects of interleukin-1β inhibition on incident anemia. *Ann Intern Med*. 2020;172(8):523-532.
18. Gold L, Ayers D, Bertino J, et al. Aptamer-based multiplexed proteomic technology for biomarker discovery. *PLoS One*. 2010;5(12):e15004.
19. Ridker PM, MacFadyen JG, Thuren T, Libby P. Residual inflammatory risk associated with interleukin-18 and interleukin-6 after successful interleukin-1beta inhibition with canakinumab: further rationale for the development of targeted anti-cytokine therapies for the treatment of atherothrombosis. *Eur Heart J*. 2020;41(23):2153-2163.
20. Zhang L, Cunningham JW, Claggett BL, et al. Aptamer proteomics for biomarker discovery in heart failure with reduced ejection fraction. *Circulation*. 2022;146(18):1411-1414.
21. Weeks LD, Niroula A, Neuberg D, et al. Prediction of risk for myeloid malignancy in clonal hematopoiesis. *NEJM Evid*. 2023;2(5):EVIDoa2200310.
22. Rossi M, Meggendorfer M, Zampini M, et al. Clinical relevance of clonal hematopoiesis in persons aged ≥80 years. *Blood*. 2021;138(21):2093-2105.
23. Howard C, Noe A, Skerjanec A, et al. Safety and tolerability of canakinumab, an IL-1beta inhibitor, in type 2 diabetes mellitus patients: a pooled analysis of three randomised double-blind studies. *Cardiovasc Diabetol*. 2014;13:94.

24. Wang Y, Sano S, Yura Y, et al. Tet2-mediated clonal hematopoiesis in nonconditioned mice accelerates age-associated cardiac dysfunction. *JCI Insight*. 2020;5(6):e135204.

25. Wu AHB, Omland T, Wold Knudsen C, et al. Relationship of B-type natriuretic peptide and anemia in patients with and without heart failure: a substudy from the Breathing Not Properly (BNP) Multinational Study. *Am J Hematol*. 2005;80(3):174-180.

26. Wollert KC, Kempf T, Wallentin L. Growth differentiation factor 15 as a biomarker in cardiovascular disease. *Clin Chem*. 2017;63(1):140-151.

27. Berry M, Galinier M, Delmas C, et al. Proteomics analysis reveals IGFBP2 as a candidate diagnostic biomarker for heart failure. *IJC Metab Endocr*. 2015;6:5-12.

28. Weiss G, Ganz T, Goodnough LT. Anemia of inflammation. *Blood*. 2019;133(1):40-50.

29. Pretre V, Papadopoulos D, Regard J, Pelletier M, Woo J. Interleukin-1 (IL-1) and the inflammasome in cancer. *Cytokine*. 2022;153:155850.

30. DeZern AE, Malcovati L, Ebert BL. CHIP, CCUS, and other acronyms: definition, implications, and impact on practice. *Am Soc Clin Oncol Educ Book*. 2019;39:400-410.

31. Mindell J, Moody A, Ali A, Hirani V. Using longitudinal data from the Health Survey for England to resolve discrepancies in thresholds for haemoglobin in older adults. *Br J Haematol*. 2013;160(3):368-376.

32. Michalak SS, Rupa-Matysek J, Gil L. Comorbidities, repeated hospitalizations, and age ≥ 80 years as indicators of anemia development in the older population. *Ann Hematol*. 2018;97(8):1337-1347.

33. van Zeventer IA, de Graaf AO, Wouters H, et al. Mutational spectrum and dynamics of clonal hematopoiesis in anemia of older individuals. *Blood*. 2020;135(14):1161-1170.

34. Williams SA, Ganz P, Hinterberg MA, et al. A proteomic surrogate for cardiovascular outcomes that is sensitive to multiple mechanisms of change in risk. *Sci Transl Med*. 2022;14(665):eadd1355.

35. Ferraro F, Miller CA, Christensen KA, et al. Immunosuppression and outcomes in adult patients with de novo acute myeloid leukemia with normal karyotypes. *Proc Natl Acad Sci U S A*. 2021;118(49):e2116427118.

36. SanMiguel JM, Eudy E, Loberg MA, et al. Distinct tumor necrosis factor alpha receptors dictate stem cell fitness versus lineage output in Dnmt3a-mutant clonal hematopoiesis. *Cancer Discov*. 2022;12(12):2763-2773.

37. Lenox LE, Perry JM, Paulson RF. BMP4 and Madh5 regulate the erythroid response to acute anemia. *Blood*. 2005;105(7):2741-2748.

38. Goldman DC, Bailey AS, Pfaffle DL, Al Masri A, Christian JL, Fleming WH. BMP4 regulates the hematopoietic stem cell niche. *Blood*. 2009;114(20):4393-4401.

39. Crisan M, Kartalaei PS, Vink CS, et al. BMP signalling differentially regulates distinct haematopoietic stem cell types. *Nat Commun*. 2015;6(1):8793.

40. Paulson RF, Shi L, Wu DC. Stress erythropoiesis: new signals and new stress progenitor cells. *Curr Opin Hematol*. 2011;18(3):139-145.

41. Shin TH, Zhou Y, Chen S, et al. A macaque clonal hematopoiesis model demonstrates expansion of TET2-disrupted clones and utility for testing interventions. *Blood*. 2022;140(16):1774-1789.

42. Haring B, Reiner AP, Liu J, et al. Healthy lifestyle and clonal hematopoiesis of indeterminate potential: results from the Women's Health Initiative. *J Am Heart Assoc*. 2021;10(5):e018789.

43. Phase Ib study of select drug combinations in patients with lower risk MDS. ClinicalTrials.gov identifier: NCT04810611. Accessed 18 November 2023. https://clinicaltrials.gov/ct2/show/NCT04810611

44. Canakinumab with darbepoetin alfa in PTs with lower-risk MDS who have failed ESA (2021-2022). ClinicalTrials.gov identifier: NCT04798339. Accessed 18 November 2023. https://clinicaltrials.gov/ct2/show/NCT04798339

45. Canakinumab and anacitidine for the treatment of low or intermediate risk myelodysplastic syndrome and chronic myelomonocytic leukemia; 2020-2022. ClinicalTrials.gov identifier: NCT04239157. Accessed 18 November 2023. https://clinicaltrials.gov/ct2/show/NCT04239157

46. Dose optimization and expansion study of DFV890 in adult patients with myeloid diseases (2022-2025). ClinicalTrials.gov identifier: NCT05552469. Accessed 18 November 2023. https://clinicaltrials.gov/ct2/show/NCT05552469

47. Canakinumab for the prevention of progression to cancer in patients with clonal cytopenias of unknown significance, IMPACT study. ClinicalTrials.gov identifier: NCT05641831. Accessed 18 November 2023. https://classic.clinicaltrials.gov/ct2/show/NCT05641831