TO THE EDITOR:

Clonal hematopoiesis is associated with improved survival in patients with metastatic colorectal cancer from the FIRE-3 trial

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Clonal hematopoiesis (CH), defined by the acquisition of somatic mutations in hematopoietic stem cells, occurs in 20% to 30% of individuals >60 years and most frequently affects epigenetic regulator genes (*DNMT3A, TET2*, and *ASXL1*). CH is associated with a higher overall mortality and an approximately 10-fold risk for the development of hematologic malignancies.[1,2] Reduced overall survival (OS) in individuals with CH is mainly caused by an increased rate of cardiovascular events.[2,3] A causal relation was found in preclinical models, showing accelerated development of atherosclerosis driven by an altered function of the NLRP3/IL1β inflammasome of mutated monocytes/macrophages.[3-5] These results pinpoint toward pleiotropic effects of mutated clones, not only affecting self-renewal and differentiation of hematopoietic stem cells but also inflammatory signaling of mature blood cells.

In patients with cancer, CH was reported to be associated with adverse outcomes such as a higher rate of therapy-associated myeloid neoplasia and reduced OS.[6,7] However, current knowledge mainly derives from retrospective patient cohorts with marked heterogeneity in terms of cancer entity, disease stage, and applied therapy. Keeping unexpected findings associated with CH in mind, caution is needed to predict clinical consequences of CH to consider this phenomenon in all its bearings. Thus, we investigated CH in 237 available peripheral blood (PB) samples from the FIRE-3 study (AIO KRK-0306), a phase 3 landmark trial comparing FOLFIRI plus cetuximab or bevacizumab as first-line treatment in metastatic colorectal cancer (mCRC).[8]

In accordance with the Declaration of Helsinki and with approval of the local ethics committee of the University of Munich, PB samples collected as part of the screening process were available from 237 patients with mCRC of the FIRE-3 intention-to-treat (ITT) population.[8] With respect to demographic and clinical characteristics, our subcohort is representative of the entire FIRE-3 ITT cohort (supplemental Table 1, available on the *Blood* Web site). PB DNA was subjected to error-corrected targeted sequencing using a customized sequencing panel covering 45 genes recurrently mutated in CH (supplemental Table 2). Somatic variants with a variant allele frequency (VAF) ≥ 1% were called using our in-house variant calling pipeline (detailed in supplemental Methods).[9,10] The primary end point of our study was OS, and secondary end points included the occurrence of adverse events and various response measurements such as early tumor shrinkage. Statistical analysis was performed in R version 4.0.1 as described in the supplemental Material. Because of the hypothesis-generating character of the study, no correction for multiple testing was implemented, and reported *P* values must be interpreted as exploratory.

We identified 119 mutations in 86 patients, corresponding to a CH prevalence of 36%. The most frequently mutated genes were *DNMT3A*, *TET2*, *PPM1D*, and *ASXL1*, accounting for 72% of all mutations (Figure 1A). In 24 (10%) patients, more than 1 mutation was detected (Figure 1B). Comparisons of baseline demographic and clinical characteristics according to CH status confirmed the age-related association of CH as reported previously (median age: 62 vs 68 years in $CH^{negative}$ vs $CH^{positive}$ patients, respectively; $P < .001$; supplemental Table 3; Figure 1C). Baseline blood counts did not differ between groups (supplemental Figure 1). Prior exposure to chemotherapy was associated with a higher CH mutation rate (50% vs 33%; $P = .04$; Figure 1D). Genes related to the DNA damage repair machinery such as *TP53*, *PPM1D*, *CHEK2*, *RAD21*, *BRCC3*, and *ATM* were particularly affected (24% vs 8%; $P = .006$; supplemental Figure 2).

Next, we observed a longer but not statistically significant OS for patients who were $CH^{positive}$ (median OS: 43.7 vs 33.2 months in patients who were $CH^{negative}$; Figure 2A). Because of the unbalanced age distribution, we presumed patient age to be a relevant confounder of the OS estimate. In a Cox proportional hazards model including age, treatment arm, liver-limited disease, synchrone vs metachrone metastasis, and Eastern Cooperative Oncology Group (ECOG) performance status as covariates, CH was an independent predictor of OS (hazard ratio [HR], 0.64; 95% confidence interval [CI], 0.46-0.89; $P = .007$; Figure 2C). This association was even more pronounced for patients with multiple mutations (HR, 0.37; 95% CI, 0.21-0.64; $P < .001$; supplemental Figure 3). In an exploratory analysis on single gene and gene group level for most frequently mutated genes, particularly *DNMT3A*-mutated patients (CH-*DNMT3A*) showed longer OS compared with *DNMT3A* wild-type patients: median OS was 51.1 vs 33.2 months ($P = .044$ in log-rank test; Figure 2B; supplemental Figure 4). CH-*DNMT3A* remained an independent predictor of OS in multivariate analysis (HR, 0.53; 95% CI, 0.36-0.80; $P = .002$; Figure 2D). In line with this, patients with CH-*DNMT3A* more frequently showed early tumor shrinkage,[11] defined by a reduction of a reference tumor lesion of at least 20% after 6 or 8 weeks of treatment (75% vs 56%; $P = .033$; supplemental

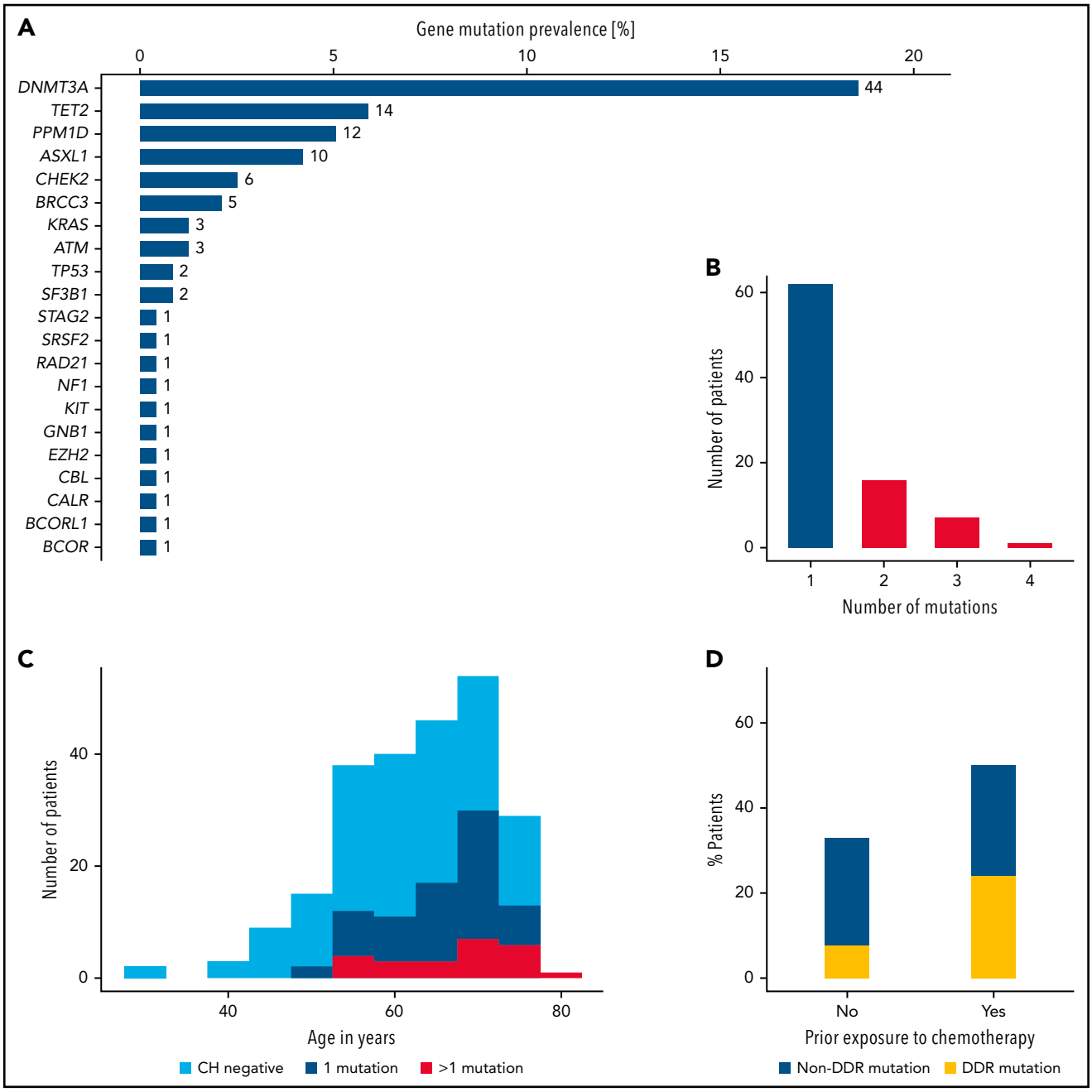
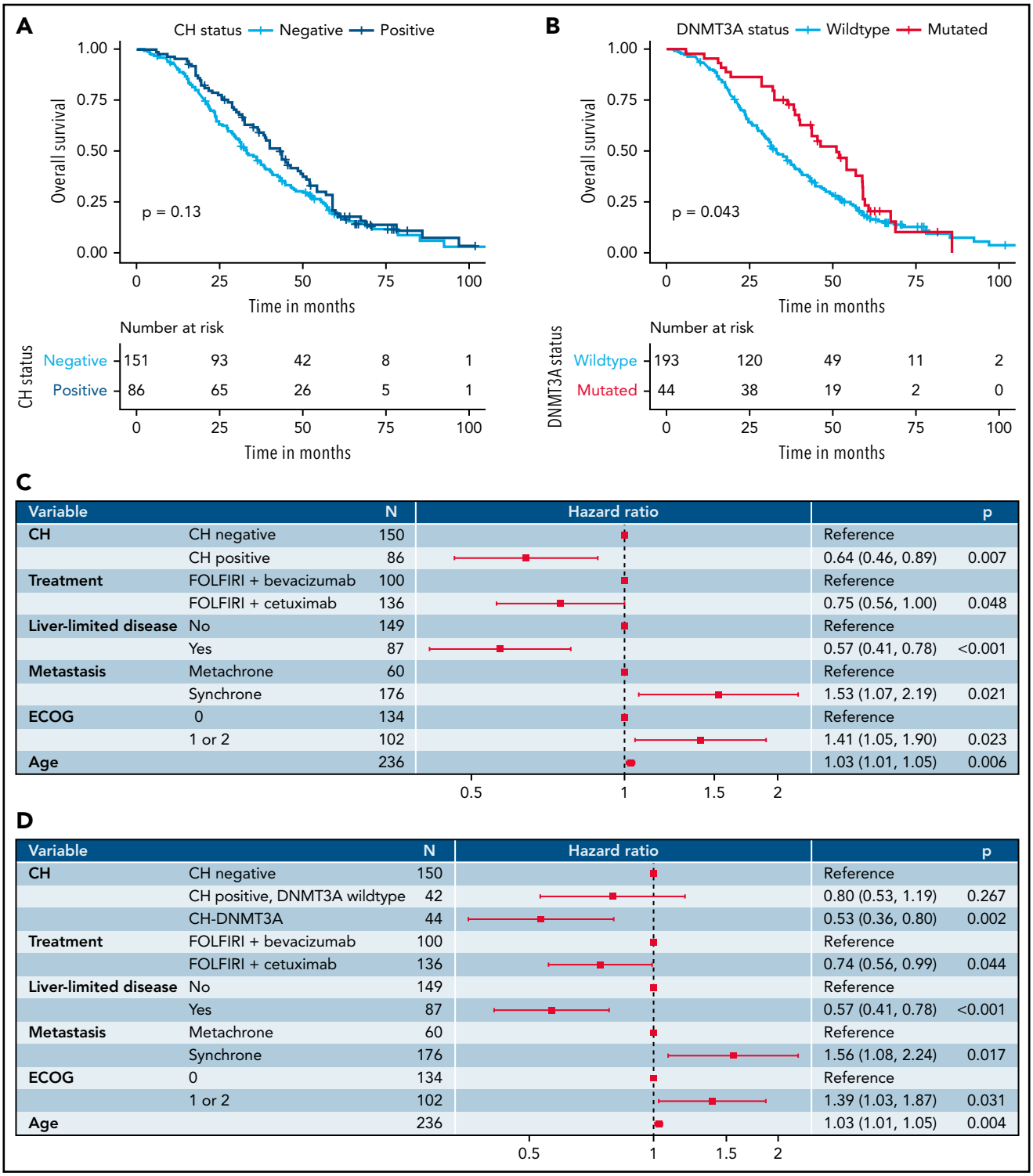


Figure 1. Overview of mutations identified by targeted sequencing. (A) Gene-specific prevalence of CH mutations. (B) Number of patients with 1, 2, 3, or 4 mutations. (C) Age distribution of 237 patients according to CH status. (D) CH prevalence according to prior exposure to chemotherapy. DDR, DNA-damage repair machinery.

Table 4). Of note, the association with improved OS also holds for the more conservative definitions of *clonal hematopoiesis of indeterminate potential*[12] (n = 65), with VAF ≥ 2%, and clonal hematopoiesis with potential driver mutations (n = 65) as defined in Coombs et al[6] (supplemental Figures 5 and 6). In fact, VAF as a continuous measure of clone size is significantly associated with improved OS, hinting toward a potential dose-response relationship (supplemental Figure 7).

Regarding secondary end points in the FIRE-3 cohort, no association of CH with progression-free survival in first-line or subsequent treatment was found (supplemental Figure 8). Although there was no difference in time on first-line treatment, time on subsequent treatment lines was significantly longer for CH and CH-*DNMT3A* (supplemental Table 5). CH was associated with the occurrence of grade 3/4 diarrhea (17.4% vs 6.6%; $P = .014$) and the occurrence of thromboembolic events (7.0% vs 1.3%; $P = .028$; supplemental Tables 6 and 7; supplemental Figure 9). Importantly, neither association of CH with hematotoxic therapy complications nor with tumor mutational status[13] nor with consensus molecular subgroups based on gene expression signatures[14] was found (supplemental Tables 8 and 9).



C

Variable		N	Hazard ratio	p
CH	CH negative	150	Reference	
	CH positive	86	0.64 (0.46, 0.89)	0.007
Treatment	FOLFIRI + bevacizumab	100	Reference	
	FOLFIRI + cetuximab	136	0.75 (0.56, 1.00)	0.048
Liver-limited disease	No	149	Reference	
	Yes	87	0.57 (0.41, 0.78)	<0.001
Metastasis	Metachrone	60	Reference	
	Synchrone	176	1.53 (1.07, 2.19)	0.021
ECOG	0	134	Reference	
	1 or 2	102	1.41 (1.05, 1.90)	0.023
Age		236	1.03 (1.01, 1.05)	0.006

0.5 1 1.5 2

D

Variable		N	Hazard ratio	p
CH	CH negative	150	Reference	
	CH positive, DNMT3A wildtype	42	0.80 (0.53, 1.19)	0.267
	CH-DNMT3A	44	0.53 (0.36, 0.80)	0.002
Treatment	FOLFIRI + bevacizumab	100	Reference	
	FOLFIRI + cetuximab	136	0.74 (0.56, 0.99)	0.044
Liver-limited disease	No	149	Reference	
	Yes	87	0.57 (0.41, 0.78)	<0.001
Metastasis	Metachrone	60	Reference	
	Synchrone	176	1.56 (1.08, 2.24)	0.017
ECOG	0	134	Reference	
	1 or 2	102	1.39 (1.03, 1.87)	0.031
Age		236	1.03 (1.01, 1.05)	0.004

0.5 1 1.5 2

Figure 2. Impact of clonal hematopoiesis on clinical outcome. (A) Kaplan-Meier analysis of OS stratified by CH status in the FIRE-3 cohort. (B) Kaplan-Meier analysis of OS stratified by *DNMT3A* mutation status in the FIRE-3 cohort. (C) Cox proportional hazard model of OS with CH status, treatment arm, liver-limited disease status, history of metastasis, ECOG performance status, and age as covariates. (D) Cox proportional hazard model of OS with CH-*DNMT3A* status, treatment arm, liver-limited disease status, history of metastasis, ECOG performance status, and age as covariates.

To our knowledge, this is the first study of CH in a well-defined cohort of patients with cancer with prospectively collected clinical data. Surprisingly, CH and, in particular, CH-*DNMT3A* were associated with improved OS in this patient collective. Coombs et al[6] reported shorter OS in 5650 patients with cancer, encompassing 51 cancer types with varying disease stages, treatment regimens, and blood sampling time points, making general conclusions for one cancer entity difficult.

Given the important role of CH in inflammatory processes, it is conceivable that CH mutations and, in particular, *DNMT3A* mutations in mature immune cells modulate the tumor microenvironment. Increased activity of the NLRP3 inflammasome has been linked to *DNMT3A* deficiency[15] and was shown to play a role in the suppression of metastatic growth via interleukin-18 and activation of natural killer cells.[16] Moreover, the polarization of protumorigenic and antitumorigenic phenotypes of tumor-infiltrating leukocytes (TILs) is in part controlled by epigenetic mechanisms.[17] In fact, *DNMT3A* regulates the polarization of TH1 and TH2 cells via silencing of the interferon γ promoter.[18,19] Along these lines, we hypothesize that *DNTM3A* mutations shift the polarization of TILs toward the antitumorigenic phenotype, leading to improved tumor control and OS.

Whether the observed association is tumor entity specific, therapy specific, or an independent factor in this context remains to be investigated in future mechanistic studies. In addition, confirmatory studies in large independent mCRC datasets are warranted in the future.

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Authorship

Contribution: C.M.A. and F.D. designed the study; C.M.A., S.D., A.S., R.H., P.M.S., C.M.S., M.T., D.P.M., S.S., V.H., and F.D. provided cases, data, and/or clinical annotation; C.M.A., S.D., A.S., R.H., P.M.S., C.M.S., M.T., and F.D. carried out experiments and data analysis; C.M.A., S.D., V.H., and F.D. wrote the manuscript; and all authors reviewed and approved the final manuscript.

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Footnotes

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Detailed information regarding the gene panel and DNA sequencing is available from the corresponding author upon reasonable request.

The online version of this article contains a data supplement.

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