

Elevated Clonal Hematopoiesis in 9/11 First Responders Has Distinct Age-related Patterns and Relies on IL1RAP for Clonal Expansion



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ABSTRACT

Environmental exposures are linked to precancerous hematologic conditions, but studies in cohorts with well-defined exposures are limited. We sequenced blood samples from a large cohort of first responders exposed to the aerosolized dust and carcinogens from the 9/11 World Trade Center (WTC) disaster and observed a significantly higher prevalence of clonal hematopoiesis (CH) mutations when compared with two sets of control cohorts after controlling for age, race, and sex. Younger exposed first responders exhibited unconventional CH mutations, with defective DNA repair signatures. Leukemia risk was elevated (3.7% vs. 0.6%; OR = 5.73) in WTC-exposed responders with CH versus without CH. Exposure to particulate matter collected from WTC site impaired healthy stem cells while expanding *Tet2*-mutant CH clones in mice. The inflammation sensor IL1RAP was overexpressed in murine CH, and genetic knockdown inhibited mutant clone growth *in vivo*. This study links discrete environmental exposure to hematopoietic mutations and leukemia, identifying IL1RAP as a novel therapeutic target in CH.

SIGNIFICANCE: This study identifies elevated CH with distinct, nonclassical mutations in 9/11 first responders, particularly younger individuals, and links CH to increased leukemia risk. Findings suggest broader relevance to other genotoxic environmental exposures and highlight IL1RAP as a critical driver of CH expansion and a promising therapeutic target for inflammation-driven malignancies.

See related commentary by Safina and van Galen, p. 2406

INTRODUCTION

Environmental exposures to chemicals such as polychlorinated biphenyls, dioxins, polycyclic aromatic hydrocarbons, asbestos, and smoke have been postulated as risk factors for various diseases and cancers (1–5). There have been few human studies that have evaluated the effects of well-defined environmental exposures with premalignant and malignant diseases of the blood (6–9). On September 11, 2001, the collapse of the World Trade Center (WTC) generated particulate matter that contained a potent mixture of carcinogens and genotoxic substances (10–12). It has been estimated that more than 400,000 responders and area workers/residents were exposed to this unprecedented environmental disaster (13–15).

Epidemiologic studies have documented elevated risks of cancer, cardiovascular diseases, and other health complications among individuals exposed to WTC-particulate matter (WTC-PM), particularly among firefighters and other responders (16–20). Furthermore, in the Fire Department of the City of New York (FDNY) WTC Health Program surveillance reports with more current data, we have noted that the incidence of leukemia is greater among WTC-exposed responders compared with the US general population [standardized incidence ratio = 1.43; 95% confidence interval (CI), 1.13–1.81; Internal report: WTCHP FDNY Data Center CLIN 3 Special Project Annual Report, Option Year 4, 2022]. In addition to elevated risk of cancer, we observed an increased prevalence of monoclonal gammopathy of unknown significance (17, 18) in

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Table 1. Demographics of WTC-exposed responders, non-WTC-exposed firefighters, and non-WTC-exposed controls.

	WTC-exposed responders (N = 988)	non-WTC-exposed firefighters (N = 255)	non-WTC-exposed controls (N = 198)
Sex			
Male	969 (98.1%)	244 (95.7%)	188 (94.9%)
Female	19 (1.9%)	11 (4.3%)	10 (5.1%)
Age at blood draw			
20–39	70 (7.1%)	0 (0%)	0 (0%)
40–49	272 (27.5%)	22 (8.6%)	0 (0%)
50–59	463 (46.9%)	82 (32.2%)	55 (27.8%)
60–69	141 (14.3%)	86 (33.7%)	77 (38.9%)
70–79	39 (4.0%)	48 (18.8%)	48 (24.2%)
80+	3 (0.3%)	17 (6.7%)	18 (9.1%)
Race/ethnicity			
White	872 (88.3%)	242 (94.9%)	188 (94.9%)
African American	49 (5.0%)	11 (4.3%)	10 (5.1%)
Hispanic	57 (5.7%)	2 (0.8%)	0 (0%)
Asian	9 (0.9%)	0 (0%)	0 (0%)
Occupation			
Firefighter	912 (92.3%)	255 (100%)	0 (0%)
Emergency Service Providers (EMS)	76 (7.7%)	0 (0%)	0 (0%)
Unknown	0 (0%)	0 (0%)	198 (100%)
Smoking			
Ever	342 (34.6%)	102 (40.0%)	101 (51.0%)
Never	646 (65.4%)	101 (39.6%)	97 (49.0%)
Unknown	0 (0%)	52 (20.4%)	0 (0%)

WTC-exposed responders (19), providing initial insights into the hematologic effects of WTC-PM exposure. We also performed a pilot sequencing analysis in relatively older WTC-exposed responders demonstrating increased clonal hematopoiesis (CH; ref. 21). CH is characterized by the acquisition of somatic mutations in blood cells. *TET2*, *DNMT3A*, and *SF3B1* are the most commonly mutated genes, and the same pattern of mutations was seen in our pilot analysis (22–24).

Building upon this preliminary evidence, we conducted a sequencing analysis of a larger cohort of FDNY-WTC responders. To comprehensively determine the association between WTC exposure and CH, we expanded our analysis to include younger exposed individuals and a broader set of age-matched controls including a new cohort of individuals who were without experience as firefighters. Importantly, the inclusion of a larger proportion of younger WTC-exposed responders reflects the diverse demographic profile of people and rescue workers affected by this and other environmental disasters. The objectives of the current study were multifaceted. Our primary goal was to identify novel pathways, potential biomarkers, and associated malignancies linked to environmentally induced CH, with the broader aim of informing precision medicine approaches for risk assessment and management in this vulnerable population. Specifically, we first sought to determine the association between WTC exposure and CH using a well-curated cohort of WTC-exposed

responders compared against two non-WTC-exposed control groups. We also assessed whether CH mutations were associated with blood count abnormalities or the development of hematologic malignancies. Finally, we used murine models to explore the underlying mechanisms of CH development following environmental exposure.

RESULTS

WTC-exposed Responders Have High Burden of CH

We evaluated CH status in FDNY responders who were exposed to the WTC disaster ($n = 988$; 912 firefighters and 76 EMS) and non-WTC-exposed firefighters ($n = 255$) and non-WTC-exposed controls ($n = 198$). The majority of participants across both WTC-exposed and non-WTC-exposed groups was male (98.1%) and identified as White (88.3%; Table 1). This cohort is predominantly composed of younger WTC-exposed responders, with 81.4% being under the age of 60 years at blood draw (Table 1). Each participant had bulk DNA isolated from their total blood, which was used for deep targeted sequencing of 237 genes frequently mutated in hematologic malignancies in a clinical-grade Clinical Laboratory Improvement Amendments–certified lab (Supplementary Table S1). Clinically significant somatic mutations with a variant allele frequency (VAF) between 2% and 40% were designated as somatic mutations indicative of CH. Furthermore, CH mutations

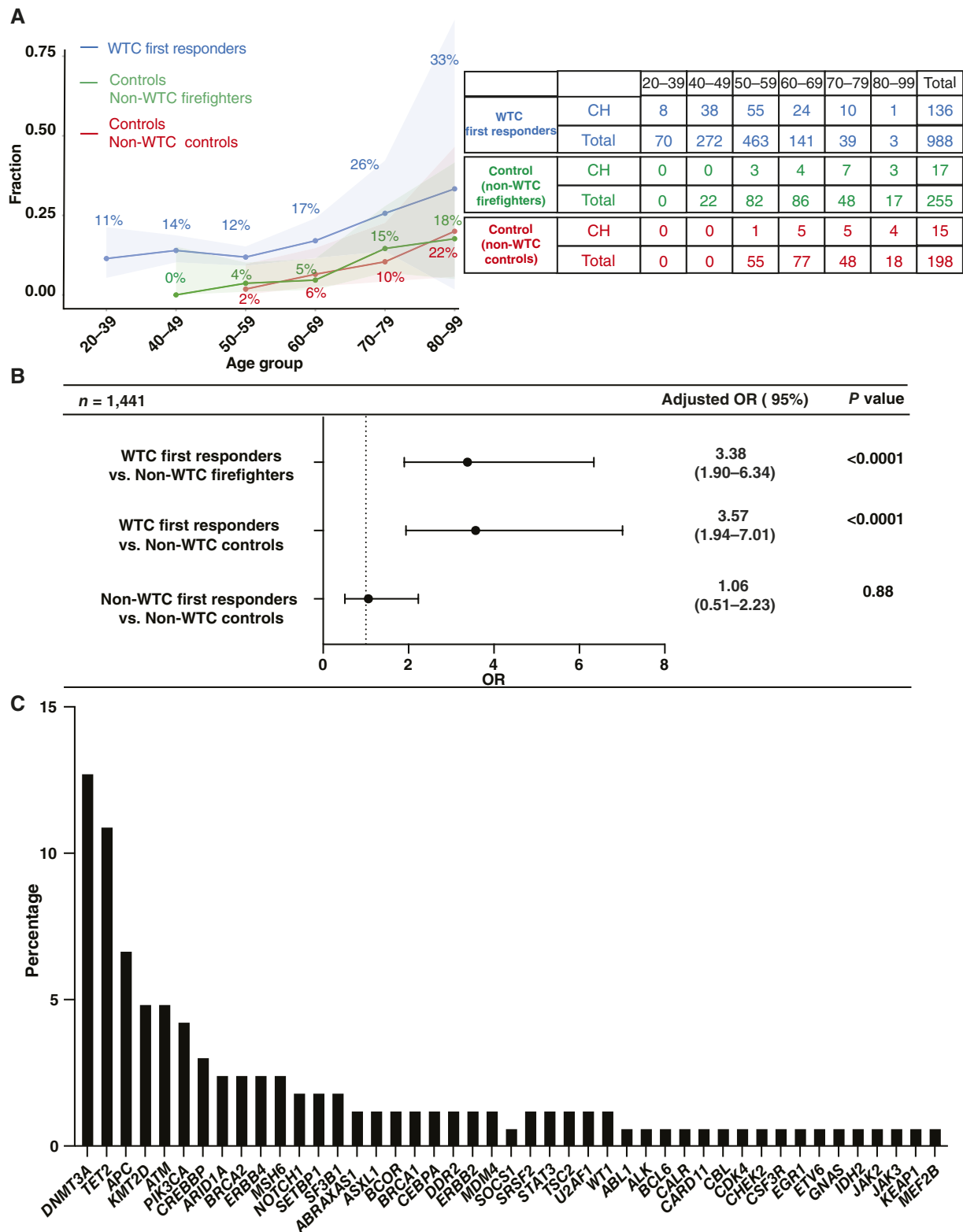


Figure 1. Prevalence of somatic mutation in WTC-exposed responders. **A**, The number of somatic mutations in 988 WTC-exposed responders (blue), 255 non-WTC-exposed firefighters (green), and 198 non-WTC-exposed (general population) controls (red) cohort in respective age group. In respective shading, 95% confidence intervals (CIs) are shown. **B**, Forest plot displaying the odds ratio (OR) and 95% CIs of CH incidence in WTC-exposed responders vs. non-WTC-exposed firefighters, WTC-exposed responders vs. non-WTC-exposed control, and non-WTC-exposed firefighters vs. non-WTC-exposed controls. Data were analyzed using a multivariable logistic regression model and exact method. **C**, Percentage of WTC-exposed responders with mutations for specific genes.

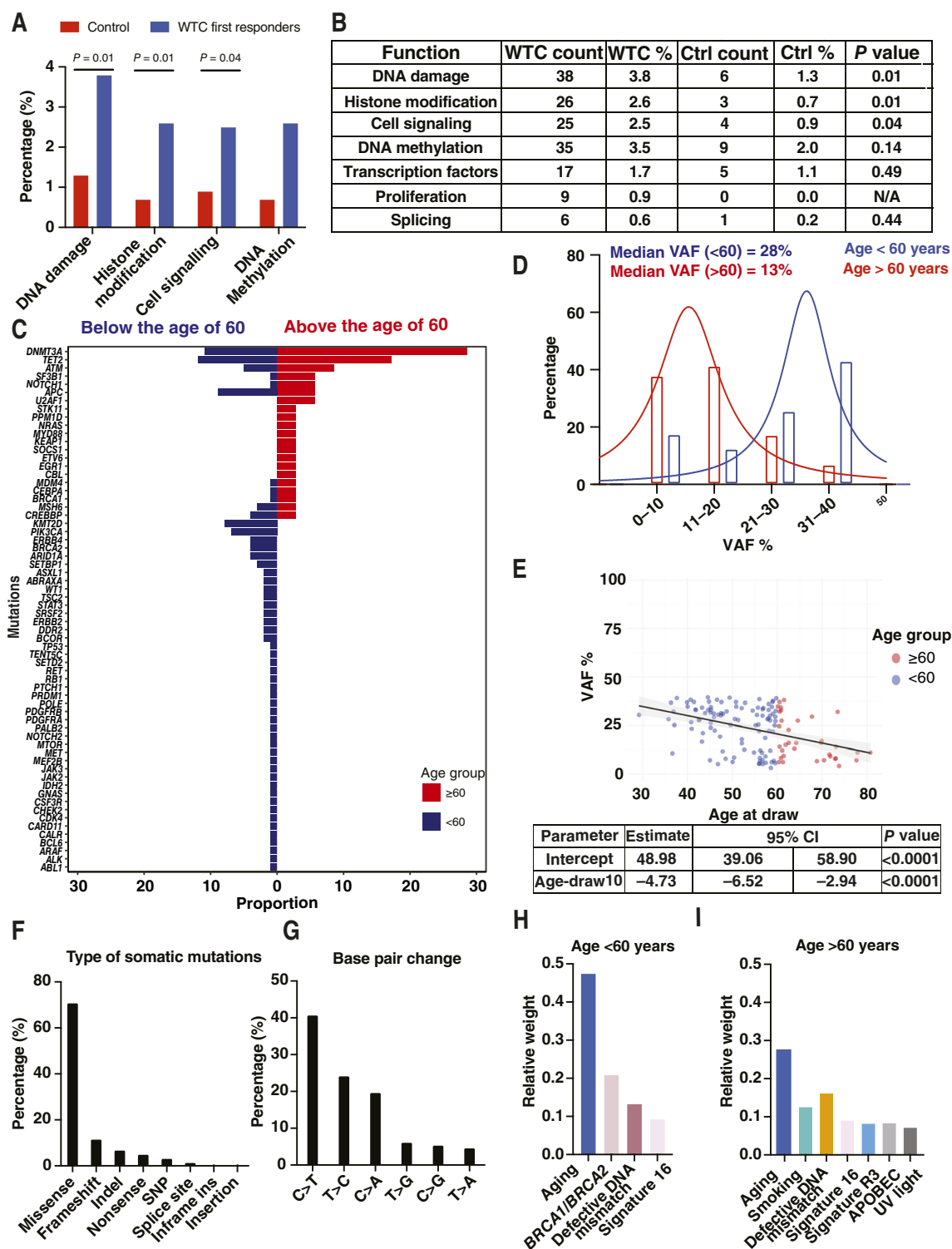


Figure 2. Age-dependent differential characteristics of somatic mutations/CH are observed in WTC-exposed responders. **A** and **B**, Bar graph (**A**) and table (**B**) showing the percentage and number of WTC-exposed responders (blue) and non-WTC-exposed controls (Ctrl; red) with CH mutations, stratified by gene function. **C**, Graph illustrating the proportions of WTC-exposed responders, categorized by age (below 60 years in blue and above 60 years in red), harboring somatic mutations in various genes. **D**, Percentage of WTC-exposed responders showing median variant allele frequency (VAF), categorized by age (blue: below 60 years and red: above 60 years). **E**, Scatter plot with simple linear regression modeling VAF as a function of age. Blue dots represent individuals aged <60 years, and red dots represent individuals aged >60 years. **F**, Percentage of specific types of somatic mutations in CH+ WTC-exposed responders. **G**, Percentage of specific base pair changes observed in CH+ WTC-exposed responders. **H** and **I**, Bar graph depicting the distribution of different mutational signatures detected from all somatic mutations in WTC-exposed responders below the age of 60 years (**H**) and WTC-exposed responders above the age of 60 years (**I**).

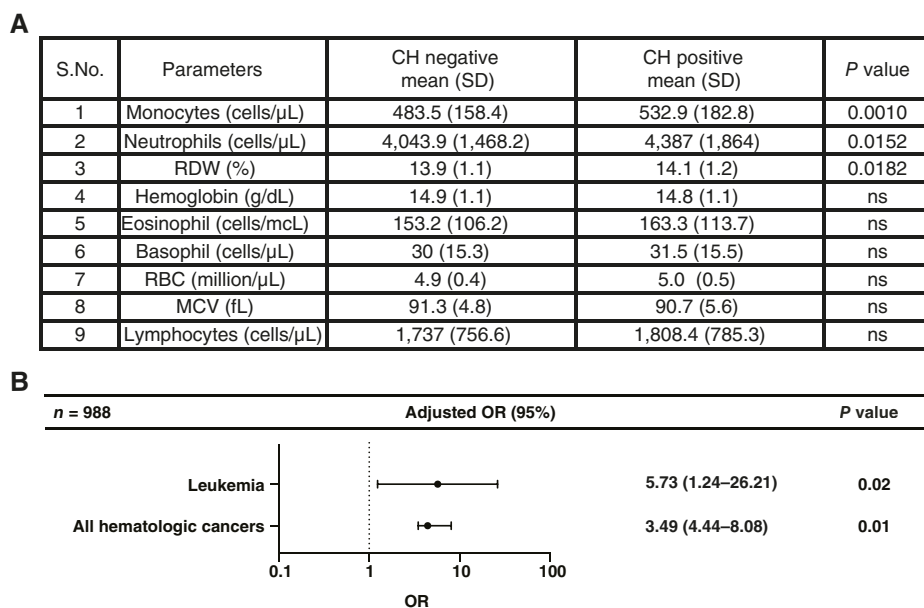


Figure 3. Increased burden of leukemia and inflammation in CH+ WTC-exposed responders. **A**, Differential blood count in CH+ ($n = 136$) and CH- ($n = 852$) WTC-exposed responders. Data depict mean values with SD (two tailed t test). **B**, Forest plot illustrating the adjusted OR (exact method) and 95% CIs for the association of leukemia and all hematologic cancers in CH+ WTC-exposed responders. Models control for age (continuous variable), sex, race (White vs. other), WTC arrival time (day 1 vs. later), smoking (ever vs. never), and work assignment (firefighter vs. EMS).

were annotated as significant based on reference databases, including the Catalog of Somatic Mutations in Cancer (COSMIC), Single Nucleotide Polymorphism Database, and Exome Aggregation Consortium.

In the WTC-exposed cohort, we identified 136 individuals with 165 total somatic mutations of expected pathogenic potential (Supplementary Table S2) for an overall prevalence of 13.8%. Of those with CH, 22 individuals (22 of 136, 16%) carried more than one CH mutation. The prevalence of CH was 6.7% (17 of 255) in the non-WTC-exposed firefighters and 7.6% (15 of 198) in the non-WTC-exposed controls (Supplementary Table S3); the prevalence of CH was similar

between these two control groups. In those with mutation, four individuals (four of 17, 23%) in the non-WTC-exposed firefighter group and two individuals (two of 14, 14%) in the non-WTC-exposed control group carried more than one CH mutation. Among both the WTC-exposed cohort and the non-WTC-exposed cohort, the crude age-specific prevalence of CH increased with age (Fig. 1A). We conducted multivariable logistic regression analyses to determine the association between WTC exposure and CH status controlling for age, sex, and race/ethnicity. In these analyses, we restricted the logistic models to those older than 50 years because of sparse data in both of the comparison populations. In models comparing WTC-exposed responders with non-WTC-exposed firefighters, WTC exposure was associated with over a three-fold increased odds of CH (OR = 3.38; 95% CI, 1.90–6.34; $P < 0.01$; Fig. 1B). Similarly, in models comparing the WTC-exposed responders with non-WTC-exposed controls, WTC exposure was associated with a 3.5-fold increase in CH (OR = 3.57; 95% CI, 1.94–7.01; $P < 0.01$; Fig. 1B). Among the WTC-exposed cohort, with each subsequent decade, we noted a significant increase in the prevalence of pathogenic somatic mutations (OR = 1.23 for each increase in age group decade, 95% CI, 1.00–1.50; $P = 0.048$). There was no difference of CH burden when comparing control groups non-WTC-exposed firefighters with non-WTC-exposed controls (OR = 1.06; 95% CI, 0.51–2.23; $P = 0.88$; Fig. 1B). In multivariable analyses restricted to those with smoking information, when controlling for smoking as well as age, sex, and race/ethnicity, the association between WTC-exposure and CH remained 3.3-fold for WTC-exposed responders versus non-WTC-exposed firefighters (OR = 3.37; 95% CI, 1.85–6.49; $P < 0.01$) and 3.5-fold for WTC-exposed responders versus the non-WTC-exposed controls (OR = 3.58;

Table 2. Diagnoses of hematologic malignancies in WTC-exposed responders.

Cancer type	N
Multiple myeloma	8
Lymphoma - non-Hodgkins	7
Chronic lymphocytic leukemia	5
Chronic myeloid leukemia	<5
Myelodysplastic syndrome	<5
Lymphoma - Hodgkins	<5
Myelodysplastic/myeloproliferative neoplasm	<5
Chronic myelomonocytic leukemia	<5
Polycythemia vera	<5
Leukemia, unspecified	<5
Essential thrombocythemia	<5

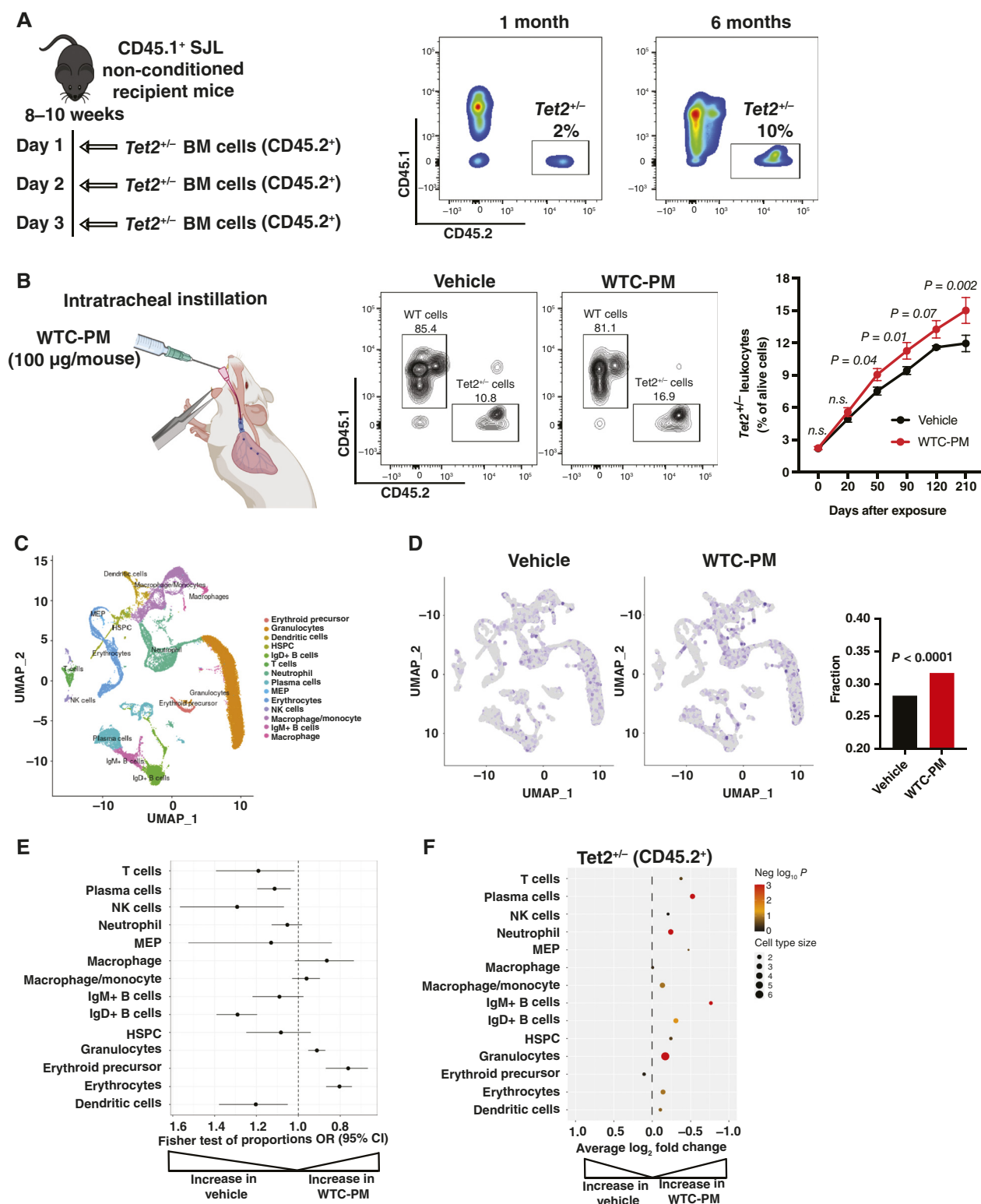


Figure 4. WTC-PM exposure expands Tet2-dependent CH and induces inflammation. **A**, Schematic representation of the murine model for CH dependent on Tet2^{+/-} mutant cells and representative flow cytometer plot showing the percentage of CD45.2⁺ Tet2^{+/-} mutant cells in the peripheral blood of CH mice at 1 and 6 months after transplantation. **B**, Representative flow cytometer plot and quantified graph showing the percentage of CD45.2⁺ Tet2^{+/-} mutant cells in peripheral blood of WTC-PM (red) and vehicle (black)-treated CH mice at the indicated time ($n = 5$ –6 per group, two-way ANOVA test, individual P values from Benjamini multiple comparison test). **C**, CITE-seq analysis reveals distinct clusters of differentiated and stem progenitor blood cells in a Uniform Manifold Approximation and Projection (UMAP) plot. **D**, UMAP plot showing the expression of CD45.2 (Tet2^{+/-} cells, blue dots) across various stem and differentiated cell populations in vehicle- and WTC-PM-treated mice. (continued on following page)

95% CI, 1.94–7.04; $P < 0.01$). Even with a more stringent, lower VAF threshold for somatic CH calling, we consistently observed a significantly increased burden of CH in WTC-exposed responders (Supplementary Figs. S1 and S2).

Differential Mutational Landscape in Younger WTC-exposed Responders

Genes typically involved in CH and myeloid malignancies were found to be mutated in WTC-exposed responders. Even though *DNMT3A* (12.7%) and *TET2* (10.9%) were the top two most mutated genes in WTC-exposed responders, we observed somatic mutations in genes that were not heavily involved in previous CH studies, including *APC* (6.6%), *KMT2D* (4.8%), *ATM* (4.8%), *PIK3CA* (4.2%), and *CREBBP* (3.0%; Fig. 1C). Common fragile sites (CFS) are genomic hotspots of replication stress that detect basal levels of stress and DNA damage in the cell. We previously observed in an *in vitro* system that WTC-PM exposure perturbs DNA replication dynamics at CFS locations in cells and makes the cells more susceptible of DNA damage and mutation accumulation (19). Consistent to our previous observation, we observed that these nonclassical CH mutations either overlap or occur in the close proximity of the fragile sites (Supplementary Fig. S3A). We further stratified the CH mutations/genes based on their biological function (Supplementary Fig. S3B) and observed that the mutations involved in DNA damage, histone modification, and cell signaling were significantly increased in WTC-exposed responders (Fig. 2A and B). We also stratified the proportion of CH mutations by age and observed that there is an enrichment of nonclassical CH mutations in WTC-exposed responders below the age of 60 years (Fig. 2C). Moreover, CH in younger responders was characterized by a higher VAF (median VAF of 28% vs. 13%), with 70% of the mutations being missense mutations (Fig. 2D–F). The most common base-pair change was the cytosine to tyrosine (C → T) mutation, associated with aging and DNA damage (Fig. 2G). Analysis of overall mutational signatures (25–27) in WTC-exposed responders demonstrated an enrichment of aging-related (Signature 1A) and defective homologous recombination-based repair or BRCA1/BRCA2 mutation-associated (Signature 3) signatures in individuals below the age of 60 years compared with those above the age of 60 years (Fig. 2H and I; Supplementary Figs. S4 and S5).

Increased Risk of Blood Count Abnormalities and Blood Cancers Among WTC-exposed Responders with CH-Associated Mutations

Among the WTC-exposed responders, we further investigated the association between CH mutations and variations in blood counts and leukemia. We observed that monocytes and neutrophil counts were significantly higher in CH cases,

suggesting an increased inflammatory milieu in WTC-exposed responders with CH versus without CH (Fig. 3A). Even though the red cell counts did not differ by CH status, we observed a significant increase in red cell width, a marker of marrow inflammation (28), in CH cases. Among the 988 WTC-exposed responders, 32 were diagnosed with a post-9/11 blood cancer, 10 of which were a leukemia diagnosis. After controlling for age, race, and sex, individuals with CH had 3.49-fold higher odds (95% CI, 4.44–8.08; $P = 0.01$) of developing any blood cancer compared with those without CH. We found that the WTC-exposed responders with CH were nearly six time more likely to develop leukemia than those without CH (3.7% vs. 0.6%; OR = 5.73; 95% CI, 1.24–26.21; $P = 0.02$; Fig. 3B; Table 2).

WTC-PM Exposure Promotes Inflammation and Mutant Clonal Growth *In Vivo*

To better understand exposure-related CH mechanisms, we created an *in vivo* model mimicking an 8-hour work shift at the WTC site for responders by administering a single dose of WTC-PM (chemically characterized matter obtained from Ground Zero) to mice via intratracheal instillation (29, 30). We employed a non-conditioned *Tet2*^{+/-} bone marrow (BM) transplantation-based model of CH (31) and investigated the impact of WTC-PM on the CH development. Loss-of-function mutations in the *TET2* gene are among the most common mutations in CH, occurring in about one third of cases (24, 32). We transplanted total BM cells freshly isolated from CD45.2⁺ *Tet2*^{+/-} mutant mice into non-conditioned CD45.1⁺ wild-type (WT) mice for three consecutive days (Fig. 4A). The chimeric BM transplant (BMT) produces recipient mice with BM comprised of both the mutant *Tet2*^{+/-} clone as well as normal hematopoietic cells. We were able to recapitulate the low chimerism (2%–3%) of CD45.2⁺ *Tet2*^{+/-} cells 4 weeks after the transplant in WT mice, similar to human CH (Fig. 4A). We exposed these transplanted mice to vehicle or WTC-PM via intratracheal administration and followed the effect of the exposure on the clonal expansion of *Tet2*^{+/-} clones. We observed significant expansion of *Tet2*^{+/-} clones in WTC-PM-exposed mice over time compared with the vehicle-exposed control mice (Fig. 4B; Supplementary Fig. S6A). We further expanded the analysis with another commonly occurring CH mutation *Dnmt3a*^{R878H}. We utilized an *in vitro* methylcellulose colony-forming assay, in which we cultured total BM cells from *Dnmt3a*-mutant (*Dnmt3a*^{R878H}) mice in the presence of vehicle or WTC-PM in clonogenic assay and counted the colonies produced 7 to 10 days after plating. We observed significantly increased colony formation capacity of WTC-PM-treated *Dnmt3a*-mutant cells compared with vehicle-treated *Dnmt3a*-mutant cells (Supplementary Fig. S6B). We further tested the effects of WTC-PM on a nonclassical CH mutation, *Apc*, in an *in vitro* clonogenic assay. We cultured total BM cells from *Apc*-mutant (*Apc*^{min}) and WT mice in the

Figure 4. (Continued) The accompanying bar graph shows the overall proportion of CD45.2⁺ cells in vehicle-treated (black) and WTC-PM-treated (red) mice. Proportion is defined as the fraction of cells with nonzero expression of the CD45.2 antibody probe within each treatment group, calculated using Seurat's FindMarkers function. *P* values were obtained using the Wilcoxon test; $n = 2$ biological replicates were used for both vehicle- and WTC-PM-treated mice. **E**, Forest plot representing OR with 95% CI (Fisher test) for the enrichment of different cell clusters in vehicle- and WTC-PM-treated *Tet2*-CH mice. **F**, Plot indicating significant enrichment of *Tet2*-mutant-derived cell types in WTC-PM-treated mice (highlighting neutrophils and granulocytes in red dots).

presence of vehicle or WTC-PM in methylcellulose assay and counted the colonies produced 7 to 10 days after plating. We observed significantly increased colony formation capacity of WTC-PM-treated *Apc*-mutant cells compared with vehicle-treated *Apc*-mutant cells, as well as WTC-PM-treated WT cells, whereas we did not observe any difference in the number of colonies of vehicle-treated *Apc*-mutant or non-mutant cells (Supplementary Fig. S6C).

To investigate the cellular and molecular consequences of WTC-PM exposure in CH mice, we performed single-cell RNA sequencing (scRNA-seq) on total BM cells isolated from both vehicle- and WTC-PM-treated *Tet2*^{+/-} CH mice. We identified various clusters of hematopoietic cell types, including hematopoietic stem and progenitor cells (HSPC), monocytes, and granulocytes (Fig. 4C), and confirmed an increased number of *Tet2*^{+/-} cells in WTC-PM-treated mice using scRNA-seq (Fig. 4D). We observed a significant increase in the size of myeloid-derived cell clusters in WTC-PM-exposed mice (Fig. 4E; Supplementary Fig. S6D). Additionally, there was an enlargement in the cell cluster size of *Tet2*^{+/-}-derived neutrophils and granulocytes (Fig. 4F). Altogether, these data suggested an increased inflammatory milieu in WTC-PM-treated CH mice.

WTC-PM Exposure Expands *Tet2*-Mutant Cells through IL1RAP

To identify the inflammatory mediators potentially responsible for the expansion of *Tet2*-mutant cells in WTC-PM-exposed mice, we analyzed differentially expressed genes in *Tet2*-mutant HSPCs. We observed significantly increased expression of IL-1 receptor accessory protein (*Il1rap*) on WTC-PM-exposed *Tet2*-mutant HSPCs (Fig. 5A). Similarly, we observed significantly increased expression of IL1RAP protein on *Tet2*-mutant myeloid cells from WTC-PM-exposed mice (Fig. 5B). IL1RAP is a cell surface accessory receptor that potentiates signaling downstream of IL-1 β , IL-33, FMS-like tyrosine kinase 3, and KIT receptor kinase (33). Consistently, we observed a significantly increased amount of IL-1 β in the plasma of WTC-PM-exposed mice (Fig. 5C).

To assess the functional significance of IL1RAP in the expansion of *Tet2*-mutant cells in WTC-PM-exposed mice, we generated *Tet2*^{+/-}; *Il1rap*^{+/-} double mutant mice, carrying heterozygous deletion for both *Tet2* and *Il1rap*. Subsequently, we generated CH models using either single mutant *Tet2*^{+/-} cells or double mutant *Tet2*^{+/-}/*Il1rap*^{+/-} cells. We exposed these mice to vehicle or WTC-PM and tracked the growth of mutant cells. We observed a significantly reduced expansion of double mutant cells compared with single mutant cells in WTC-PM-treated mice and did not observe any difference in the growth capacity of single and double mutant cells in vehicle-treated mice (Fig. 5D; Supplementary Fig. S7A–S7H). These findings indicate that IL1RAP-dependent signals play a crucial role in the expansion of *Tet2*-mutant cells under environmental exposure conditions.

As IL1RAP co-dimerizes with IL1R1 to activate IL-1 β -dependent downstream signaling, we further tested the therapeutic efficacy of IL1R1 blockade using anakinra in an *in vivo* *Tet2*-CH model. WTC-PM-exposed *Tet2*-mutant CH mice were co-treated with either vehicle or anakinra. We observed a nonsignificant trend toward reduced *Tet2*-mutant cell expansion in anakinra-treated mice (Fig. 5E). These findings

imply that IL1RAP potentially exerts a stronger effect on *Tet2*-mutant CH progression under environmentally exposed conditions.

WTC-PM Exposure Impairs Hematopoietic Stem Cells

Furthermore, we investigated the impact of WTC-PM on the normal hematopoiesis. We did not find any significant changes in the complete blood count (CBC) of WTC-PM-exposed mice, although a modest increase in eosinophils was observed after 3 months of exposure (Supplementary Fig. S8A). Additionally, there was no difference in the frequency or absolute number of WT HSPCs or hematopoietic stem cells (HSC) in WTC-PM-exposed mice (Fig. 6A; Supplementary Fig. S8B). However, we observed impaired reconstitution potential of WTC-PM-exposed HSCs in competitive and serial BMT assays, suggesting a functional decline of HSCs after exposure to WTC-PM (Fig. 6B and C; Supplementary Fig. S9A–S9H). To further investigate the mechanisms underlying HSC decline following WTC-PM exposure, we performed gene set enrichment analysis using previously generated cellular indexing of transcriptomes and epitopes by sequencing (CITE-seq) data from WT HSPCs and observed enrichment of hallmark pathways, including E2F targets, glycolysis, and hypoxia, which are previously implicated in the regulation of HSC function (Fig. 6D). Given the important role of IL1RAP in environmentally induced *Tet2*-mutant CH, we next assessed its role in HSC function following environmental exposure. WT and *Il1rap*^{+/-} mice were exposed to vehicle or WTC-PM, and primary and secondary methylcellulose assays were performed using total BM cells harvested 3 months after exposure. We observed a significant reduction in colony numbers in both WTC-PM-exposed WT and *Il1rap*^{+/-} mice compared with their respective vehicle-treated controls (Fig. 6E). As *Il1rap* haploinsufficiency did not rescue the colony-forming capacity of BM cells from WTC-PM-exposed mice, it suggested that WTC-PM-induced HSC impairment occurs independently of IL1RAP. We also observed mutations in nonclassical CH genes in HSPCs of WTC-PM-exposed mice, similar to those observed in younger WTC-exposed responders (Supplementary Fig. S9I). Taken together, our *in vivo* results demonstrate that exposure to WTC-PM leads to an inflammatory milieu, reduced healthy HSC functionality, and faster clonal growth of CH clones.

DISCUSSION

Our study demonstrates the profound health implications of a large-scale environmental exposure, highlighting the elevated burden of developing CH and increased odds to develop blood malignancies in those who harbor CH. The observed increase in CH prevalence among WTC-exposed responders, after adjusting for demographic factors and smoking history, underscores the significant inflammatory effects of WTC-PM exposure on HSCs. Importantly, our findings reveal a distinct spectrum of mutations in younger WTC-exposed responders, indicating potential mechanisms specific to the environmental exposure. Furthermore, the enrichment of nonclassical CH mutations among younger responders suggests an accelerated aging process and impaired DNA damage pathways, potentially driven by the genotoxic substances present in

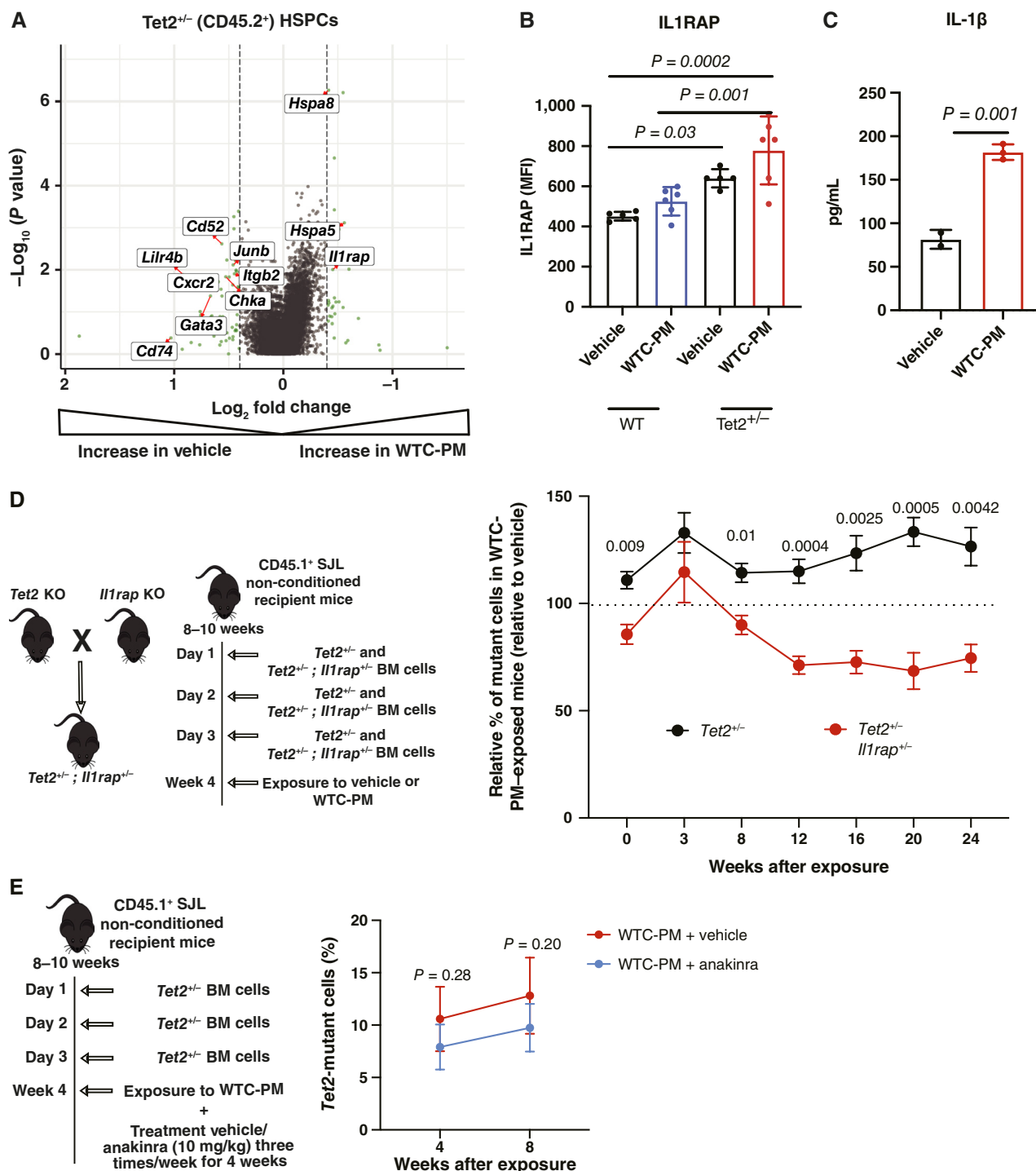


Figure 5. *Il1rap* deletion rescues the expansion of WTC-PM-dependent *Tet2*-mutant cells in vivo. **A**, Volcano plot highlighting significant differentially expressed genes (DEGs) in *Tet2*-mutant stem progenitor cells, featuring *Il1rap* (fold change threshold 0.4). **B**, Mean fluorescence intensity (MFI) of IL1RAP on CD45.1⁺ (WT) and CD45.2⁺ (*Tet2*-mutant; *Tet2*^{+/-}) CD11b⁺ myeloid cells in vehicle- and WTC-PM-treated mice after 20 days of exposure ($n = 5$ –6/group, one-way ANOVA; $P = 0.0002$, individual P values from the Tukey multiple comparison test). **C**, IL-1 β levels in plasma of WTC-PM (red)– and vehicle (black)–treated WT mice after 3 weeks of exposure ($n = 3$ /group, t test, $P = 0.001$). **D**, Schema of the experiment followed by a graph comparing *Tet2*^{+/-}-mutant cells (black, $n = 7$) and *Tet2*^{+/-}; *Il1rap*^{+/-} double mutant cells (red, $n = 7$) in peripheral blood of WTC-PM-exposed mice at the indicated time points. Mutant cells are shown relative to the average mutant cells from the vehicle-treated *Tet2*^{+/-} and *Tet2*^{+/-}; *Il1rap*^{+/-} CH mice for both groups ($n = 7$ for each group), as indicated by the dotted line. P values are calculated via two-way ANOVA, individual P values from the Šidák multiple comparison test. KO, knockout. **E**, Schema of the experiment followed by a graph depicting the percentage of *Tet2*^{+/-}-mutant cells in peripheral blood of WTC-PM-exposed mice treated with vehicle (red) or anakinra (blue, 10 mg/kg, three times a week; $n = 5$ /group, two-way ANOVA, individual P values from the Šidák multiple comparison test).

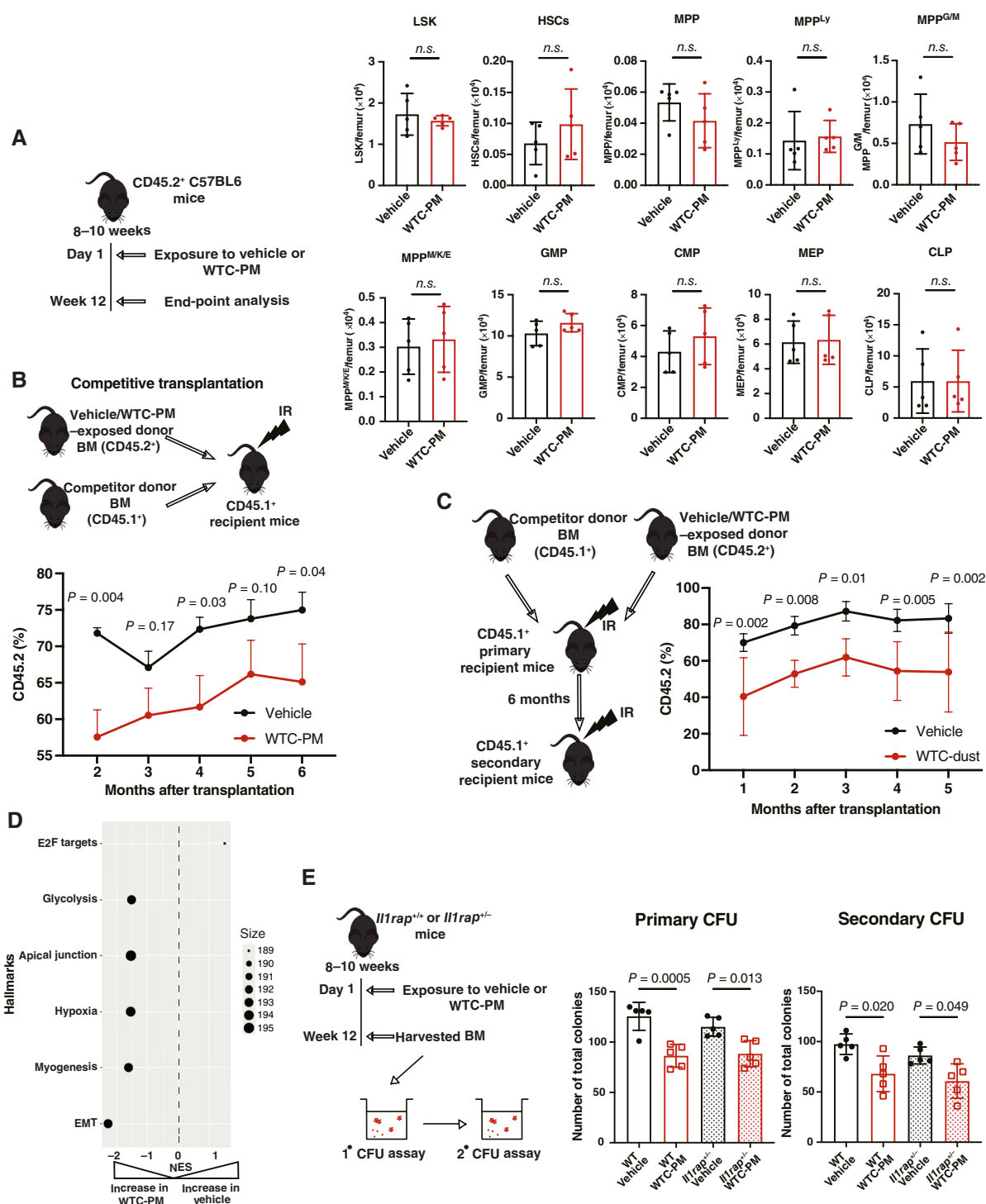


Figure 6. Environmental exposure with WTC-PM impairs HSC function. **A**, Absolute number of indicated cell types in the BM of vehicle- ($n = 5$; black) and WTC-PM ($n = 5$; red)-exposed WT mice after 12 weeks of exposure. **B**, Percentage of CD45.2⁺ cells 2–6 months after competitive transplantation of CD45.2⁺ total donor BM cells from vehicle (black) and WTC-PM (red), mixed with CD45.1⁺ competitor BM cells, and transplanted into lethally irradiated CD45.1⁺ recipient mice ($n = 5$, analyzed using two-way ANOVA, individual P values from the Benjamini, Krieger, and Yekutieli multiple comparison test). **C**, Percentage of CD45.2⁺ cells 1–5 months after secondary transplantation of total BM cells from primary competitively transplanted recipient mice (vehicle treated, black and WTC-PM treated, red) into lethally irradiated CD45.1⁺ recipient mice ($n = 5$, analyzed using two-way ANOVA, individual P values from the Sidák multiple comparison test). **D**, Dot plot showing reanalysis of CITE-seq data (Fig. 4) from WT HSPCs, depicting differential hallmark pathways enriched in DEGs from vehicle- or WTC-PM-exposed HSPCs. **E**, Schema of the experiment followed by a graph depicting the total number of colonies formed 7–10 days after primary or secondary plating of 50,000 total BM cells from WT (clear) or *Il1rap*^{+/-} (shaded) mice exposed to vehicle (black) or WTC-PM (red) 3 months prior to the assay. No additional WTC-PM was added to the methylcellulose media ($n = 5$ per group; one-way ANOVA, individual P values from the Tukey multiple comparison test). CFU, colony-forming unit; IR, irradiation; EMT, epithelial-mesenchymal transition.

WTC-PM. Consistently, we also observed the enrichment of mutations implicated in DNA damage response mechanisms (*TP53*, *CHEK2*, *BRCA1*, *BRCA2*, and *PPM1D*), as well as an enrichment of aging and defective homologous recombination-based repair/*BRCA1/2* mutational signatures in younger WTC-exposed responders. CFSs are genomic regions highly susceptible to replication stress, specially after exposure to genotoxins. We previously reported that WTC-PM exposure disrupts DNA replication dynamics and induces damage preferentially at CFSs (19). In the present study, we examined the genomic locations of nonclassical CH mutations (*APC*, *ATM*, *PIK3CA*, *KMT2C*, *KMT2D*, and *BRCA2*) and found that many of them either overlap with or are in close proximity to CFSs. This provides an additional mechanistic basis for the accumulation of novel CH mutations in WTC-exposed individuals. As most CH studies have been conducted in predominantly older cohorts without clearly defined environmental exposures, our findings suggest that CH in this context may involve a distinct set of genes in younger subjects.

The association between CH and elevated levels of inflammatory markers underscores the role of inflammation in driving hematopoietic alterations following WTC-PM exposure. scRNA-seq data provide mechanistic insights into the inflammatory milieu, implicating IL1RAP and IL-1 β inflammatory cytokine signaling in the expansion of mutant cells. Although IL1RAP has been recognized as a crucial marker for leukemic stem cells in malignant acute myeloid leukemia (33–35), its significance in the progression of nonmalignant hematopoietic conditions, including CH, has been understudied. Our *in vivo* models support the link between WTC-PM exposure and *Tet2* CH expansion via IL1RAP. Additionally, *in vitro* experiments using two different CH models harboring *Dnmt3a* and *Apc* mutations also demonstrated an increased expansion capacity of mutant cells following WTC-PM exposure. The genetic heterozygous deletion of *Il1rap* rescued the expansion of *Tet2*^{+/−} cells in the CH model following WTC-PM exposure. However, loss of IL1RAP is dispensable for normal HSC function (33). Strikingly, the previously reported (36–39) resistance to inflammation-mediated toxicity exhibited by *Tet2*-mutant cells was lost in *Il1rap*-deficient *Tet2*-mutant cells. The identification of IL1RAP-dependent signals as potential therapeutic targets may offer novel avenues for intervention to mitigate CH-associated risks among environmentally exposed individuals. IL1RAP has also been reported as a potential key molecule for driving CH-associated cardiovascular disease (40). CH is associated with the development of various illnesses, such as cancers, inflammation-related, and cardiovascular-related conditions (41). Additionally, we observed an increased incidence of leukemia in CH⁺ responders in our current study. IL1RAP could be a potential intervention point for the targeting of CH in these disease contexts.

In addition to toxic environmental exposures, other factors such as chronic infection and trauma (42) can also induce inflammation, which may contribute to the development of hematopoietic defects or cancers (43, 44). Such inflammation can accelerate stem cell aging and exhaustion (45) that can lead to CH development (46–48). We have observed that WTC-PM exposure induces functional stem cell defects. The enrichment of hallmark pathways such as E2F targets,

glycolysis, and hypoxia in differentially expressed genes from WTC-PM-exposed HSPCs suggests potential mechanisms underlying HSC dysfunction following toxic environmental exposure. We plan to further investigate these pathways in future studies. Although previous studies have explored the association of CH occurrence with smoking and chemotherapy treatment (49–51), they have not adequately addressed the impact of environmental factors on CH development. Moreover, understanding the mechanisms by which environmental exposures like WTC-PM influence CH is crucial for developing targeted interventions. Future research should focus on validating these results in larger cohorts and exploring potential interventions that could inhibit IL1RAP activity to prevent or reduce CH progression.

Overall, our study underscores the critical need for continued monitoring and comprehensive healthcare support for environmentally exposed individuals, including WTC-exposed responders, given the heightened risk of hematopoietic abnormalities and associated malignancies. By combining genomic analysis of human samples with murine models, we investigated the mechanisms underlying CH development following environmental exposures. Our findings provide insights into the long-term health impacts of environmental disasters and lay the groundwork for targeted interventions and personalized healthcare strategies, aiming to improve outcomes for those affected by the WTC disaster or future environmental disasters.

MATERIALS AND METHODS

WTC-exposed Participants

In a previous investigation, CH was examined in 481 WTC-exposed responders. Subsequently, our study expanded to include 988 participants. Detailed methodologies have been previously documented (19). Briefly, between December 2013 and October 2015, whole blood samples were collected from these 988 responders as part of their clinical evaluations. The study protocol was approved by the Institutional Review Board of Albert Einstein College of Medicine (07-09-320). All participants provided written informed consent, and the studies were conducted in accordance with recognized ethical guidance (Declaration of Helsinki).

Data Collection and Definitions. Participant demographic information, including age at sample collection, race/ethnicity, and sex, was obtained from the FDNY employee database. CBC data were ascertained at the time of blood draw. Smoking history was self-reported (ever vs. never).

Hematologic Cancer Case Ascertainment. FDNY Bureau of Health Services confirms all cancer diagnoses via medical review and/or through state cancer registry linkages (Arizona, Connecticut, Florida, New Jersey, New York, North Carolina, Pennsylvania, South Carolina, and Virginia), ensuring each case was counted only once from either data source. Diagnoses from state cancer registries were categorized in accordance with the International Classification of Diseases for Oncology, specifically including cases classified as malignant cancers (International Classification of Diseases for Oncology behavior code 3). Cancer types (Surveillance Epidemiology and End Results recode) that were included were myeloma (34000), leukemia (35011-35043), non-Hodgkin lymphoma (33041-33042), Hodgkin lymphoma (33011-33012), myelodysplastic syndrome, and myeloproliferative neoplasms. All post-9/11 cancers diagnosed through 2024 were included in analyses.

Non-WTC-exposed Comparison Populations

We included two comparison groups in this study: non-WTC-exposed firefighters and non-WTC-exposed controls (individual from the general population). The non-WTC-exposed firefighter comparison group comprised 52 US firefighters recruited during the annual International Association of Firefighters convention and an additional 203 Nashville firefighters. Demographic data for the 52 firefighters from the International Association of Firefighters were obtained at the time of blood draw. Data for the 203 Nashville firefighters were obtained from the Synthetic Derivative, an Electronic Health Record connected with a paired biorepository (BioVU) at Vanderbilt University Medical Center, utilizing natural language-processing methods. Both the occupational status as firefighters and smoking history of these 203 selected individuals were manually validated. The 198 non-WTC-exposed controls were also obtained from the BioVU at Vanderbilt University Medical Center, utilizing natural language-processing methods. Demographic data were obtained at the time of blood draw, and smoking history of these individuals was manually validated.

Clonal Hematopoiesis Status

The blood samples were extracted using the Promega Maxwell RSC instrument with a Maxwell RSC Whole Blood DNA Kit for extraction of genomic DNA. Targeted sequencing was conducted at a New York State Clinical Laboratory Improvement Amendments–certified facility operated by Neogenomics. Next-generation sequencing involved alignment to the hg19 genome build using the Burrows–Wheeler aligner, with read quality and depth assessed via GATK. Mutect, Pindel, and VarDict were used for variant calling. Variants were excluded if present in the Exome Aggregation Consortium/gnomAD databases with $\geq 1\%$ minor allele frequency.

Single-nucleotide variants required a minimum sequence depth of $250\times$ and a VAF threshold of 5%, whereas indel mutations required a 10% VAF threshold. Variants detected between 50 and $250\times$ depth were analyzed based on a proprietary CI. All variants underwent annotation by molecular geneticists trained in clinical genetics.

The median sequencing coverage depth in the study was $839\times$, with a mean coverage of $908\times$. Variants with VAFs $>40\%$ were excluded to minimize potential inclusion of germline variants. Genes for deep targeted sequencing are included in Supplementary Table S1.

Statistical Analyses

Descriptive characteristics were computed using counts/proportions for the cohort exposed to WTC conditions and the two comparison cohorts. We determined the prevalence of somatic mutations among WTC-exposed participants, using exact methods with binomial assumptions within 10-year age categories (20–39, 40–49, 50–59, 60–69, 70–79, and 80 years and older), and calculated associated 95% CIs.

Descriptive analyses were conducted to examine the distribution of CH mutations across different age groups. Participants were also stratified into those aged 60 years and older and those younger than 60 years to assess the prevalence of mutations in each age category. We chose 60 as the cutoff as CH is reported to increase significantly with age after 60 years (41). Next, the proportion of each mutation was calculated by dividing the number of participants exhibiting a specific mutation by the total number of participants with mutations within each respective age group. This is presented as a two-way bar chart to depict the distribution among older (≥ 60) and younger (<60) participants.

We performed multivariable logistic regression analysis on all participants to investigate the association between WTC exposure and CH. For the primary analyses, somatic mutations were defined as a VAF below 40%. As a secondary analysis, we applied stricter thresholds of $\leq 35\%$ and $\leq 25\%$ to confirm the associations between CH and

WTC exposure under more stringent criteria for ruling out germline variants. The outcome variable was the presence of CH, and the exposure variable included three categories: WTC-exposed responders, non-WTC-exposed firefighters, and non-WTC-exposed controls. These models controlled for age at blood collection, race/ethnicity (non-Hispanic White, non-Hispanic Black, non-Hispanic Asian, non-Hispanic other races, and Hispanic), and sex.

Next, among the WTC-exposed responders, we analyzed CBC values at the time of blood draw for individuals with CH by calculating mean values and SDs. We utilized an independent sample *t* test to compare CBC counts between participants with and without CH.

We evaluated the association between WTC exposure and CH across mutation groupings, including cell cycle, DNA methylation, histone modification, transcription factors, cell signaling, proliferation, and splicing. Unadjusted logistic regression with Fisher exact test *P* values compared the WTC cohort with the combined comparison populations. To assess the association between age and VAF, we generated a scatter plot and performed a simple linear regression, modeling VAF as a function of age. Finally, also among the WTC-exposed responders, we conducted four analyses to explore the relationship between CH and different health conditions. The presence of CH was included as the independent variable. Models assessed the following conditions: overall incidence of blood cancer and leukemia. Logistic regression was used for cancer outcomes.

All *in vivo* and *in vitro* experiments were analyzed using GraphPad Prism software. Statistical significance was determined using the Student *t* test or one-way/two-way ANOVA, as indicated in the respective figure legends.

Mice

Seven- to ten-week-old C57BL/6/N (CD45.2) or SJL (CD45.1; B6.SJL-Ptprca Pepcb/BoyJ), *Tet2*^{+/−} (JAX: 023359), *Il1rap*^{+/−} (B6.129S1-*Il1rap*^{tm1Roml}/J; JAX: 003284, mice were backcrossed for over 10 generations to a C57BL/6J background) and *Tet2*^{+/−}; *Il1rap*^{+/−}; APC^{min} (JAX: 002020) and Vav-Cre (JAX:035670); Dnmt3a^{R878H} (JAX: 031514) mice were used for these experiments. All mice were bred in our mouse facility. All animal studies were approved by the Institutional Animal Care and Use Committee of Albert Einstein college of Medicine.

Transplantation-based Murine Model of *Tet2*^{+/−} CH

We used 8- to 10-week-old CD45.2⁺ *Tet2*^{+/−} or CD45.2⁺ *Tet2*^{+/−}; *Il1rap*^{+/−} of either gender as donor. Mice were euthanized and long bones were flushed, followed by red blood cell (RBC) lysis. We conducted daily BM cell transplants of 3×10^6 total BM cells from mutant mice into non-conditioned 8- to 10-week-old CD45.1⁺ WT male mice over a period of three consecutive days. This chimeric BMT approach resulted in recipient mice possessing BM consisting of both the mutant *Tet2*^{+/−} clone and normal hematopoietic precursors. These two populations can be easily distinguished based on their CD45.1 and CD45.2 alleles via flow cytometer-based analysis, allowing for precise analysis and purification of both CH-mutant and normal cells.

Anakinra Treatment

Tet2-CH mice were exposed to a single dose of WTC-PM 4 weeks after transplantation of *Tet2*^{+/−} cells. To inhibit IL1R1 signaling, mice were treated intraperitoneally with either vehicle or anakinra (10 mg/kg) three times per week for 4 weeks.

BMT and In Vivo Assays

C57BL/6N (CD45.2) donor mice were treated with single dose of vehicle or WTC-PM (100 μ g/mouse) via oropharyngeal aspiration, 3 months after mice were euthanized and long bones were flushed,

followed by RBC lysis (Gibco, Thermo Fisher Scientific). For competitive transplantation, 2×10^6 total BM cells from C57BL/6N mice were co-transplanted with 1×10^6 CD45.1⁺ BM competitor cells into lethally irradiated (900 cGy in equal-split dose) SJL (CD45.1⁺) recipient mice. For secondary transplantation, 1×10^6 total BM cells from primary transplanted mice (after 6 months) were transplanted into lethally irradiated SJL (CD45.1⁺) recipient mice. Cell engraftment and chimerism were assessed by flow cytometry of peripheral blood leukocytes for CD45.1 and CD45.2 after 4, 8, 12, 16, 20, and 24 weeks after transplantation (Supplementary Table S4).

Murine Oropharyngeal Aspiration Model

WTC-PM was collected, following previously established methods, from five different locations within a 0.5-mile radius of Ground Zero on September 13, 2001 (52). The composition of WTC-PM was analyzed using X-ray fluorescence, as detailed in prior publications (52). To deliver the PM, we employed oropharyngeal aspiration, a method comparable with intratracheal instillation in terms of deposition efficiency, as described in earlier studies (29, 53). Each mouse aspirated 100 μ g of WTC-PM suspended in sterile PBS. To avoid batch bias, littermate mice housed together were exposed to both PBS and WTC-PM on the same day. The single 100 μ g dose of WTC-PM was selected based on its estimated capacity to induce adverse pulmonary effects in mice, akin to those experienced by rescue workers exposed to 425 μ g/m³ of WTC-PM over an 8-hour shift (52). This exposure level is consistent with the PM concentrations measured at the 9/11 debris site.

Analysis of Mice

We assessed the CBC of vehicle- or WTC-PM-exposed mice using a complete blood count analyzer (ADVIA 2020i hematology system, Siemens Healthineers). Peripheral blood and BM samples were stained with antibodies according to Supplementary Table S4 for flow cytometry, which was performed on a BD LSRII (BD Biosciences). The lineage antibody cocktail contained antibodies to B220, CD5, Ter119, F4/80, and CD11b. HSCs were assessed based on the expression of the following markers: lineage⁻/lo c-kit^{high} Sca-1^{hi} CD48⁻ CD150^{high}. HSPCs were assessed based on the expression of the following markers: MPP^{Ly}; lin⁻/lo c-kit^{high} Sca-1^{hi} CD150⁻ Flk2⁺, MPP^{G/M}; lin⁻/lo c-kit^{high} Sca-1^{hi} CD48⁺ CD150⁻, MPP^{Mk/E}; lin⁻/lo c-kit^{high} Sca-1^{hi} CD48⁺ CD150⁺, MPP⁺; lin⁻/lo c-kit^{high} Sca-1^{hi} CD48⁻ CD150⁻, CLP⁺; lin⁻ Sca-1^{low} c-kit^{low} IL-7R α ⁺, CMP⁺; lin⁻ Sca-1⁻ c-kit⁺ CD16/32^{lo} CD34⁺, MEP⁺; lin⁻ Sca-1⁻ c-kit⁺ CD16/32^{lo} CD34⁻, and GMP⁺; lin⁻ Sca-1⁻ c-kit⁺ CD16/32^{hi} CD34⁺.

Methylcellulose Assay

A total of 50,000 BM cells from vehicle- or WTC-PM-exposed WT or *Il1rap*^{+/-} mice were seeded per well of a six-well plate. For treating BM cells *in vitro* with vehicle and WTC-PM, BM cells from *Vav-Cre*; *Dnmt3a*^{R878H}; or *APC*^{min} were mixed with vehicle or specified doses of WTC-PM in methylcellulose media from STEMCELL Technologies (MethoCult, cat. #M3434) supplemented with 1% penicillin-streptomycin. Plates were kept for 7 to 10 days in a humidified incubator containing 5% CO₂ and maintained at 37°C, followed by counting the total number of colonies. In the culture, 150 to 200 μ g/mL WTC-PM was added.

IL-1 β Detection

Plasma was collected by centrifuging whole blood, which was drawn into EDTA-coated tubes, at 10,000 rpm for 10 minutes. The upper liquid layer (plasma) was then collected and stored at -80°C. IL-1 β levels were determined in vehicle- and WTC-PM-exposed mice using the Mouse Luminex Discovery Assay (Biotechne, R&D Systems) according to the manufacturer's instructions.

Single-Cell Sequencing (CITE-seq)

For performing CITE-seq with the 10x Genomics platform (54), BM cells were collected from *Tet2*-CH mice after 3 months of exposure to either vehicle or WTC-PM. The BM cells were collected by flushing the long bones, followed by RBC lysis. Cell viability of >95% was confirmed using trypan blue exclusion.

The cells were stained for 30 minutes with antibodies from the Total-seq A mouse universal cocktail (cat. #199901, lot #B374086, BioLegend) and sample-specific hashing oligos. Single-cell CITE-seq libraries were generated using the 10x Genomics Chromium Next GEM Single Cell 3' Reagent Kit v3.1 (PN-1000268; 10x Genomics). BM cells were stained and pooled in equal amounts before chip loading. Quality control was assessed with the Agilent 2100 Bioanalyzer to ensure the integrity of the libraries.

For preparing the gene expression library (GEX), the Dual Index Kit TT Set A (PN-1000215) was used. For the CellPlex hashing [cell multiplexing oligo (CMO)] library, the Dual Index Kit NN Set A (PN-1000243) was used. The CITE antibody [antibody-derived tag (ADT)] library was single indexed using i7 indices. Libraries were constructed according to the manufacturer's instructions, purified, and sequenced using NovaSeq (PE150) from Medgenome.

Analysis of CITE-seq Data

Cell multiplexing was performed using the 10x CellRanger multi pipeline to generate filtered matrices for GEX and ADT. The input included fastq files from GEX, ADT, and CMO libraries. The CMO libraries were used to demultiplex the samples. The cell feature count matrix from all samples was then merged and analyzed with Seurat (v5) following the established pipeline.

The Uniform Manifold Approximation and Projection (UMAP) algorithm was applied to visualize cell clustering using principal components. Clustering and cell type identification were performed using the *clustifyR* package in R.

R script for the analysis is uploaded on the public repository Github, which can be assessed at <https://github.com/kpradhan2025/VermaWTC2025>.

Tet2^{+/-} cells were detected by the positivity of CD45.2. For CD45.2 positivity, we plotted the ADT densities of all cells and noticed two peaks. One centered at 0 and the other centered close to 0.5. A cutoff threshold was determined to be 0.2. Every cell with a CD45.2 ADT value above 0.2 was labeled as CD45.2 positive.

More information on the tools and pipelines used can be found at <https://www.10xgenomics.com/support/software/cell-ranger/latest> and <https://satijalab.org/seurat/>.

Mutational Signature

The trinucleotide matrix was constructed for all samples from WTC-exposed responders with somatic mutations using chromosomal positions, locations, reference alleles, and VAFs. The analysis was performed using the R package "deconstructSigs" (RRID: SCR_026286) (<https://github.com/raerose01/deconstructSigs>). We computed trinucleotide count matrices and reconstructed them using previously known single-base substitution (SBS) signatures (25, 27). In WTC-exposed responders below the age of 60 years, we observed *de novo* signatures closely matched to COSMIC SBS1A, SBS3, SBS16, and SBS21. For those above the age of 60 years, *de novo* signatures closely matched to COSMIC SBS1A, SBS7, SBS4, SBS13, SBS15, SBS16, and SBSR3. We matched the etiology of COSMIC SBS signatures as published previously (25, 27).

Mouse DNA Sequencing

This analysis represents a re-evaluation of DNA sequencing data originally reported in our prior publication (19), now with a focused analysis on nonclassical mutational gene sets identified in

WTC-exposed responders. For downstream analysis, we restricted the dataset to include only mutations classified as high or moderate impact. Mutations were then counted in a curated list of genes previously identified as relevant in our study of WTC-exposed first responders. The frequency and distribution of these mutations were compared between vehicle-treated and WTC-PM-exposed mice.

Data Availability

All sequencing data for WTC-exposed first responders and non-WTC-exposed control are deposited to the European Variation Archive Project with accession ID PRJEB49193, which can be assessed at <https://www.ebi.ac.uk/eva/>. R script for the analysis of CITE-seq data is uploaded on the public repository Github, which can be assessed at <https://github.com/kpradhan2025/VermaWTC2025>.

Authors' Disclosures

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Authors' Contributions

D. Verma: Conceptualization, resources, data curation, software, formal analysis, supervision, funding acquisition, validation, investigation, visualization, methodology, writing—original draft, project administration, writing—review and editing. **R. Zeig-Owens:** Formal analysis. **D.G. Goldfarb:** Formal analysis. **L. Kravets:** Supported the

murine experiments. **K. Pradhan:** Analyzed CITE-seq and mutational signature data and generated the associated graphs. **B. Rockwell:** Supported maintenance of blood biobank from WTC-exposed. **S. Sahu:** Supported the experiments. **S. Kelly:** Data curation. **O. Giricz:** Data curation. **S. Jasra:** Data curation. **Y. Zou:** Supported the performance of the murine intratracheal instillation technique. **C. Prophete:** Supported the murine experiments. **L.S. Torres:** Supported the murine experiments. **S. Aluri:** Supported lab experiments. **S. Chakraborty:** Supported the murine experiments. **R. Kumari:** Supported the murine experiments. **S. Gordon-Mitchell:** Supported the murine experiments. **J. Wang:** Supported the performance of the murine intratracheal instillation technique. **A.J. Silver:** Supported collection of samples from control group. **T.M. South:** Supported experiments. **S.D. Olmstead:** Supported the murine experiments. **C.B. Hall:** Investigation. **S. Sidoli:** Methodology. **R. Bender:** Investigation. **O. Landgren:** Investigation. **L.M. Greenberger:** Resources. **A. Wickrema:** Resources. **A. Madireddy:** Formal analysis. **A. Shastri:** Investigation. **E.M. Pietras:** Intellectual discussion. **L.M. LaFave:** Supported the performance of the murine intratracheal instillation technique. **A. Nolan:** Provided WTC-dust. **M.D. Cohen:** Resources. **M.R. Savona:** Resources, provided blood biobank from the control group. **U. Steidl:** Resources, supervised CH murine experiments with Tet2 and Il1rap. **D.J. Prezant:** Resources, provided support in generating blood biobank from WTC-exposed responders. **A. Verma:** Conceptualization, resources, funding acquisition.

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