A. OVERALL COVER PAGE

Project Title: EARLY DETECTION OF CLONAL HEMATOPOIESIS AND LEUKEMIA ASSOCIATED MUTATIONS IN WTC EXPOSED FIREFIGHTERS AFTER THE 9/11 ATTACKS	
Grant Number: 5U01OH012271-03	**Project/Grant Period:** 07/01/2021 - 06/30/2024
Reporting Period: 07/01/2023 - 06/30/2024	**Requested Budget Period:** 07/01/2023 - 06/30/2024
Report Term Frequency: Final	**Date Submitted:** 01/03/2025
Program Director/Principal Investigator Information: AMIT K VERMA , MD **Phone Number:** 718-430-8761 **Email:** amit.verma@einsteinmed.org	**Recipient Organization:** ALBERT EINSTEIN COLLEGE OF MEDICINE 1300 MORRIS PARK AVE BRONX, NY 104611900 **UEI:** H6N1ZF5HJ2G3 **EIN:** 1830621846A1 **RECIPIENT ID:**
Change of Contact PD/PI: NA	
Administrative Official: SUZANNE LOCKE 1300 Morris Park Ave Bronx, NY 10461 **Phone number:** 7184302786 **Email:** Suzanne.Locke@einsteinmed.edu	**Signing Official:** ROSE RAMUNNO 1300 Morris Park Avenue Bronx, NY 10461 **Phone number:** 718-430-8846 **Email:** rose.ramunno@einsteinmed.edu
Human Subjects: NA	**Vertebrate Animals:** NA
hESC: No	**Inventions/Patents:** No

B. OVERALL ACCOMPLISHMENTS

B.1 WHAT ARE THE MAJOR GOALS OF THE PROJECT?

Aim 1: Will determine the prevalence of Clonal Hematopoiesis (CH)/leukemia associated mutations in WTC-exposed firefighters and two unique, matched control cohorts: CH and other preleukemic conditions are associated with genotoxic exposures. Based on our preliminary data demonstrating increased single nucleotide variants in WTC-exposed firefighter samples, we propose to conduct deep targeted sequencing in a larger cohort of WTC exposed firefighter and control samples (1500 WTC-exposed firefighters, 250 non-WTC exposed firefighters and 250 age matched non-firefighter controls). The non-WTC firefighter and non-firefighter controls will be provided by MPI (Savona) from a well annotated biobank at Vanderbilt University. Mutations will be correlated with level of WTC dust exposure, age, serial blood counts, smoking history and other clinical characteristics. Early detection of CH will enable potential disease altering interventions in first responders.
Aim 2: Will determine the functional effects of WTC particulate matter on development of clonal hematopoiesis and inflammation in vivo: We have acquired samples of fine particulate matter from WTC site (WTC-PM) and have used these to mimic WTC exposure among firefighters in mice by oropharyngeal administration. WTC-PM exposure led to altered hematopoietic differentiation with myeloid bias in pilot murine experiments. We will now use mouse models with commonly mutated CH/leukemia genes (TET2 and DNMT3A) and competitive transplantation to determine whether WTC dust exposure in vivo can accelerate the development of CH and myeloid neoplasms. We will also systematically evaluate WTC-PM dose response effects on inflammation in the niche, stem cell self-renewal, differentiation and cell cycle kinetics. Stem cells from mice exposed to WTC-PM will also be sequenced to determine the impact of exposure to mutational events.
Aim 3: Will determine the effect of WTC-PM exposure on cellular changes at the single molecule level: Our preliminary studies suggest that cells exposed to WTC-PM display cell cycle alterations, widespread dysregulation of DNA replication at common fragile sites and increased DNA damage. To evaluate these events comprehensively, we will first profile the magnitude and chromosomal locations of DNA damage in WTC-PM exposed cells. Next, we will determine the mechanistic basis for damage accumulation by investigating cell cycle checkpoint cascades activated in response to replication stress. In addition, we will evaluate the integrity of DNA replication, an essential basic cellular mechanism required for normal cell function, at single DNA strand resolution at defined genomic hotspots. These results will determine the contribution of defects in basic cellular mechanisms to genomic instability in WTC-PM exposed cells.

B.1.a Have the major goals changed since the initial competing award or previous report?

No

B.2 WHAT WAS ACCOMPLISHED UNDER THESE GOALS?

File Uploaded : B.2 Accomplishments.pdf

B.3 COMPETITIVE REVISIONS/ADMINISTRATIVE SUPPLEMENTS

For this reporting period, is there one or more Revision/Supplement associated with this award for which reporting is required?

No

B.4 WHAT OPPORTUNITIES FOR TRAINING AND PROFESSIONAL DEVELOPMENT HAS THE PROJECT PROVIDED?

NOTHING TO REPORT

B.5 HOW HAVE THE RESULTS BEEN DISSEMINATED TO COMMUNITIES OF INTEREST?

NOTHING TO REPORT

B.6 WHAT DO YOU PLAN TO DO DURING THE NEXT REPORTING PERIOD TO ACCOMPLISH THE GOALS?

Not Applicable

B.2 (B.2 Accomplishments.pdf)

Aim 1: Will determine the prevalence of Clonal Hematopoiesis (CH)/leukemia associated mutations in WTC-exposed firefighters and two unique, matched control cohorts:

We have finished deep targeted sequencing in a larger cohort of WTC exposed firefighter and control samples (1000 WTC-exposed firefighters, 250 non-WTC exposed firefighters and 250 age matched non-firefighter controls). The results are being analyzed presently and we expect the analysis to be finished in the next two months. Early detection of CH will enable potential disease altering interventions in first responders.

Aim 2: Will determine the functional effects of WTC particulate matter on development of clonal hematopoiesis and inflammation in vivo:

We have established a mouse model of CH with tet2 +/- clone and shown that exposure to WTC dust leads to expansion of the malignant clone. We are now treating these mice with inhibitors of IL-1b to evaluate the effect on the malignant clone.

C. OVERALL PRODUCTS

C.1 PUBLICATIONS

Are there publications or manuscripts accepted for publication in a journal or other publication (e.g., book, one-time publication, monograph) during the reporting period resulting directly from this award?

Yes

Publications Reported for this Reporting Period

Public Access Compliance	Citation
N/A: Not NIH Funded	Davis T, Fedorov K, Gregos PS, Shapiro LC, Shastri A, Gritsman K, Shah N, Sica RA, Konopleva M, Feldman E, Mantzaris I, Braunschweig I, Verma A, Cooper D, Kornblum N, Goldfinger M. High Dose Cyclophosphamide for the Treatment of Severe Immune Checkpoint Inhibitor Related Adverse Events. Journal of oncology research and therapy. 2023;8(4). PubMed PMID: 39371330; PubMed Central PMCID: PMC11451324; DOI: 10.29011/2574-710x.10194.
N/A: Not NIH Funded	Nanoparticle STING Agonist Reprograms the Bone Marrow to an Anti-Tumor Phenotype and Protects Against Bone Destruction. Cancer research communications. DOI: 10.1158/2767-9764.crc-22-0180.

C.2 WEBSITE(S) OR OTHER INTERNET SITE(S)

NOTHING TO REPORT

C.3 TECHNOLOGIES OR TECHNIQUES

NOTHING TO REPORT

C.4 INVENTIONS, PATENT APPLICATIONS, AND/OR LICENSES

Have inventions, patent applications and/or licenses resulted from the award during the reporting period? No

If yes, has this information been previously provided to the PHS or to the official responsible for patent matters at the grantee organization? No

C.5 OTHER PRODUCTS AND RESOURCE SHARING

NOTHING TO REPORT

D. OVERALL PARTICIPANTS

D.1 WHAT INDIVIDUALS HAVE WORKED ON THE PROJECT?

Commons ID	Sr/Key	Name	Degree(s)	Role	Cal	Aca	Sum	Foreign Org	Country	SS
AMIT72	Y	Verma, Amit K.	MD	PD/PI	1.2	0.0	0.0			NA
DPREZANT	Y	PREZANT, DAVID J	BS,MD	PD/PI	0.1	0.0	0.0			NA
SAVONAM	Y	Savona, Michael R	MD	PD/PI	0.6	0.0	0.0			NA
AMONTEITH6	N	Monteith, Andrew		Postdoctoral Scholar, Fellow, or Other Postdoctoral Position	3.9	0.0	0.0			NA
AMADIREDDY	N	Madireddy, Advaitha	PHD	Collaborator	1.2	0.0	0.0			NA
RZEIGOWENS	N	Zeig-Owens, Rachel	DPH,MPH	PD/PI	1.2	0.0	0.0			NA
	N	Pradhan, Kith		Biostatistician	1.2	0.0	0.0			NA
	N	Aluri, Srinivas		Associate	12.0	0.0	0.0			NA
	N	Gordon, Shanisha		Technician	3.8	0.0	0.0			NA
	N	Arrate, Maria	MS	Research Assistant	2.6	0.0	0.0			NA

Glossary of acronyms:
Sr/Key - Senior/Key
Cal - Person Months (Calendar)
Aca - Person Months (Academic)
Sum - Person Months (Summer)
Foreign Org - Foreign Organization Affiliation
SS - Supplement Support
RS - Reentry Supplement
DS - Diversity Supplement
OT - Other
NA - Not Applicable

D.2 PERSONNEL UPDATES

D.2.a Level of Effort

Not Applicable

D.2.b New Senior/Key Personnel

Not Applicable

D.2.c Changes in Other Support

Not Applicable

D.2.d New Other Significant Contributors

Not Applicable

D.2.e Multi-PI (MPI) Leadership Plan

Not Applicable

E. OVERALL IMPACT

E.1 WHAT IS THE IMPACT ON THE DEVELOPMENT OF HUMAN RESOURCES?

Not Applicable

E.2 WHAT IS THE IMPACT ON PHYSICAL, INSTITUTIONAL, OR INFORMATION RESOURCES THAT FORM INFRASTRUCTURE?

NOTHING TO REPORT

E.3 WHAT IS THE IMPACT ON TECHNOLOGY TRANSFER?

Not Applicable

E.4 WHAT DOLLAR AMOUNT OF THE AWARD'S BUDGET IS BEING SPENT IN FOREIGN COUNTRY(IES)?

NOTHING TO REPORT

G. OVERALL SPECIAL REPORTING REQUIREMENTS SPECIAL REPORTING REQUIREMENTS

G.1 SPECIAL NOTICE OF AWARD TERMS AND NOTICE OF FUNDING OPPORTUNITIES REPORTING REQUIREMENTS

NOTHING TO REPORT

G.2 RESPONSIBLE CONDUCT OF RESEARCH

Not Applicable

G.3 MENTOR'S REPORT OR SPONSOR COMMENTS

Not Applicable

G.4 HUMAN SUBJECTS

G.4.a Does the project involve human subjects?

Not Applicable

G.4.b Inclusion Enrollment Data

NOTHING TO REPORT

G.4.c ClinicalTrials.gov

Does this project include one or more applicable clinical trials that must be registered in ClinicalTrials.gov under FDAAA?

G.5 HUMAN SUBJECTS EDUCATION REQUIREMENT

NOT APPLICABLE

G.6 HUMAN EMBRYONIC STEM CELLS (HESCS)

Does this project involve human embryonic stem cells (only hESC lines listed as approved in the NIH Registry may be used in NIH funded research)?

No

G.7 VERTEBRATE ANIMALS

Not Applicable

G.8 PROJECT/PERFORMANCE SITES

Not Applicable

G.9 FOREIGN COMPONENT

No foreign component

G.10 ESTIMATED UNOBLIGATED BALANCE

Not Applicable

G.11 PROGRAM INCOME

Not Applicable

G.12 F&A COSTS

Not Applicable

I. OVERALL OUTCOMES

I.1 What were the outcomes of the award?

To comprehensively determine the association between WTC exposure and CH, we conducted a sequencing analysis of a larger cohort of FDNY-WTC-responders including younger, exposed individuals, and two non-WTC-exposed comparison populations. 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.

We evaluated CH status in FDNY responders who were exposed to the WTC disaster (n=988; 912 firefighters, 76 EMS) and non-WTC-exposed firefighters (n=255) and non-WTC-exposed controls (n=195). The majority of participants across both WTC-exposed and non-WTC-exposed groups were male (98.1%) and identified as White (88.3%). This cohort is predominantly composed of younger WTC-exposed responders, with 81% being under the age of 60. WTC-exposed responders. 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 CLIA certified lab. 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 were annotated as significant based on reference databases, including the Catalog of Somatic Mutations in Cancer (COSMIC), dbSNP database, and Exome Aggregation Consortium (ExAC).

In the WTC-exposed cohort, we identified 136 individuals with 165 total somatic mutations of expected pathogenic potential for an overall prevalence of 13.8%. Of those with CH, 22 individuals (22/136, 16%) carried more than one CH mutation. The prevalence of CH was 6.7% (17/255) in the non-WTC-exposed firefighters and 7.2% (14/195) in the non-WTC-exposed controls; the prevalence of CH was similar between these two control groups. Four individuals (4/17, 23%) in the non-WTC-exposed firefighter group and two individuals (2/14, 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 cohorts, the crude age-specific prevalence CH increased with age (Fig. S1A). 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 due to 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 3-fold increased odds of CH (OR, 3.38; 95% CI, 1.90-6.34; P<0.01) (Fig. S1B). 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. S1B). 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 decile (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, 0.95; 95% CI, 0.45-1.97; P=0.88) (Fig. S1B). 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 (OR=3.37, 95% CI: 1.85–6.49, P<0.01 for WTC-exposed responders vs non-WTC exposed firefighters and OR=3.58, 95% CI: 1.94–7.04, P<0.01 for WTC-exposed responders vs the non-WTC-exposed controls). Smoking history was not significantly associated with CH in either multivariable model.