A. OVERALL COVER PAGE

Project Title: Impact of WTC dust on immune functions and prostate cancer promotion	
Grant Number: 5U01OH011328-05	**Project/Grant Period:** 09/01/2016 - 08/31/2021
Reporting Period: 09/01/2020 - 08/31/2021	**Requested Budget Period:** 09/01/2020 - 08/31/2021
Report Term Frequency: Final	**Date Submitted:** 12/30/2022
Program Director/Principal Investigator Information: STUART A AARONSON , MD **Phone Number:** 212-659-5400 **Email:** stuart.aaronson@mssm.edu	**Recipient Organization:** ICAHN SCHOOL OF MEDICINE AT MOUNT SINAI ICAHN SCHOOL OF MEDICINE AT MOUNT SINAI 1 GUSTAVE L. LEVY PL NEW YORK, NY 100296574 **DUNS:** 078861598 **UEI:** C8H9CNG1VBD9 **EIN:** 1136171197A1 **RECIPIENT ID:**
Change of Contact PD/PI: NA	
Administrative Official: JESSICA RUTH MOISE One Gustave L. Levy Place Box 1075 1 GUSTAVE L. LEVY PL, BOX 1075 NEW YORK, NY 10029 **Phone number:** (212) 824-8300 **Email:** jessica.moise@mssm.edu	**Signing Official:** JESSICA RUTH MOISE One Gustave L. Levy Place Box 1075 1 GUSTAVE L. LEVY PL, BOX 1075 NEW YORK, NY 10029 **Phone number:** (212) 824-8300 **Email:** jessica.moise@mssm.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?

Aim1. Investigate tumor promotion by WTC dust in genetic and metastatic prostate cancer models.
Aim 2. Elucidate host inflammatory responses that may contribute to prostate tumor development and metastases in vivo.
Aim 3: Identify human prostate tumor tissue biomarkers from WTC dust exposed rescue and recovery workers.

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?

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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

Final RPPR report
B.2 What was accomplished under these goals?

This project addressed adverse health effects to World Trade Center (WTC) rescue and recovery workers resulting from exposure to dust containing asbestos and toxic components. Studies were aimed at testing mechanisms that may be responsible for the increased incidence of prostate cancer in World Trade Center (WTC)-exposed responders. For this purpose, we utilized mouse genetic models of pre-initiated tumors reflecting genetic alterations observed commonly in human prostate tumors. Immune and/or other biomarkers identified were also applied in association studies with prostate tumors from WTC workers and from non WTC prostate cancers. Monthly meetings of our investigators were very effective in the planning and implementation of this research. A summary of accomplishments in achieving the major goals of the aims are summarized below:

Aim1. Investigate tumor promotion by WTC dust exposure in genetically modified prostate cancer mouse models. To demonstrate potentially causative associations between WTC dust exposure and prostate cancer we assessed the organ distribution of WTC cancer causing components after exposure. In two major dosing studies to determine the organ distribution of WTC dust components in mice. *First*, cohorts of normal C57BL/6 mice were exposed to WTC dust (3 mg/30 μl, 4 consecutive doses over 4 days) or served as PBS controls. Mass spectrometry analysis showed that the highest levels in a large metal panel (lead, cadmium etc.) were detected in lung but above background levels, found in the prostate at 21 days post exposure. Using analytic electron microscopy, we determined that cancer causing asbestos fibers showed substantial retention in the lungs but with minimal distribution to other organs including prostate, thyroid, spleen and liver with similar observations at 4 months. *Second*, using mice with homozygous loss of Pten in the prostate (*Pb-Cre*$^{+}$*;Pten*$^{loxp/loxp}$ GEMs, we conducted WTC dosing and organ resection 1 year after dust exposure to demonstrate long term retention of dust components enriched in the lungs. Collectively, these data indicate that in WTC dust exposed mice, the majority of cancer-causing fibers and heavy metals are retained in the lungs.

We also observed that WTC dust exposure can promote prostate cancer progression**.** For these studies, we used the *Pb-Cre*$^{+}$*;Pten*$^{loxp/loxp}$ primary tumor model and a transplant model of prostate tumorigenesis. In mice exposed to WTC dust (nasal installation, 4x doses of 4 mg/30 μl) we evaluated primary GEM tumors at 6-12 months at the histological level. Using immuno stain values quantified with QuPath software analysis, we observed three significant progression changes in dust exposed primary tumors including: (1) increased epithelial proliferation (Ki67+ cells), (2) increased invasiveness (SMA+ staining regions) and (3) increased GU block weight. Using a transplant model established by the Mulholland laboratory and consisting of surgically implanted Pten-null cells to the prostates of male C57BL/6 mice, we observed that WTC exposed mice exhibited significantly reduced overall survival compared to PBS treated mice (media survival: WTC = 17.5 w *vs.* PBS = 55 w). Similar observations were made in WTC dust exposed mice with venous (lung) injected prostate cancer cells (median survival: WTC = 17 w, PBS = 41.5 w). These, results were extended using Ras activated Pten deficient mouse tumor lines having metastatic potential. In summary, our recently published findings (Wang et al, 2022) indicate that WTC dust exposure can lead to increased prostate cancer progression in a genetically primed prostate epithelial model directly relevant to the human disease.

Aim 2. Elucidate host inflammatory responses that may contribute to prostate tumor development and metastases in vivo. We used a dosing strategy consisting of either one or 4 consecutive daily exposures of WTC solubilized dust (3 mg/30 μl PBS) and assayed for changes in immune and inflammatory gene expression after short term (7 days post exposure) and longer time points (3 weeks post exposure). RNAs from normal prostate, spleen, lung and blood were assessed using RT2 Profiler PCR Arrays (Immunity and Inflammation Panel). Using the above dosing schemes, mouse modeling showed that WTC dust exposure results in marked increases in inflammatory cytokine detection in prostate (*Ctla4*, *Cxcl5*, *IL17α*, *Il6*), lung (*Cxcl5*, *Cxcr1*, *Bcl2l1,* $p < 0.05$), spleen (*Ccl20*, *Cxcl11*, *Ccl28*, *Il17a,* $p<0.05$) and blood (*Il6,* $p<0.05$) when normalized to PBS treated controls (>1.0 log2 fold change dust/PBS treated samples). We also identified marked increases in *Il6* (IL-6), a cytokine that has been implicated in prostate cancer growth and metastasis. Moreover, WTC exposed mice showed increased content of CD19+ B cells, NK1.1+ cells and decreased content of CD3+ T cells in WTC dust treated Wt ($p=0.0153$) and Pten mutant ($p=0.023$) prostates. Collectively, these data demonstrate that WTC dust promotes a strong inflammatory response including increased levels of cytokines associated with tumorigenesis (Wang et al., 2022).

B.2 (FRPPR.pdf)

Aim 3: Identify human prostate tumor tissue biomarkers from WTC dust exposed rescue and recovery workers. WTC and non-WTC prostate cancer samples: Using an updated list of newly diagnosed prostate cancer patients from the WTC Data Center, we continued the accrual of patients and samples initiated under a previous project (U01 OHO10396). To date, we consented 206 WTC patients, requested 127 tissue samples and acquired prostate tumor tissue samples with a total of 75 from WTC prostate cancers. Additional prostate tumor samples from Mount Sinai patients and other hospitals were also collected. One set of 25 blinded samples from WTC and non WTC prostate tumors, frequency matched on race, age (+/- 5 years) and Gleason score, were assessed for immune markers by 'Hyperion' imaging mass spectrometry. This state of the art immunological approach uniquely combines time-of-flight mass spectrometry with metal-labeling technology to enable breakthrough discovery and comprehensive functional profiling (135 available detection channels). Immunostaining with as many as 30-40 antibodies can be performed in combination on the same slide, allowing detailed analysis of interactions between inflammatory cells and tumor cells. Conditions were optimized for analysis of most of the antibodies under study. Of note, this technology is critical in view of the limited number of prostate cancer tissue sections that are retrievable in some cases. Wilcoxon-rank sum analysis (8 WTC exposed; 12 non WTC exposed controls) of markers tested in a coded manner indicates that the prostate tumors of WTC dust exposed responders/survivors exhibit statistically significant increases in CD4 (median cells/mm^2: 530.40 vs. 184.67; p=0.0073), a marker seen on the surface of a subset of T helper cells. WTC dust exposed responders/survivors also had significant increases in CD56 (median cells/mm^2: 189.06 vs. 53.43; p=0.0096), a phenotypic marker of natural killer cells also expressed on some other immune cells. Although not statistically significant, those with WTC dust exposures, also trended towards higher CD68 (median cells/mm^2: 263.50 vs. 163.70; p=0.1153), a marker also expressed by monocytes and tissue macrophages; and decreases in CD20 (median cells/mm^2: 26.56 vs. 92.95; p=0.1349). There was no significant difference in expression of CD1c (p=0.3837), CD8 (p=0.2703), or CD15 (p=0.6239). These findings indicate that prostate tumors of WTC responders/survivors exhibit an increased inflammatory/immune phenotype, providing complementary, confirmatory as well as more detailed understanding to our recently published analysis of gene expression by nanostring of relevant cytokine and other RNAs from this same set of tumors (Wang et al, 2022; Gong et al, 2019).

We also sought to explore the epigenetics of WTC associated PC. Patients were recruited from the World Trade Center Health Program. Non-WTC PC samples were frequency matched on age, race/ ethnicity and Gleason score. Bisulfite-treated DNA was extracted from tumor tissue blocks and used to assess global DNA methylation with the MethylationEPIC BeadChip. Differential and pathway enrichment analyses were conducted. RNA from the same tumor blocks was used for gene expression analysis to further support DNA methylation findings. Methylation data were generated for 28 samples (13 WTC and 15 non-WTC). Statistically significant differences in methylation were observed for 3,586 genes; on average WTC samples were statistically significantly more hypermethylated (P = 0.04131). Pathway enrichment analysis revealed hypermethylation in epithelial mesenchymal transition (EMT), hypoxia, mitotic spindle, TNFA signaling via NFKB, WNT signaling, and TGF beta signaling pathways in WTC compared to non-WTC samples. The androgen response, G2M and MYC target pathways were hypomethylated. These results correlated well with RNA gene expression. In conclusion, long-term epigenic changes associated with WTC dust exposure were observed in PC tissues. These occurred in genes of critical pathways, likely increasing prostate tumorigenesis potential and warranting analysis of larger WTC groups and other cancer types (Yu et al 2022).

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	Yu H, Tuminello S, Alpert N, van Gerwen M, Yoo S, Mulholland DJ, Aaronson SA, Donovan M, Oh WK, Gong Y, Wang L, Zhu J, Taioli E. Global DNA methylation of WTC prostate cancer tissues show signature differences compared to non-exposed cases. Carcinogenesis. 2022 June 27;43(6):528-537. PubMed PMID: 35239955; PubMed Central PMCID: PMC9234756; DOI: 10.1093/carcin/bgac025.

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

NOTHING TO REPORT

C.3 TECHNOLOGIES OR TECHNIQUES

Category	Explanation
Models	Application of mouse genetic models with tumor-initiating lesions targeted to the prostate to assess the effects of WTC dust exposure on tumor promotion. These technologies are being shared through the publications listed that were generated with the support of this U01.
Research Material	1. Use of Mass spec mediated immunostaining technology for analysis of multiple antigens on a single tissue slide 2. Application of epigenetic analysis to tumor tissue sections using global DNA methylation and gene expression analysis on the same sample associated with bioinformatic analysis of pathway enrichment.

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

Category	Explanation
Data or Databases	Helped to support banking of prostate tumor sections from WTC responders and nonexposed controls and collection of

	related clinical data.

D. OVERALL PARTICIPANTS

D.1 WHAT INDIVIDUALS HAVE WORKED ON THE PROJECT?

Commons ID	S/K	Name	Degree(s)	Role	Cal	Aca	Sum	Foreign Org	Country	SS
SAAARONSON	Y	Aaronson, Stuart A	MD	PD/PI	2.4	0.0	0.0			NA
MULHOLLAND2	Y	Mulholland, David	MOTH	Co-Investigator	1.2	0.0	0.0			NA
ESPOSITO.DAVIDE	N	Esposito, Davide		Postdoctoral Scholar, Fellow, or Other Postdoctoral Position	2.4	0.0	0.0			NA
SANDHUHARKIRAT	N	Sandhu, Harkirat Singh	BS,MS,PHD	Postdoctoral Scholar, Fellow, or Other Postdoctoral Position	4.0	0.0	0.0			NA
ILAPANT	N	Pant, Ila	PhD	Postdoctoral Scholar, Fellow, or Other Postdoctoral Position	8.4	0.0	0.0			NA
	N	Yao, Shen		Technician	1.8	0.0	0.0			NA
	N	Portman, Kensey		Technician	4.8	0.0	0.0			NA
	N	Alpert, Naomi		Research Coordinator	2.4	0.0	0.0			NA

Glossary of acronyms:
S/K - 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 FUNDING OPPORTUNITIES ANNOUNCEMENT 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

File(s) uploaded:
CumulativeInclusionEnrollmentReport 2022 final.pdf

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

Cumulative Inclusion Enrollment Report

This report format should NOT be used for collecting data from study participants.

Study Title: Impact of WTC dust inhalation on immune functions and prostate tumor promotion

Comments:

Racial Categories	Ethnic Categories									Total
	Not Hispanic or Latino			Hispanic or Latino			Unknown/Not Reported Ethnicity			
	Female	Male	Unknown/ Not Reported	Female	Male	Unknown/ Not Reported	Female	Male	Unknown/ Not Reported	
American Indian/ Alaska Native										**0**
Asian		2								**2**
Native Hawaiian or Other Pacific Islander										**0**
Black or African American		9			6					**15**
White		58			15					**73**
More Than One Race		4								**4**
Unknown or Not Reported								5		**5**
Total	**0**	**73**	**0**	**0**	**21**	**0**	**0**	**5**	**0**	**99**

PHS 398 / PHS 2590 (Rev. 08/12 Approved Through 8/31/2015)

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OMB No. 0925-0001/0002
Cumulative Inclusion Enrollment Report

I. OVERALL OUTCOMES

I.1 What were the outcomes of the award?

Epidemiological evidence indicates an increased incidence of prostate cancer and some other tumors in WTC responders. We showed that in mouse genetic models with initiating oncogenic lesions in prostate, WTC dust exposure induced acute pulmonary inflammation with persistence of inflammatory cytokines in lung, peripheral blood and in prostate. WTC dust exposure did not detectably increase prostate tumor incidence in wild type mice but did promote in vivo growth of initiated prostate epithelial cells and transplanted tumor cells. These findings are consistent with growth promotion by an inflammatory/immune mechanism. When age/race/tumor grade matched prostate tumor sections from WTC responders versus unexposed individuals were analyzed by mass spec multiplex imaging, global DNA methylation and gene expression analysis, we observed that immune/inflammatory biomarkers were enriched in WTC prostate cancers. Further studies are needed to confirm and extend these findings and to test whether the levels of dust exposure at the WTC sites correlate with the biomarkers identified. These studies have resulted in dissemination of results to the scientific community and the public at large through 3 publications to date.