A. OVERALL COVER PAGE

Project Title: Thyroid Cancer Aggressiveness in WTC Responders	
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Program Director/Principal Investigator Information: EMANUELA TAIOLI , MD MS PHD **Phone Number:** 212 659 9590 **Email:** Emanuela.taioli@mountsinai.org	**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 **UEI:** C8H9CNG1VBD9 **EIN:** 1136171197A1 **RECIPIENT ID:** 18-2626
Change of Contact PD/PI: NA	
Administrative Official: AMANDA AMESCUA One Gustave L. Levy Place, Box 1075 New York, NY 100296574 **Phone number:** 646-605-8659 **Email:** amanda.amescua@mssm.edu	**Signing Official:** AMANDA AMESCUA One Gustave L. Levy Place, Box 1075 New York, NY 100296574 **Phone number:** 646-605-8659 **Email:** amanda.amescua@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?

Specific Aims
An increased risk of thyroid cancer has been reported in the Mount Sinai World Trade Center (WTC) responders, WTC-exposed fire fighters, and the NYC Department of Health exposed residents, with an excess risk in the range of 2 to 3 times the incidence reported by Cancer Registries after 9/11. Over-diagnosis because of enhanced surveillance of the WTC cohort has been suggested as possible explanation for this excess risk, but this was contradicted by our preliminary evidence which showed no excess of false-positives thyroid cancer diagnoses among the WTC cohort compared to the non- WTC cohort. Although most of the agents and carcinogens found at Ground Zero after the planes crashed and the towers collapsed have no known carcinogenic effect on the thyroid, our preliminary evidence of in-vitro studies showed that exposure of rodents to WTC dust is associated with an inflammatory reaction in distant organs such as prostate tissue. Furthermore, prostate cancer tissue from WTC responders showed a distinct pattern of gene expression different from non WTC responders, with downregulation of genes involved in innate immunity response and an upregulation of genes related to apoptosis. This suggests that inflammation caused by the inhalation of WTC dust may act as a tumor promoter by disrupting the immune-regulatory response.
The objectives of this project are to move further along the path to understanding the reasons for the observed significant increased incidence of thyroid cancer among WTCHP responders by first investigating the inflammatory response to WTC dust in thyroid tissue and by assessing if the thyroid cancers found in the WTC participants are more aggressive than thyroid cancer observed in non-WTC patients, either as a consequence of inflammation caused by the dust exposure or by some other mechanism.

The specific aims of the project are:
Aim 1: To analyze inflammatory markers in the thyroid gland using an animal model. We hypothesize that rodents exposed to WTC dust have an inflammatory response coupled with decreased immune-response in the thyroid. We will use the thyroids of rodents exposed to WTC dust currently stored in the WTC tissue biobank. To characterize the inflammatory and immune microenvironment, we will use the nanostring technology to perform multiplex gene expression analysis of 770 genes from 24 different immune cell types, common checkpoint inhibitors, CT antigens, and genes which cover the adaptive and the innate immune response.

Aim 2: To analyze inflammatory markers in thyroid cancer. We hypothesize that thyroid cancer in WTC responders arise in a microenvironment characterized by an inflammatory response coupled with immune-dysregulation in comparison with non-WTC responders. We will use thyroid cancer cases identified within the WTCHP cohort and thyroid cancer cases collected from the Mount Sinai tumor bank who serve as age, gender, and histology frequency matched controls. To characterize the inflammatory microenvironment, we will use the same nanostring technology as used in aim 1 of this research protocol. Differences in the inflammatory markers identified via gene expression profiling will be evaluated in WTCHP thyroid cancer cases versus age, gender and histology matched non-WTC thyroid cancer controls.

Aim 3: To investigate the aggressiveness of WTC thyroid cancer compared to non-WTC thyroid cancer. We hypothesize that WTC cancers are inherently more aggressive than non-WTC thyroid cancers as a consequence of WTC exposure. We will isolate DNA from the Formalin-Fixed Paraffin-Embedded (FFPE) tissue slides that have already been acquired and perform whole exome sequencing (WES). The aggressiveness of the genetic alterations found will be compared between the WTC and non-WTC thyroid cancer cohorts. WES will also allow us to identify novel driver mutations in the WTC group; we will use a validation cohort of known aggressive and non-aggressive thyroid cancer samples to evaluate if these new mutations are associated with aggressiveness, and if they drive thyroid cancer initiation or growth.

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

October 23, 2024

Final Progress Report

Title: Thyroid Cancer Risk in WTC Responders

Principal Investigator: Emanuela Taioli, MD, PhD
Director, Institute for Translational Epidemiology
Associate Director for Population Science, Tisch Cancer Institute
Director, Center for the Study of Thoracic Diseases Outcome
Professor, Population Health Science & Policy and Thoracic Surgery
Icahn School of Medicine at Mount Sinai
Email- Emanuela.Taioli@mountsinai.org

Gregory Riggins, MD, PhD
Director of the Brain Cancer Biology and Therapy Research Laboratory
Professor, Oncology and Neurosurgery
Johns Hopkins Medicine
Email- griggin1@jhmi.edu

Institution: Icahn School of Medicine at Mount Sinai
One Gustave L. Levy Place. New York, NY 10029

Co-Investigators: Eric Gender, MD, Rachel Brody, MD, PhD, Maaike van Gerwen, MD, PhD Icahn School of Medicine at Mount Sinai. Janette Cerutti, PhD, Thais Biude Mendes, PhD, Gabriel Gama PhD University of Sao Paulo

Project Director: James Yiin, PhD

Sponsor: National Institute for Occupational Safety and Health (NIOSH) Centers for Disease Control and Prevention (CDC)

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List of Terms and Abbreviations

B-Raf proto-oncogene	BRAF
Deoxyribonucleic acid	DNA
Formalin-fixed paraffin-embedded tissue	FFPE
Follicular thyroid carcinoma	FTC
Follicular variant papillary thyroid carcinoma	FVPTC
Hematoxylin and eosin	H&E
Isoflurane anesthesia	ISO
New York City	NYC
Odds ratio	OR
Papillary thyroid carcinoma	PTC
Ribonucleic acid	RNA
Ribonucleic acid sequencing	RNAseq
Single-nucleotide polymorphism	SNP
Standardized incidence ratio	SIR
Telomerase reverse transcriptase	TERT
World Trade Center	WTC
World Trade Center Health Program	WTCHP

Abstract

The health consequences of exposure to the World Trade Center (WTC) disaster site have been an area of active research due to the significant increase in various health conditions among responders and nearby residents. This report summarizes findings of the work we conducted on both animal models and human samples.

In Aim 1 we proposed to determine the molecular response in rat thyroids as a result of exposure to the dust collected at the WTC site. Using total RNA sequencing and whole genome sequencing, we have generated preliminary results showing some key markers being overexpressed in thyroids of rats after exposure to WTC dust.

Aim 2 was designed to determine the inflammatory and immune-response in WTC thyroid cancers. By employing total RNA sequencing on WTC and non-WTC thyroid cancers from Formalin-Fixed Paraffin-Embedded (FFPE) tissue slides, we observed distinct molecular profiles.

Aim 3 was to investigate the genetic mutational profile of WTC thyroid cancers compared to non-WTC exposed thyroid cancers. We performed whole exome sequencing on DNA from FFPE tissue slides, identifying a high frequency of *TERT* promoter mutations in WTC thyroid cancers. These findings are presented in the publication *TERT and BRAF V600E mutations in thyroid cancer of World Trade Center Responders*, which underscored the role of these genetic alterations in the pathogenesis of thyroid cancer in this population.

Collectively, the work provides a comprehensive overview of the multifaceted impact of WTC exposure on thyroid cancer risk, from epidemiological patterns to molecular mechanisms. The findings underscore the need for ongoing surveillance, targeted screening, and further research to mitigate the long-term health consequences faced by WTC responders.

Section 1
Significant or Key Findings

The primary scope of aim 1 of this study was to determine whether exposure to WTC dust resulted in significant molecular responses in the rat thyroid. The present work follows our published work on prostates of rats exposed to WTC dust, where through total RNA sequencing and whole genome sequencing we identified key markers that were significantly overexpressed in rodents exposed to the dust. These findings suggest a strong link between environmental exposure at the WTC site and inflammatory responses, paving the way for further understanding the health risks faced by those exposed.

In aim 2, we have studied thyroid cancer gene expression in WTC responders in comparison to thyroid cancers not WTC related. We observed 5741 differentially expressed transcript at 0.99 level; 4186 protein coding transcripts. These results are in the format of a draft article that will be submitted for publication shortly. We are now in the middle of comparing gene expression profiling of areas of normal thyroid tissue in the WTC samples and non-WTC samples, to better understand the carcinogenesis process after WTC dust exposure.

The third aim involved the investigation into the genetic mutational profile of WTC thyroid cancers, using whole exome sequencing on DNA from FFPE tissue slides, and revealed that although there was no significant difference in *BRAF V600E* mutation found, there were significantly more prevalent *TERT* promoter mutations found in WTC thyroid cancer versus non-exposed thyroid cancer cases (P=0.021). These results may suggest that exposure to pollutants present in WTC dust contributes to excess thyroid cancer risk, and a potentially more aggressive thyroid cancer. We are now collaborating with NCI to compare the mutation profile of the WTC thyroid cancers with the profile of Chernobyl thyroid cancers, to identify specific hotspots associated with WTC dust exposure.

Translation of Findings

The findings from this research project have substantial implications for both clinical practice and public health. The identification of significant markers and genetic profiles specific to WTC thyroid cancers suggests that enhanced screening and early detection protocols are crucial for individuals exposed to WTC dust. These insights can lead to more targeted and effective monitoring strategies, ultimately improving early intervention and treatment outcomes.

Furthermore, the discovery of a high prevalence of the *TERT* promoter mutation in WTC thyroid cancers highlights the need for surveillance in this high-risk population. Understanding these genetic alterations allows for the development of personalized therapeutic approaches, potentially increasing the efficacy of treatments and reducing morbidity.

Collectively, these findings underscore the importance of continuous surveillance and tailored healthcare strategies to address the long-term health impacts faced by WTC responders, ultimately guiding future research and healthcare policy.

Research Outcomes/Impact

The outcomes of this project have significantly advanced our understanding of the impact of WTC exposure on thyroid cancer. Our findings from Aim 1, which identified specific signatures and markers in rodents exposed to WTC dust through RNA sequencing and whole genome sequencing, underlining the environmental exposure's role in possibly triggering a carcinogenesis process. This foundational research provides a critical link between environmental pollutants at the WTC site and potential cancer risk, informing future studies on environmental carcinogenesis.

Through Aim 2, we discerned distinct genomic profiles in WTC thyroid cancers compared to non-WTC thyroid cancers. By employing total RNA sequencing on FFPE, we observed unique gene expression patterns associated with WTC exposure. These results emphasize the importance of understanding the specific molecular and immunological changes in thyroid cancer caused by exposure to pollutants in the WTC dust. This knowledge can guide personalized treatment approaches and enhance early detection strategies for affected individuals.

Aim 3's exploration of the genetic mutational profiles revealed a high frequency of *TERT* promoter mutation in WTC thyroid cancers. These genetic alterations, identified through whole exome sequencing, provide crucial insights into the aggressive nature of thyroid cancer among WTC responders. This research highlights the necessity of targeted genetic screening and the development of tailored

therapeutic interventions to improve outcomes for this high-risk population. Ultimately our research underscores the long-term health impacts of WTC exposure and the importance of continued surveillance and targeted research to mitigate these effects.

Section 2

Scientific Report

Background

An increased risk of thyroid cancer has been reported in the Mount Sinai World Trade Center (WTC) responders. Similar results have also been reported in two other cohorts, the WTC-exposed firefighters and the NYC Department of Health exposed residents. The excess risk is in the range of 2-3 times the incidence reported by the Cancer Registries. It is unclear whether the excess is associated with WTC-related exposures or represents an artifact. There are several possible explanations for an increase in thyroid cancer incidence, and the ability to disentangle the roles of the various contributors would have major clinical and preventive consequences. One possibility is an over-diagnosis of thyroid cancer cases due to enhanced surveillance (surveillance bias), and evidence for this phenomenon would represent an important reassuring message to WTC responders and workers. On the other hand, if thyroid cancer clinical and molecular characteristics support a specific carcinogenic effect of WTC exposures on the thyroid, that would argue for implementing specific screening activities among the exposed cohorts, with the intent of early detection and early treatment. The WTCHP Responders who participated as rescue, recovery, and cleanup efforts at the WTC sites have been enrolled at Mount Sinai in the World Trade Center Health Program (WTCHP), which is funded under the James Zadroga 9/11 Health and Compensation Act of 2010, on the basis of eligibility criteria including type of duties, site location, and dates and hours worked. The medical protocol for the monitoring program includes self- administered physical and mental health questionnaires, as well as a physical examination, laboratory tests, spirometry, and a chest radiograph. Over 27,000 responders have had a least one monitoring visit in the WTCHP and have consented to aggregation of their data. A total of 20,984 responders have consented to have their records used for medical research. Over one third of WTCHP members belong to minority groups; policemen and other protective service workers represent the largest occupational group.

Increased incidence of thyroid cancer among WTC responders: In the first analysis of thyroid cancer incidence of subjects included in the WTCHP cohort, a standardized incidence ratio (SIR) of 3.12 (95% CI: 2.04 to 4.57) was observed. The results, confirmed by partially overlapping cohorts comprised of both workers and residents, suggest that WTC responders experienced a 2 to 3 fold increased incidence of thyroid cancer. Under previous funding, we concluded that 1) surveillance bias may play a role, but does not solely explain the excess risk of thyroid cancer in WTC responders and 2) physician bias resulting in a false-positive thyroid cancer diagnosis does not explain increased thyroid cancer incidence in the WTC population. We were able to confirm through molecular markers, that during the yearly screening visits, true cases of thyroid cancer were identified.

We proposed here to elucidate the mechanism between WTC dust exposure and thyroid cancer by studying both animal models exposed to WTC dust, and thyroid cancer tissues from WTC responders.

Specific Aims

The specific aims of the project were:

Aim 1. **Determine the inflammatory and immune-response in the rat thyroid as a result of exposure to the dust collected at the WTC site.** We have used the thyroids of rodents exposed to WTC dust currently stored in the WTC tissue biobank through a collaboration with NYU. To characterize the inflammatory and immune microenvironment, we originally proposed to use the nanostring technology to perform multiplex gene expression analysis of 770 genes from 24 different immune cell types, common checkpoint inhibitors, CT antigens, and genes which cover the adaptive and the innate immune response. However, we realized that due to technology advances, we were able to conduct RNA-seq on the samples, thus having a complete picture of gene function after exposure.

Aim 2. **Determine the inflammatory and immune-response in WTC thyroid cancers.** We have used thyroid cancer cases identified within the WTCHP cohort and thyroid cancer cases collected from the Mount Sinai tumor bank who serve as age, gender, and histology frequency matched controls. To characterize the inflammatory microenvironment, we originally proposed to use the same nanostring technology as used in aim 1 of this research protocol. However, we realized that due to technology advances, we were able to conduct RNA-seq on the samples, and be aligned with Aim 1.

Aim 3. **Investigate the genetic mutational profile of WTC thyroid cancers compared to non-WTC exposed thyroid cancers.** We have isolated DNA from the Formalin-Fixed Paraffin-Embedded (FFPE) tissue slides and have performed whole exome sequencing (WES). The frequencies of the genetic alterations found were compared between the WTC and non-WTC thyroid cancer cohorts. WES will also allow us to identify novel driver mutations in the WTC group; we are using a validation cohort of known aggressive and non-aggressive thyroid cancer samples to evaluate if these new mutations are associated with aggressiveness, and if they drive thyroid cancer initiation or growth.

Methodology

Aim 1. **Determine the inflammatory and immune-response in the rat thyroid as a result of exposure to the dust collected at the WTC site.**

In collaboration with NYU and Ohio University, we analyzed rats thyroids collected after controlled exposure to WTC dust, and compared to thyroids collected after sham exposure (controls). We also compared the results to what was observed in rats prostates.
Spontaneously hypertensive rats (SHR) were exposed to the WTC dust or isoflurane (ISO) anesthesia alone in an intratracheal inhalation integrated system as described previously. Sets of rats were exposed to WTC53 dust at 33 mg dust/m3 under ISO anesthesia for 2 hours/day on two consecutive days. Control rats were exposed to ISO only (1.5%–1.8% in carrier O2 gas). Rat thyroids were dissected and snap-frozen in liquid nitrogen, after animals were euthanized by injection with Sleepaway. Thyroid RNA was extracted using Automated Robotic System with Beckman's Agencourt RNA dv Advance tissue kit. RNA quality was assessed by bioanalyzer nanochips and must have a minimal RIN of 7. RNA sequencing was performed after in-house RNA library preparation with RiboMinus Eukaryote System v2 and NEXTflex Rapid Directional.
Statistical analysis: For each gene, we compared the mean expression level (log2- transformed RPKM value) between the WTC group (case) and ISO group (control) using R package limma. We reported both the group mean difference (log fold change) and the nominal P value of significance. For each cell type in each sample, we calculated an enrichment score (ES) based on cell type–specific gene sets using the

GSVA method. We then compared the ES between WTC dust–exposed and ISO groups for each cell type and report the difference of the group mean, nominal P-value of significance (limma package), and FDR (Bonferroni–Holm method).

Aim 2. **Determine the inflammatory and immune-response in WTC thyroid cancers.**

A follow-up for cancer incidence is routinely performed within the WTCHP. The current number of thyroid cases is 203, of which 148 have agreed to be part of a research program. Newly diagnosed, incident cases of thyroid cancer were identified through this mechanism, and asked for permission to access their medical and epidemiologic records and their tissue sample. Samples were retrieved in collaboration with the WTC cancer tissue biobank (Dr. Taioli, PI). Information on tumor clinical characteristics, personal and medical history, prevalence of thyroid cancer risk factors, and medical history specific to thyroid cancer diagnosis were compared between cases diagnosed among WTC responders, and a group of gender-, and race-frequency matched thyroid cancer cases treated at Mount Sinai (control group) during the same period. The Department of Otolaryngology has an active research database of all treated thyroid cancer cases (Dr Genden, Chairman of the Department of Otolaryngology is a co-investigator in this grant) from which we obtained the non-WTC control group.
We studied 119 paraffin-embedded thyroid tissues, which were micro-dissected, and in case of two tumor locations on the same slide, the mean of gene expression was calculated.
Preprocessing of RNA-seq expression data: The software TopHat2 was used to map the RNA-seq reads to the genome and to calculate the Reads Per Kilobase of transcript, per Million mapped reads (RPKM) value for each gene, after removing samples of low quality (read count per sample less than 5 million). Expression profiles were further quantile-normalized by R package limma.
Differential gene expression analysis and pathway enrichment analysis Differential analysis of gene expression was carried out through R package limma. For each gene, its expression level was compared between WTC samples and non-WTC samples to get t-statistics and the P-values. The P-values of all genes were corrected by Benjamini-Hochberg multiple-comparison correction. Derived gene lists underwent pathway enrichment analysis using the Molecular Signatures Database (MSigDB) hallmark gene set. To identify the enriched pathways, the t- statistics of genes within a pathway of interest were compared with those outside the pathway through Mann-Whitney rank-sum test. P-values from Mann-Whitney rank-sum tests were corrected by Benjamini-Hochberg multiple-comparison correction. We set a FDR of 0.05, and used EBSeq for analysis, since it specifically deals with transcript differential expression and can detect both statistically equally expressed and differentially expressed transcripts.

Aim 3. **Investigate the genetic mutational profile of WTC thyroid cancers compared to non-WTC exposed thyroid cancers.**

WTC responders enrolled in the World Trade Center Health Program (WTCHP) at Mount Sinai Hospital prior to their cancer diagnosis were eligible to enroll in the WTC Biobank. Thyroid cancer diagnosis was validated through linkage with the cancer registries of New York, New Jersey, Pennsylvania and Connecticut, as these states accounted for 98% of the responder's residencies at time of WTCHP enrollment. The full methodology of patient recruitment and consenting has been described previously. In summary, eligible patients were contacted by phone and then mailed a consent form if interested in participating. After obtaining consent, a cancer tissue sample was obtained from the hospital where the patient received thyroid cancer surgery and stored in the WTC Biobank, together with de-identified demographic and clinical data. For the current study, 30 eligible WTC thyroid cancer patients were identified in the WTC Biobank and frequency matched by sex, age at diagnosis (±5 years), race and histology to 30 non-WTC thyroid cancer patients from the Mount Sinai Cancer Biorepository. The study

was conducted under the approval of the Icahn School of Medicine at Mount Sinai´s Institutional Review Board (IRB-17-01323).

The following variables were collected for all WTC and non-WTC thyroid cancer patients: age at diagnosis, sex, histology, tumor size, extrathyroidal extension, vascular invasion, presence of lymph nodes measuring >3 cm, presence of more than five lymph nodes measuring 0.2–3 cm, TNM stage and American Joint Committee on Cancer (AJCC) staging (8th edition). Following the 2015 American Thyroid Association Management Guidelines, thyroid cancers were stratified into low (intrathyroidal differentiated thyroid cancer, ≤5 lymph node micrometastasis (<0.2 cm), intermediate (aggressive histology, minor extrathyroidal extension, vascular invasion or >5 involved lymph nodes (0.2–3 cm) and high risk (gross extrathyroidal extension, incomplete tumor resection, distant metastases or lymph node >3 cm).

After sample de-identification, formalin-fixed paraffin-embedded tissue (FFPE) samples (4 µm) of the 60 patients were sent to John Hopkins University for analysis, as described previously. For each patient, whose slides were accessible, areas of interest were circled on a hematoxylin and eosin (H&E)-stained slide by an expert pathologist and the corresponding areas from extra slides were manually macrodissected using a razor blade, to remove contaminating normal cells. The tissue fragments were placed in a 1.5 ml microcentrifuge tube, deparaffinized with xylene, vortexed and centrifuged at 14000 rpm × 5 min. The tissue pellet was washed twice with 100% ethanol and centrifuged for 3 min at 20 000*g*. The DNA was extracted using the kit Gene Read DNA FFPE tissue kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions and quantified using a NanoDrop 2000c spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA).

To detect the *BRAF V600E* mutation, TaqMan Mutation Detection Assays (Thermo Fisher Scientific), a competitive allele-specific assay which reliably discriminate the *BRAF V600E* (Hs00000111_mu) and *BRAF* wild-type (Hs00000110_wt), was used. PCR (polymerase chain reaction) was performed in a 20 µl final volume containing 20 ng of DNA, 1× TaqMan Genotyping Master Mix and 1× TaqMan *BRAF* assay (*BRAF V600E* or wild-type). PCR reaction was performed in QuantStudio 12K Flex Real-Time PCR System (Applied Biosystems, Thermo Fisher Scientific), with following cycling condition: 95°C for 10 min, 5 cycles of 92°C for 5 s and 58°C for 1 min and 40 cycles of 92°vC for 15 s and 60°C for 1 min. The analysis was according to the manufacturer's instructions.

The most prevalent *TERT* promoter mutations (C250T or C228T) were assessed using two TaqMan SNP (single-nucleotide polymorphism) genotyping assays (Hs000000092_rm and Hs000000093_rm), which reliably discriminate the mutant from the wild-type alleles (Cat#A44177; Thermo Fisher Scientific), as described previously. Briefly, the PCR reaction for each assay consisted of 30 ng of DNA, 1× TaqMan Genotyping Master Mix and 1× Custom TaqMan *TERT* mutation assay (C228T or C250T) and run on QuantStudio 12K Flex Real-Time PCR System (Applied Biosystems, Thermo Fisher Scientific). *TERT* promoter mutation was considered present when either C250T or C228T mutation was present or both.

Continuous variables were summarized using mean ± standard deviation, whereas categorical variables were summarized as frequency (%). To compare the clinical and pathological features between the WTC and non-WTC thyroid cancer groups, two-sided *t*-tests for continuous variables and chi-square or Fisher's exact tests for categorical variables were performed.

Unadjusted logistic regression modeling was conducted to compare *BRAF V600E* status and *TERT* status between WTC thyroid cancers and non-WTC thyroid cancers, followed by adjusted logistic regression modeling adjusting for age, sex, race (White and non-White) histology and tumor size. The interaction between *BRAF V600E* status and *TERT* status was explored.

All statistics presented here were done using SAS 9.4 (SAS Institute, Cary, NC).

Results

Specific Aim 1:

In collaboration with NYU and Ohio University, we analyzed rats thyroids collected after controlled exposure to WTC dust, and compared to thyroids collected after sham exposure (controls). The distribution of RNA seq data in exposed and non-exposed rates is shown in figure 1, where a distinct pattern associated with exposure is observed.

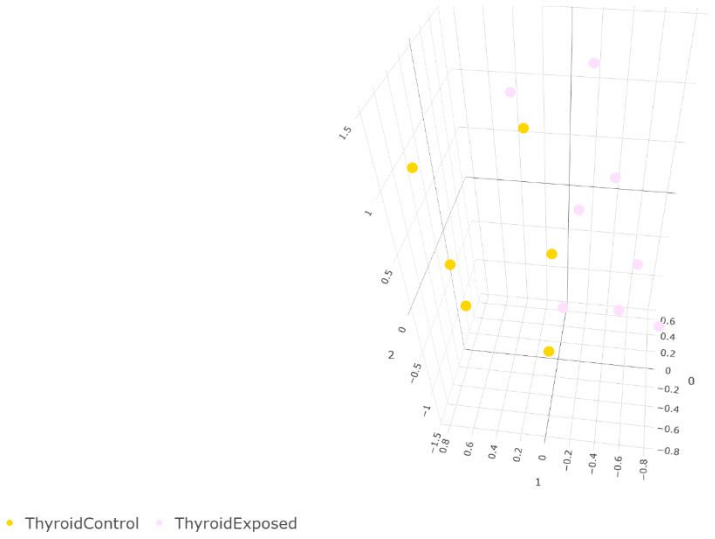

Figure 1 – distribution of samples according to exposure status

We also compared the results to what was observed in rats' prostates. In rats' thyroids, we observed 62 differentially expressed genes (reported in Table 1). We are now conducting pathways and enrichment analyses to understand the significance of these overexpressed genes.

Table 1 – genes overexpressed in thyroids of rats exposed to WTC dust in comparison to control rats

Row.names	ThyroidCc	ThyroidEx	theta	prob	log2FC	length	GC	Chrom	GeneStart	GeneEnd	Biotype	external_
ENSRNOG00000002632	417.0515	215.0581	1.397336	0.962605	0.955499	7120	47.51	7	469723	481992	protein_c	Naca
ENSRNOG00000004078	169.616	52.47569	1.348942	0.950258	1.692551	2404	54.67	10	55366975	55375921	protein_c	Eno3
ENSRNOG00000005269	93.47932	26.49688	1.415001	0.963243	1.818825	5065	49.15	10	11034035	11078101	protein_c	Srl
ENSRNOG00000006224	150.4259	39.66272	1.590735	0.971639	1.923198	6166	42.62	8	78515514	78538873	protein_c	Klhl31
ENSRNOG00000006527	9.292642	0.379881	1.458444	0.957075	4.612471	4054	51.71	4	1.47E+08	1.47E+08	protein_c	Slc6a1
ENSRNOG00000006783	2910.492	807.5195	1.588249	0.971227	1.849694	31319	42.54	3	36613716	36811574	protein_c	Neb
ENSRNOG00000006930	174.6133	46.46635	1.629613	0.971966	1.909906	1848	50.38	13	84670649	84680339	protein_c	Casq1
ENSRNOG00000008170	63.1905	15.5273	1.446687	0.959151	2.024901	4169	50.22	3	1.52E+08	1.52E+08	protein_c	Jph2
ENSRNOG00000008210	46.84094	9.81612	1.512394	0.953679	2.254545	5498	50.01	8	1.03E+08	1.03E+08	protein_c	Ky
ENSRNOG00000008235	74.44005	19.42147	1.353457	0.951678	1.938427	2111	53.38	3	1.41E+08	1.41E+08	protein_c	Mylk2
ENSRNOG00000010079	617.9326	254.8717	1.443762	0.959671	1.277678	2018	41.27	2	86770420	86784280	protein_c	Ca3
ENSRNOG00000010830	155.6694	69.67021	1.357001	0.952791	1.159872	1229	49.09	16	46072939	46076733	protein_c	Slc25a4
ENSRNOG00000011227	219.6843	70.66026	1.639133	0.970258	1.636461	2975	52.88	10	54318701	54324933	protein_c	Atp1b2
ENSRNOG00000011306	13.43659	2.112779	1.364609	0.955144	2.668953	1742	56.35	1	96884948	96887554	protein_c	Myod1
ENSRNOG00000011624	177.2844	68.12037	1.518246	0.954169	1.379907	6076	51.25	3	1.68E+08	1.68E+08	protein_c	Eef1a2
ENSRNOG00000011624	177.2844	68.12037	1.518246	0.954169	1.379907	6076	51.25	3	1.68E+08	1.68E+08	protein_c	Eef1a2
ENSRNOG00000011754	108.0258	30.39921	1.430525	0.961803	1.82927	5360	46.94	16	74520157	74592772	protein_c	Myom2
ENSRNOG00000011912	96.28244	29.87124	1.415317	0.96323	1.688516	2097	50.7	16	17258164	17273415	protein_c	Tmem38a
ENSRNOG00000012609	204.8809	61.39673	1.411575	0.963328	1.738552	4485	36.86	1	23955651	24410595	protein_c	Trdn
ENSRNOG00000013262	389.8155	105.1589	1.58363	0.970342	1.89022	2185	39.78	9	68437517	68458261	protein_c	Myl1
ENSRNOG00000013532	91.25143	24.75678	1.425308	0.962455	1.882024	842	53.72	14	80681776	80683940	protein_c	Pgam2
ENSRNOG00000014025	26.46763	0.962273	1.648735	0.96794	4.781638	6309	46.36	8	90445154	90574269	protein_c	Rasgrf1
ENSRNOG00000014867	158.031	51.17426	1.390383	0.961611	1.626718	5862	43.24	2	2.11E+08	2.11E+08	protein_c	Synpo2
ENSRNOG00000015155	140.334	39.44265	1.38467	0.960506	1.831036	749	54.98	3	1.54E+08	1.54E+08	protein_c	Tnnc2
ENSRNOG00000016151	95.13111	28.78955	1.406401	0.963271	1.724372	3007	54.5	9	38773240	38779839	protein_c	Ankrd23
ENSRNOG00000016714	156.6347	45.41838	1.387135	0.961013	1.786056	5486	47.98	1	2.55E+08	2.55E+08	protein_c	Nrap
ENSRNOG00000016731	224.84	58.13891	1.434225	0.961263	1.951323	4746	52.74	5	57770864	57779992	protein_c	Tpm2
ENSRNOG00000016837	631.6265	181.8682	1.437995	0.960661	1.796178	1444	51	1	79061456	79071720	protein_c	Ckm
ENSRNOG00000016983	67.55985	10.08455	1.405166	0.963223	2.744019	5923	51.6	15	28446550	28468217	protein_c	Myh7
ENSRNOG00000017645	171.5347	53.88126	1.38291	0.960118	1.670645	975	49.88	1	1.82E+08	1.82E+08	protein_c	Mylpf
ENSRNOG00000017786	1819.906	434.9498	1.636508	0.970794	2.064943	1453	56.94	19	51883715	51886742	protein_c	Acta1
ENSRNOG00000018184	335.0822	139.7849	1.548523	0.960268	1.261306	4554	47.36	8	67635479	67662802	protein_c	Tpm1
ENSRNOG00000018220	398.6916	174.1971	1.349504	0.950435	1.194553	10385	44.95	2	1.85E+08	1.85E+08	protein_c	Pde4dip
ENSRNOG00000018656	71.74503	19.63625	1.364472	0.955102	1.869359	2987	46.48	2	1.91E+08	1.91E+08	protein_c	Ampd1
ENSRNOG00000019627	279.109	91.24054	1.544443	0.959106	1.613082	4099	51.29	1	94994104	95017584	protein_c	Mybpc2
ENSRNOG00000019745	498.283	138.3645	1.572129	0.967505	1.848492	2867	50.39	1	2.02E+08	2.02E+08	protein_c	Actn3
ENSRNOG00000019780	57.10945	15.09034	1.409543	0.963333	1.920104	3403	47.54	2	1.96E+08	1.96E+08	protein_c	Sypl2
ENSRNOG00000019810	170.6836	52.22526	1.410192	0.963336	1.708504	3292	52.62	9	76850982	76858699	protein_c	Des
ENSRNOG00000020276	244.0304	70.71979	1.460974	0.956654	1.786875	1402	57.57	1	1.98E+08	1.98E+08	protein_c	Tnni2
ENSRNOG00000020332	628.0973	163.5405	1.545528	0.959407	1.94134	2871	51.99	1	1.98E+08	1.98E+08	protein_c	Tnnt3
ENSRNOG00000020557	705.3285	177.021	1.604106	0.973058	1.994375	15427	50.61	1	84292578	84423812	protein_c	Ryr1
ENSRNOG00000020922	195.2113	64.30149	1.402815	0.963096	1.602113	1775	55.16	1	85806146	85809071	protein_c	Hspb6

ENSRNOG00000021090	795.7655	224.3468	1.584513	0.970523	1.826613	2975	51.15	1	2.04E+08	2.04E+08	protein_c	Pygm
ENSRNOG00000023803	877.9875	228.4457	1.678569	0.959263	1.942349	12622	44.8	2	24279528	24378364	protein_c	Cmya5
ENSRNOG00000033134	104.1371	40.30791	1.421139	0.962862	1.369349	3245	38.93	2	13993438	14132880	protein_c	Mef2c
ENSRNOG00000034258	571.7383	142.7841	1.653364	0.966665	2.00152	12660	38.15	3	51870092	52213091	protein_c	Xirp2
ENSRNOG00000046231	161.2337	41.56582	1.522812	0.954695	1.955684	6500	50.04	13	47493949	47564318	protein_c	Cacna1s
ENSRNOG00000047124	2710.439	739.3595	1.590674	0.971629	1.874179	3831	50.43	1	1.81E+08	1.81E+08	protein_c	AABR0700
ENSRNOG00000047186	187.9045	59.05893	1.356204	0.952541	1.669772	2209	37.54	18	36705314	36724841	protein_c	Myot
ENSRNOG00000049056	86.71987	34.13494	1.349539	0.950446	1.345113	2172	54.29	3	15912485	15923041	protein_c	AABR0705
ENSRNOG00000049695	5166.752	1357.638	1.602477	0.972957	1.928159	12070	43.3	10	51885913	51946295	protein_c	Myh4
ENSRNOG00000049695	5166.752	1357.638	1.602477	0.972957	1.928159	12070	43.3	10	51885913	51946295	protein_c	Myh4
ENSRNOG00000049942	68.38039	15.61733	1.39786	0.962664	2.130434	7381	49.02	16	7910433	7961958	protein_c	RGD15648
ENSRNOG00000056493	316.3103	81.23129	1.489661	0.95347	1.961233	4131	42.65	7	22930350	23015957	protein_c	Mybpc1
ENSRNOG00000057701	103.6209	29.92383	1.353365	0.951649	1.791948	5720	43.04	9	1.11E+08	1.11E+08	protein_c	Myom1
ENSRNOG00000058068	470.9089	127.6218	1.481098	0.95405	1.883574	22708	51.29	10	43789293	43919723	protein_c	Obscn
ENSRNOG00000059350	149.1206	42.4185	1.459934	0.956825	1.813714	7975	36.2	4	42939599	42980638	protein_c	Ppp1r3a
ENSRNOG00000062678	258.3443	76.33354	1.404234	0.963178	1.758906	2097	56.63	1	1.98E+08	1.98E+08	protein_c	H19
ENSRNOG00000065740	309.8067	43.91286	1.629543	0.971975	2.818653	6041	42.79	10	51856738	51883236	protein_c	Myh2
ENSRNOG00000069271	25144.35	7167.55	1.55502	0.962253	1.810682	113560	43.07	3	61652439	61924741	protein_c	Ttn
ENSRNOG00000070083	174.981	46.73677	1.497051	0.953257	1.904569	4180	41.64	17	31661513	31744544	protein_c	RGD15653
ENSRNOG00000070083	174.981	46.73677	1.497051	0.953257	1.904569	4180	41.64	17	31661513	31744544	protein_c	RGD15653

We also compared the gene expression pattern observed in thyroids to what observed in prostates, and recorded several genes that are exclusively overexpressed in thyroid (Table 2). this may represent some specific mechanisms of WTC dust carcinogenesis that is relevant for thyroid. We are in the middle of analyzing the functional significance of each one of these genes using the methods described above.

Table 2- genes that are overexpressed in rat thyroids in comparison to rat prostates after WTC dust exposure

log2FC	GC	Chrom	GeneStart	GeneEnd	Biotype	external_gene_name
-7.610366	43.57	6	46698414	46768199	protein_coding	Tpo
-7.534515	46.57	3	7185723	7242363	protein_coding	Pax8
-7.520839	45.01	11	64235251	64304811	protein_coding	Casr
-7.558156	44.03	7	98418293	98603210	protein_coding	Tg
-7.755064	57.00	6	73996601	73999791	protein_coding	Nkx2-1
-7.661328	53.05	6	121696051	121707398	protein_coding	Chga
-6.505741	43.30	3	54189308	54346708	protein_coding	Lrp2
-7.658503	46.69	1	168878214	168883105	protein_coding	Calca
-7.598565	53.71	1	94808276	94855824	protein_coding	Shank1
-6.897085	46.02	11	34577363	34621952	protein_coding	Kcnj15
-7.166827	50.53	9	105833504	105862550	protein_coding	Ddx11
-7.385546	54.37	5	162626560	162660256	protein_coding	Espn
-7.752257	38.32	1	167508598	167511530	protein_coding	Pth
-7.443761	51.47	3	9070185	9126946	protein_coding	Ccdc187

Specific aim 2:

We observed 5741 differentially expressed transcript at 0.99 level; 4186 protein coding transcripts. These results are in the format of a draft article that will be submitted for publication shortly. We are now in the middle of comparing gene expression profiling of areas of normal thyroid tissue in the WTC samples and non-WTC samples, to better understand the carcinogenesis process after WTC dust exposure.

Specific Aim 3:

Of the 60 thyroid cancers tissue samples sent for DNA extraction, 17 (10 WTC and 7 non-WTC thyroid cancers) did not contain enough material or DNA was degraded thus leaving 20 WTC thyroid cancers and 23 non-WTC thyroid cancers to be included in the analysis. Of the 10 WTC cases excluded from this analysis because of lack of DNA, 2 were follicular thyroid carcinoma and 1 was oncocytic thyroid carcinoma and vascular/capsular invasion was observed, no vascular invasion was observed in 3 papillary cases; information was not available from the pathology report for 3 cases and the pathology report was missing for 1 case. In the non-exposed group, vascular invasion was only noted for 1 follicular thyroid carcinoma case.

There was no difference in age (*P* = 0.880), sex (*P* = 0.486), histology (*P* = 0.331) and tumor size (*P* = 0.376) between the two groups (Table 1). Although vascular invasion was significantly more present in the WTC than non-WTC thyroid cancer group (*P* = 0.038), there was no significant difference in risk stratification (*P* = 0.295). No significant difference in the presence of *BRAF V600E* mutation was found between the two groups, although the frequency was slightly higher in the WTC than in the non-WTC thyroid cancer group, with 15 patients (75%) and 14 patients (60.9%) having this mutation, respectively (*P* = 0.324). A *TERT* promoter mutation was significantly more prevalent in WTC thyroid cancers (70%; 14/20) compared with non-WTC thyroid cancer patients (34.8%; 8/23) (*P* = 0.021). The C250T *TERT* mutation was far more prevalent (83%; 19/23) than C228T *TERT* mutation (17%; 4/23). The presence of combined mutations was not significantly different between the two groups (*P* = 0.058) (Table 3)

Table 3. Characteristics of study participants

	WTC thyroid cancers (*n* = 20)	**Non-WTC thyroid cancers (*n* = 23)**	***P* value**
Age at diagnosis (years)	47.4 (±7.1)	46.9 (±11.4)	0.880
Sex, *n* (%)			0.486
Male	15 (75)	15 (65.2)	
Female	5 (25)	8 (34.8)	
Race, *n* (%)			0.272
White	11 (55)	17 (73.9)	
Black	2 (10)	3 (13)	
Hispanic	1 (5)	1 (4.3)	
Asian/Pacific Islander	1 (5)	2 (8.7)	
Multiracial	2 (10)	0 (0)	
Unknown	3 (15)	0 (0)	

Histology, *n* (%)			0.331
FTC	0 (0)	1 (4.35)	
FVPTC	3 (15)	6 (26.1)	
Micro PTC	4 (20)	1 (4.35)	
PTC	13 (65)	15 (65.2)	
Tumor size[a] (cm)	1.58 (± 0.99)	1.34 (± 0.74)	0.376
Extrathyroidal extension, *n* (%)[b]			0.661
0	12 (63.2)	16 (69.6)	
1	7 (36.8)	7 (30.4)	
Vascular invasion, *n* (%)[a]			**0.038**
0	15 (78.9)	22 (100)	
1	4 (21.1)	0 (0)	
Lymph nodes >3 cm			0.092
0	17 (85.0)	23 (100)	
1	3 (15.0)	0	
More than five lymph nodes of 0.2–3 cm			0.440
0	15 (75.0)	20 (87.0)	
1	5 (25.0)	3 (13.0)	
ATA Risk Stratification[d]			0.295
Low	10 (50.0)	16 (69.6)	
Intermediate	2 (10.0)	3 (13.0)	
High	8 (40.0)	4 (17.4)	
T-stage[a], *n* (%)			0.909
1	10 (55.5)	14 (60.9)	
2	5 (27.8)	5 (21.7)	
3	3 (16.7)	4 (17.4)	
N-stage[c], *n* (%)			0.281
0	1 (7.7)	3 (30.0)	
1	12 (92.3)	7 (70.0)	
AJCC staging[b], *n* (%)			1.000
I	16 (84.2)	20 (87.0)	
II and III	3 (15.8)	3 (13.0)	
***BRAF V600E* mutation, *n* (%)**			0.324
No	5 (25.0)	9 (39.1)	
Yes	15 (75.0)	14 (60.9)	
***TERT* promoter mutation, *n* (%)**			**0.021**
No	6 (30.0)	15 (65.2)	
Yes	14 (70.0)	8 (34.8)	
Combined mutations, *n* (%)			0.058

***TERT* and *BRAF V600E* WT**	1 (5)	5 (21.7)	
1 WT and 1 mutation	9 (45)	14 (60.9)	
***TERT* and *BRAF V600E* mutation**	10 (50)	4 (17.4)	

FTC, follicular thyroid carcinoma; FVPTC, papillary thyroid carcinoma, follicular subtype; PTC, papillary thyroid carcinoma; WT, wild-type.

[a]Two missing tumor size; T stage; vascular invasion.

[b]One missing AJCC staging/extrathyroidal extension.

[c]20 N stage missing.

[d]Following the 2015 American Thyroid Association Management Guidelines, thyroid cancers were stratified into low (intrathyroidal differentiated thyroid cancer, ≤5 lymph node micrometastasis (<0.2 cm), intermediate (aggressive histology, minor extrathyroidal extension, vascular invasion or >5 involved lymph nodes (0.2–3 cm) and high risk (gross extrathyroidal extension, incomplete tumor resection, distant metastases, lymph node >3 cm, *TERT* or *TERT* + *BRAF V600E* mutation). The results in bold are statistically significant.

Although no difference in the presence of *BRAF V600E* mutation was found between WTC and non-WTC-exposed thyroid cancers [OR: 1.93 (95% CI: 0.52–7.17)], the odds of a *TERT* promoter mutation was significantly greater in the WTC versus the non-WTC thyroid cancer group [OR: 4.38 (95% CI: 1.21–15.81)]. After adjustment, the odds of *TERT* promoter mutation remained significantly greater in the WTC versus the non-WTC thyroid cancer group [OR_{adj}: 7.11 (95% CI: 1.21–41.83)] (Table 4). There was no interaction between *BRAF V600E* and *TERT* promoter mutations (P = 1.00).

Table 4. Association of the presence of *BRAF V600E* or TERT promoter mutations in WTC thyroid cancers versus non-WTC thyroid cancers

	Unadjusted analysis OR (95% CI)	**Adjusted analysis[a] OR_{adj} (95% CI)**
***BRAF V600E* mutation**	1.93 (0.52–7.17)	1.12 (0.23–5.48)
***TERT* promoter mutation**	**4.38 (1.21–15.81)**	**7.11 (1.21–41.83)**

[a]Adjusted for age at diagnosis, sex, race, histology and tumor size. The results in bold are statistically significant.

Discussion

Specific Aim 1

Because of advances in gene expression, we were able to conduct whole genome RNAseq on rats exposed to WTC dust and compare them to control, unexposed rats, for the same cost of a nanostring analysis. This approach has several advantages, mainly the ability to study the whole genome instead of the immune and inflammatory compartment alone. The results are promising, and show distinct signatures elicited by exposure to WTC dust, that are present in thyroid of exposed rats but not in thyroids of control rats. We are now completing a pathway enrichment analysis to better understand the significance of such signatures, and what specific biological pathways are stimulated by the exposure to WTC dust. We are also comparing the profiles to the profile obtained in past experiments from prostates of rats exposed to WTC dust, to assess if there is a common set of markers, or rather each organ has a

distinct response to exposure. Ultimately, the results will be compared to RNAseq obtained in human samples (aim 2), to link the biological effects of exposure to thyroid cancer occurrence in this population.

Specific Aim 2

As for aim 1, through collaborations with NCI and Johns Hopkins, we were able to contain the costs of the laboratory work, and to complete RNAseq on the human thyroid cancer samples. This is a great advantage because it allows a complete picture of the thyroid cancer genome, as well as to compare the results of the human study with the results of the animal exposure study.

We were very pleased to observe molecular signatures in WTC thyroid cases in comparison to non-WTC cases, and are now diving into the study of the significance of these gene expression changes. Next steps include path enrichment analysis, the comparison of the human (aim 2) and animal (aim 1) results, as well as the comparison of RNAseq in the tumor versus the surrounding normal tissue. This last step should shed light on the effect of exposure on the normal tissue, and the carcinogenesis process (on the caner tissue).

Lastly, we are collaborating with NCI to compare the mutational profile of the WTC thyroid cancers with the profile of the Chernobyl cohort, to better understand if there are unique markers of exposure in the WTC population. All these efforts combined should culminate in a more complete interpretation of the causation of these thyroid cancers.

Specific Aim 3

This mutational analysis of thyroid cancers developed following exposure to a mixture of pollutants present in the dust cloud following the WTC disaster provides the first evidence that *TERT* promoter mutations are more prevalent in these cancers compared with non-WTC-exposed thyroid cancers, potentially indicating a pathway responsible for the excess in thyroid cancer risk found in WTC-exposed populations. *BRAF V600E* mutations are the most commonly found genetic alterations in adult papillary thyroid cancer, ranging between 27 and 83%. Analysis of 500 adult papillary thyroid cancers in The Cancer Genome Atlas (TCGA) found that a *BRAF* V600E mutation was present in 59.7% of the cancers. The *BRAF* gene is a member of the RAF family of serine/threonine protein kinases, which have an important role in cell proliferation, differentiation, and programmed cell death. Because RAF proteins activate the mitogen-activated protein kinase pathway, inappropriate activation of the mitogen-activated protein kinase pathway following a *BRAF* mutation may result in abnormal proliferation and differentiation. It has been shown that activation of these pathways is predominantly implicated in the pathogenesis of papillary thyroid cancer, and associated with high-risk clinicopathological characteristics and thus thyroid cancer aggressiveness in adults. In present study, 96% of included thyroid cancers had papillary histology. It is therefore not surprising that both groups had high prevalence of *BRAF V600E* mutations, 75 and 61% for the WTC and the non-WTC thyroid cancer group, respectively.

Telomerase reverse transcriptase (*TERT*), a subunit of the catalytic core of human telomerase, controls the activity of telomerase. Although telomerase is responsible for elongation of the telomeric DNA, it can also lead to infinite malignant cell proliferation by stabilizing the telomere length. *TERT* promoter mutations, which have been associated with reactivation of *TERT* RNA expression, have been associated with cancer progression as it enhances cell proliferation. Two *TERT* promoter mutations of interest, namely chr5:1,295,228C>T (C228T) and chr5:1,295,250C>T (C250T), were found to be prevalent in aggressive thyroid cancers, including tall cell papillary thyroid cancer, poorly differentiated thyroid cancer, anaplastic thyroid cancer and *BRAF*-positive papillary thyroid cancer. Although an association between *TERT* promoter mutation and vascular invasion has been suggested, two recent meta-analyses did not confirm this association: pooled OR: 1.78 (95% CI: 0.83–3.84) and pooled OR: 1.38 (95% CI: 0.84–3.33). *TERT* inhibition in two PTC carcinoma cells (BCPAP and TPC1) decreased cell invasion, migration

and angiogenesis. The author suggested that BIBR1532 (*TERT*-specific inhibitor) and *TERT* siRNA significantly repress *TERT* expression and reduce PTC cell invasion, migration and angiogenesis by downregulating *TERT* target gene expression such as MMP-2, MMP-9 and VEGF.
Additionally, *TERT* inhibited angiogenesis in these PTC cells, explaining, at least in part the clinical observation. Present study is the first to describe an increased prevalence of *TERT* promoter mutations in WTC-exposed thyroid cancers, which may partially explain the thyroid cancer risk excess found in this population because of *TERT* mutation-associated aggressive cancers.

WTC responders have been exposed to a mixture of pollutants present in the WTC dust cloud. Although multiple of these pollutants has been classified as carcinogens, including asbestos, benzene, dioxins, chromium and polychlorinated biphenyls, research into the association between exposure to one of these pollutants and specific mutations has been limited. Asbestos exposure may potentially be associated with *TERT* promoter mutations as this mutation has been identified in malignant pleural mesothelioma, an aggressive tumor arising from the pleural cavities with as major risk factor past exposure to asbestos. Another hypothesis is that *TERT* overexpression may play a non-canonical role in cancer, which includes inflammatory response, activation of pro-cancer genes expression, reactive oxygen species generation, invasion, and metastasis. Further analysis needs to better understand the role of *TERT* promoter mutation in WTC-exposed thyroid cancer cases.

Conclusion

Specific Aim 1: Our study utilized RNAseq to reveal distinct genetic signatures in the thyroids of rats exposed to WTC dust. Our ongoing pathway enrichment analysis aims to clarify the significance of these findings. Future comparisons with human RNAseq data will help explain these biological effects in relation to WTC-exposed thyroid cancer.

Specific Aim 2: Our study's RNAseq analysis of human thyroid cancer samples revealed significant molecular signatures in WTC cases compared to non-WTC cases. These findings allow us to strengthen our results with the animal study and enhances our understanding of the carcinogenesis process following WTC exposure. Our ongoing efforts will include pathway enrichment analysis, as well as evaluating the mutational profile against the Chernobyl cohort.

Specific Aim 3: In conclusion, the increased prevalence of *TERT* promoter mutations in the WTC-exposed thyroid cancer may indicate that exposure to the mixture of pollutants present in the WTC dust cloud following the 9/11 disaster resulted in an excess thyroid cancer risk and potentially more aggressive thyroid cancer. This result warrants questioning WTC responders about potential thyroid-associated symptoms as well as physical examination of the thyroid gland during the yearly screening visits. Future studies should also include long-term follow-up of the WTC-exposed thyroid cancer cohort to provide important insights in whether thyroid cancer-specific survival is negatively affected by exposure to the WTC dust cloud pollutants, potentially because of the presence of one or more driver mutations.

Publications

1. Maaike van Gerwen, Janete Maria Cerutti, Thais Biude Mendes, Rachel Brody, Eric Genden, Gregory J Riggins, Emanuela Taioli, *TERT* and *BRAF V600E* mutations in thyroid cancer of World Trade Center Responders, *Carcinogenesis*, Volume 44, Issue 4, April 2023, Pages 350–355.

2. Tuminello S, Durmus N, Snuderl M, et al. DNA Methylation as a Molecular Mechanism of Carcinogenesis in World Trade Center Dust Exposure: Insights from a Structured Literature Review. *Biomolecules*. 2024;14(10):1302. doi:10.3390/biom14101302

3. Tuminello S, Nguyen E, Durmus N, Alptekin R, Yilmaz M, Crisanti MC, Snuderl M, Chen Y, Shao Y, Reibman J, et al. World Trade Center Exposure, DNA Methylation Changes, and Cancer: A Review of Current Evidence. *Epigenomes*. 2023; 7(4):31.

4. van Gerwen M, Cerutti JM, Rapp J, Genden E, Riggins GJ, Taioli E. Post-9/11 excess risk of thyroid cancer: Surveillance or exposure? *Am J Ind Med*. 2021; 64: 881-884.

Cumulative (Actual)

Racial Categories	Ethnic Categories									
	Not Hispanic or Latino			Hispanic or Latino			Unknown/Not Reported Ethnicity			**Total**
	Female	**Male**	**Unknown/ Not Reported**	**Female**	**Male**	**Unknown/ Not Reported**	**Female**	**Male**	**Unknown/ Not Reported**	
American Indian/ Alaska Native	0	0	0	0	0	0	0	0	0	0
Asian	0	0	0	0	0	0	0	0	0	0
Native Hawaiian or Other Pacific Islander	0	0	0	0	0	0	0	0	0	0
Black or African American	0	17	0	0	9	0	0	0	0	26
White	0	92	0	0	8	0	0	0	0	100
More than One Race	0	13	0	0	0	0	0	0	0	13
Unknown or Not Reported	0	0	0	0	0	0	0	4	0	4
Total	0	122	0	0	17	0	0	4	0	143

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?

No

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
TAIOLI	Y	TAIOLI, EMANUELA	MS,PHD,MD	PD/PI	2.4	0.0	0.0			NA
GRIGGIN1	Y	RIGGINS, GREGORY Joseph	BS,MS,PHD,MD	PD/PI	0.1	0.0	0.0			NA
	N	Anderson, Christina		Statistician	2.4	0.0	0.0			NA
	N	Alpert, Naomi		Biostatistician	4.4	0.0	0.0			NA
	N	Rapp, Joseph		Technician	2.9	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

File(s) uploaded:
CumulativeInclusionEnrollmentReport.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: Thyroid Cancer Risk in WTC Responders

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	0	0	0	0	0	0	0	0	**0**
Asian	0	0	0	0	0	0	0	0	0	**0**
Native Hawaiian or Other Pacific Islander	0	0	0	0	0	0	0	0	0	**0**
Black or African American	0	17	0	0	9	0	0	0		**26**
White	0	92	0	0	8	0	0	0	0	**100**
More Than One Race	0	13	0	0	0	0	0	0	0	**13**
Unknown or Not Reported	0	0	0	0	0	0	0	4	0	**4**
Total	**0**	**122**	**0**	**0**	**17**	**0**	**0**	**4**	**0**	**143**

I. OVERALL OUTCOMES

I.1 What were the outcomes of the award?

The outcomes of this project have significantly advanced our understanding of the impact of WTC exposure on thyroid cancer. Our findings from Aim 1, which identified specific signatures and markers in rodents exposed to WTC dust through RNA sequencing and whole genome sequencing, underlining the environmental exposure's role in possibly triggering a carcinogenesis process. This foundational research provides a critical link between environmental pollutants at the WTC site and potential cancer risk, informing future studies on environmental carcinogenesis.
Through Aim 2, we discerned distinct genomic profiles in WTC thyroid cancers compared to non-WTC thyroid cancers. By employing total RNA sequencing on FFPE, we observed unique gene expression patterns associated with WTC exposure. These results emphasize the importance of understanding the specific molecular and immunological changes in thyroid cancer caused by exposure to pollutants in the WTC dust. This knowledge can guide personalized treatment approaches and enhance early detection strategies for affected individuals.
Aim 3's exploration of the genetic mutational profiles revealed a high frequency of TERT promoter mutation in WTC thyroid cancers. These genetic alterations, identified through whole exome sequencing, provide crucial insights into the aggressive nature of thyroid cancer among WTC responders. This research highlights the necessity of targeted genetic screening and the development of tailored therapeutic interventions to improve outcomes for this high-risk population. Ultimately our research underscores the long-term health impacts of WTC exposure and the importance of continued surveillance and targeted research to mitigate these effects.