Initial Whole-Genome Sequencing of Plasma Cell Neoplasms in First Responders and Recovery Workers Exposed to the World Trade Center Attack of September 11, 2001

Francesco Maura[1,2], Benjamin Diamond[1], Kylee H. Maclachlan[1], Andriy Derkach[3], Venkata D. Yellapantula[1], Even H. Rustad[1], Malin Hultcrantz[1], Urvi A. Shah[1], Jessica Hong[4], Heather J. Landau[5], Christine A. Iacobuzio-Donahue[6], Elli Papaemmanuil[3], Shani Irby[5], Laura Crowley[7], Michael Crane[7], Mayris P. Webber[8,9,10], David G. Goldfarb[7,9], Rachel Zeig-Owens[8,9,10], Orsi Giricz[11], Amit Verma[11], David J. Prezant[9,12], Ahmet Dogan[13], Sohrab P. Shah[3], Yanming Zhang[14], and Ola Landgren[1,2]

ABSTRACT

Purpose: The World Trade Center (WTC) attack of September 11, 2001 created an unprecedented environmental exposure to known and suspected carcinogens. High incidence of multiple myeloma and precursor conditions has been reported among first responders to the WTC disaster. To expand on our prior screening studies, and to characterize the genomic impact of the exposure to known and potential carcinogens in the WTC debris, we were motivated to perform whole-genome sequencing (WGS) of WTC first responders and recovery workers who developed a plasma cell disorder after the attack.

Experimental Design: We performed WGS of nine CD138-positive bone marrow mononuclear samples from patients who were diagnosed with plasma cell disorders after the WTC disaster.

Results: No significant differences were observed in comparing the post-WTC driver and mutational signature landscapes with 110 previously published WGSs from 56 patients with multiple myeloma and the CoMMpass WGS cohort ($n = 752$). Leveraging constant activity of the single-base substitution mutational signatures 1 and 5 over time, we estimated that tumor-initiating chromosomal gains were windowed to both pre- and post-WTC exposure.

Conclusions: Although limitations in sample size preclude any definitive conclusions, our findings suggest that the observed increased incidence of plasma cell neoplasms in this population is due to complex and heterogeneous effects of the WTC exposure that may have initiated or contributed to progression of malignancy.

[1]Myeloma Service, Department of Medicine, Memorial Sloan Kettering Cancer Center, New York, New York. [2]Myeloma Program, Sylvester Comprehensive Cancer Center, University of Miami, Miami, Florida. [3]Department of Epidemiology and Biostatistics, Memorial Sloan Kettering Cancer Center, New York, New York. [4]Center for Hematological Malignancies, Department of Medicine, Memorial Sloan Kettering Cancer Center, New York, New York. [5]Adult Bone Marrow Transplant Service, Department of Medicine, Memorial Sloan Kettering Cancer Center, New York, New York. [6]Human Oncology & Pathogenesis Program, Memorial Sloan Kettering Cancer Center, New York, New York. [7]Mount Sinai School of Medicine, New York, New York. [8]Department of Medicine, Montefiore Medical Center, Bronx, New York. [9]Bureau of Health Services, Fire Department of the City of New York, Brooklyn, New York. [10]Department of Epidemiology and Population Health, Albert Einstein College of Medicine, Bronx, New York. [11]Division of Hemato-Oncology, Department of Oncology, Albert Einstein College of Medicine and Montefiore Medical Center, Bronx, New York. [12]Division of Pulmonary Medicine, Department of Medicine, Montefiore Medical Center and Albert Einstein College of Medicine, New York, New York. [13]Hematopathology Service, Department of Pathology, Memorial Sloan Kettering Cancer Center, New York, New York. [14]Cytogenetics Laboratory, Department of Pathology, Memorial Sloan Kettering Cancer Center, New York, New York.

Note: Supplementary data for this article are available at Clinical Cancer Research Online (http://clincancerres.aacrjournals.org/).

Corresponding Authors: Ola Landgren, Department of Medicine, Sylvester Comprehensive Cancer Center, University of Miami, 1120 NW 14th Street, Miami, FL 33136. Phone 305-243-6578; E-mail: col15@miami.edu; and Francesco Maura; Phone: 305-243-7687, E-mail: fxm557@med.miami.edu

Clin Cancer Res 2021;27:2111–8

doi: 10.1158/1078-0432.CCR-20-2245

Introduction

Multiple myeloma, a clonal neoplasm of post-germinal center B cells, is one of the most prevalent hematologic malignancies among adults in the United States. It is always preceded by an asymptomatic precursor condition [i.e., monoclonal gammopathy of undetermined significance (MGUS) or smoldering multiple myeloma (SMM); refs. 1, 2]. The evolution from a B cell's first germinal center encounter to multiple myeloma is driven by the acquisition of different genomic drivers and is shaped by the activity of different mutational processes (1, 2). Using whole-genome sequencing (WGS) data, seven main mutational processes (i.e., single-base substitution signatures) have been described in newly diagnosed multiple myeloma, six of which are associated with a recognized etiology: SBS1 and SBS5 (aging), SBS2 and SBS13 [apolipoprotein B mRNA editing enzyme, catalytic polypeptide-like (APOBEC)], SBS18 (damage by reactive oxygen species), and SBS9 [non-canonical activity of activation-induced cytidine deaminase (nc-AID); refs. 3–8].

Although multiple myeloma and myeloma precursor condition appear to occur sporadically without a known underlying etiology, an increased incidence of the disease has been reported in those exposed to a variety of carcinogens, including agent orange (with dioxin contaminant), polychlorinated biphenyls, and polycyclic aromatic hydrocarbons (PAH; refs. 2, 9, 10).

On September 11, 2001, the World Trade Center (WTC) attacks created an environmental exposure of unprecedented scale. Firefighters of the Fire Department of the City of New York were among the first responders met with aerosolized dust, gases, and debris containing many of the above known and other potential carcinogens (11). Some

Translational Relevance

The World Trade Center (WTC) attack of September 11, 2001 created an unprecedented environmental exposure to known and potential carcinogens and first responders have demonstrated a higher risk of developing multiple myeloma. We were motivated to identify distinct genetic signatures and temporal patterns responsible for this increased risk and so we performed the first whole-genome sequencing characterization of plasma cell neoplasms in first responders and recovery workers exposed to the WTC attack. While we did not observe any unifying genomic events or mutational signatures, we were able to estimate that the exposure to the debris may have had a role either in initiating the first aberrant cell or in promoting the progression of a preexisting clone. The existence of premalignant clonal entities at the time of WTC exposure may, therefore, be relevant for future WTC-related studies.

of these substances (e.g., PAHs) have been reported to leave unique mutational signatures on the genome of human cells, allowing for quantification of the mutational burden for which they are responsible (12). It has since been documented that first responders and recovery workers with exposure to the WTC attack have higher than expected rates of malignancy (13–15) and, specifically, there are reports of increased incidence of multiple myeloma and precursor conditions following WTC exposure (16). However, the mutational impact of this unique exposure has never been investigated or quantified in any cancer type so far.

Whole-genome, whole-exome, and target sequencing have been extensively used to characterize the genomic landscape of multiple myeloma cases diagnosed in the general population. Discoveries have included a description of 81 potential driver genes, and the cataloguing of structural variations (SV), including complex events, such as chromothripsis, and copy-number abnormalities, with negative prognostic implications (4, 7, 8, 17–20). However, potential common themes in the genomic characteristics of plasma cell neoplasms developing following a shared carcinogenic event, such as the WTC attack, have never been investigated.

To expand on our prior screening study (16), we were motivated to perform WGS for nine first responders and recovery workers who developed a plasma cell disorder after being exposed to the disaster. The key aims of this study were specifically: (i) to identify distinct genetic signatures (i.e., mutational signatures) of increased risk of multiple myeloma and precursor conditions among WTC survivors and (ii) to establish whether the WTC disaster initiated the preneoplastic clone or accelerated the progression of a preexisting one. While we did not observe any unifying distinct genomic events, using mutational signatures and the molecular clock (7), we were able to estimate that the exposure to the WTC attack may have had a role either in initiating the first aberrant cell or in promoting the progression of a preexisting clone.

Materials and Methods

Thirty-one WTC-exposed patients who developed plasma cell disorders after the WTC attack are currently in follow-up in the Myeloma Service at the Memorial Sloan Kettering Cancer Center (New York, NY; Supplementary Table S1). Samples and data were obtained and managed in accordance with the Declaration of Helsinki. We performed WGS of nine cases that had sufficient CD138-positive bone marrow mononuclear cells: four MGUSs, two SMMs, two multiple myelomas, and one patient with plasma cell leukemia (PCL; **Table 1**; ref. 16). Eight patients (88%) were first responders and one was a recovery worker (IID_H135225). The study involved the use of human samples, which were collected after written informed consent was obtained (institutional review board 14-276 and 06-107). Plasma cell selection was performed by CD138-positive magnetic bead–selected bone marrow mononuclear cells. All sequencing investigations were performed at Memorial Sloan Kettering Cancer Center's (New York, NY) Integrated Genomics Operation (21). Peripheral blood mononuclear cells were used as a normal match. For the only PCL sample, peripheral blood granulocytes were used as normal match to avoid tumor plasma cell contamination.

WGS

After PicoGreen quantification and quality control by Agilent Bioanalyzer, 500 ng of genomic DNA was sheared using a LE220-plus Focused-ultrasonicator (Covaris, catalog no., 500569) and sequencing libraries were prepared using the KAPA Hyper Prep Kit (Kapa Biosystems, KK8504) with modifications. Briefly, libraries were subjected to a 0.5 × size select using aMPure XP Beads (Beckman Coulter, catalog no., A63882) after post-ligation cleanup. Libraries not amplified by PCR (07652_C) were pooled equivolume and were quantitated on the basis of their initial sequencing performance. Libraries amplified with five cycles of PCR (07652_D, 07652_F, and 07652_G) were pooled equimolar. Samples were run on a NovaSeq 6000 in a 150 bp/150 bp paired-end run, using the NovaSeq 6000 SBS v1 kit and an S4 Flow Cell (Illumina), as described previously (22).

Whole-genome analysis pipeline

The median coverage for tumor and normal samples was 50.9 × (range, 47–76) and 37 × (range, 35–41), respectively (**Table 1**). Short-insert paired-end reads were aligned to the reference genome (GRCh37) using the Burrows–Wheeler Aligner (v0.5.9; ref. 17). Somatic mutations were identified by CaVEman (22). Copy-number analysis and tumor purity (i.e., cancer cell fraction) were evaluated using Battenberg (https://github.com/Wedge-Oxford/battenberg). SVs were defined by merging calls from SvABA (23), BRASS (https://github.com/cancerit/BRASS), and GRIDSS (https://github.com/PapenfussLab/gridss). Complex events (i.e., templated insertion, chromothripsis, and chromoplexy) were defined and annotated as described previously (19, 20). The phylogenetic tree of each case was reconstructed using the Dirichlet process (https://github.com/Wedge-Oxford/dpclust).

Mutational signatures were investigated by combining and comparing the WTC cohort with 110 WGSs from 56 patients with multiple myeloma and myeloma precursor condition (4, 7, 17, 19–21). All WGSs were characterized by using the same pipeline described above. To estimate the activity of mutational signatures, we followed our recently published workflow based on three steps: *de novo* extraction, assignment, and fitting (5). For the first step, we ran SigProfiler and hierarchical Dirichlet process (3). All extracted signatures were then compared with the latest Catalogue of Somatic Mutations in Cancer reference (https://cancer.sanger.ac.uk/cosmic/signatures/SBS/) to define which known mutational processes were active in our cohort. Finally, we applied mmsig (https://github.com/evenrus/mmsig), a fitting algorithm designed for multiple myeloma, to confirm the presence and estimate the contribution of each mutational signature in each sample (7). Confidence intervals were generated by drawing 1,000 mutational profiles from the multinomial distribution,

Table 1. Sequencing, demographic, and clinical profile of the 9/11 WTC cohort.

Sample	WTC exposure	Age at diagnosis	Year of diagnosis	Stage	Sex	Isotype	IGH translocations	Coverage	Purity	Ploidy
IID_H196059	First responder	67	2019	MGUS	M	IgG kappa/lambda[a]	HRD	49.19	0.35	2.45
IID_H135336	First responder	50	2014	SMM	M	IgG lambda	HRD	76.54	0.40	2.18
IID_H196060	First responder	48	2017	PCL	M	IgG lambda	t(14;16)	48.76	0.96	1.9
IID_H130588	First responder	57	2018	MM	M	IgG kappa	HRD	50.39	0.86	2.29
IID_H196061	First responder	52	2019	MGUS	M	IgA kappa	t(11;14)	51.73	0.45	2.48
IID_H196062	First responder	58	2019	SMM	M	IgG lambda	t(6;14)	47.57	0.31	1.99
IID_H196063	First responder	61	2019	MGUS	M	IgG lambda	HRD	47.85	0.5	2.81
IID_H196064	First responder	65	2019	MGUS	M	IgG lambda	—	51.46	0.86	1.9
IID_H135225	Rescue worker	47	2015	MM	M	IgG kappa	t(2;16)(IGL-MAF)	76.22	0.98	1.89

Abbreviations: HRD, hyperdiploid; MM, multiple myeloma.
[a]Biclonal.

each time repeating the signature fitting procedure, and finally taking the 2.5th and 97.5th percentile for each signature. To analyze the contribution of each mutational signature over time, we explored all Dirichlet process clusters with more than 50 mutations using mmsig as described above (4, 7).

To exclude the contribution of environmental agents detected in the WTC debris with recognized mutational signatures (12), we ran mmsig in each post-WTC case, including and forcing the extraction of these mutational signatures (7).

The landscape of recurrent genomic drivers and complex events was then compared with 752 patients with multiple myeloma enrolled in the CoMMpass trial with available whole-exome and low-coverage long-insert WGS data (IA15; NCT01454297; refs. 7, 20). As recurrent and driver genomic events, we selected the most relevant copy-number changes, translocations, complex events, and a catalog of 81 driver genes involved by mutations derived from combining two large driver analyses (6, 18, 19).

Molecular time

The relative timing of each multi-chromosomal gain event was estimated using the R package, mol_time (https://github.com/nicos-angelopoulos/mol_time; refs. 7, 19). Correcting the ratio between duplicated mutations [variant allele frequency (VAF), ∼66%, acquired before the chromosomal duplication] and nonduplicated mutations (VAF, 33%, acquired on either the nonduplicated allele or on one of the two duplicated ones), this approach allows to estimate the relative timing of acquisition of all large (>1 Mb) chromosomal gains (e.g., trisomy in hyperdiploid patients with myeloma) with more than 50 clonal mutations as estimated by the Dirichlet process (6, 7, 19, 24). Tetrasomies, with both alleles duplicated, were removed given the impossibility of defining whether the two chromosomal gains occurred in close temporal succession, or in two discrete time windows (2).

Overall, the molecular time approach allowed the definition of chromosomal gains that were acquired in the same time window. Next, to convert the relative molecular time estimate into an absolute estimate, we combined chromosomal gains acquired in the same time window and calculated the molecular time based only on the mutational burden of single bases, SBS1 and SBS5 (3, 7, 25). Considering that these mutational processes are known to be constant in multiple myeloma (as in all cancers and normal tissues; refs. 3, 7, 25), we could convert the SBS1 and SBS5 molecular clock into an absolute time estimate for the acquisition of these events in each patient's life. Confidence intervals were generated by bootstrapping the molecular time estimate. Only multi-gain events with more than 50 SBS1 and SBS5 mutations were included. The PCL case was excluded because of its mutational profile characterized by high mutational burden (>10,000) and hyper-APOBEC contribution (7).

Data analysis and statistical analysis

Data analysis was carried out in R version 3.6.1. Standard statistical tests are mentioned consecutively in the article, while more complex analyses are described above. All reported *P* values are two-sided, with a significance threshold of <0.05.

Data availability

Sequence files are available at the European Genome-phenome and dbGaP archive under the accession codes:

(i) EGAD00001003309: 67 WGS data from 30 patients with multiple myeloma.

(ii) EGAS00001004404: 21 WGSs data from 4 patients.

(iii) phs000748.v1.p1: whole-exome sequencing and low-coverage/long-insert WGS data from 752 patients with newly diagnosed multiple myeloma included in this study (CoMMpass trial; IA 15).

(iv) phs000348.v2.p1: WGS data from 22 patients with multiple myeloma.

(v) EGAS00001004467: WTC WGS data.

Results

A total of 56,682 single-nucleotide variants (SNV) were detected, with a median of 5,115 SNVs per sample (range, 1,164–19,658). Among 433 nonsynonymous mutations detected [median, 40/sample (range, 4–198)], only 12 involved known multiple myeloma drivers [median, 1/sample (range, 0–4); **Fig. 1**]. Across 9 patients, a total of 277 SVs were called, with a median of 17 SVs per patient (range, 0–93). Deletions were the most common SV type (31%), followed by inversions (29%), tandem duplications (21%), and translocations (19%). A total of 174 of 277 SVs (63%) were part of a complex event (i.e., chromothripsis, chromoplexy, templated insertion, or unclassifiable complex event; refs. 19, 20). Four patients had a chromothripsis and 2 patients had a templated insertion involving two or more chromosomes. Overall, 5 patients had at least one complex event. We did not observe any significant differences in multiple myeloma driver SNVs, SVs, complex events, or copy-number alterations (CNA) between the WTC cohort and the CoMMpass series (**Fig. 1**). Of interest, three of four MGUSs were characterized by low SV burden and absence of complex events, and all followed an indolent clinical course. In contrast, 1 patient with MGUS, having intermediate-high risk for

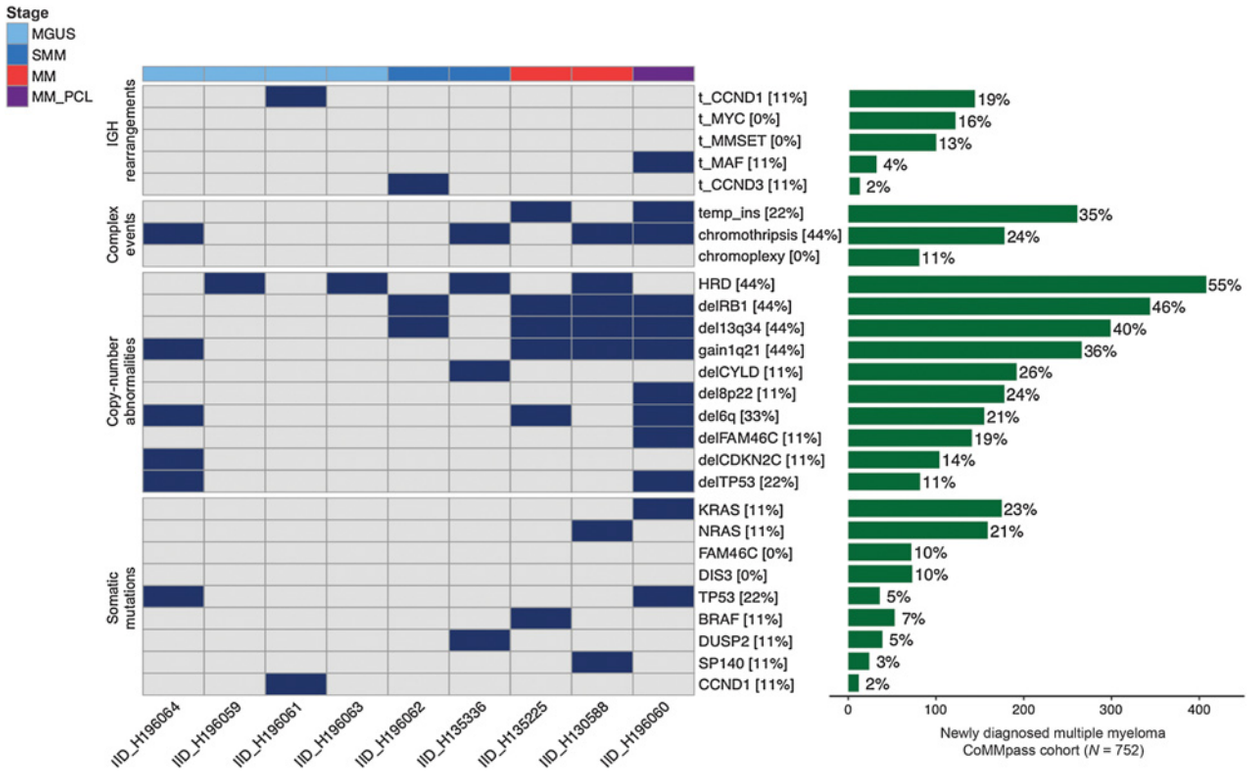

Figure 1.

Multiple myeloma genomic driver landscape in the WTC cohort. The prevalence of each genomic driver is compared with that observed in 752 patients with multiple myeloma enrolled in the CoMMpass trial with whole-exome and low-coverage long-insert WGS data available. Only fully clonal copy numbers were reported for the WTC cohort. HRD, hyperdiploid; MM, multiple myeloma; MM_PCL, plasma cell leukemia; SMM, smoldering multiple myeloma.

progression at diagnosis, a monoclonal protein spike of 2.1 g/dL, and a bone marrow plasma cell infiltration of 5%, was characterized by chromothripsis, high APOBEC signature contribution, and biallelic *TP53* inactivation; within 2 years, this patient had progression into multiple myeloma.

Mutational signature landscape in WTC-exposed patients

In comparison with 110 previously published WGSs from 56 patients with multiple myeloma and precursor conditions (7, 17, 19), we did not observe any new mutational signatures among WTC-exposed patients. Three WTC cases (one MGUS, one multiple myeloma, and the one PCL) showed relatively high APOBEC contribution with only the PCL having t(14;16)(*MAF*;*IGH*) (8, 26). To rule out any undetected or low mutational contribution from exposure to WTC toxic substances, we used the mmsig mutational signature fitting approach, including and forcing the extraction of five mutational signatures associated with environmental agents detected in the WTC debris (e.g., PAHs; Supplementary Table S2; ref. 12). There were no significant contributions from any of these described mutational signatures. When refitting mutational signatures in the absence of those related to environmental exposure, the signature profile of the WTC cohort recapitulated previous observations for multiple myeloma and precursor conditions (**Fig. 2A**).

We recently defined four main temporal patterns of mutational signatures activity in multiple myeloma (7): (i) where nc-AID activity is limited to the first phase of cancer development, and APOBEC is only active during later phases; (ii) where APOBEC is active since the beginning without any significant nc-AID contribution; (iii) where nc-AID activity is prolonged over time contributing to the subclonal diversification; and (iv) where nc-AID is active only during the first phase of cancer development, and APOBEC is always absent. Reconstructing the temporal activity of each mutational process, we sought to explore whether WTC-exposed patients had differing patterns in mutational signature timelines (Materials and Methods; **Fig. 2B**; ref. 7). Among the five cases where the subclonal mutational load allowed for a reliable mutational signature estimation (4, 7), four cases showed a reduction in nc-AID from clonal to subclonal mutations. In three cases, APOBEC increased from clonal to subclonal mutations. In the PCL case, nc-AID was not detectable and APOBEC was the major mutational process in both clonal and subclonal variants, in line with the temporal pattern of hyper-APOBEC mutational signatures (#2 above; ref. 7). Overall, these data revealed that the mutational signature activity over time in post-WTC plasma cell dyscrasia is heterogenous and reflects what has been observed previously in multiple myeloma without WTC exposure.

Timing the initiation of plasma cell dyscrasias in WTC-exposed patients

We, and others, have recently shown that a cancer-initiating event can be acquired 30–40 years before its clinical diagnosis, often between the second and third decade of life (7, 25, 27). These estimates are made possible due to distinct mutational processes that are stably active over time (i.e., clock-like; ref. 28). Without mutational contributions directly linked to exposure, we leveraged

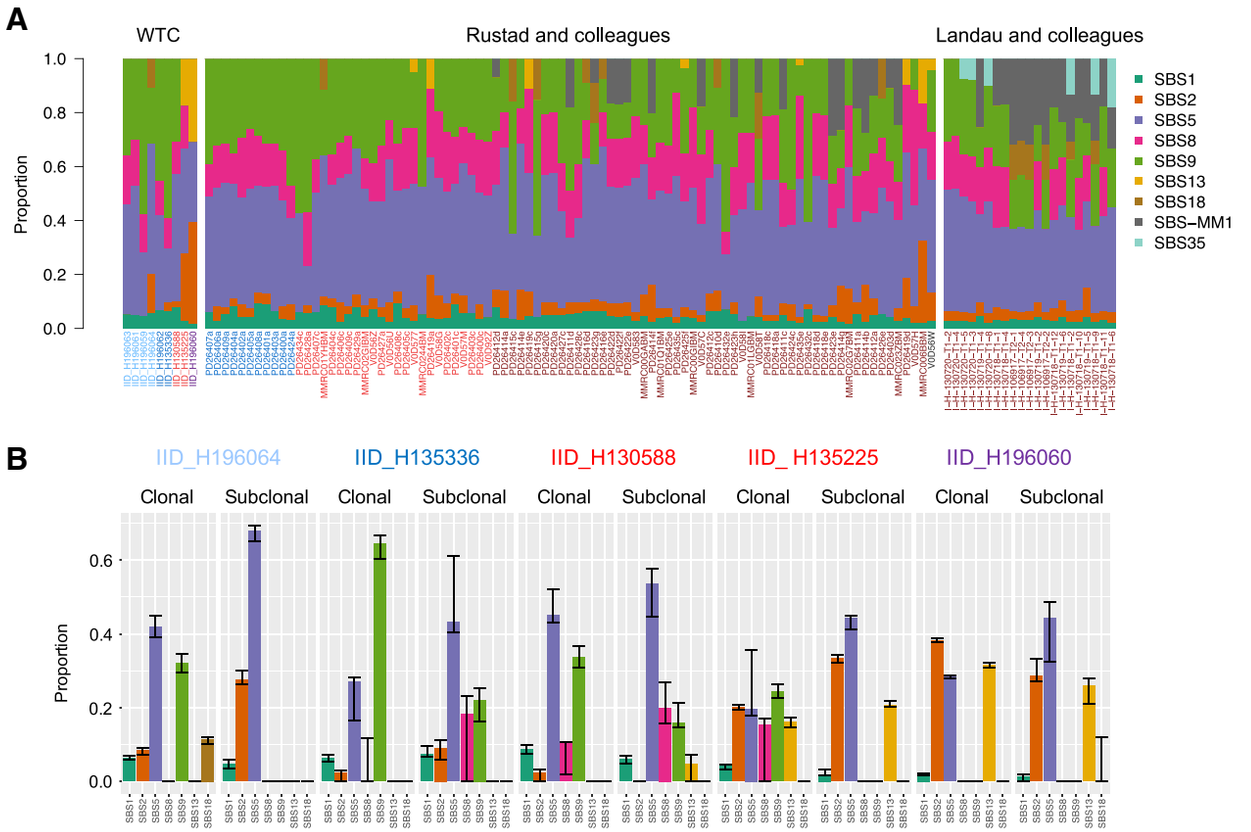

Figure 2.

Mutational signature landscape in first responders and recovery workers exposed to the WTC disaster. **A,** Relative contribution of each mutational signature in the WTC cohort and in a validation set including 110 available WGSs from 56 patients. Label colors reflect the clinical stage at the time of sample collection. Specifically light blue, dark blue, red, brown, purple, and black reflect MGUS, SMM, newly diagnosed multiple myeloma, relapsed myeloma, plasma cell leukemia, and cases without clinical annotation, respectively. **B,** Mutational signatures' differences between clonal and subclonal copy-number variations (CNV) in each case with more than 50 subclonal mutations. The confidence interval of each mutational signature estimate was generated by drawing 1,000 mutational profiles from the multinomial distribution, each time repeating the signature fitting procedure (mmsig), and finally taking the 2.5th and 97.5th percentile for each signature.

the cancer molecular clock concept to ascertain whether exposure to the WTC attack might have either accelerated progression of a preexisting clonal entity, served as an initiating carcinogenic event, or a combination of both.

For each patient with at least one large chromosomal gain in the WTC cohort (8/9), we applied our recently published molecular time workflow (19) to estimate the relative order and time window of its acquisition (Materials and Methods; **Fig. 3A**). Then, following the molecular clock concept (7, 25, 28), we collapsed together chromosomal gains acquired in the same time window and used the pre- and post-gain SBS1 and SBS5 mutation burden to convert relative time estimates into absolute ones (i.e., the age at which these events were acquired in each patient's life). One case was excluded because of low SBS5 mutational burden (IID_H196064) and another due to high mutational burden and hyper-APOBEC activity (H196060; PCL), each of which affect the accuracy of timing predictions (Materials and Methods; **Fig. 3B**; ref. 7). According to our time estimates, one multiple myeloma case (IID_H130588), one SMM case (IID_H135336), and one MGUS case (IID_H196059) showed evidence of a preexisting clone before the WTC attack. The remaining two MGUS cases showed evidence of multi-chromosomal gain acquisition after the attack (H196061 and H196063). Finally, in the last multiple myeloma case (IID_H13225), the 1q gain was acquired around the time of the WTC attack. Overall, these data are consistent with the recently proposed model that the first genomic driver precedes myeloma diagnosis by decades (7).

Discussion

Exposure to environmental carcinogens can promote cancer development through various mechanisms. As a canonical example, tobacco smoke stimulates hundreds of mutations in directly exposed cells, leaving evidence of its effect through signature SBS4 (3, 29, 30). While this evidence is seen directly in virtually all tobacco smoke–related lung cancers, there are other tobacco-associated cancers without any evidence of SBS4 contribution, suggesting that tobacco-mediated carcinogenesis is also promoted through other mechanisms (29). The exposure to the WTC debris is known to promote several cancers, including multiple myeloma and precursor conditions. The lack of a homogeneous and distinct mutational signature among WTC-exposed patients who subsequently developed either multiple myeloma or myeloma precursor condition suggests that the carcinogens present in the WTC debris may promote myelomagenesis through alternate evolutionary trajectories and combination of drivers, without

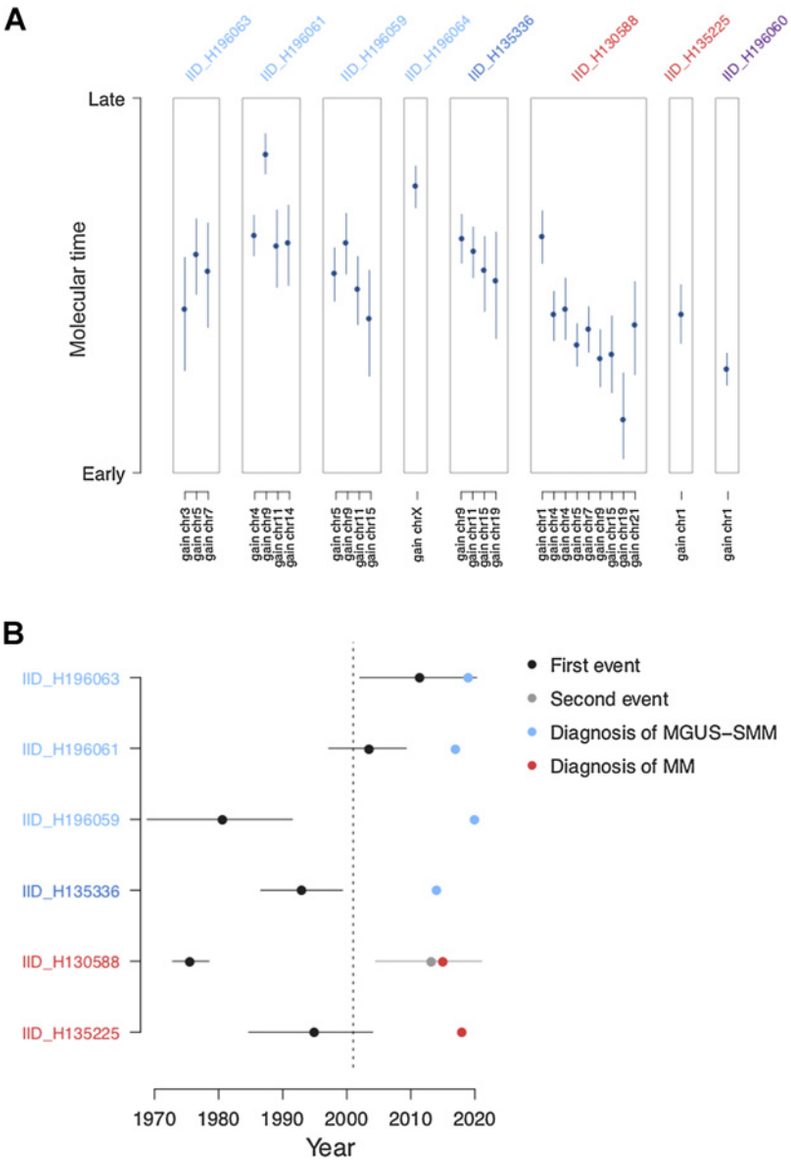

Figure 3.

Timing multi-chromosomal gain events in first responders and recovery workers exposed to the WTC disaster with multiple myeloma and precursor conditions. **A,** Molecular time estimated for each clonal gain and copy-neutral LOH in the WTC cohort. Blue dots and lines represent the molecular time estimates and the 95% confidence intervals, respectively. Only large chromosomal gains (>1 Mb) with more than 50 clonal SNVs were considered. **B,** Absolute timing of each multi-gain event in relation to the WTC attack and the patient's age at diagnosis for 6 evaluable patients. Dark blue and gray dots represent the first and second multi-gain events, respectively, with 95% confidence intervals. Blue dots represent MGUS diagnosis and red dots represent multiple myeloma diagnosis. The dotted line represents September 11, 2001. Label colors reflect the clinical stage at the time of sample collection. Specifically light blue, dark blue, and red reflect MGUS, SMM, and newly diagnosed multiple myeloma, respectively.

leaving direct mutagenic evidence. While the small sample size might have limited the power of genomic driver discovery, likely it did not affect the analysis of mutational signatures. In fact, 9 patients, all exposed to the same carcinogenic event, had enough power to potentially detect a new WTC-related mutational process.

While we did not observe any distinct genomic link between the WTC exposure and multiple myeloma or precursor conditions, leveraging the molecular time concept, we were able to show that the WTC exposure might, in some cases, have had a role in promoting a preexisting clonal entity (i.e., progression from preexisting myeloma precursor condition to frank malignancy) and, in others, it may have contributed to creating the conditions required for initiating the clonal entity. It is also possible that (at least some) myeloma precursors were acquired independent of the WTC attack, as in patient IID_H196061, where the first datable event was acquired approximately 12 years after the attack. Because of the limited sample size, if all patients have had the same temporal pattern (i.e., first multi-gain events uniformly acquired either before or after the WTC attack), we would not have been able to claim a universal WTC-related temporal model due to power limitation. However, the observation of different/dichotomous patterns supports the concept that WTC exposure affected individuals in different ways.

Overall, in our study, we provided the first WGS characterization of first responders and recovery workers exposed to the WTC attack, who developed multiple myeloma and myeloma precursor conditions. The observed genomic and temporal heterogeneity herein suggests that the observed increased incidence of plasma cell neoplasm in WTC-exposed patients is due to complex and heterogenous effects that may have initiated or promoted subsequent disease development.

Authors' Disclosures

M. Hultcrantz reports grants from Memorial Sloan Kettering and the Memorial Sloan Kettering Cancer Center NCI Core Grant (P30 CA 008748) during the conduct of the study. M. Hultcrantz also reports grants from Swedish Blood Cancer

Foundation and Karolinska Institute Foundations, and personal fees from Curio Science and Intellisphere, LLC, outside the submitted work. U.A. Shah reports grants from Parker Institute for Cancer Immunotherapy, Celgene/BMS, and Janssen, and personal fees from Physicians Education Resources outside the submitted work. H.J. Landau reports grants from Genzyme Corporation, Amgen, Takeda Pharmaceuticals, Janssen Scientific Affairs LLC, HCA Healthcare, Spectrum Pharmaceuticals, Inc, Caelum Biosciences, Inc, Celgene, Clinical Care Options, and Karyopharm outside the submitted work. C.A. Iacobuzio-Donahue reports grants from Bristol Myers Squibb outside the submitted work. E. Papaemmanuil reports a patent for algorithm for WGS analytics pending to Isabl and equity holder of Isabl, a WGS analytics company that licenses the technology used at least, in part, to generate the data used in this study. L. Crowley reports other from World Trade Center Health Program during the conduct of the study and outside the submitted work. D.J. Prezant reports grants from National Institute of Occupation Safety & Health and CDC during the conduct of the study. A. Dogan reports grants from Roche, grants and personal fees from Takeda, nonfinancial support from AbbVie, and personal fees from Seattle Genetics, PER, EUSA Pharma, and PeerView outside the submitted work. S.P. Shah reports personal fees from Canexia Health Inc outside the submitted work. O. Landgren reports grants from Memorial Sloan Kettering and the Memorial Sloan Kettering Cancer Center NCI Core Grant (P30 CA 008748) during the conduct of the study. O. Landgren also reports grants from Amgen, Celgene, Janssen, Takeda, Glenmark, Seattle Genetics, Karyopharm, FDA, Multiple Myeloma Research Foundation, International Myeloma Foundation, Leukemia and Lymphoma Society, Perelman Family Foundation, and Rising Tide Foundation; personal fees from Adaptive Biotech, Amgen, Binding Site, Bristol Myers Squibb, Celgene, Cellectis, Glenmark, Janssen, Juno, and Pfizer; and other from Takeda, Merck, Janssen, and Theradex outside the submitted work. No disclosures were reported by the other authors.

Authors' Contributions

F. Maura: Conceptualization, resources, data curation, software, formal analysis, supervision, funding acquisition, validation, investigation, visualization, methodology, writing–original draft, project administration, writing–review and editing. **B. Diamond:** Conceptualization, data curation, formal analysis, writing–original draft. **K.H. Maclachlan:** Data curation. **A. Derkach:** Formal analysis, supervision. **V.D. Yellapantula:** Data curation, software. **E.H. Rustad:** Software. **M. Hultcrantz:** Data curation. **U.A. Shah:** Data curation. **J. Hong:** Project administration. **H.J. Landau:** Data curation. **C.A. Iacobuzio-Donahue:** Data curation. **E. Papaemmanuil:** Data curation, software. **S. Irby:** Data curation. **L. Crowley:** Data curation. **M. Crane:** Data curation. **M.P. Webber:** Data curation. **D.G. Goldfarb:** Data curation. **R. Zeig-Owens:** Data curation. **O. Giricz:** Data curation. **A. Verma:** Data curation. **D.J. Prezant:** Data curation. **A. Dogan:** Resources, data curation. **S.P. Shah:** Data curation. **Y. Zhang:** Resources, data curation. **O. Landgren:** Conceptualization, resources, data curation, software, supervision, funding acquisition, investigation, visualization, methodology, writing–original draft, project administration, writing–review and editing.

Acknowledgments

This work was supported by the Society of Memorial Sloan Kettering, the Sylvester Comprehensive Cancer Center NCI Core Grant (P30 CA 240139), and the Memorial Sloan Kettering Cancer Center NCI Core Grant (P30 CA 008748). F. Maura was supported by the American Society of Hematology, the International Myeloma Foundation, and The Society of Memorial Sloan Kettering Cancer Center. B. Diamond was supported by Conquer Cancer and the ASCO Foundation. K.H. Maclachlan was supported by the Haematology Society of Australia, New Zealand New Investigator Scholarship, the Royal College of Pathologists of Australasia Mike, and Carole Ralston Travelling Fellowship Award. We thank Integrated Genomics Operation for the sequencing support.

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Received June 9, 2020; revised December 21, 2020; accepted January 22, 2021; published first January 27, 2021.

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