original reports

PRECISION MEDICINE

Mutant *PPM1D*- and *TP53*-Driven Hematopoiesis Populates the Hematopoietic Compartment in Response to Peptide Receptor Radionuclide Therapy

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abstract

PURPOSE Hematologic toxic effects of peptide receptor radionuclide therapy (PRRT) can be permanent. Patients with underlying clonal hematopoiesis (CH) may be more inclined to develop hematologic toxicity after PRRT. However, this association remains understudied.

MATERIALS AND METHODS We evaluated pre- and post-PRRT blood samples of patients with neuroendocrine tumors. After initial screening, 13 cases of interest were selected. Serial blood samples were obtained on 4 of 13 patients. Genomic DNA was analyzed using a 100-gene panel. A variant allele frequency cutoff of 1% was used to call CH.

RESULT Sixty-two percent of patients had CH at baseline. Persistent cytopenias were noted in 64% (7 of 11) of the patients. Serial sample analysis demonstrated that PRRT exposure resulted in clonal expansion of mutant DNA damage response genes (*TP53, CHEK2*, and *PPM1D*) and accompanying cytopenias in 75% (3 of 4) of the patients. One patient who had a normal baseline hemogram and developed persistent cytopenias after PRRT exposure showed expansion of mutant *PPM1D* (variant allele frequency increased to 20% after exposure from < 1% at baseline). In the other two patients, expansion of mutant *TP53*, *CHEK2*, and *PPM1D* clones was also noted along with cytopenia development.

CONCLUSION The shifts in hematopoietic clonal dynamics in our study were accompanied by emergence and persistence of cytopenias. These cytopenias likely represent premalignant state, as *PPM1D*-, *CHEK2*-, and *TP53*-mutant clones by themselves carry a high risk for transformation to therapy-related myeloid neoplasms. Future studies should consider CH screening and longitudinal monitoring as a key risk mitigation strategy for patients with neuroendocrine tumors receiving PRRT.

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ASSOCIATED CONTENT

Data Supplement

Author affiliations and support information (if applicable) appear at the end of this article.

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INTRODUCTION

Cancer-related mortality is decreasing each year in the United States.[1] Therapy-related myeloid neoplasms (t-MNs) are expected to rise in parallel among cancer survivors.[2] t-MNs constitute 10%-20% of newly diagnosed acute myeloid leukemia and myelodysplastic syndrome cases and are associated with dismal outcomes.[3] Radiation therapy and DNA-damaging chemotherapy are linked to increased risk of t-MNs.[2] With the application of sensitive sequencing technologies, the putative origin of t-MN driver mutations such as those involving DNA damage response (DDR) genes like *TP53* can be detected even at time of the primary malignant diagnosis. Detection of clonal hematopoiesis (CH) mutations offers the first potential biomarker for risk of subsequent t-MN.[4,5] t-MN's biology is a complex interplay of dose; type and duration of cytotoxic therapy; and age, environment, and genetic make-up of the host.[2] Comprehensive genomic analysis of at-risk patients undergoing potentially leukemogenic therapies will aid in understanding biology of t-MN and might help to devise a mitigation strategy for t-MN risk.

t-MN historically has been considered a consequence of DNA damage induced in normal hematopoietic stem or progenitor cells by the effect of genotoxic therapy (Data Supplement). However, this model is falling out of favor as not all patients exposed to genotoxic therapies develop t-MN and in those who develop a t-MN, there is a wide range of latency periods. There is increasing evidence outlining the role of the presence of somatic mutations in hematopoietic stem cells (HSCs) before the exposure to genotoxic therapy as precursors for t-MN.[2] As CH and its

CONTEXT

Key Objective

Do patients with neuroendocrine tumors (NETs) who experience short-term and long-term hematologic adverse events after peptide receptor radionuclide therapy (PRRT) exposure harbor any underlying clonal predisposition for altered or malignant hematopoiesis?

Knowledge Generated

Blood samples from patients with NETs demonstrated a high prevalence, 62% with clonal hematopoiesis (CH) at the time of diagnosis. On serial sample analysis, CH in DNA damage response genes, ie, *TP53*, *CHEK2*, and *PPM1D*, expanded on longitudinal monitoring after PRRT therapy in three of four patients. The clonal expansions in DNA damage response genes after PRRT were associated with varying degrees of prolonged cytopenias in all the patients with *TP53*, *CHEK2*, and *PPM1D* mutations.

Relevance

Under the stress of potentially leukemogenic therapy (PRRT), *TP53*, *CHEK2*, and *PPM1D* clones preferentially repopulated the hematopoietic compartment and were associated with prolonged cytopenias. Screening and longitudinal monitoring of CH in patients with NET receiving PRRT can be an important risk-modifying strategy for these patients.

evolution may serve as a surrogate predictive marker for subsequent t-MN development, we sought to study hematopoietic clonal dynamics and fitness in the context of a modern and potentially leukemogenic therapy.

Neuroendocrine tumors (NETs) are frequently diagnosed in younger patients, have a relatively indolent clinical course, and are associated with germline predisposition in some cases.[6] Several studies have demonstrated varying degrees of t-MN risk after radionuclide treatment including peptide receptor radionuclide therapy (PRRT).[7-11] PRRT is a tumor-targeted treatment that uses radiation to induce tumor cell death via β particle–emitting radionuclide.[9] The lower rates of progression of primary tumors treated with this agent[12] led to US Food and Drug Administration approval of PRRT for somatostatin receptor–positive gastroenteropancreatic NETs in 2018. Hematologic toxicity was the major adverse event with the nadir of cytopenias occurring about a month after therapy.[13] Permanent hematologic dysfunction has been reported after therapy, indicating significant injury to hematopoietic precursors and potentially an increased risk for development of t-MNs.[11,14,15] To date, the precise stress induced by PRRT on the HSC compartment is largely elusive because of the novelty of this drug.[16,17]

The aim of our study is to better elucidate the role of PRRT in exerting selective pressure on specific mutant HSCs and their clonal progeny and assess the overall clonal fitness of hematopoietic cells under PRRT-induced stress.

MATERIALS AND METHODS

Patients who received PRRT therapy between March 2018 and August 2019 were identified through our NET Clinic database and the biorepository at our institution, Roswell Park Comprehensive Cancer Center (RPCCC). After initial screening, 20 individuals were identified. Thirteen cases were selected on the basis of availability of at least one whole blood or DNA sample preceding PRRT treatment. Of the 13 cases, serial peripheral blood samples could be obtained for four patients after an informed consent. Clinical and laboratory variables were collected through retrospective chart review and included CBC counts obtained at time of initial sample collection (±30 days). Associations with prior chemotherapy or radiotherapy exposure were based on receipt of any NET-directed systemic therapy (excluding hormone therapy with somatostatin analog) or radiation therapy before the date of initial blood DNA sampling. None of the patients in our analysis had another active hematologic malignancy or a precursor state such as monoclonal gammopathy of undetermined significance or monoclonal B-cell lymphocytosis at the time of peripheral blood next generation sequencing. Patients were followed for the development of subsequent hematologic adverse events with the last follow-up date of January 1, 2021. The study was approved by the Institutional Review Boards of both participating sites (RPCCC and Weill Cornell Medical College). Chi-square tests and Wilcoxon rank-sum tests were used to compare patient and clinical characteristics among patients with and without CH. Patients were stratified on the basis of age, sex, and smoking status. Logistic regression models were used to study the association of covariates with mutational events. All analyses were conducted at a significance level of 0.05.

Mutational Analysis

Method details. Genomic DNA from mononuclear cells or buffy coats was extracted. Targeted enrichment using a custom pool of biotinylated baits directed to 100 genes involved in CH, cancer, and CVD was performed. After filtering out potential germline and artifacts, variants with a variant allele frequency (VAF) ≥ 1% were reported. A detailed technical description is provided in the Data Supplement.

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TABLE 1. Baseline Patients' Characteristics

Characteristic	Value
Total No. of patients (%)	13 (100)
Median age (years)	58 (41-75)
Female, No. (%)	4 (30.8)
Male, No. (%)	9 (69.2)
White, No. (%)	13 (100)
NET type, No. (%)	
Small bowel	6 (46.2)
Pancreatic	3 (23.1)
Others	4 (30.7)
Tumor grade, No. (%)	
Low	8 (61.5)
Intermediate	5 (38.5)
Stage IV, No. (%)	13 (100)
Prior chemotherapy exposure, No. (%)	1 (7.7)
Prior radiotherapy exposure, No. (%)	3 (23.1)
History of CAD or CVA, No. (%)	5 (38)
Tobaccos use history, No. (%)	4 (30.7)

Abbreviations: CAD, coronary artery disease; CVA, cerebrovascular accident; NET, neuroendocrine tumor.

Quantification and statistical analysis. Analysis was conducted in R environment v4.0.2 (R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria).[18] Figures were produced using the package *ggplot2 v3.3.3*[19] and *ComplexHeatmap v2.4.3*.[20] Data summarization and reshaping were performed using *plyr v1.8.6*, *dplyr v1.0.1*, and *reshape2 v1.4.4* packages. Statistical significance was defined as $P < .05$. Statistical tests were performed using R software.

The list of genes in the CH panel is provided in the Data Supplement.

RESULTS

Patient Characteristics

Table 1 shows the baseline characteristics of the 13 patients before PRRT exposure. As noted, this cohort had low exposure to prior chemotherapy and radiotherapy (7.7% and 23.1%, respectively).

Commonly used therapies after baseline sample collection included temozolomide (23%), everolimus (38%), capecitabine (8%), and nintedanib (8%). Patients received a median of four PRRT cycles (range 2-4 cycles). Seventy-five percent (9 of 12) of the cohort developed cytopenias after PRRT exposure. Persistent cytopenias (defined as cytopenia that persisted at least 3 months beyond last PRRT exposure, with no chemotherapy or targeted therapy exposure during those 3 months) were observed in 54% of patients (Table 2). The median laboratory follow-up time after last PRRT dose was 7 months (range 0-23 months). Three patients had dose interruptions or cancelations because of various reasons. One patient's last dose of PRRT was canceled because of persistent thrombocytopenia (patient 3). Cycle 3 was delayed for patient 8 because of gastroenterologic side effects, and the patient never received the remaining two cycles because of sudden death. Patient 11 received only two cycles because of lack of clinical benefit. The median time for development of cytopenias from first exposure to PRRT was 3 months. Table 2 demonstrates the development of neutropenia, thrombocytopenia, and anemia determined from the CBC obtained at baseline (pre-PRRT exposure), 6 months after PRRT exposure, at the last follow-up after PRRT exposure (graded using the NCI CTCAE v5.0 hematologic toxicity scale) and other details regarding PRRT exposure.

CH Prevalence Before PRRT Exposure

The overall prevalence of CH in this cohort with minimal prior chemotherapy and radiotherapy exposure was 62% (8 of 13). *TET2* was the most commonly mutated gene, followed by mutations in *ASXL1*, *CHEK2*, *PPM1D*, and *TP53*. *DNMT3A* mutations, often considered an advanced age-related clonal event,[21] were not common in this cohort, perhaps because of the younger age of this group. Figure 1 shows the mutational landscape of myeloid neoplasm–associated genes in the pre-PRRT exposure cohort, along with clinical and laboratory parameters. Table 3 shows gene mutations identified, types of mutations, VAFs, and associated nucleotide and protein changes as a result of these mutations (the detailed call set report is provided in the Data Supplement). Analysis of age and cytopenias in the CH+ group versus CH– group (Data Supplement) showed significant association of age with CH ($P = .047$) and a trend toward significant association between absolute neutrophil count and CH, $P = .093$.

Serial Sampling Samples After PRRT Exposure

Serial sampling samples after PRRT exposure were obtained for four patients in the baseline cohort (Fig 2). Clinical features and clinical course of these four patients along with clonal trajectory of CH genes during therapy are described in detail below.

Case 1: Patient 2

A 59-year-old individual with metastatic intermediate-grade small bowel NET with liver and bony metastases presented initially with carcinoid syndrome 4 years ago. Before initiating treatment with a somatostatin analog, they had initial blood sampling. Subsequent therapies included SIR-spheres to the right and left hepatic arteries, palliative radiation to the thoracic spine at T4-T7, treatment on a clinical trial with nintedanib, everolimus, and temozolomide, and subsequently four cycles of PRRT in 2018. The most recent treatment was hepatic arterial embolization. This patient did not develop cytopenias during or after PRRT treatment.

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TABLE 2. Development of Neutropenia, Thrombocytopenia, and Anemia Determined From the CBC Obtained at Baseline (pre-PRRT exposure), 6 Months After PRRT Exposure, and at the Last Follow-Up After PRRT Exposure (graded using the NCI CTCAE v5.0 hematologic toxicity scale)

Patient ID	Presence of Cytopenias on Baseline Hemogram	Cytopenias 6 Months After PRRT Exposure	Cytopenias at the Time of Last Follow-Up After Last PRRT Exposure	Persistent Cytopenias (yes/no) After PRRT	Total No. of PRRT Cycles	Time (in months) From Last PRRT Dose to Last Laboratory Follow-Up	Other Chemotherapeutic Exposure Within or After 6 Months of PRRT	Baseline CH (yes *v* no); If Yes, Mutations Noted in Table 3
1	No	Grade 1 anemia	Grade 1 anemia	Yes	4	6	No	No
2	Grade 1 anemia and thrombocytopenia	No	Grade 1 anemia	Yes	4	23	No	Yes
3	Grade 1 anemia	NA	Grade 2 anemia and thrombocytopenia	Yes	3	5	No	Yes
4	No	Grade 1 thrombocytopenia	Grade 1 thrombocytopenia	Yes	4	18	No	Yes
5	Grade 1 anemia	Grade 1 anemia and thrombocytopenia	Grade 2 anemia and recovered thrombocytopenia	Yes	4	22	Yes (6+ months after PRRT)	No
6	No	NA	No	No	4	7	No	Yes
7	No	NA	No	No	4	10	No	Yes
8	No	NA	No	No	1	2	No	Yes
9	Grade 1 anemia	NA	Grade 1 trilineage cytopenias after cycle 3	No	3	0	NA (lost to follow-up after cycle 3)	No
10	Grade 2 anemia	Grade 1 anemia and thrombocytopenia	Grade 1 trilineage cytopenias	Yes	4	21	Yes (everolimus 5+ months after PRRT)	Yes
11	No	Grade 1 anemia and thrombocytopenia	Grade 2 anemia and neutropenia approaching Grade 1	Yes	2	10	Yes (CapeTem[a] 7+ months after PRRT)	Yes
12	No	Grade 3 neutropenia and Grade 2 anemia and thrombocytopenia	NA	Unknown	4	6	No	No
13	No	NA	Grade 1 trilineage cytopenia	NA	4	2	Unknown (chose care closer to home)	No

Abbreviations: CH, clonal hematopoiesis; NA, not available; PRRT, peptide receptor radionuclide therapy.
[a]Capecitabine plus temozolomide.

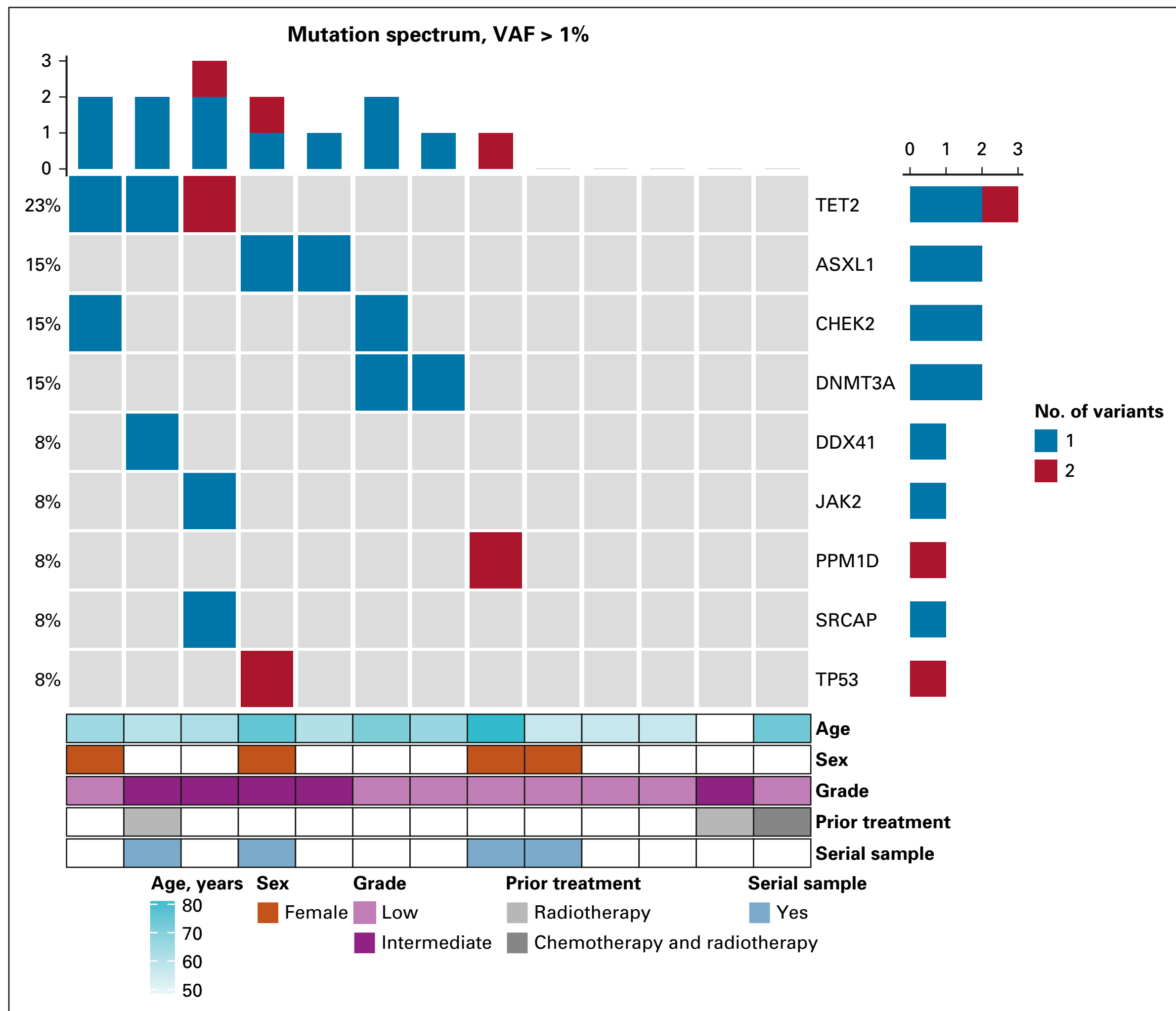

FIG 1. Oncoprint for patients with neuroendocrine tumors along with clinical and laboratory parameters. VAF, variant allele frequency.

Clonal trajectory. In 2015, at the time of initial sample collection, this patient had mutational events involving the *TET2* and *DDX41* genes with a VAF of about 2.5%. In 2018, after exposure to PRRT, both clones diminished in size to undetectable or very low levels. Subsequent sampling from 2020 demonstrated re-emergence of the *TET2*-mutant clone.

Case 2: Patient 4

A 77-year-old individual with low-grade NET of the small bowel presented with liver, lymph nodes, and bony metastasis 2 years ago. A baseline peripheral blood sample for the biorepository was collected before treatment with a somatostatin analog. The patient was subsequently treated for progressive disease with four cycles of PRRT. There were no cytopenias before PRRT initiation; however, during and after PRRT, the patient developed mild thrombocytopenia (range, 100-130 × 10^9/L), which persists to date.

Clonal trajectory. At the time of initial sample collection, this patient had mutations in *TP53* (two variants, VAFs 1.78% and 1.67%) and *ASXL1* at a VAF of 1% (Table 3). After exposure to PRRT, the two *TP53* variants expanded to VAFs of 6.33% and 6.12%, respectively, whereas the mutant *ASXL1* clone diminished in size to a level undetectable by our assay. A mutation in *CHEK2* was detected after therapy that has persisted. During and after exposure to PRRT, this patient developed new thrombocytopenia (118 × 10^9/L) that has persisted long term. These findings suggest that the mutant *TP53* and *CHEK2* clones (and not *ASXL1*) were selectively expanded after exposure to PRRT, with ensuing cytopenia related to expansion of primarily *TP53*-mutant population.[22,23]

Case 3: Patient 5

A 74-year-old individual with stage IV low-grade pancreatic-NET with widespread metastases diagnosed initially 15 years ago presented with a pancreatic mass and synchronous liver metastasis. Treatment was initiated with a somatostatin analog and interferon before initial peripheral blood sampling for our biorepository. This patient had a

TABLE 3. Patients With Mutational Events in Specific Genes, Associated Nucleotide and Protein Change, and Baseline and Serial Sample VAFs %

Patient ID	Gene	Nucleotide Change	Protein Change	Baseline VAF (%)	Post-Therapy VAF at Timepoint 1 (%)	Post-Therapy VAF at Timepoint 2 (%)
2*	DDX41	c.878G>A	p.Arg293His	2.65	0.22	0.19
2*	TET2	c.3732_3733del	p.Tyr1245Leufs*22	2.6	ND	0.66
3	CHEK2	c.1037+1G>A	NA	8.73		
3	TET2	c.4618C>T	p.Gln1540*	8.74		
4*	ASXL1	c.1954G>A	p.Gly652Ser	1.04	ND	ND
4*	CHEK2	c.814G>A	p.Gly272Ser	ND	1	0.63
4*	TP53	c.842A>T	p.Asp281Val	1.78	5.36	6.33
4*	TP53	c.586C>G	p.Arg196Gly	1.67	5.6	6.12
5*	ATM	c.6486C>G	p.Ser2162Arg	ND	2	1.29
5*	PPM1D	c.1579G>T	p.Glu527*	ND	7.82	18.78
6	JAK2	c.1849G>T	p.Val617Phe	2.18		
6	SRCAP	c.5750_5751insA	p.Tyr1918Valfs*4	11.08		
6	TET2	c.3812dup	p.Cys1271Trpfs*29	10.51		
6	TET2	c.4527dup	p.Gln1510Thrfs*68	1.35		
7	CHEK2	c.671G>A	p.Arg224His	1.76		
7	DNMT3A	c.1907T>G	p.Val636Gly	2.45		
8	ASXL1	c.1210C>T	p.Arg404*	11.93		
10*	CHEK2	c.460G>T	p.Asp154Tyr	0.82	1.53	1.42
10*	PPM1D	c.1440dup	p.Ala481Serfs*8	ND	3.69	3.57
10*	PPM1D	c.1508C>A	p.Ser503*	1.65	2.39	3.26
10*	PPM1D	c.1654C>T	p.Arg552*	0.57	1.4	1.51
10*	PPM1D	c.1709C>G	p.Ser570*	1.24	2.52	2.68
11	DNMT3A	c.1481G>A	p.Cys494Tyr	3.04		

NOTE. Patient IDs with * denote patients who had serial samples available for analysis. The detailed call set is provided in the Data Supplement.
Abbreviations: NA, not available; ND, not detected; VAF, variant allele frequency.

long disease course marked by treatments with temozolomide and capecitabine followed by surgical resection with stable disease for a few years, after which everolimus, an mTOR inhibitor, was used for 6 months. At further disease progression, a blood sample for the biorepository was obtained before four cycles of PRRT in 2018. Before PRRT, the patient had normal blood cell counts and an unremarkable differential. At the end of treatment with PRRT, the hemoglobin and platelet counts were decreased to 10.9 g/dL and 94 × 10^9/L, respectively. Another sample was obtained in follow-up at which point the patient demonstrated evidence of progressive cytopenias.

Clonal trajectory. In 2006, at the time of initial sample collection, the patient had no detectable clonal events. In 2018, after exposure to multiple other therapies, new clonal events were detected with the appearance of a *PPM1D* mutation with a VAF of about 7.5%. During and after exposure to PRRT therapy, the patient was noted to develop cytopenias. As seen in Figure 2A, cytopenias were associated with a steep rise in the *PPM1D*-mutant VAF to 20%. These findings suggest that the worsening cytopenias after PRRT exposure arose because of repopulation of the hematopoietic compartment with less functional mutant *PPM1D* stem cells.

Case 4: Patient 10

An 82-year-old individual with low-grade metastatic NET with synchronous primaries in the midgut and pancreas presented with metastases to liver and lymph nodes 6 years ago. After initial surgery for a small bowel obstruction, she was treated with a somatostatin analog, chemoembolization of multiple right liver lobe lesions, and subsequently PRRT. This patient had baseline anemia (hemoglobin 10 g/dL), with no other abnormalities of the hemogram. During and after PRRT treatment, she had an ongoing anemia with the development of mild leukopenia (3-4 × 10^9/L) and thrombocytopenia (80-120 × 10^9/L range).

Clonal trajectory. Samples drawn shortly after completion of PRRT demonstrated distinct mutational events in *PPM1D* gene at low initial VAFs. Six and 12 months after exposure to PRRT, initially identified *PPM1D* clones had expanded to higher VAFs and in addition, few additional *PPM1D* variants were also identified (Fig 2A). Mutation in *CHEK2* showed clonal expansion as well. These findings

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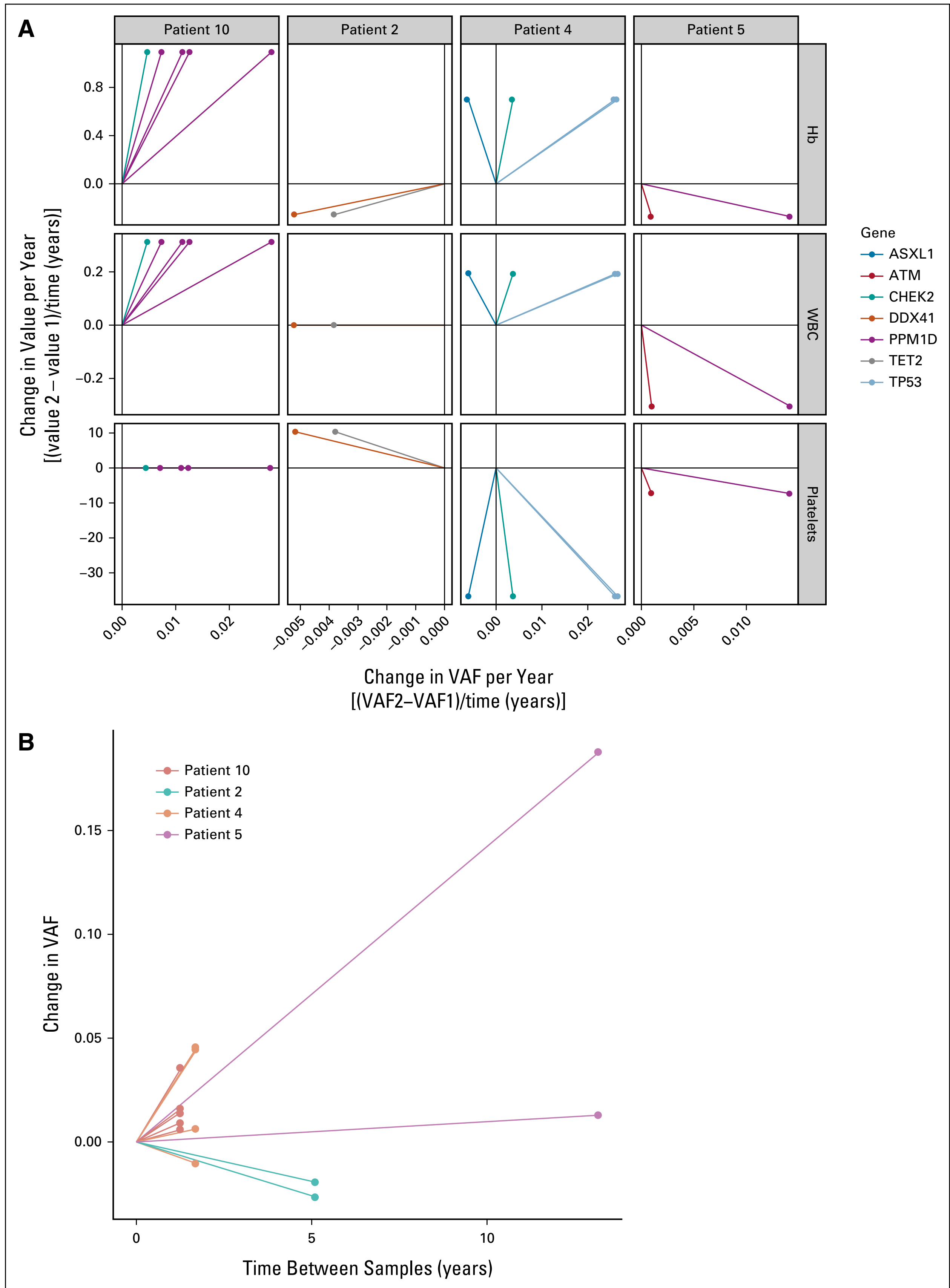

FIG 2. The clonal trajectories of mutation events. Serial analysis of patients exposed to PRRT. DNA specimens from four patient cases (patients 10, 2, 4, and 5) exposed to PRRT and serial samples after PRRT exposure were analyzed at serial time points. (A) Line graphs indicate change in VAF per year and change in blood parameters as a result of change in VAF, eg, in patient 5, with increasing (continued on following page)

FIG 2. (Continued) PPM1D clone (in purple), there was a notable change in HgB, WBC, and platelet count (all decreased; the *y*-axis in (A) depicts changes in blood parameters over time). Different colors indicate different gene mutations. (B) Different colors indicate different patients and depict change in VAFs over time. Seen in example of patient 5 (purple), dramatic changes in VAF were noted over time. In the Data Supplement, additional associated changes in blood parameters are depicted, which highlight the considerable drop in all three blood parameters, particularly patient 5. HgB, hemoglobin; PRRT, peptide receptor radionuclide therapy; VAF, variant allele frequency. PRRT exposure leads to mutant TP53, CHEK2, and PPM1D expansion in blood, contributing to prolonged cytopenias.

reinforce the suggestions that *PPM1D* and *CHEK2* clones (both DDR pathway genes) expand with PRRT exposure and can populate the hematopoietic compartment contributing to the development of cytopenias.

In summary, we identified recurrent mutations in *TP53*, *PPM1D*, and *CHEK2* in three of the above four cases with NETs irrespective of prior exposure to genotoxic stressors, ie, chemotherapy and radiation. These mutations appear to opportunistically populate the hematopoietic compartment in response to the stress induced by PRRT, particularly in older individuals.

DISCUSSION

The goal of cancer therapies is to create an environment conducive to favor cancer cell death. The extrinsic perturbations by cancer therapies affect HSCs (mutated and wildtype) and create a bone marrow microenvironment suited to select for mutant clones that are able to survive the stress event. Cell-intrinsic factors, perhaps acquisition of fitter somatic mutations, increase resistance to cell death from external stressors (in this case, PRRT) and allow for the emergence of clonally dominant mutant HSCs, thereby contributing to the fate of the individual HSCs. This clonal dominance and expansion in aging individuals underscore the findings that in addition to cell-intrinsic properties of mutant clones, cell-extrinsic factors such as an aging marrow environment may lead to alterations in hematopoiesis.

Here, to our knowledge, for the first time, we report a high baseline prevalence of CH in patients with NETs, in the setting of minimal prior chemotherapy and radiotherapy exposure. Furthermore, we demonstrate the clonal expansion of hematopoietic cells with *PPM1D* and *TP53* mutations in response to radionuclide therapy, thereby corroborating on the growing evidence that pretreatment somatic genetic alterations may contribute to malignant hematopoiesis in a more dynamic way (Fig 3) rather than the previously thought linear phenomenon (Data Supplement). After this cytotoxic therapy, we observed expansion of clones carrying mutations in genes involved in the DDR. Many healthy individuals have low-VAF mutant DDR clones that can remain stable for many years without clinical consequences. Additional selective pressures or external perturbations are therefore likely essential for progression to t-MN as a majority of individuals with CH do not develop an overt hematologic malignancy.[24]

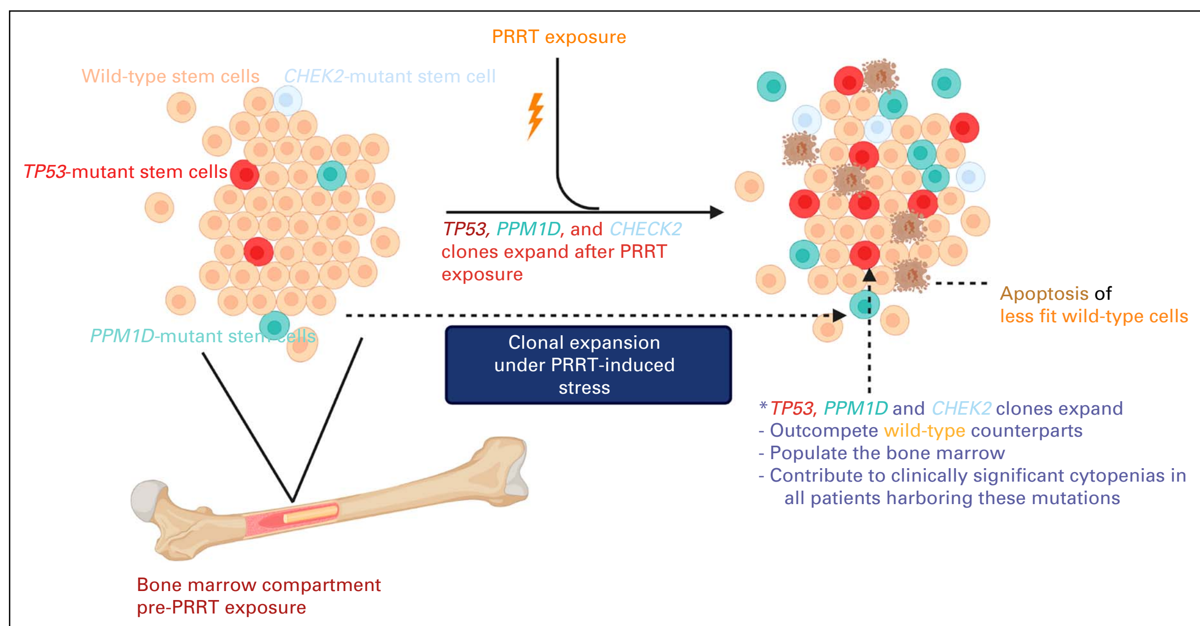

FIG 3. Dynamic model of clonal evolution and PRRT-induced carcinogenesis. The image was generated using BioRender. PRRT, peptide receptor radionuclide therapy.

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Our analysis suggests that cell-extrinsic perturbation conferred by PRRT selects for HSCs harboring DDR mutations such as *PPM1D* and *TP53*, because of their ability to preferentially withstand the stress induced by PRRT. It is known that detection of CH after chemotherapy or radiotherapy for a nonmyeloid neoplasm can increase the risk of tMNs, especially if the emergent clones bear a *TP53* or *PPM1D* mutation.[5,25,26] It is, therefore, conceivable that these surviving HSCs preferentially repopulate the hematopoietic compartment, leading to dysfunctional hematopoiesis and ultimately an increased risk for transformation to t-MN (although we did not report the latter in our cohort because of smaller sample size and shorter follow-up). Previous genomic analyses of samples from patients with t-MNs and analysis of samples years before the overt development of t-MN have identified mutations in *TP53*.[23,27] In addition, studies have reported an increased incidence of CH in cancer cohorts by virtue of previous exposure to genotoxic stressors.[5,26,28,29] The presence of therapy-related CH has been implicated as a mean of predicting an increased risk of hematologic malignances and inferior survival.[28,29] Growing knowledge suggests that t-MNs are often driven by expansion of pre-existing mutant clones in response to chemotherapy.[5,26,30] These studies in addition to our findings here provide robust evidence that specific mutant clones can be selected for various cancer therapies.

PRRT remains an excellent option for treatment of patients with progressive NETs, not only prolonging survival but also improving quality of life. These patients unfortunately do seem to be at particular risk for the development of t-MN.[31] As patients treated with PRRT have extended survival, the risk of clonal expansion from PRRT-induced selective pressures will become evident as overt development of t-MNs. It is therefore imperative to develop screening or surveillance strategies for such individuals to evaluate and mitigate risks of harmful clonal expansion and subsequent t-MN development. Devising chemoprevention research strategies to mitigate the lethal adverse consequences of promising life-prolonging therapies is the next key step in therapeutic development.

Limitations to our study include the small sample size and lack of serial samples on all 13 patients. Although a smaller sample, acquisition of baseline and serial samples with adequate follow-up was feasible because of our center's expertise in treating NETs and early adoption of PRRT since its approval. In addition, certain mutations seen in our analysis, such as those in the genes DDX*41* or *CHEK2*, could be germline mutational events or related to the primary NET. Unfortunately, their origin cannot be clearly delineated in our patients because of a lack of paired tumor and germline sample sequencing. However, the presence of these mutations at lower VAFs and appearance of *CHEK2* mutation only after exposure to PRRT would argue against that.

In conclusion, we have identified baseline DDR pathway gene defects in patients with NETs that are known to increase the risk for development of future hematologic malignancy. *TP53*-, *CHEK2*-, and *PPM1D*-mutant clonal expansion was temporally associated with exposure to PRRT, suggestive of positive selection of these clones in response to PRRT. These mutations likely provide a survival advantage to HSCs, which harbor them and preferentially repopulate the hematopoietic compartment. An improved understanding of the impact of baseline CH and expansion in response to genotoxic stressors will inform future studies to evaluate importance of screening, surveillance, and early detection to decrease the risk of t-MN in patients undergoing cancer treatments. There is a pressing need for recommendations to guide our management of these patients undergoing PRRT, who harbor mutations in DDR genes.

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