Prognostic relevance of clonal hematopoiesis in myeloid neoplastic transformation in patients with follicular lymphoma treated with radioimmunotherapy

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Abstract

While novel radioisotope therapies continue to advance cancer care, reports of therapy-related myeloid neoplasms (t-MN) have generated concern. The prevalence and role of clonal hematopoiesis (CH) in this process remain to be defined. We hypothesized that: (i) CH is prevalent in relapsed follicular lymphoma and is associated with t-MN transformation, and (ii) radiation in the form of radioimmunotherapy (RIT) plays a role in clonal progression. In this retrospective cohort study, we evaluated the prevalence and prognostic impact of CH on clinical outcomes in 58 heavily pre-treated follicular lymphoma patients who received RIT. Patients had been given a median of four lines of therapy before RIT. The prevalence of CH prior to RIT was 46%, while it was 67% (P=0.15) during the course of RIT and subsequent therapies in the paired samples. Fourteen (24%) patients developed t-MN. Patients with t-MN had a higher variant allele fraction (38% *vs*. 15%; P=0.02) and clonal complexity (P=0.03) than those without. The spectrum of CH differed from that in age-related CH, with a high prevalence of DNA damage repair and response pathway mutations, absence of spliceosome mutations, and a paucity of signaling mutations. While there were no clear clinical associations between RIT and t-MN, or overall survival, patients with t-MN had a higher mutant clonal burden, along with extensive chromosomal abnormalities (median survival, afer t-MN diagnosis, 0.9 months). The baseline prevalence of CH was high, with an increase in prevalence on exposure to RIT and subsequent therapies. The high rates of t-MN with marked clonal complexities and extensive chromosomal damage underscore the importance of better identifying and studying genotoxic stressors accentuated by therapeutic modalities.

Introduction

Clonal hematopoiesis (CH) is defined by the acquisition and subsequent expansion of somatic DNA variants, including somatic mutations and copy number alterations in hematopoietic stem and progenitor cells (HSPC).[1-4] When CH mutations occur in leukemia-associated genes, with a variant allele fraction (VAF) ≥2% in individuals without a diagnosed hematologic disorder, the condition is termed CH of indeterminant potential. While CH is ubiquitous with aging, context-specific development of CH is heterogeneous and dependent on clonal selection pressures. CH mutations have differential rates of fitness and stability and expand based on clonal selection pressures.[5] Retrospective series have demonstrated the role of CH in therapy-related myeloid neoplasms (t-MN) and have documented associations with inferior overall survival (OS) in the setting of prior cytotoxic therapies.[6]

CH of indeterminate potential is considered the first step in a multi-hit model for the development of t-MN.[7] DNA-damage-inducing therapies such as chemotherapy or radiation used in primary cancers can lead to collateral alterations in HSPC, resulting in clonal populations with enhanced fitness and propagation potential,[6,8,9] particularly when they involve DNA damage response and repair (DDR) genes, such as *TP53* and *PPM1D*. [6,8,10-12] Although the impact of CH on t-MN has been extensively investigated in settings of chemoradiation therapy,[6,8,9] autologous stem cell transplantation (ASCT),[13-15] and chimeric antigen receptor (CAR) T-cell therapy,[16,17] the prevalence and impact of CH in the context of systemic radioisotope therapy

remains to be further clarified.[18] The need for these data is particularly relevant, given the increasing use of radioisotopes.[19-22] A few examples include lutetium dotatate for neuroendocrine tumors and metastatic prostate cancer and radioimmunotherapy (RIT) ^{90}Y ibritumomab tiuxetan (^{90}YIT, Acrotech Biopharma) for relapsed. low-grade and follicular non-Hodgkin lymphoma (FL).

It has been reported that the incidence of t-MN is between 5-10% after chemotherapy with ASCT and 2-20% in the context of radioisotope therapy combined with cytotoxic chemotherapy in non-Hodgkin lymphoma. [23-29] Given the evidence of a higher risk of t-MN in patients with CH of indeterminate potential, we hypothesized that exposure to β radiation would enhance the prevalence and growth of CH in HSPC, resulting in a higher prevalence of t-MN, with CH negatively impacting OS. The group of patients that we studied was unique because all patients had normal bone marrow morphology for t-MN and normal cytogenetics prior to RIT and had a very mature follow-up duration, providing valuable data on the role of clonal progression in the context of cancer-directed therapies.

Methods

Cohort of patients

After institutional review board approval, we identified 58 patients with relapsed FL treated with RIT who had cryopreserved peripheral blood DNA for CH assessment at the Mayo Clinic. Of note, all samples were banked in the relapsed or refractory disease phase. Among these, 24 (group 1) had paired samples before and after RIT exposure; 22 (group 2) had one or more samples after RIT exposure, and 13 (group 3) had samples only before RIT exposure.

Given our hypothesis that RIT could cause clonal expansion secondary to its genotoxic effects, we compared clonal VAF and complexity in group 1 (n=24 patients with paired samples); we also identified 13 patients in group 2 who had two serial cryopreserved samples after RIT exposure (group 2a) and five patients in group 3 who had two serial samples

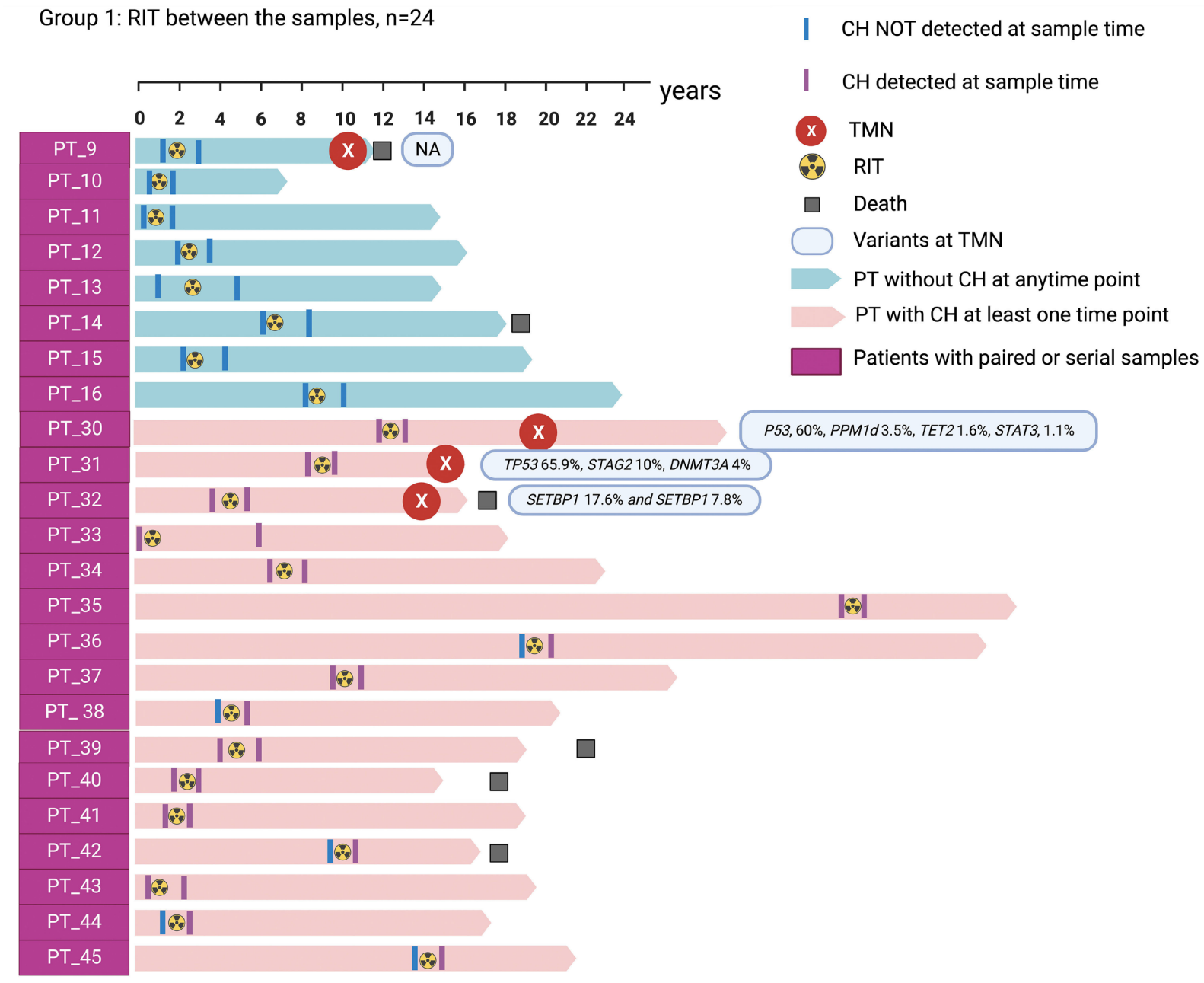

Figure 1. Swimmer plot for group 1 patients (patients with paired samples, with radioimmunotherapy administered between samples). PT_9, 30, 31, and 32 received a total of 6, 6, 3, and 5 different therapies, respectively. RIT: radioisotope therapy; PT: patient; NA: not available; CH: clonal hematopoiesis; TMN: treatment-related myeloid malignancy.

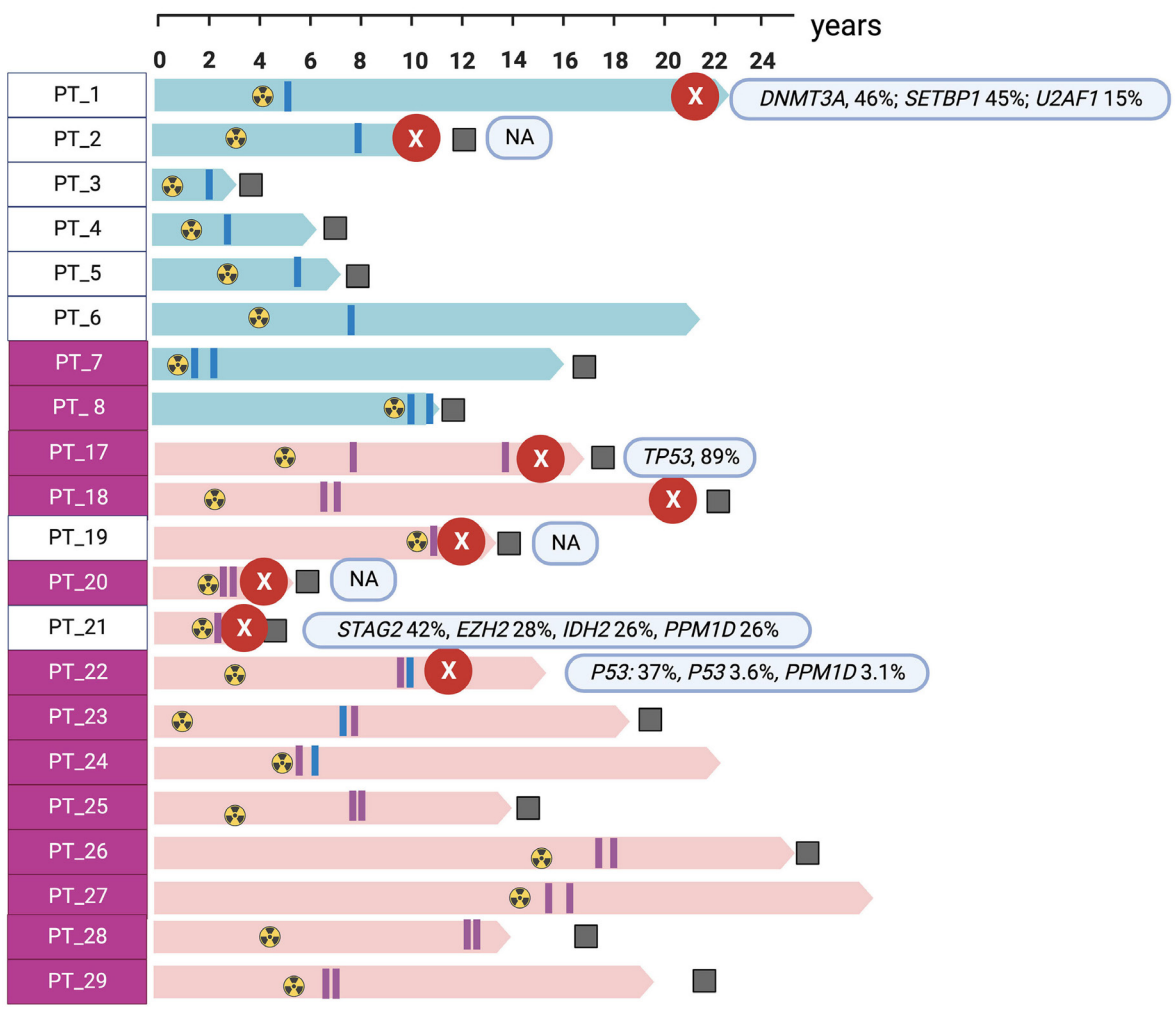

Figure 2. Swimmer plot for group 2 patients (patients with one or more samples taken after radioimmunotherapy). Symbols and abbreviations as in Figure 1.

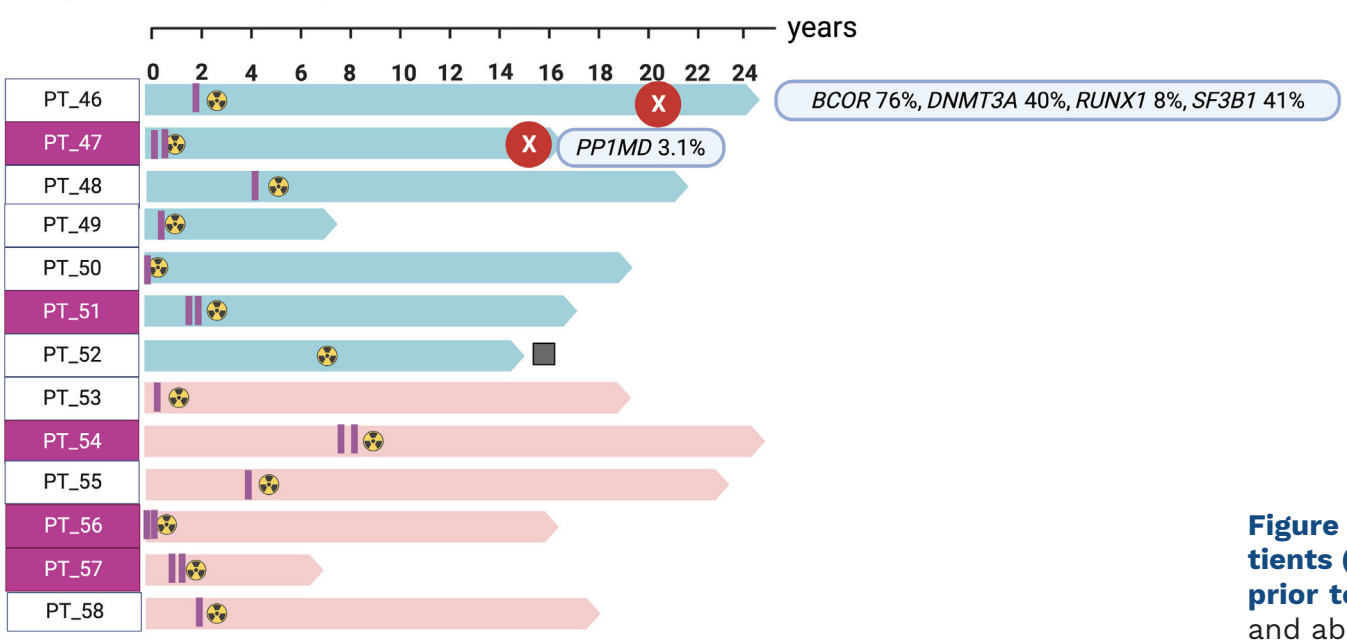

Figure 3. Swimmer plot for group 3 patients (patients with samples taken only prior to radioimmunotherapy). Symbols and abbreviations as in Figure 1.

prior to RIT exposure (group 3a), and we compared clonal evolution in subgroups 2a and 3a (Figures 1-3).

Clinical data, including prior therapy regimens, were retrospectively abstracted from clinical records. Outcomes of interest included the spectrum and diversity of CH, clonal dynamics, the development of t-MN, and OS.

Detection of clonal hematopoiesis

DNA was extracted from peripheral blood mononuclear cells and subjected to a customized, targeted next-generation sequencing assay, as previously described[30] (*Online Supplementary Material, Online Supplementary Table S1*). A VAF ≥2% was considered as CH. Cryopreserved bone marrow DNA, collected at the time of t-MN diagnosis, was also available for eight patients and was subjected to sequencing with the same panel. Based on the recent description of the involvement of genes in lymphoproliferative disorders, we considered *ARID1A, ARID1B, CARD11, CD79B, CREBBP, EP300, EZH2* (gain-of-function variants only), *HIST1H1C, HIST1H1D, KMT2D, NOTCH1, STAT6*, and *TNFRSF14* mutations

as lymphoid CH (L-CH) and the rest as myeloid CH (M-CH), including loss-of-function *EZH2* mutations.[31,32] We classified our observations based on the following categories: L-CH, M-CH, both L-CH and M-CH (LM-CH), DDR mutations and mutations in *DNMT3A, TET2,* and *ASXL1* (the so-called DTA genes). For patients with multiple pathogenic variants, we used the maximum VAF for VAF comparisons. Mutation patterns were analyzed using ProteinPaint.[33]

Statistical analysis

We compared clinical characteristics, mutation patterns, VAF, and outcomes of patients with and without t-MN. Continuous variables are presented as a median with interquartile range (IQR) or mean with standard deviation, and categorical variables as frequency (percentage). Differences in the distribution of nonparametric continuous variables between categories were compared using the Wilcoxon matched pairs signed rank test for paired samples. Categorical variables were compared using the χ^2 or Fisher exact test. OS was measured from the date of RIT exposure to the date of death from any cause; data were censored at the time patients were last known to be alive. The univariable logistic regression model was used to evaluate potential risk factors for outcomes. The median point estimate and 95% confidence interval (95% CI) for follow-up time, t-MN, and OS were estimated using the Kaplan-Meier method. All *P* values were two-sided tests. All statistical calculations were carried out using R version 4.0.1. Considering the hypothesis-generating character of the study, no multiple testing correction was implemented, and the reported *P* values should be interpreted as exploratory.

Results

Patients' characteristics and clinical outcomes

Fifty-eight patients with relapsed FL were included. Their median age was 48.5 years (range, 28-79) and 23 (40%) were females. The median follow-up duration was 17.8 years (IQR, 15.7-21.9). At the last follow-up, 14 (24%) patients had developed t-MN, and 23 (40%) had died, including eight deaths from t-MN. Eleven (19%) patients had diffuse large B-cell lymphoma transformation and in this group, three (17.5%) developed t-MN. The median time from FL diagnosis to t-MN was 14.4 years (IQR, 11.3, 18.6), with the median latency from RIT to t-MN diagnosis being 8.8 years (IQR, 6.7-12.6) and the median OS after t-MN diagnosis being 0.9 years (IQR, 0.24-2.2). All 58 patients had significant exposures to chemo-immunotherapy or involved field radiation therapy with a median of four prior regimens (Table 1). There were no significant differences in the total number of therapies received between patients with and without CH (median 5 *vs*. 4; *P*=0.5). Twenty-eight patients (48%) had received purine/pyrimidine analogs, 23 (57%) DNA topoisomerase

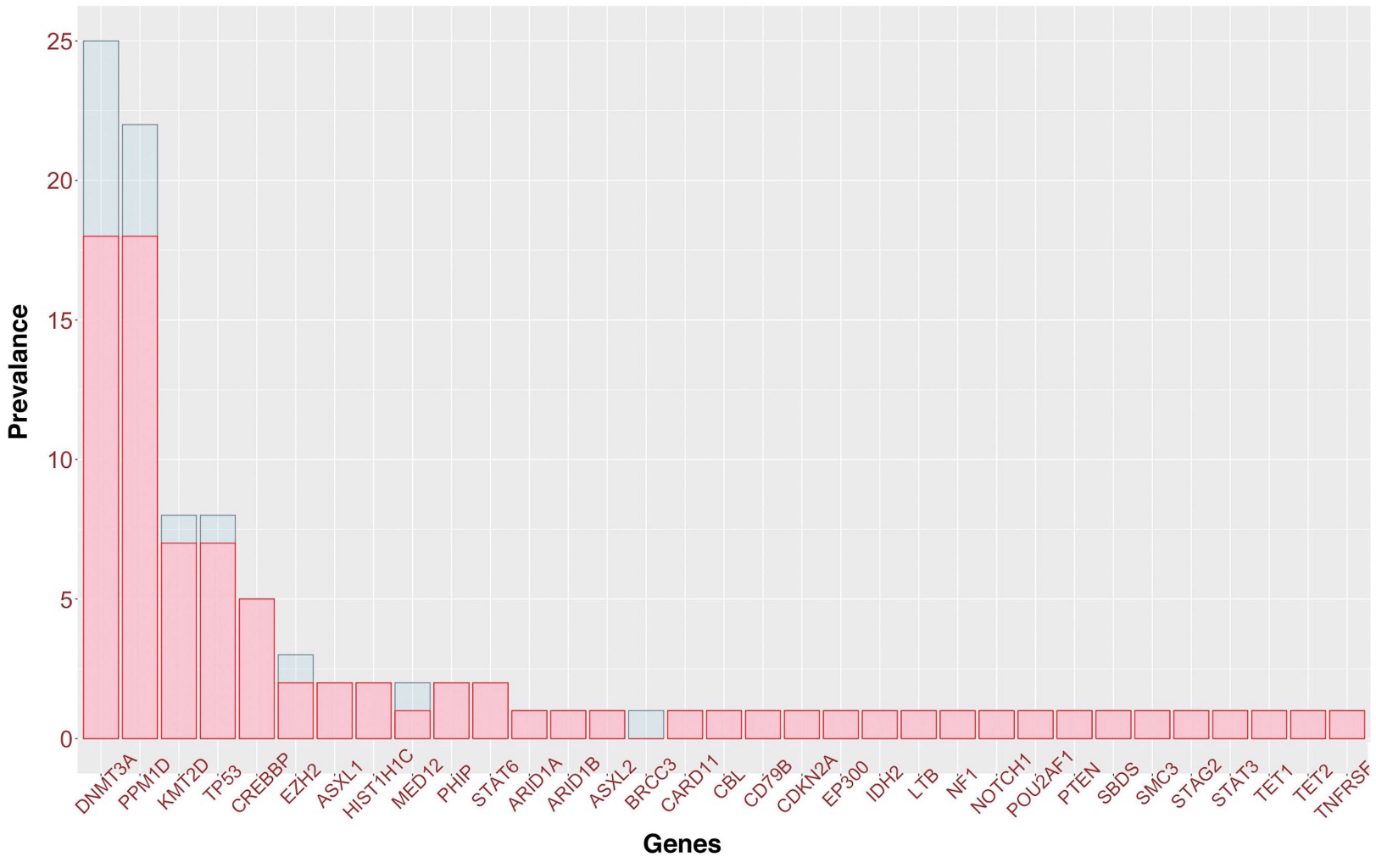

Figure 4. Mutation prevalence and co-mutation status. The light blue color indicates the number of patients with the specific mutation, and the light pink color indicates the number of patients with other mutations.

inhibitors, 52 (90%) alkylating agents, and 21 (36%) ionizing radiation therapy. In the 14 patients who developed t-MN, all had been previously exposed to chemotherapy, including 11 (79%) with exposure to alkylating agents and eight (57%) with exposure to topoisomerase inhibitors. In addition, eight (57%) had undergone prior conventional radiation, including six (43%) exposed to alkylating agents/topoisomerase inhibitors and radiation therapy.

Prevalence and mutational spectrum of clonal hematopoiesis

Immediately prior to RIT administration, all 58 patients had a bone marrow biopsy in which no morphological atypia was found, and all patients had a normal karyotype. Despite these normal findings, the prevalence of CH at any timepoint was 60% (35/58), with 97 somatic variants identified. The most frequent mutations were *DNMT3A* (25%), followed by *PPM1D* (23%), *KMT2D* (8%), and *TP53* (8%) (Figure 4, *Online Supplementary Figure S1*). Among patients with CH, 12 (34%) had one mutation, and 23 (66%) had two or more mutations. The median VAF was 19% (IQR, 4-39%). The co-mutation status and VAF are shown in Figure 4 and *Online Supplementary Figure S2*. In the entire cohort, 21 (60%) patients had M-CH only, two (6%) had L-CH only, and 12 (34%) had LM-CH. The pathogenic variants for each patient are listed in *Online Supplementary Table S2*. The oncoplot for the entire cohort is shown in Figure 5. The common mutation patterns were missense, followed by nonsense and frameshift mutations.

Clonal expansion after radioimmunotherapy

In patients with paired samples (n=24; group 1), the median time interval between the samples was 1 year. The prevalence of CH before and after RIT exposure was 46% *versus* 67% (*P*=0.15) and the prevalence of M-CH was 42% *versus* 61% (*P*=0.19). There were 17 and 33 variants identified in the pre- and post-exposure samples, respectively, with *DNMT3A* being the most frequent (47% and 30%), followed by *PPM1D* (12% and 21%). Five patients without CH prior to RIT had M-CH in the post-RIT samples. (*Online Supplementary Figure S2*: PT_36, PT_42, and PT_44 with *DNMT3A* mutations and PT_38 with mutations in *DNMT3A* and *PPM1D*, and PT_45 with a *MED12* mutation). The median VAF was not significantly different between the paired

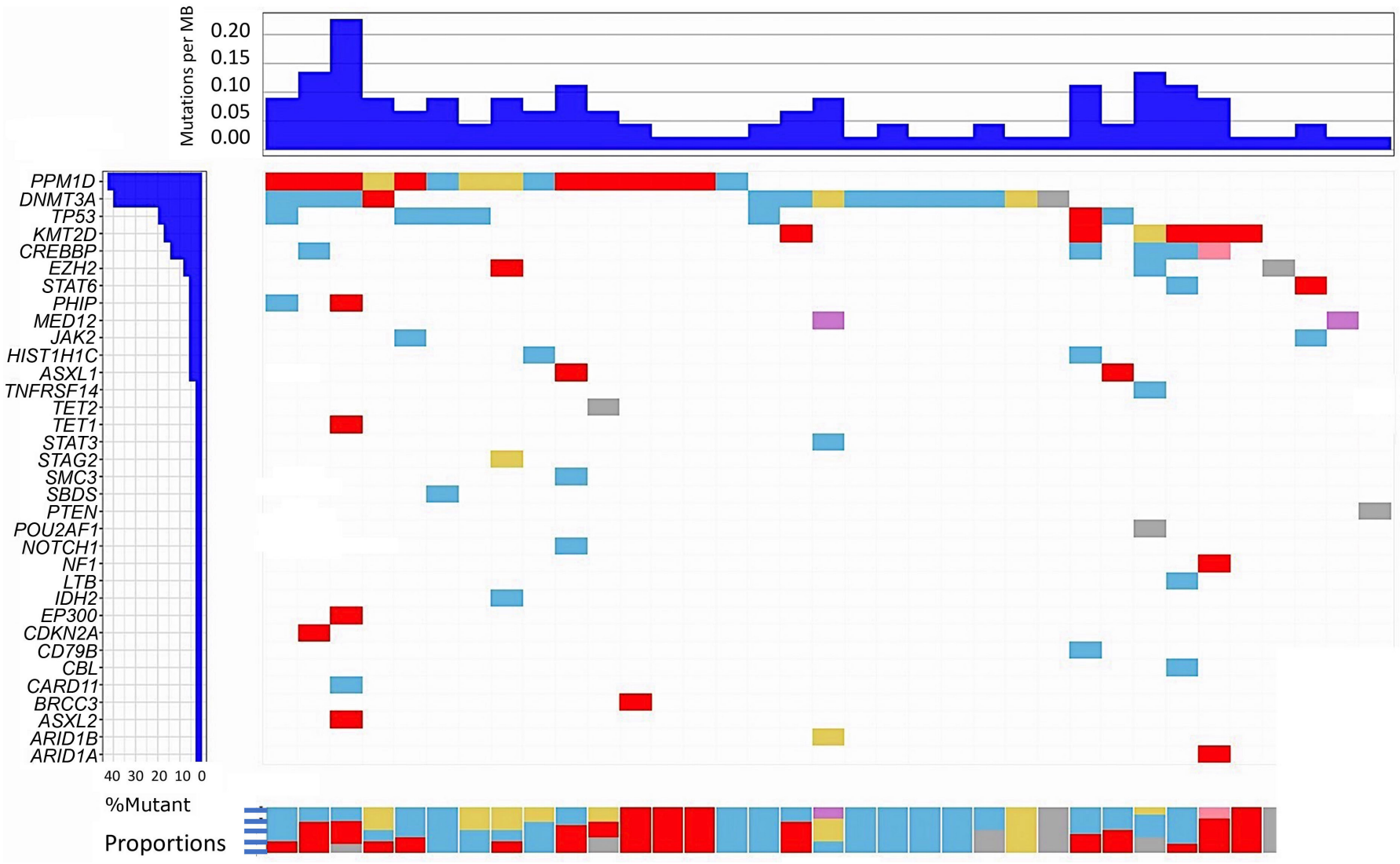

Figure 5. Co-mutation plot showing mutations present in 35 patients with somatic mutation(s). Each column represents a single patient. The top row denotes the translational effect and mutations per megabase. The bar graph on the left designates the prevalence of mutations (count for once regardless of the prevalence/patient). The mutation subtypes are represented by colors, red indicates nonsense, light-blue indicates missense; yellow indicates frameshift; gray indicates splice effect; purple indicates duplication, pink indicates deletion. The bar at the bottom designates the proportion of the mutation subtypes for each patient. Proportions are from 0 (bottom) to 25%, 50%, 75%, and 100% (top). MB: megabase.

samples (19.1% *vs*. 14.3%; *P*=0.27) (Figure 6). The median annual change in VAF for *DNMT3A* (n=15) was 2% (range, -8% to 14%), and that for DDR mutations (n=8) was 4.5% (range, 2% to 19%). Four patients developed t-MN in this group, three with existing CH before the diagnosis of t-MN and one without (Figure 1, *Online Supplementary Table S3*). *Online Supplementary Figures S3* and *S4* demonstrate the changes in clone VAF and complexity in this group.

In patients with two serial samples from subgroups 2a and 3a (Figures 2 and 3), there were no differences in the prevalence of CH or M-CH between the samples at the two timepoints in either group. In subgroup 2a the prevalence of CH at the two timepoints was 69% *versus* 75% (*P*=0.75) and the prevalence of M-CH was 62% *versus* 67% (*P*=0.79). In subgroup 3a, at both timepoints the prevalence of CH was 60% and that of M-CH was 40%. Furthermore, there were no significant difference in VAF between the two timepoint samples in subgroups 2a or 3a (VAF in subgroup 2a, 15.5% *vs*. 14.8%, *P*=0.73; VAF in subgroup 3a, 29% *vs*. 23.8%, *P*>0.99) (Figure 6). These results may be limited by the smaller sample size and shorter time interval between sampling points (time intervals were 1, 0.48, and 0.04 years for group 1, subgroup 2a, and subgroup 3a, respectively). These time intervals were statistically not different (*P*=0.32) and hence we were not able to draw additional conclusions on the impact of time differences on CH prevalence and CH-VAF changes.

CREBBP and *KMT2D* are FL-associated genes and were annotated as L-CH in our cohort: these clones decreased with lymphoma-directed therapy and could in fact represent circulating tumor cells that were inadvertently included in the peripeheral blood mononuclear cell fraction. In addition, all CH mutations became undetectable in one patient who underwent allogeneic stem cell transplantation (*Online Supplementary Figure S3;* PT_43).

Clinical association with therapy-related myeloid neoplasms

Fourteen (24%) patients developed t-MN (7 with acute myeloid leukemia, 7 with myelodysplastic syndromes). Their median age was 51.5 years (range, 33-79) and nine (64%) were males. There were no significant differences in baseline age (*P*=0.44), gender (*P*=0.72), FL stage (*P*=0.54), grade (*P*=0.89), Follicular Lymphoma International Prognostic Index (FLIPI) score (*P*=0.71), or the number of prior therapies (median, 4 in both groups) (*P*=0.66), including prior ASCT (*P*=0.18), between the t-MN and non-t-MN groups.

Among the 97 identified somatic variants, 27 (28%) were in the t-MN group and 70 (72%) in the non-t-MN group. The most frequent variants seen in the t-MN group included *PPM1D* (n=7, 28%), *DNMT3A* (n=3, 11%), and *TP53* (n=2, 8%), whereas the most frequent variants seen in the non-t-MN group included *DNMT3A* (n=16, 23%), *PPM1D* (n=15, 21%), *KMT2D* (n=7, 10%), and *TP53* (n=6, 9%). The prevalence of CH was not significantly different between the t-MN (64%) and non-t-MN (59%) groups (*P*=0.97). However, the t-MN group had a higher CH complexity (M-CH other than DDR and DTA mutations) (*P*=0.03) and a higher VAF compared to those without (38% *vs*. 15%; *P*=0.02). (Table 1). The presence of CH prior to RIT or after therapy was not associated with t-MN development (*P*=0.6 and *P*=0.73, respectively).

Detailed clinical, molecular, and cytogenetic information on patients with t-MN are provided in Table 2. Thirteen of the 14 evaluable patients with t-MN had an abnormal karyotype. These abnormal karyotypes comprised seven (50%) monosomal karyotypes, one (7%) complex karyotype, four chromosome 17 abnormalities, eight (57%) chromosome 5 abnormalities, and 11 (79%) chromosome 7 abnormalities. Twenty-one variants were found in the eight sequenced t-MN bone marrow samples, with 19 new pathogenic variants emerging that were not present in the pre-t-MN assessments (Table 2). Among these eight patients, four (50%) had no CH in the prior samples. Common mutations gained at t-MN transformation were *TP53* (n=6, 32%), with four patients having biallelic *TP53* inactivation. *PPM1D* and *SETBP1*

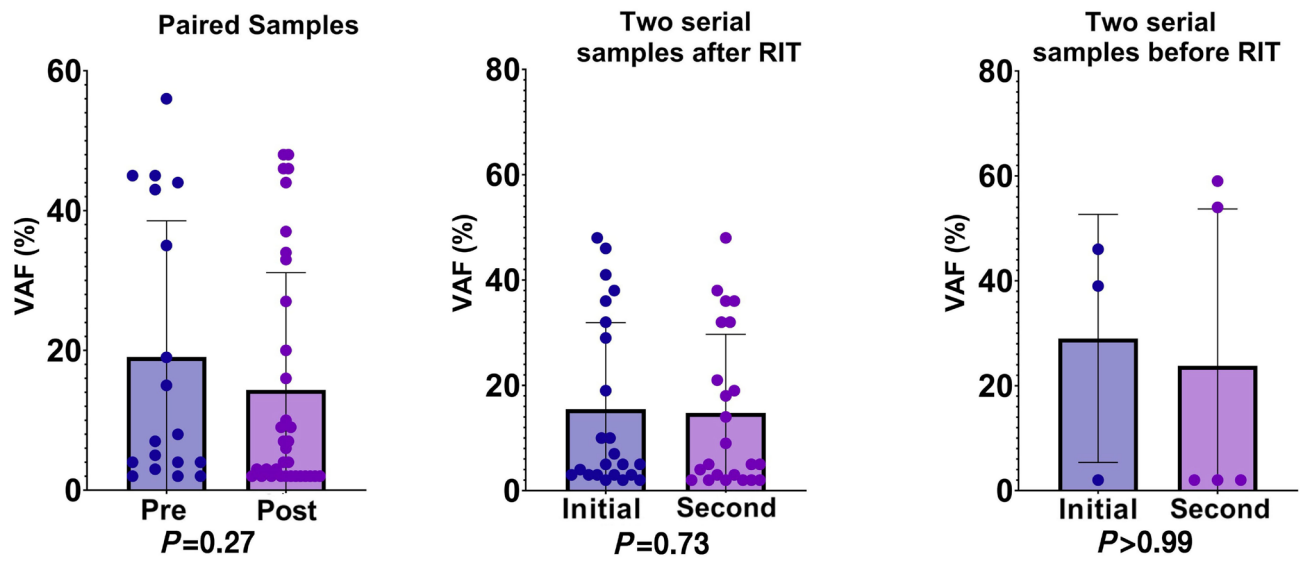

Figure 6. Changes in variant allele fraction in patients with paired samples in group 1, with two paired serial samples in subgroup 2a; and with two paired serial samples in subgroup 3a. VAF: variant allele fraction; RIT: radioimmunotherapy.

Table 1. Clinical and demographic comparisons between patients with or without therapy-related myeloid neoplasms.

Characteristic	t-MN	Non-t-MN	*P*
N	14	44	
Age in years, median (IQR)	51.5 (44.3-63)	48 (41- 61)	0.46
Sex, N (%)			0.97
Female	5 (36)	18 (41)	
Male	9 (64)	26 (59)	
FNHL grade, N (%)			0.89
1	7 (50)	22 (50)	
2	5 (36)	17 (39)	
3	0 (0)	1 (2)	
Missing information	4 (29)	2 (4)	
FNHL stage, N (%)			0.53
I	1 (7)	4 (9)	
II	2 (14)	4 (9)	
III	0 (0)	8 (18)	
IV	9 (64)	26 (59)	
Missing information	1 (7)	2 (4)	
FLIPI score, N (%)			0.54
0	1 (7)	4 (9)	
1	3 (21)	6 (14)	
2	5 (36)	24 (55)	
3	4 (29)	6 (14)	
4	0 (0)	1 (2)	
Missing information	1 (7)	3 (7)	
CH, N of pts (%)	9 (64)	26 (59)	0.97
DTA mutations, N of pts (%)	4 (15)	18 (26)	0.14
DNMT3A	3 (11)	16 (23)	0.19
TET2	0 (0)	1 (1)	-
AXSL1	1 (4)	1 (1)	0.48
DDR mutations, N of pts (%)	9 (33)	21 (30)	0.75
TP53	2 (7)	6 (9)	0.85
PPM1D	7 (26)	15 (21)	0.63
Other myeloid CH, N of pts (%)	9 (33)	10 (14)	0.03
ASXL2	0 (0)	1 (1)	-
BRCC3	1 (4)	0 (0)	-
CBL	1 (4)	1 (1)	0.48
CDKN2A	0 (0)	0 (0)	-
IDH2	1 (4)	0 (0)	-
LTB	1 (4)	0 (0)	-
MED12	1 (4)	1 (1)	0.48
NF1	0 (0)	1 (1)	0.48
PHIP	0 (0)	2 (3)	-
POU2AF1	0 (0)	1 (1)	-
PTEN	0 (0)	1 (1)	-
SBDS	0 (0)	1 (1)	-
SMC3	1 (4)	0 (0)	-
STAG2	1 (4)	0 (0)	-
STAT3	1 (4)	0 (0)	-
TET1	0 (0)	1 (1)	-
Lymphoid CH, N of pts (%)	0 (0)	21 (30)	0.38
ARID1A	1 (4)	1 (1)	-
ARID1B	0 (0)	0 (0)	-
CAR11	0 (0)	1 (1)	-
CD79B	1 (4)	1 (1)	-
CREBBP	0 (0)	4 (6)	0.68
EP300	1 (4)	1 (1)	-
EZH2	0 (0)	2 (3)	0.83
HIST1HIC	1 (4)	2 (3)	-
KMT2D	1 (4)	7 (10)	0.31
STAT6	0 (0)	1 (1)	0.48
TNFRSF14		1 (1)	-
N of therapies, median (IQR)	4 (3-6)	4 (2-6)	0.9
Death, N (%)	8 (48)	15 (34)	0.22

t-MN: therapy-related myeloid neoplasms; IQR: interquartile range; FNHL: follicular, non-Hodgkin lymphoma; FLIPI: Follicular Lymphoma International Prognostic Index; CH: clonal hematopoiesis; pts: patients; DTA: *DNMT3A*, *TET2*, and *ASXL1*; DDR: DNA damage response and repair.

were the next most frequent mutations seen (n=3, 16%). Within the limitations of the small sample size, we did not see an impact of CH on t-MN development; nine (26%) of 35 patients with CH developed t-MN, compared to five (22%) of 23 without CH (*P*=0.97). We were also limited in identifying potential risk factors for t-MN development, including CH subtypes, e.g., *TP53* (*P*=0.53) or *PPM1D* (*P*=0.4), and the impact of prior therapies (*Online Supplementary Figure S5*).

Risk factors for overall survival

Patients with t-MN had a trend towards a shorter OS compared to those without (median OS: 5.16 years *vs*. 17.8; *P*=0.1). Age ≥50 years was identified as a risk factor for shorter OS (15-year survival probability: 42% *vs*. 82%; *P*=0.001) (*Online Supplementary Figure S6*). Other variables, including CH (*Online Supplementary Figure S7*) and its subtype, mutation numbers, and nature and the number of prior cytotoxic therapies, had no impact on OS. The relevance of these findings is limited by the small sample size and the retrospective nature of the study that was not powered to detect a difference.

Discussion

In this retrospective study, we focused on a unique cohort of patients with low-grade relapsed FL, which represents a very particular clinical situation as patients have long survivals, punctuated by multiple treatment modalities.[34] This provides a great scenario to study CH in the context of evolving selection pressures. ^{90}Y ibritumomab tiuxetan for FL was the first RIT approved by the US Food and Drug Administration[35] in 2002 and, although not currently in use, provides a valuable database with a long follow-up that can serve as a model to inform risk for patients now receiving newer RIT for solid tumors and potentially the re-emergence of RIT for lymphoma. Using paired pre- and

Table 2. Clinical, cytogenetics, and somatic mutations in patients who developed therapy-related myeloid neoplasms.

Pt #	Age in years at FL diagnosis	Sex	N of prior therapies	Topo-isomerase inhibitor	Alkylators	Purine/ pyrimidine nucleoside analog	Radiation therapy	MDS/AML subtype	Cytogenetics	Baseline CH mutations and VAF (%)	Pathogenic variants (VAF %) at t-MN diagnosis
1	43	F	5	Y	Y	Y	N	t-MDS	46, XX, -7[3]/46, idem,+r[17]	No CH at baseline	*DNMT3A*, (46) *SETBP1* (45) *U2AF1* (15)
2	57	F	4	Y	Y	N	N	t-MDS → AML	42-45, XX, add(5)(q11.2), -7,add(14)(q32),ins(15;?)(q13;?)[3], -18, -21,-21[3],+0-2mar[cp16]/46, XX[4]5q del	No CH at baseline	NA
3	54	F	4	Y	Y	Y	Y	t-MDS (MDS-EB-2) → t-AML	t-MDS 46,XX,del(1)(p32p36.1), del(5)(q22q35), -7,+8[16]/46,XX[4] progressed to t-AML 46,XX,del(1)(p32p36.1),del(5) (q22q35),-7,+8[20]	*PPM1D* (2) *TP53* (5)	NGS: negative in 2019 and 2020
4	53	M	10	Y	Y	Y	Y	t-AML	40-43,X,-Y, 3,add(3)(q21), add(5) (q13),del(5)(q13q33), -7,-10, -12, del(12)(q13q24.1), der(15)t(1;15) (q21;q22),-16,inv(16)(p13.3q22),-17,add(17)(p11.2),add(18)(p11.2),-19,+22,mar[cp20]	*ASXL1* (3) *NOTCH1* (48) *PPM1D* (2) *PPM1D* (3) *SMC3* (2)	*TP53* (89)
5	65	M	7	Y	Y	Y	Y	t-MDS (EB-2)	del(5q) and monosomy 7	*PPM1D* (19) *TP53* (9)	NA
6	48	M	4	Y	Y	N	N	t-MDS	45,XY,add(5)(q11.2),add(7)(q22), add(10)(q24),-12, add(13)(p11.2),-17, -18,+2mar[19]/46, XY[1]	*CBL* (18) *CREBBP* (32) *KMT2D* (36) *LTB* (38) *STAT6* (36)	NA
7	68	M	3	N	Y	Y	Y	Pure erythroid leukemia	NA (CG was normal 2 years ago before t-MN diagnosis)	*PPM1D* (45)	NA
8	48	M	1	N	N	N	Y	t-AML	46, XY,del(7)(q22)[10]/46,XY[10]	*EZH2* (30) *IDH2* (37) *PPM1D* (32) *STAG2* (36)	NA
9	79	M	6	N	Y	N	Y	t-MDS	40-41,XY,add(5)(q11.2),-7,add(8) (p11.2),add(12)(q13),-16, -17,-18,-20,-22, add(22)(q11.2),+0-4mar[cp15]/78-81,idemx2[2]/46,XY[3]	No CH at baseline	*TP53* (37) *TP53* (3.6) *PPM1D* (3.1)

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Pt #	Age in years at FL diagnosis	Sex	N of prior therapies	Topo-isomerase inhibitor	Alkylators	Purine/ pyrimidine nucleoside analog	Radiation therapy	MDS/AML subtype	Cytogenetics	Baseline CH mutations and VAF (%)	Pathogenic variants (VAF %) at t-MN diagnosis
10	40	F	6	N	Y	Y	N	t-AML	45-46,XX,del(1)(p32p36.1),del(5)(q13q33),-7, der(9;18)(p10;q10),add(15)(q22),+r, +0-1mar[cp13]/46,XX[7]	*ARIDIB* (9) *MED12* (33) *STAT3* (10)	*STAT3* (1.1) *TP53* (60) *PPM1D* (3.5) *TET2* (1.6)
11	50	M	3	N	N	N	Y	t-AML	46,XY,add(2)(p11.2), 3,add(3)(p21),del(5)(q22q35), der(7)t(3;7)(p13;p22),add(8)(q13), der(8)t(8;10)(p21;q11.2),der(10)t(9;10)(q13;q11.2),+11, der(15;17)(q10;q10), +mar[3]/49 53,XY,add(2)(p11.2),add(3)(p13),t(3;7)(p13;p22), del(5)(q22q35), +6,+add(8)(q13)x2,der(8)t(8;10)(p21;q11.2)x2der(10)t(9;10)(q13;q11.2),+11,+13, +19,+0-1r[cp17]	*DNMT3A* (3) *DNMT3A* (48)	*DNMT3A* (4) *TP53* (65.9) *STAG2* (10)
12	67	F	5	Y	Y	Y	N	t-AML	46, XX,-7,+r[20]	*PPM1D* (4)	*SETBP1* (17.8) *SETBP1* (7.8)
13	36	M	1	Y	N	N	N	t-MDS (MDS-EB-2)	46,XY,del(20)(q11.2q13.3)[17]/46,sl,t(3;17)(p21;q25)[3]	No CH at baseline	*BCOR* (76) *DNMT3A* (40) *RUNX1* (8) *SF3B1* (41)
14	33	M	6	N	Y	Y	Y	t-AML with monocytic differentiation	Complex, details unknown, treated elsewhere	No CH at baseline	*PPM1D* (3.1)

Pt#: patient's number; FL: follicular lymphoma; MDS: myelodysplastic syndrome; AML: acute myeloid leukemia; CH: clonal hematopoiesis; VAF: variant allele fraction; t-MN: therapy-related myeloid neoplasms; F: female; M: male; Y: yes; N: no; t-MDS: therapy-related MDS; EB-2: excess blasts-2; t-AML: therapy-related AML; NA: not available/applicable; NGS: next-generation sequencing; CG: cytogenetics.

post-RIT samples, we found that 42% of patients with relapsed FL had CH despite normal conventional cytogenetic studies at the time of RIT, with an eventual 67% prevalence rate for CH after RIT. In the context of a median follow-up duration of 17.8 years, 24% of patients had t-MN with high clonal burdens and with extensive chromosomal damage. The median latency of t-MN development was 8.8 years after RIT exposure, which highlights the difficulty of fully assessing bone marrow toxicity in the short term for any new agent being tested for the treatment of indolent non-Hodgkin lymphomas. The survival after t-MN diagnosis of less than a year also underscores the dismal outlook and a key unmet need for better treatment for patients with t-MN.

The spectrum of CH seen in this group was different from that in classical age-related CH, with a high prevalence of DDR pathway mutations, absence of spliceosome mutations, and a paucity of signaling mutations, including at t-MN diagnosis. We observed clonal evolution in the paired samples before and after RIT without a clear inference of causality between exposure to RIT and clonal expansion. Furthermore, CH was not associated with an increased risk of t-MN or inferior OS. This is likely due to the small sample size and the high prevalence of CH in both the t-MN and non-t-MN groups. In fact, while new CH mutations were encountered at t-MN diagnosis, including somatic *TP53* mutations, all patients had extensive chromosomal damage, highlighting the genomic instability seen in this heavily treated patient population.

There are several unique findings in our study. First, we found a significantly higher incidence of t-MN in heavily treated FL patients who received RIT, higher than stated in previous reports.[23-28,36] Two reports on RIT suggested that the cumulative incidence of t-MN was 2.5% at 5 years[37] and 10% at 10 years, with a median latency of 6.6 years.[38] In our study, the incidence of t-MN was 24% after 17.8 years of follow-up, with the median latency being 8.8 years. The latency is also strikingly longer than that which can be seen after exposure to alkylating agents (5-7 years) or topoisomerase II inhibitors (1-3 years),[39] suggesting mechanistic differences that might contribute to t-MN development.

Secondly, in patients with non-Hodgkin lymphoma undergoing ASCT or CAR T-cell therapy, the prevalence of CH was estimated to be 25-50%, with the most common mutations being *DNMT3A, PPM1D, TET2*, and *TP53*.[13-17,40] In our cohort, the prevalence of CH was higher, at 60%. One explanation is that our CH panel was larger (>200 genes) and was able to detect mutations with greater sensitivity (VAF of >0.5%) compared to other studies.[16,41] Consistent with other studies, the most frequent mutations were *DNMT3A* (25%), *PPM1D* (23%), *KMT2D* (8%), and *TP53* (8%), which reflect the effect of cytotoxic therapy and the FL genetic landscape.[31,42-44] *PPM1D* mutations were highly enriched in this cohort, suggesting that these mutations represent convergent mechanisms of clonal fitness in the constraints of oncogenic exposures.

Third, in paired samples taken 1 year apart before and after exposure to RIT, we observed clonal evolutionary changes as reflected by an increase, albeit not statistically significant, in the prevalence of CH. In addition, five (21%) patients without CH prior to RIT had CH after RIT. There were no significant changes in mutational VAF, a phenomenon not seen in the other two groups either. These findings may be limited by the retrospective nature of cohort assignment, smaller sample size, and the impact of natural clonal growth rates, which are hard to approximate for individual patients.[5]

We found that all evaluable patients with t-MN had extensive cytogenetic abnormalities at the time of t-MN, with 79% demonstrating chromosome 5 and/or 7 abnormalities and 36% having chromosome 17 abnormalities, all of which are associated with poor outcomes.[45] Our data suggest that radioisotope therapy may play a role in chromosomal damage/alterations; however, it is difficult to parse out the role of RIT on t-MN development as all patients in our study had multiple cumulative exposure to chemoradiation therapy. Our study highlights the somatic genomic landscape in FL patients treated with cytotoxic chemotherapy, ionizing radiation, and radioisotope therapy, demonstrating the impact of these treatment modalities on CH, chromosomal damage, and clonal evolution. The strikingly high prevalence of CH and t-MN highlights the oncogenic potential of such therapies, while the exact mechanisms of oncogenesis and the impact of CH remain to be elucidated. This study has important implications as different types of RIT in cancer are rapidly gaining approval for a variety of malignancies. It has been reported that the incidence of t-MN in recipients of peptide receptor radionuclide therapy ranges between 1.8-5.4%, although the latency of t-MN development in such patients was shorter, suggesting different mechanisms of t-MN development.[46,47] Recently, radioligand therapy with 177Lu-PSMA-617 was approved for prostate-specific membrane antigen-positive, metastatic, castration-resistant prostate cancer[22] and CAR-T therapy with purine nucleoside analog lymphodepletion has been approved for relapsed and refractory FL.[48] Lastly, the widening long-term use of poly ADP ribose polymerase inhibitors in patients with breast, ovarian, and prostate cancers has brought increasing awareness to the problem of t-MN and acute myeloid leukemia.[49] It will, therefore, be important to monitor the impact of CH and clonal evolution closely in such patients.

The advantages of our study include the baseline bone marrow studies with conventional analysis, the extensive molecular analysis, and the long follow-up duration, all of which confirm the need for long-term monitoring and the relevance to solid tumor oncology in which these agents are gaining popularity. The limitations of our study include the relatively small sample size, retrospective study design, and heterogeneity regarding prior therapies.

In summary, we report the prevalence of CH and t-MN in FL patients who relapsed after conventional therapy and then received radiation therapy with RIT. With a

long follow-up, we found a high prevalence of CH and t-MN in this cohort. The spectrum of CH was unique and different from that in age-related CH, with no clear causality between RIT exposure and clonal expansion or t-MN development. All patients with t-MN had high clonal burdens and demonstrated extensive chromosomal damage, with very poor survival outcomes. Our study provides the rationale for future prospective studies with paired samples before and after RIT interventions, evaluating the impact of CH by specific CH subtype and by clonal complexity, on t-MN occurrence and non-relapse mortality, given the rapidly growing indications for this type of treatment.

Disclosures

MMP has received research funding from Stem Line Pharmaceuticals and Kura Oncology and has served on an advisory board for CTI Pharmaceuticals. TEW and the Mayo Clinic received research support from IDEC, Spectrum and Acrotech Biopharma for clinical trials of 90-yttrium ibritumomab tiuxetan. ZX has no conflicts of interest to disclose.

Contributions

ZX and MMP designed the study using samples from the radioimmunotherapy database (TEW). ZX, MMP and TEW wrote, reviewed, and edited the manuscript. All authors approved the final version.

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Data-sharing statement

Please contact the author for correspondence to discuss data sharing.

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