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

Authors: Zhuoer Xie\textsuperscript{1,2}, Terra Lasho\textsuperscript{1}, Arushi Khurana\textsuperscript{1}, Alejandro Ferrer\textsuperscript{1}, Christy Finke\textsuperscript{1}, Abhishek A. Mangaonkar\textsuperscript{1}, Stephen Ansell\textsuperscript{1}, Jenna Fernandez\textsuperscript{1}, Mithun Vinod Shah\textsuperscript{1}, Aref Al-Kali\textsuperscript{1}, Naseema Gangat\textsuperscript{1}, Jithma Abeykoon\textsuperscript{1}, Thomas E. Witzig\textsuperscript{1}, Mrinal M. Patnaik\textsuperscript{1}

Affiliations:

1. Mayo Clinic, Department of Internal Medicine, Hematology Division, Rochester, MN, United States
2. Malignant Hematology Department, H. Lee Moffitt Cancer Center & Research Institute, FL, United States

Corresponding author:

Dr. Mrinal Patnaik, M.B.B.S.
Division of Hematology
Mayo Clinic
200 1st St. SW
Rochester, MN 55904
Email: Patnaik.Mrinal@mayo.edu

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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 (1) CH is prevalent in relapsed follicular lymphoma (FL) and is associated with t-MN transformation. (2) 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 FL patients who received RIT. Patients received a median of 4 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 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 0.9 months). The baseline prevalence of CH is high, with a subsequent increase in prevalence on exposure to RIT and subsequent therapies. The high rates of t-MN with high clonal complexities and extensive chromosomal damage underscore the importance of better identifying and studying genotoxic stressors accentuated by these agents.
Introduction

Clonal hematopoiesis (CH) is defined by the acquisition and subsequent expansion of somatic DNA variants, including somatic mutations and copy number alterations in the hematopoietic stem and progenitor cells (HSPCs).\(^1\)\(^-\)\(^4\) When CH mutations occur in leukemia-associated genes, with variant allele fraction (VAF) \(\geq 2\%\) in individuals without a diagnosed hematologic disorder, the condition is termed CH of indeterminant potential (CHIP). 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.\(^6\)

CHIP 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 HSPCs, 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 T-cell therapy (CAR-T),\(^16\)\(^,\)\(^17\) 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 CHIP, we hypothesized that beta radiation exposure would enhance the prevalence and growth of CH in HSPCs resulting in a higher prevalence of t-MN, with CH negatively impacting OS. This group 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. 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 5 patients in Group 3 who had two serial samples prior to RIT exposure (group 3a), and we compared clonal evolution in subgroup 2a and subgroup 3a, respectively. (Figures 1-3)

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

CH detection. DNA was extracted from peripheral blood mononuclear cells (PBMC) and subjected to a customized, targeted next-generation sequencing assay, as previously described and can be found in Supplementary material and table S1. VAF ≥ 2% was considered as CH. Cryopreserved bone marrow DNA was also available on 8 patients at the time of t-MN diagnosis 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. 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 (DTA). For patients with multiple pathogenic variants, we used the maximum VAF for VAF comparisons. Mutation patterns were analyzed using ProteinPaint.
**Statistical analysis.** We compared clinical characteristics, mutation patterns, VAF, and outcomes of those with and without t-MN. Continuous variables were presented as median (interquartile range, IQR), mean (standard deviation, SD), 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 χ² 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 on outcomes. The median point estimate and 95% confidence interval (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**

**Patient characteristics and clinical outcomes.** Fifty-eight patients with relapsed FL were included, with a median age of 48.5 (range: 28-79) years; 23 (40%) females. The median follow-up duration was 17.8 (IQR: 15.7-21.9) years. At the last follow-up, 14 (24%) patients developed t-MN, and 23 (40%) deaths were documented, including 8 deaths from t-MN. Eleven (19%) patients had DLBCL transformation and in this group, 3 (17.5%) developed t-MN. The median time from FL diagnosis to t-MN was 14.4 (IQR: 11.3, 18.6) years, with the median latency from RIT to t-MN diagnosis being 8.8 (IQR: 6.7-12.6) years and the median OS after t-MN diagnosis being 0.9 (IQR: 0.24-2.2) years. All 58 patients had significant exposures to chemo-immunotherapy or involved field radiation therapy with a median of 4 prior regimens (Table 1).

There were no significant differences between 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 inhibitors, 52 (90%) alkylating agents, and 21 (36%) ionizing radiation therapy. In the 14 patients who developed t-MN, all had prior chemotherapy exposure, including 11 (79%) with exposure to alkylating agents and 8 (57%) with exposure to topoisomerase inhibitors. In addition, 8 (57%) had prior conventional radiation exposure, including 6 (43%) with exposure to alkylating agents/topoisomerase inhibitors and radiation therapy.
**Prevalence and mutational spectrum of CH.** Immediately prior to RIT administration, all 58 patients had a bone marrow biopsy with no morphological atypia, 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 and Fig S1). Among patients with CH, 12 (34%) patients had 1 mutation, and 23 (66%) had ≥2 mutations. The median VAF was 19% (IQR: 4-39%). The co-mutation status and VAF are shown in Figure 4 and Fig S2. In the entire cohort, 21 (60%) patients had M-CH only, 2 (6%) had L-CH only, and 12 (34%) had LM-CH. The pathogenic variants for each patient are listed in Table S2. Figure 5 demonstrates the oncoplot for the entire cohort. The common mutation patterns were missense, followed by nonsense and frameshift.

**Clonal expansion after RIT.** 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% vs. 67% (p=0.15); and the prevalence of M-CH was 42% vs. 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. (Supplementary Figure 2, PT_36, PT_42, 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 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 for DDR mutations (n=8) was 4.5% (range: 2% to 19%). Four patients developed t-MN in this group, 3 with existing CH before t-MN diagnosis and 1 without (Figure 1 and table S3). Fig S3 and S4 demonstrate the changes in clone VAF and complexity in this group.

In patients with 2 serial samples from group 2a and subgroup 3a (Figures 2 and 3), there were no differences in the prevalence of CH or M-CH between the two-time points in either group. (group 2a: CH prevalence at the 2-time points was 69% vs. 75%, p=0.75, M-CH: 62% vs. 67%, p=0.79; Subgroup 3a CH prevalence was 60% and M-CH was 40% at both time points). Further, there was no significant difference in VAF between the two-time points samples in groups 2a or 3a (VAF of group 2a: 15.5% vs. 14.8%, p=0.73; subgroup 3a: 29% vs. 23.8% p>0.99) (Figure 6). These results may be limited by the smaller sample size and a shorter time interval between sampling points (time intervals were 1 vs. 0.48 vs. 0.04
years for Group 1, group 2a, and 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, with a decrease in these clones with lymphoma-directed therapy and could in fact represent circulating tumor cells that were inadvertently included in the PBMC cell fraction. In addition, all CH mutations became undetectable in one patient who underwent allogeneic stem cell transplant (Fig S3, PT_43).

Clinical association with t-MN. Fourteen (24%) patients developed t-MN (7 each for AML and MDS); median age 51.5 years (range: 33-79) and 9 (64%) patients were male. There were no significant differences in baseline age (p=0.44), gender (p=0.72), FL stage (p=0.54), grade (p=0.89), FL international prognostic index (FLIPI) scores (p=0.71), and the number of prior therapies (median, 4 in both groups) (p=0.66), including prior ASCT (p=0.18) between the t-MN vs. non-t-MN groups.

Among the 97 identified somatic variants, 27 (28%) were in the t-MN 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%), and 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 post-therapy was not associated with t-MN development (p=0.6 and 0.73, respectively).

Detailed clinical, molecular, and cytogenetic information on patients with t-MN are outlined in Table 2. Thirteen of 14 evaluable patients with t-MN (7 each with t-MDS and t-AML) had an abnormal karyotype. Among these, 7 (50%) had monosomal karyotype, 1 (7%) complex karyotype, 4 chromosome 17 abnormalities, 8 (57%) chromosome 5 abnormalities, and 11 (79%) chromosome 7 abnormalities, respectively. Twenty-one variants were found in the 8 sequenced t-MN BM samples, with 19 new pathogenic variants emerging that were not present in the pre-t-MN assessments (Table 2). Among these 8 patients, four (50%) had no CH in the prior samples. Common mutations gained at t-MN transformation
were TP53 (n=6, 32%), with 4 patients having biallelic TP53 inactivation. PPM1D and SETBP1 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; 9 (26%) of 35 patients with CH developed t-MN, compared to 5 (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 (Fig S5).

Risk factors for OS. 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 years survival probability: 42% vs. 82%, p=0.001, Fig S6). Other variables, including CH (Fig S7) and its subtype, mutation numbers, and nature and the number of prior cytotoxic therapies, had no impact on OS. These findings were limited to the small sample size and retrospective nature 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 represent a unique clinical situation as patients have long survivals, punctuated by multiple treatment modalities.\textsuperscript{34} This provides a great scenario to study CH in the context of evolving selection pressures.\textsuperscript{90}Y ibritumomab tiuxetan for FL was the first RIT approved by the US Food and Drug Administration (FDA)\textsuperscript{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 RITs for solid tumors and potentially the re-emergence of RIT for lymphoma. Using paired samples pre- and post-RIT, we show 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 post-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 – emphasizing the difficulty of fully assessing bone marrow toxicity in the short term in any new agent being tested for indolent NHL. 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 classical age-related CH (ARCH), with a high prevalence of DDR pathway mutations, absence of spliceosome mutations, and a paucity of signaling mutation, including at t-MN diagnosis. We observed clonal evolution in the paired samples pre- and post-RIT exposure without a clear causality inference between RIT exposure and clonal expansion. Further, 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 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. Two reports on RIT suggested that the cumulative incidence of t-MN was 2.5% at 5 years and 10% at 10 years, with a median latency of 6.6 years. 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 what can be seen with exposure to alkylating agents (5-7 years) or topoisomerase II inhibitors (1-3 years), suggesting mechanistic differences that might contribute towards t-MN development.

Secondly, In NHL patients undergoing ASCT or CART, the prevalence of CH was estimated at 25-50%, with the most common mutations being DNMT3A, PPM1D, TET2, and TP53. In our cohort, the CH prevalence 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. 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 exposure and the FL genetic landscape. PPM1D was highly enriched in this cohort, suggesting that PPM1D represent convergent mechanisms of clonal fitness in the constraints of oncogenic exposures.

Third, with paired samples pre- and post-RIT exposure one year apart, we observed clonal evolutionary changes as reflected by an increase in the CH prevalence, albeit not statistically significant. In addition, five (21%) patients without CH prior to RIT had CH post-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.\textsuperscript{5}

Further, 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\% demonstrating chromosome 17 abnormalities; all of which are associated with poor outcomes.\textsuperscript{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 chemoradiation therapy exposures.

Our study highlights the somatic genomic landscape in FL patients treated with cytotoxic chemotherapy, ionizing radiation, and radioisotope therapy, demonstrating the impact of these agents 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 RIT in cancer are rapidly gaining approvals for a variety of malignancies. It has been reported that the incidence of t-MN in peptide receptor radionuclide therapy (PRRT) ranges between 1.8-5.4\%, although the latency of t-MN development in such therapy was shorter, suggesting different mechanisms of t-MN development.\textsuperscript{46,47} Recently, radioligand therapy with 177Lu-PSMA-617 has been approved for PSMA-positive metastatic castration-resistant prostate cancer\textsuperscript{22} and CAR-T therapy with purine nucleoside analog lymphodepletion has been approved for relapsed and refractory FL.\textsuperscript{48} Lastly, the widening long-term use of poly ADP ribose polymerase (PARP) inhibitors in patients with breast, ovarian, and prostate cancers has brought increasing awareness to the problem of t-MN and AML.\textsuperscript{49} Therefore, it will be important to closely monitor the impact of CH and clonal evolution in such patients. The advantages of our study include the baseline marrow studies with conventional analysis, the extensive molecular analysis, the long follow-up duration, all of which confirm the need for long-term monitoring and the relevance to solid tumor oncology where 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 this long follow-up, we demonstrate a high
prevalence of CH and t-MN in this cohort. The spectrum of CH was unique and different from ARCH, with no clear causality inferences 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 complexities, on t-MN occurrence and non-relapse mortality, given the rapidly growing indications for these agents.
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48. FDA grants accelerated approval to axicabtagene ciloleucel for relapsed or refractory follicular lymphoma | FDA. Accessed December 17, 2022.

Table 1. Clinical and demographic comparisons between patients with t-MN and without t-MN. **Abbreviation. CH:** clonal hematopoiesis. **DTA:** DNMT3A, TET2, and ASXL1. **DDR:** DNA damage response and repair. **FLIPI:** follicular lymphoma international prognostic index. **MT:** mutation. **t-MN:** treatment-related myeloid neoplasm. **VAF:** Variant allele fraction.

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<td>TNFRSF14</td>
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**Number of therapies, median (IQR)**

| Death, N (%) | 8 (48) | 15 (34) | 0.22 |
Table 2. Clinical, cytogenetics, and somatic mutations in patients who developed t-MN. **Abbreviations.** FL: follicular lymphoma; t-MDS: treatment-related myelodysplastic syndrome; t-AML: treatment-related acute myelogenous leukemia. VUS: variant of unknown significance.

<table>
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<th>No.</th>
<th>Age at FL diagnosis</th>
<th>Gender</th>
<th>Number of prior therapies</th>
<th>Topoisomerase inhibitor</th>
<th>Alkytators</th>
<th>Purine/Pyrimidine nucleoside analog</th>
<th>Radiation therapy</th>
<th>MDS/AML Subtype</th>
<th>Cytogenetics</th>
<th>Baseline CH mutations and Variant allele fraction</th>
<th>Pathogenic variants at t-MN diagnosis</th>
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<td>1</td>
<td>43</td>
<td>F</td>
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<td>Y</td>
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<td>N</td>
<td>t-MDS</td>
<td>46, XX, -7(3)[46], idem,+r(17)</td>
<td>No CH at baseline</td>
<td>DNMT3A 46%; SETBP1 45%; U2AF1 19%</td>
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<td>2</td>
<td>57</td>
<td>F</td>
<td>5</td>
<td>Y</td>
<td>Y</td>
<td>N</td>
<td>t-MDS→AML</td>
<td>42-45, XX, add(3)(q11.2), -7,add(14)(q32),ins(15;7)(q13.7)[3], -18, -21,-23[3], -6,mar(1p16)(46,XX)[4] Sq del</td>
<td>No CH at baseline</td>
<td>NA</td>
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<td>3</td>
<td>54</td>
<td>F</td>
<td>4</td>
<td>Y</td>
<td>Y</td>
<td>Y</td>
<td>t-MDS (MDS-EB-2)→t-AML</td>
<td>46,XX,del(1)(q32p36.1),del(5)(q22q35), -7,-q(16)[46,XX][4] progressed to t-AML</td>
<td>PPM1D 2%; TP53 5%</td>
<td>NGS: negative on 2019 and 2020</td>
<td>TP53: 89%</td>
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<td>4</td>
<td>53</td>
<td>M</td>
<td>10</td>
<td>Y</td>
<td>Y</td>
<td>Y</td>
<td>t-AML</td>
<td>40-43, X,Y, 3.add(3)(q21),add(5)(q13),del(5)(q34q33), -7,-10,-12,del(12)(q13p24.1), der(15)(q13)<a href="q21.22">15</a>,-16,inv(16)(p1 3.3q22), -17,add(17)(p11.2),add(16)(p11.2), -19, +2mar(1p20)</td>
<td>No CH at baseline</td>
<td>NA</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>65</td>
<td>M</td>
<td>7</td>
<td>Y</td>
<td>Y</td>
<td>Y</td>
<td>t-MDS (EB-2)</td>
<td>del(5q) and monosomy 7.</td>
<td>PPM1D 19%</td>
<td>TP53 9%</td>
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<tr>
<td>7</td>
<td>68</td>
<td>M</td>
<td>3</td>
<td>N</td>
<td>Y</td>
<td>Y</td>
<td>Pure erythroid leukemia</td>
<td>NA</td>
<td>PPm1D 45%</td>
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<tr>
<td>8</td>
<td>48</td>
<td>M</td>
<td>1</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>t-AML</td>
<td>46, XY,del(7)(q22)[10]/46,XY[10]</td>
<td>EZH2 30%, IDH2 37%, PPM1D 32%, STAG2 36%</td>
<td>NA</td>
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<tr>
<td>9</td>
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<td>6</td>
<td>N</td>
<td>Y</td>
<td>Y</td>
<td>t-MDS</td>
<td>49-41,XY,add(5)(q11.2), -7,add(8)(p11.2),add(12)(q13)-16, -17, -18, 20, 22,add(22)(q11.2),-6,mar(15)p78-81, idem[2]/46,XY[3]</td>
<td>No CH at baseline</td>
<td>TP53 37%; TP53 3.6%; PPM1D 3.1%</td>
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<td>10</td>
<td>40</td>
<td>M</td>
<td>6</td>
<td>N</td>
<td>Y</td>
<td>Y</td>
<td>t-AML</td>
<td>45-46,XX,del(1)(q32p36.1),del(5)(q13p33)-7, der(9)(18)(p10)(q10),add(15)(q22),-16, -19, +2mar(1p13)[46,XX][7]</td>
<td>ARIDIB 9%, MED12 33%, STAT3 16%</td>
<td>STA53 1.1%; TP53 60%; PPM1D 3.5%; TET2 1.6%</td>
<td></td>
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<tr>
<td>12</td>
<td>67</td>
<td>F</td>
<td>5</td>
<td>Y</td>
<td>Y</td>
<td>N</td>
<td>t-AML</td>
<td>46, XX,-7,+r(20)</td>
<td>PPM1D 4%</td>
<td>SETBP1 17.8%; SETBP1 7.8%</td>
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<tr>
<td>13</td>
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<td>Y</td>
<td>N</td>
<td>N</td>
<td>t-MDS (MDS-EB-2)</td>
<td>46,XY,del(20)(q11.2)[13.3][17]/46,sl,t(3;17)(p21q2 5)[3]</td>
<td>No CH at baseline</td>
<td>BCOR 76%, DNMT3A 40%, RUNX1 8%, SF31B 41%</td>
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<tr>
<td>14</td>
<td>33</td>
<td>M</td>
<td>6</td>
<td>N</td>
<td>Y</td>
<td>Y</td>
<td>t-AML with monocytic differentiation</td>
<td>Complex, details unknown, treated elsewhere</td>
<td>No CH at baseline</td>
<td>PPM1D 3.1%</td>
<td></td>
</tr>
</tbody>
</table>
Figure legends

**Figure 1.** Swimmer plot for group 1 patients. Abbreviation: PT: patients; RIT: radioisotope therapy; OS: overall survival; TMN: treatment-related myeloid malignancy; VAF: variant allele fraction. PT_9, 30, 31, and 32 received a total of 6, 6, 3, 5 different therapies, respectively.

**Figure 2.** Swimmer plot for group 2 patients. Abbreviation: PT: patients; RIT: radioisotope therapy; OS: overall survival; TMN: treatment-related myeloid malignancy; VAF: variant allele fraction.

**Figure 3.** Swimmer plot for group 3 patients. Abbreviation: PT: patients; RIT: radioisotope therapy; OS: overall survival; TMN: treatment-related myeloid malignancy; VAF: variant allele fraction.

**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.

**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 MB. The bar graph of the left designates the prevalence of mutations (count for once regardless of the prevalence/patient). The mutation subtypes were highlighted in colors, red indicates nonsense, light-blue indicates missense; yellow indicates frameshift; grey indicates splice effect; purple indicates duplication, pink indicates deletion. The bar on the bottom designates the proportion of the mutation subtypes for each patient. Proportions are from 0 (bottom) to 25%, 50%, 75%, and 100% (top).

**Figure 6.** VAF changes in patients with paired samples in group 1; two paired serial samples in group 2a; and two paired serial samples in group 3a. Abbreviation: VAF: variant allele fraction.
Group 2: RIT before the samples, n=21

PT_1

PT_2

PT_3

PT_4

PT_5

PT_6

PT_7

PT_8

PT_17

PT_18

PT_19

PT_20

PT_21

PT_22

PT_23

PT_24

PT_25

PT_26

PT_27

PT_28

PT_29

DNMT3A, 46%; SETBP1 45%; U2AF1 15%

TP53, 89%

STAG2 42%, EZH2 28%, IDH2 26%, PPM1D 26%

P53: 37%, P53 3.6%, PPM1D 3.1%
Supplemental information.

DNA sequencing method: briefly 200 ng of target DNA was fragmented using the Covaris LE220 plus sonicator. The ends were repaired using the Sureselect End-Repair-A-Tailing enzyme mix. Adapter ligated DNA fragments were size selected to enrich for 200 bp inserts (~320 bp total library size) using AMPURE XP bead purification. The size selected adapter-modified fragments were enriched, and specific indexes were added by 12 cycles of PCR using universal Index Primers.

The Custom Capture hybrid-target enrichment probes were designed using Agilent SureSelect design software (Agilent Technologies, Santa Clara, CA). The targeted gene panel was comprised of 62962 single probes with size 1.805Mbp, and covered the coding regions, UTRs, and overlapping intron/exon regions for 205 genes described and/or enriched for CHIP mutations. The custom capture was carried out using the Agilent Bravo liquid handler following Agilent’s SureSelect XT Low. Purified capture products were then amplified using the SureSelect Post-Capture primer mix for 14 cycles. Libraries were validated and quantified on the Agilent Bioanalyzer. Samples were sequenced by 150 paired end reads, 21 samples to a Flow Cell, on an Illumina NovaSeqSP with an expected expected depth of \( \geq 1,000X \) coverage (Illumina, SanDiego, CA).

Secondary bioinformatics analysis included quality assessment and alignment to the hg19 build reference genome using Novoalign (Novo- craft Technologies, Malaysia), followed by GATK based single nucleotide and small insertion/deletion variant calling, structural variation discovery, and annotation. The quality of sequencing chemistry was evaluated using FastQC (‘FASTQC’). After alignment, PCR duplication rates and percent reads mapped on target were used to assess the quality of the sample preps. Realignment and recalibration steps were implemented in the GATK. Somatic single nucleotide variations (SNVs) were then genotyped using SomaticSniper, whereas insertions and deletions were called by GATK Somatic Indel Detector. Each variant in coding regions was Strand-Aware Variant Annotation Tool ), as well as ClinVar, dbNSFP, OMIM, and the Human Gene Annotation Database to predict biological effects. Interpretation for relevant alterations included absence in international normal variant allele databases (GnomAD, ExAC), deleterious effect on protein function by multiple phenotype prediction models, somatic and functional annotation in literature, consequence of variant (nonsense, truncating, etc.) and location proximal to important domains.
Supplementary Table 1. The CH panel used in the study covered 289 genes.

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SWAP70
TBL1XR1
TCF3
TET1
TET2
TET3
TMEM30A
TNF
TNFAIP3
TNFRSF14
TP53
TRAF3
TYW1
U2AF1
U2AF2
UBR5
VPS13B
VPS13C
VWF
WT1
XBP1
XPO1
ZNF471
ZRSR2
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<th>Amino acid change</th>
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<th>CH type</th>
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Supplementary table 2. The pathogenic variants identified in the entire cohort.
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Supplementary Table 3. Detailed information for group 1, including the time differences between the paired samples.

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Supplementary Figure 1. Lollipop plot showing type and location along the protein sequence of TP53 mutation, PPM1D mutation, and DNMT3A mutation. The number of recurrently detected alterations is indicated by the text within each disc, as well as by disc size. Colors indicate the type of mutation: blue, missense; orange, nonsense; red, frameshift. Most of the TP53 mutations were missense mutations and occurred in the DNA binding domain, while 82% of the PPM1D mutations were truncating mutations that occurred in the terminal exon. DNMT3A mutations were largely missense mutations and occurred throughout the genome, with 6 involving the catalytic methyltransferase domain, including 1 dominant negative R882 mutation that has been implicated commonly in MDS/AML.
Supplemental figure 2. Variant allele fraction is sorted from the highest to the lowest.
Supplemental figure 3. Clone evolution before and after radioisotope therapy in group 1.
Supplementary Figure 4. Oncoplot before and after radioisotope therapy in group 1.
Supplementary Figure 5. Forest plot for the variables on TMN development (univariable analysis);

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Abbreviation: DAT: DNMT3A, ASXL1, and TET2; AnytimeDAT: DAT mutations at any time points. AnytimeNonDAT: mutations other than DAT mutations at any time points. DDR: DNA damage response and repair; M-CH: myeloid CH; L-CH: lymphoid CH; LM-CH: lymphoid and myeloid CHIP; MT>2: more than 2 mutations.
Supplementary Figure 6. Age > 50 is a risk factor for shorter OS. Group 0 indicates age < 50, group 1 indicates age ≥ 50.

Strata

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Survival probability

Time

p = 0.0015
Supplementary Figure 7. Overall survival stratified by CH status.

![Survival probability graph](image)

Number at risk

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