Clonal Hematopoiesis Associated With Adverse Outcomes After Autologous Stem-Cell Transplantation for Lymphoma


ABSTRACT

Purpose
Clonal hematopoiesis of indeterminate potential (CHIP) is an age-related condition characterized by somatic mutations in the blood of otherwise healthy adults. We hypothesized that in patients undergoing autologous stem-cell transplantation (ASCT) for lymphoma, CHIP at the time of ASCT would be associated with an increased risk of myelodysplastic syndrome and acute myeloid leukemia, collectively termed therapy-related myeloid neoplasm (TMN), and other adverse outcomes.

Methods
We performed whole-exome sequencing on pre- and post-ASCT samples from 12 patients who developed TMN after autologous transplantation for Hodgkin lymphoma or non-Hodgkin lymphoma and targeted sequencing on cryopreserved aliquots of autologous stem-cell products from 401 patients who underwent ASCT for non-Hodgkin lymphoma between 2003 and 2010. We assessed the effect of CHIP at the time of ASCT on subsequent outcomes, including TMN, cause-specific mortality, and overall survival.

Results
For six of 12 patients in the exome sequencing cohort, mutations found in the TMN specimen were also detectable in the pre-ASCT specimen. In the targeted sequencing cohort, 120 patients (29.9%) had CHIP at the time of ASCT, which was associated with an increased rate of TMN (10-year cumulative incidence, 14.1% vs. 4.3% for those with and without CHIP, respectively; P = .002). Patients with CHIP had significantly inferior overall survival compared with those without CHIP (10-year overall survival, 30.4% vs. 60.9%, respectively; P < .001), including increased risk of death from TMN and cardiovascular disease.

Conclusion
In patients undergoing ASCT for lymphoma, CHIP at the time of transplantation is associated with inferior survival and increased risk of TMN.

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INTRODUCTION

Recent studies have demonstrated that at least 10% to 15% of healthy older adults harbor clonal populations of blood cells bearing one or more somatic mutations associated with myeloid malignancies.1-5 This condition, termed clonal hematopoiesis of indeterminate potential (CHIP), is associated with an elevated risk of hematologic malignancy, death from cardiovascular disease, and overall mortality.6 To date, the prevalence and implications of CHIP have not been assessed in populations of patients with cancer who have received cytotoxic therapy.

One of the most serious consequences of cytotoxic therapy is the development of a therapy-related myeloid neoplasm (TMN), which includes myelodysplastic syndrome and acute myeloid leukemia developing after prior chemotherapy or radiation.7-15 Although TMNs were once presumed to arise as a consequence of aggregate DNA damage in otherwise healthy hematopoietic stem cells, a recent study demonstrated that in four patients with TP53-mutated TMN, the TMN-associated TP53 mutation driving the myeloid neoplasm could be found at low levels in samples collected years before the development of TMN.12 It is not known whether CHIP
predicts the subsequent development of TMN or other outcomes.

In this study, we assessed the impact of CHIP in the context of autologous stem-cell transplantation (ASCT), an extreme hematopoietic stress in which autologous stem cells are harvested before the administration of myeloablative chemotherapy and then reinfused into the patient to repopulate the hematopoietic system. ASCT is used for patients with relapsed, refractory, and otherwise high-risk lymphoma and is notable for a high rate of subsequent TMN, which is a major cause of nonrelapse mortality in this population.\textsuperscript{13-16} We asked whether the presence of CHIP at the time of ASCT, after chemotherapy for lymphoma, alters the risk of TMN and other clinical outcomes.

### Methods

**Cohorts**

We collected clinical data and biologic specimens (peripheral blood and bone marrow) from adult patients who underwent ASCT for Hodgkin lymphoma or non-Hodgkin lymphoma between 1999 and 2011 (City of Hope National Medical Center, Duarte, CA; exome cohort). We collected clinical data and specimens (cryopreserved aliquots of autologous stem-cell products) from a separate cohort of patients who underwent ASCT for non-Hodgkin lymphoma between 2003 and 2010 (Dana-Farber Cancer Institute, Boston, MA; targeted sequencing cohort). All patients signed consent for sample and data collection and subsequent analysis at or before the time of ASCT, according to protocols approved by the institutional review boards at each institution. Additional details are provided in the Data Supplement.

**Exome Sequencing of Patients With TMN**

We performed whole-exome sequencing on samples from 18 patients in the exome cohort who had one sample drawn within 2 months before ASCT and another drawn simultaneously with the TMN diagnosis. Exomes were analyzed to identify mutations in genes recurrently mutated in hematologic malignancies, as described in the Data Supplement. We excluded samples with less than 50 times median coverage across the 10 most commonly mutated CHIP genes.\textsuperscript{1,2} Sequencing data from six patients did not meet this standard and were excluded from further analysis.

**Targeted Sequencing of Patients Who Received ASCT**

We performed targeted sequencing on aliquots of mobilized stem-cell products banked at the time of ASCT for 401 patients with non-Hodgkin lymphoma (targeted sequencing cohort) and on bone marrow aspirates obtained at the time of TMN diagnosis for all patients in this cohort who developed TMN and had an available specimen. We enriched target regions of 86 genes, which were selected based on pathogenic involvement in CHIP or myeloid malignancies, using the Custom SureSelect hybrid capture system (Agilent Technologies, Santa Clara, CA). A list of genes and coordinates is provided in the Data Supplement. Library construction, sequencing, and processing are detailed in the Data Supplement. We classified variants as pathogenic driver mutations based on mutation type and position and on frequency in publicly available single nucleotide polymorphism databases. Variants with variant allele fraction (VAF) less than 0.02 were excluded. We performed additional amplicon-based deep validation sequencing on selected variants with low VAF, as detailed in the Data Supplement.

**Statistical Analysis**

We compared characteristics and outcomes of those with and without CHIP in the targeted sequencing cohort at the time of ASCT. The cumulative incidence of TMN was assessed using the `cmprsk` package in R (www.r-project.org), identifying death as a competing risk and censoring patients at the time of any subsequent allogeneic stem-cell transplantation. The cumulative incidence of relapse was assessed using death without relapse as a competing risk. Overall survival was measured from the date of ASCT to the date of death from any cause; data were censored at the time patients were last known to be alive. Event-free survival was measured from date of ASCT to date of event, defined as death, lymphoma relapse, or development of TMN. Mortality as a result of TMN was assessed in a cumulative incidence model identifying death from other causes as a competing risk; mortality as a result of other causes was assessed similarly, with patients censored at the time of subsequent allogeneic stem-cell transplantation. All P values were two-sided tests. Full details of the statistical analysis are provided in the Data Supplement.

### Results

**Pre-Existing Mutations in Patients With TMN**

To examine whether CHIP commonly precedes the development of TMN, we performed whole-exome sequencing on blood or bone marrow samples from patients who developed TMN after ASCT for lymphoma. Of the 12 patients whose sequencing met quality control standards, eight had at least one mutation in a known driver of myeloid malignancy in the TMN sample. In six of these eight patients, at least one somatic mutation from the TMN sample could be detected in the pre-ASCT sample at a VAF of at least 0.02. The genes mutated in both samples have been previously reported to be mutated in CHIP, including `PPM1D` (two patients), `TP53` (three patients), and `TET2` (two patients), with one patient having two mutations (Table 1; Data Supplement). These findings indicate that CHIP at the time of ASCT for lymphoma is common in patients who subsequently develop TMN.

**CHIP in Patients Undergoing ASCT**

We next examined the clinical consequences of CHIP in an independent set of 401 patients with non-Hodgkin lymphoma who underwent ASCT. We performed targeted sequencing of 86 genes on DNA purified from aliquots of mobilized stem-cell products collected immediately before ASCT. Unlike the exome sequencing cohort, patients were included in the targeted sequencing cohort regardless of whether they were subsequently diagnosed with TMN.

Among the 401 patients in the targeted sequencing cohort, 120 patients (29.9%) had at least one pathogenic somatic mutation at the time of ASCT that met inclusion criteria (Data Supplement). Relative to CHIP in the general population, mutations in `PPM1D` and `TP53` occurred at a higher frequency in this cohort.\textsuperscript{1,2} `PPM1D` was the most commonly mutated gene (55 mutations in 48 patients; Fig 1A). Mutations in `DNMT3A`, `TET2`, and `ASXL1` were also frequent, similar to CHIP in the general population. In contrast to CHIP in the general population, in which the vast majority of patients have only one mutation, 37 (30.8%) of the 120 patients had two or more mutations (Data Supplement). Patients with `PPM1D` mutations were 5.4 times more likely to have at least one additional mutation than those with other mutations (range, 2.3 to 12 times; P < .001).

The clinical characteristics of the targeted sequencing cohort are listed in Table 2. Consistent with the finding that CHIP is associated with increasing age in the general population,\textsuperscript{1,3} the median age of those with CHIP at ASCT was 61 years, compared
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NOTE. Only mutations with a VAF greater than 0.02 in at least one sample are listed.

Abbreviations: ASCT, autologous stem-cell transplantation; BM, bone marrow; MDS-U, myelodysplastic syndrome, unclassifiable; PB, peripheral blood; RAEB, refractory anemia with excess blasts; RARS, refractory anemia with ring sideroblasts; RCMD, refractory cytopenias with multilineage dysplasia; RCUD, refractory cytopenias with unilineage dysplasia; TMN, therapy-related myeloid neoplasm; VAF, variant allele fraction.

*Mutations present in both pre-ASCT samples and samples from the time of TMN.
†Variants validated by deep sequencing (Data Supplement).
with 57 years for those without CHIP (P < .001), although CHIP was also present in younger patients (Fig 1B). Patients with CHIP were more likely to have cytogenetic abnormalities at the time of ASCT than patients without CHIP and had subtle differences in CBC parameters (Data Supplement). There was no association between the presence of CHIP and other pre-ASCT clinical variables. Patients with CHIP required more days to collect an adequate number of stem cells and were more likely to fail peripheral mobilization and require bone marrow harvest, both of which remained significant on multivariable regression (Data Supplement).

**Risk of TMN**

We next examined whether CHIP at the time of ASCT alters the risk of TMN. Eighteen of the 401 patients in our targeted sequencing cohort developed biopsy-proven myeloid neoplasm, an overall 5- and 10-year cumulative incidence of 3.6% and 7.6%, respectively (median of 5 years of follow-up; Data Supplement). Of the 18 patients who developed TMN, 12 had CHIP with a VAF at least 0.02 at the time of ASCT. At 5 years, the cumulative incidence of TMN was 7.4% for patients with CHIP, compared with 1.7% for those without CHIP; by 10 years, the difference had widened to 14.1% vs 4.3%, respectively (P = .002; Fig 2A).

Patients with more than one mutation at the time of ASCT had a particularly elevated risk of TMN (5-year cumulative incidence, 16.5% for those with more than one mutation vs 4% for those with one mutation; 10-year cumulative incidence, 25.3% vs 9.9%, respectively; P < .001; Data Supplement). On multivariable modeling using a Fine-Gray competing risks regression, the following three variables were significantly associated with an elevated
risk of TMN: having received a nucleoside analog (cytarabine, fludarabine, or gemcitabine; subdistribution hazard ratio [SHR] 2.9; 95% confidence interval, 1.1 to 7.3; \( P = .03 \)), lifetime dose of cyclophosphamide exceeding 10 g/m\(^2\) including mobilization (SHR, 4.6; range, 1.7 to 12.5; \( P = .003 \)), and CHIP (SHR, 3.7; range, 1.4 to 9.9; \( P = .009 \); Data Supplement).

We then sought to determine the relationship of mutations present at ASCT to subsequent TMNs by sequencing bone marrow aspirate samples acquired at the time of TMN diagnosis (Data Supplement). Nine of 18 patients with TMN in the targeted sequencing cohort had an available sample, five of whom had CHIP. The spectrum of mutations in these TMNs was similar to that observed in the exome cohort, with frequent mutations in TP53 and PPMID and with frequent cooperating cytogenetic abnormalities, including loss of chromosomes 5 and 7, as well as complex karyotypes. We observed two major patterns of clonal relationships between the TMN and ASCT samples. In some cases, mutations present at the time of ASCT had expanded by the time of TMN development. In other cases, mutations present at ASCT remained at low levels, and the major TMN clone included mutations that had either been present at a VAF of less than 0.02 at the time of ASCT or had not been present at all. These data demonstrate that CHIP present at the time of ASCT is a powerful risk factor for subsequent TMN and may or may not directly evolve into the myeloid neoplasm itself.

**Impact of CHIP on Survival**

Having shown that CHIP at the time of ASCT is associated with an elevated risk of TMN, we next analyzed its effect on other outcomes. We found that patients with CHIP had significantly inferior overall and event-free survival compared with patients without CHIP (Figs 2B and 2C, respectively). At 5 years after ASCT, overall survival was 59.9% for patients with CHIP, compared with 72.4% for patients without CHIP; by 10 years, the overall survival rates were 30.4% and 60.9%, respectively (hazard ratio, 1.8; 95% CI, 1.4 to 2.8; \( P < .001 \) by
log-rank test). We observed an increased risk of mortality with both higher allele burden and greater number of mutations (Data Supplement). Survival was inferior in patients with CHIP regardless of age, efficiency of mobilization, aggregate burden of prior chemotherapy, or type of prior therapy (Data Supplement).

The other variables significantly associated with overall survival in univariate analysis were age and lymphoma type (Data Supplement). Although overall survival was inferior for patients with CHIP regardless of lymphoma type, we observed that the survival trajectories with CHIP differed for those with aggressive and nonaggressive lymphomas (Data Supplement). Therefore, we tested age $\geq 60$ years and presence of CHIP in a Cox proportional hazards model stratified by lymphoma type, and both remained significant (Fig 2E).

The difference in survival was driven primarily by an increased risk of nonrelapse mortality, which at 10 years was 26.2% for patients with CHIP compared with 11.1% for patients without CHIP ($P < .001$; Fig 2D; Data Supplement). Patients with CHIP had a higher rate of death as a result of TMN, with 5- and 10-year cumulative incidence rates of 2.6% and 7.6%, compared with 0.4% and 0.4% for patients without CHIP, respectively ($P = .001$; Data Supplement). We also observed an increased risk of death from ischemic cardiovascular disease (five deaths among patients with CHIP v two deaths in patients without CHIP; 5-year cumulative incidence, 4.3% v 0.3%, respectively; $P = .03$; Data Supplement). In aggregate, CHIP at the time of ASCT is associated with significantly inferior overall survival that is driven largely by an elevated risk of nonrelapse mortality, including an increased risk of dying from TMN and ischemic cardiovascular disease.

**PPM1D Mutations**

**PPM1D** was the most commonly mutated gene in the targeted sequencing cohort. **PPM1D** encodes the WIP1 phosphatase, which regulates p53, CHK1, CHK2, and other key components of the DNA damage response pathway. Somatic **PPM1D** mutations, all of which are nonsense or frameshift mutations in exon 6, have been found in the peripheral blood of women with a history of breast cancer and are common in CHIP in the general population.

Overall survival in the targeted sequencing cohort was inferior in patients with **PPM1D** mutations ($n = 48$) compared with patients with other mutations ($n = 72$; 10-year survival, 20.8% v 39.9%, respectively; $P = .02$ by log-rank test; Fig 3A). We did not observe a similar impact on survival for other recurrently mutated genes in the cohort (Data Supplement). Compared
with patients who had other mutations, there was no significant association of PPM1D mutations with lymphoma type (Data Supplement) or age (median, 61.5 years v 60 years for patients with other mutations; $P = .39$). Patients with PPM1D mutations had received more doxorubicin than patients with other mutations (Data Supplement).

Much of the mortality risk attributable to PPM1D mutations was a result of increased nonrelapse mortality, which at 10 years was 27.7% for patients with PPM1D mutations compared with 11.1% for patients without CHIP ($P = .001$). Although nonrelapse mortality for patients with PPM1D mutations was not significantly different from that of patients with other mutations, nonrelapse deaths for patients with PPM1D mutations peaked earlier (5-year nonrelapse mortality, 19.5% v 8.5%, respectively; Fig 3B). In aggregate, PPM1D mutations are common in the setting of ASCT for non-Hodgkin lymphoma and are associated with inferior survival primarily as a result of deaths occurring in the absence of relapse.

### DISCUSSION

In this study of patients undergoing ASCT for lymphoma, we found that CHIP is associated with elevated overall mortality, an increased risk of TMN, and a predisposition to death from cardiovascular disease. CHIP in our study of patients with lymphoma is more common than in prior studies of CHIP in patients unscreened for hematologic phenotype,1,2 potentially because of prior treatment with chemotherapy, use of growth factor mobilization, or the background of lymphoma. In addition, CHIP in this context has a distinct mutational spectrum, with a higher frequency of TP53 and PPM1D mutations and an increased percentage of individuals with more than one mutation.

TMN is a devastating complication of cytotoxic therapy. Previous studies have shown that some cases of TMN are preceded by clonal hematopoiesis, whether quantified as cytogenetic abnormalities,20 skewed X chromosome inactivation,21 or somatic mutations.12 In this study, we showed that the presence of CHIP at the time of ASCT is powerfully associated with the risk of subsequent TMN. Mutations detectable at the time of ASCT are often, but not always, the ones that drive the eventual TMN. In some cases, CHIP may reflect a state of the hematopoietic system that predisposes to the development of TMN, whether as a result of a deficiency of normal hematopoietic stem cells or an aberrant bone marrow microenvironment.

PPM1D was mutated recurrently in our exome sequencing cohort and was the most commonly mutated gene in our targeted sequencing cohort. PPM1D mutations have been shown to be present in the peripheral blood of patients with breast, ovarian, and lung cancer and are significantly associated with prior exposure to chemotherapy.19,22–24 Indeed, PPM1D mutations may be a marker of prior exposures that select for cells with impaired DNA damage response. We found PPM1D mutations to be present in this cohort with high frequency at the time of ASCT; they are also detectable in TMN samples, although often at low VAF, suggesting that they may not always be part of the dominant neoplastic clone. Beyond their involvement in TMN, PPM1D mutations were associated with inferior overall survival after ASCT, even when compared with patients who had mutations in other genes.

The association between CHIP and inferior survival should prompt a prospective evaluation of CHIP in patients newly diagnosed with lymphoma, focusing on the effect of pre-ASCT therapy on clonal expansion. Given the magnitude of risk conferred by CHIP in this setting, alternatives to ASCT could be explored for patients with CHIP and high-risk lymphoma in the context of clinical trials; this could include allogeneic transplantation, which would have the potential to eliminate the premalignant CHIP clone. The population of patients who receive ASCT with CHIP is a high-risk group and could be included in early-phase trials of targeted therapies directed against frequently mutated CHIP genes. Finally, the results of this study raise the question of whether CHIP is common and has a clinical impact after treatment of other cancers.

![Fig 3](image-url)
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