ABSTRACT

BACKGROUND  Clonal hematopoiesis of indeterminate potential (CHIP) is a novel cardiovascular disease (CVD) risk factor in individuals without acute myeloid leukemia (AML).

OBJECTIVES  The aim of this study was to examine the association between mutations associated with CHIP (CHIP-related mutations) identified in patients at AML diagnosis and the risk for cardiovascular events (CVEs).

METHODS  This was a retrospective cohort study of 623 patients with AML treated between 2015 and 2018 who underwent DNA analysis. Cause-specific hazard regression models were used to study the associations between pathogenic mutations in common CHIP-related genes (DNMT3A, TET2, ASXL1, JAK2, TP53, SRSF2, and SF3B1) and the rate of CVEs (heart failure hospitalization, acute coronary syndrome, coronary artery revascularization, ischemic stroke, venous thromboembolism, and CVD death) and between CVE development and all-cause mortality.

RESULTS  Patients were 64.6 ± 15.3 years of age, 265 (42.5%) were women, and 63% had at least 1 CHIP-related mutation. Those with CHIP-related mutations were older (69.2 ± 12.3 vs 56.6 ± 16.6 years; P < 0.001) and had a greater prevalence of CVD risk factors and CVD history. In adjusted analysis, the presence of any CHIP-related mutation was associated with a higher rate of CVEs (HR: 1.74; 95% CI: 1.03-2.93; P = 0.037) among intensively treated patients (anthracycline based) but not the whole cohort (HR: 1.26; 95% CI: 0.81-1.97; P = 0.31). TP53 (HR: 4.18; 95% CI: 2.07-8.47; P < 0.001) and ASXL1 (HR: 2.37; 95% CI: 1.21-4.63; P = 0.012) mutations were associated with CVEs among intensively treated patients. Interval development of CVEs was associated with all-cause mortality (HR: 1.99; 95% CI: 1.45-2.73; P < 0.001).

CONCLUSIONS  Among patients with AML treated with intensive chemotherapy, mutations in CHIP-related genes were associated with an increased risk for developing incident CVEs after AML diagnosis. (J Am Coll Cardiol CardioOnc 2022;4:38–49) © 2022 The Authors. Published by Elsevier on behalf of the American College of Cardiology Foundation. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).
CHIP-Related Mutations and CVD in AML

Can and cardiovascular disease (CVD) share several common risk factors. Clonal hematopoiesis is a novel shared risk factor for both diseases that has garnered interest within the cardiology, hematology, and oncology communities. Clonal hematopoiesis is the clonal expansion of hematopoietic stem and progenitor cells that have acquired somatic mutations resulting in a survival and proliferative advantage. The clonal hematopoiesis spectrum extends from CH of indeterminate potential (CHIP) and clonal cytopenias of undetermined significance to myelodysplastic syndrome and acute myeloid leukemia (AML). It is estimated that about 10% of individuals >70 years of age have CHIP. CHIP is associated with an 11-fold higher relative risk for developing hematologic malignancies but a modest absolute risk.

Individuals with CHIP (who, importantly, by definition do not have evidence of hematologic malignancies) are at 2- to 4-fold increased risk for developing CVD. Considering that AML is at the end of the clonal hematopoiesis spectrum, these patients may be at higher risk for cardiovascular events (CVEs) given their long-term exposure to white blood cells harboring CHIP-related mutations and resultant clonal expansion. In fact, recent studies have demonstrated an increased risk for CVD in patients with AML, especially in the context of intensive therapy with anthracyclines. However, it is unknown whether the presence of CHIP-related mutations in patients with AML contributes to this increased risk for CVD. Moreover, the association of incident CVEs after AML diagnosis with overall survival has not been widely explored. We use the term “CHIP-related mutations” to differentiate patients with AML who have mutations in 1 of these 7 genes (DNMT3A, TET2, ASXL1, TP53, JAK2, SRSF2, and SF3B1) described in CHIP, from patients who have other clonal mutations seen in AML. We hypothesized that among patients with AML, these specific mutations might represent early drivers.

**METHODS**

**PATIENTS.** This was a retrospective cohort study of consecutive patients with AML who were treated at Princess Margaret Cancer Centre between February 2015 and April 2018 and underwent next-generation sequencing (NGS) of DNA as part of a clinical study. Patients were included if NGS analysis of DNA was available from bone marrow or peripheral blood at diagnosis or before treatment within the clinical study database and at least 1 documented follow-up visit or discharge summary within the electronic medical record. Patients with acute promyelocytic leukemia or incomplete follow-up data were excluded. The index date was that of AML diagnosis. We collected demographics, cardiovascular risk factors (CVRFs) (diabetes, smoking, dyslipidemia, hypertension, obesity), CVD history (previous coronary artery disease, heart failure [HF], atrial fibrillation, stroke, peripheral artery disease), and AML variables (risk stratification, type of AML, treatment) from electronic medical records. AML risk stratification was performed according to European Leukemia Network 2017 criteria. Intensive chemotherapy was defined as at least 1 induction with anthracycline-based chemotherapy. Follow-up data were collected up to January 1, 2020.

**DNA ANALYSIS.** DNA was extracted from bone marrow (n = 517) or peripheral blood samples (n = 106), and targeted sequencing was performed using the TruSight Myeloid Sequencing Panel (Illumina), which profiles complete consensus coding sequences (15 genes) and hotspot regions (39 genes) of 54 clinically relevant genes in AML (Supplemental Table 1). Sequencing details are provided in the Supplemental Appendix.

For this study, we considered all oncogenic mutations (Supplemental Appendix) with variant allele frequencies (VAFs) >5% in the following genes to be CHIP-related mutations: DNMT3A, TET2, ASXL1, TP53, JAK2, SRSF2, and SF3B1. Mutations in these genes were selected because they are the most common variants among individuals with CHIP, representing more than 90% of mutations found in...
otherwise healthy individuals (except for PPM1D, which was not part of our panel). Among patients with AML, these specific mutations might represent early drivers.13,14 Of note, the term “CHIP-related mutations” is used throughout this paper to differentiate patients with AML who have mutations in 1 of these 7 genes described in CHIP from patients who have other clonal mutations seen in AML. It is important to recognize that this does not imply that patients with AML harboring these variants have coexisting CHIP, which is a different clinical entity within the spectrum of CH and by definition cannot coexist with AML. Mutations in the remaining 47 genes of the panel were considered non-CHIP-related mutations. Whenever 2 or more mutations in the same gene were present, the VAF of the dominant clone (highest VAF) was used for downstream analysis. The results of NGS were not used in the decision of intensive vs nonintensive therapy for AML.

**OUTCOMES.** The primary outcome was a composite of CVEs, including HF hospitalization, acute coronary syndrome (ACS), coronary artery revascularization, ischemic stroke, venous thromboembolism, and cardiovascular death (detailed definitions are provided in the Supplemental Appendix). Death before the occurrence of a CVE was treated as a competing risk.

| TABLE 1 | Baseline Characteristics and AML Treatment for the Whole Cohort and Patients With and Without CHIP-Related Mutations |
|---------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
|         | All (n = 623)                                                                                                                                   | CHIP-Related Mutation Negative (n = 230)                                                                 | CHIP-Related Mutation Positive (n = 393)                                                                 | P Value |
| Age, y  | 64.6 ± 15.3                                                                                                                                   | 56.6 ± 16.6                                                                                               | 69.2 ± 12.3                                                                                               | <0.001  |
| Female  | 265 (42.5)                                                                                                                                   | 100 (43.5)                                                                                               | 165 (42.0)                                                                                               | 0.72    |
| Body mass index, kg/m² | 27.5 ± 6.0                                                                                                                                   | 27.1 ± 5.8                                                                                               | 27.7 ± 6.0                                                                                               | 0.28    |
| Diabetes | 125 (20.1)                                                                                                                                   | 46 (20.0)                                                                                               | 79 (20.1)                                                                                               | 0.98    |
| Obesity | 139 (22.3)                                                                                                                                   | 52 (22.6)                                                                                               | 87 (22.1)                                                                                               | 0.89    |
| Hypertension | 246 (39.5)                                                                                                                                | 74 (32.2)                                                                                               | 172 (43.8)                                                                                               | 0.004   |
| Smoking | 254 (40.8)                                                                                                                                   | 81 (35.2)                                                                                               | 173 (44.0)                                                                                               | 0.031   |
| At least 1 CVRF | 453 (72.7)                                                                                                                                | 150 (65.2)                                                                                               | 303 (77.1)                                                                                               | 0.001   |
| Atrial fibrillation | 46 (7.4)                                                                                                                                   | 14 (6.1)                                                                                               | 32 (8.1)                                                                                               | 0.34    |
| Prior HF | 12 (1.9)                                                                                                                                   | 4 (1.7)                                                                                                 | 8 (2.0)                                                                                               | 0.99    |
| Prior CAD | 88 (14.1)                                                                                                                                  | 27 (11.7)                                                                                               | 61 (15.5)                                                                                               | 0.19    |
| Prior stroke | 9 (1.4)                                                                                                                                   | 3 (1.3)                                                                                                 | 6 (1.5)                                                                                               | 0.99    |
| Prior peripheral artery disease | 136 (21.8)                                                                                                                                  | 38 (16.5)                                                                                               | 98 (24.9)                                                                                               | 0.014   |
| Baseline medications |                                                                                                                                           |                                                                                                           |                                                                                                          |         |
| Aspirin | 85 (13.6)                                                                                                                                   | 21 (9.1)                                                                                               | 64 (16.3)                                                                                               | 0.012   |
| Statin | 167 (26.8)                                                                                                                                   | 46 (20.0)                                                                                               | 121 (30.8)                                                                                               | 0.003   |
| ACE inhibitor/ARB | 165 (26.5)                                                                                                                                  | 56 (24.3)                                                                                               | 109 (27.7)                                                                                               | 0.36    |
| Beta-blocker | 115 (18.5)                                                                                                                                  | 28 (12.2)                                                                                               | 87 (22.14)                                                                                               | 0.002   |
| Any cardiac medication | 290 (46.5)                                                                                                                                  | 83 (36.1)                                                                                               | 207 (52.7)                                                                                               | <0.001  |
| Baseline LVEF, % | 65.5 ± 6.5                                                                                                                                  | 65.3 ± 6.9                                                                                               | 65.6 ± 6.21                                                                                               | 0.63    |
| AML features |                                                                                                                                           |                                                                                                           |                                                                                                          |         |
| ELN adverse risk | 306 (49.1)                                                                                                                                  | 60 (26.1)                                                                                               | 246 (62.6)                                                                                               | <0.001  |
| Normal karyotype | 235 (37.7)                                                                                                                                  | 86 (37.4)                                                                                               | 149 (37.9)                                                                                               | 0.90    |
| Therapy-related AML | 52 (8.3)                                                                                                                                   | 21 (9.1)                                                                                               | 31 (7.9)                                                                                               | 0.59    |
| Secondary AML | 71 (11.4)                                                                                                                                    | 11 (4.8)                                                                                               | 60 (15.3)                                                                                               | <0.001  |
| Induction/anthracycline | 393 (63.1)                                                                                                                                  | 174 (75.7)                                                                                               | 219 (55.7)                                                                                               | <0.001  |
| Allo-HSCT | 188 (30.2)                                                                                                                                   | 90 (39.1)                                                                                               | 98 (24.9)                                                                                               | <0.001  |
| Patients receiving intensive therapy (n = 393) |                                                                                                                                           |                                                                                                           |                                                                                                          |         |
| Cumulative anthracycline dose (Dox equivalent), mg/m² | 285 (219-319)                                                                                                                                  | 290 (224-338)                                                                                             | 262 (155-310)                                                                                             | 0.013   |
| Allo-HCT | 188 (47.8)                                                                                                                                   | 87 (50.0)                                                                                               | 95 (43.4)                                                                                               | 0.19    |
| Relapsed/refractory disease | 174 (44.3)                                                                                                                                  | 69 (39.7)                                                                                               | 105 (48.0)                                                                                               | 0.10    |
| CR1 duration, mo | 18.5 (8-34.8)                                                                                                                                  | 24 (10-40)                                                                                               | 14 (7-30)                                                                                               | <0.001  |

Values are mean ± SD, n (%), or median (IQR). aHeart failure, coronary artery disease, stroke, peripheral artery disease, or atrial fibrillation. bTotal lifetime cumulative dose. ACE = angiotensin-converting enzyme; allo-HCT = allogeneic hematopoietic cell transplantation; AML = acute myeloid leukemia; ARB = angiotensin receptor blocker; CAD = coronary artery disease; CHIP = clonal hematopoiesis of indeterminate potential; CR1 = first complete remission; CVD = cardiovascular disease; CVRF = cardiovascular risk factor (diabetes, dyslipidemia, obesity, hypertension, or smoking); Dox = doxorubicin; ELN = European Leukemia Network; HF = heart failure; LVEF = left ventricular ejection fraction.
The secondary outcome was all-cause death. The study was approved by the University Health Network research ethics board.

### STATISTICAL ANALYSIS

Continuous variables are reported as mean ± SD or median (IQR) as appropriate, while categorical variables are expressed as frequencies and percentages. Baseline and treatment characteristics were compared between patients with and those without CHIP-related mutations using independent-samples Student’s t-tests or the Mann-Whitney U test for continuous variables according to distribution. The chi-square or Fisher exact test was used to compare categorical variables.

We used cumulative incidence function curves, stratified by presence of CHIP-related mutations, to describe the incidence of CVEs after AML diagnosis, with noncardiovascular death treated as a competing risk among the whole cohort and according to whether patients did or did not receive intensive chemotherapy. Statistical significance for these comparisons was determined using the Gray test. Cause-specific hazard regression models were used to study the univariable association between the rate of CVEs after AML diagnosis and baseline characteristics, with noncardiovascular death treated as a competing risk. This was followed by a multivariable cause-specific hazard regression model that included variables that were chosen a priori on the basis of clinical importance: age, sex, diabetes, dyslipidemia, obesity, hypertension, smoking, allogeneic hematopoietic cell transplantation (allo-HCT), previous CVD, and presence of ≥1 CHIP-related mutation. Allo-HCT was modeled as a time-varying covariate. Model results are presented using HRs and 95% CIs.

We conducted subgroup analyses of patients who received intensive therapy given that anticipated survival is expected to be different. Baseline left ventricular ejection fraction (LVEF) and lifetime cumulative doxorubicin-equivalent dose were treated as continuous variables, and the latter was handled as a time-varying covariate. These 2 variables were used along with those described earlier in a cause-specific regression model with incident CVEs as the outcome.

We then conducted a set of post hoc exploratory analyses. Given that $IDH1$ and $IDH2$ mutations have been recently associated with worsening LVEF in patients with AML, we adjusted for these mutations in our models. We also restricted our cohort to patients without previous CVD to assess the effect of CHIP-related mutations on the risk for CVEs among individuals without prior CVD. We then repeated the 2 primary models with specific CHIP-related mutations. We also examined the association between CVEs and the number of CHIP-related mutations and the VAFs of individual mutations. Finally, we assessed the unadjusted association between CHIP-related mutations and major adverse cardiovascular and cerebrovascular events (MACCE) (HF, ACS, coronary revascularization, ischemic stroke, and cardiac death) and venous thromboembolism separately.

Next, we used a cause-specific hazard regression model that incorporated interval CVE development as a time-varying covariate, along with the clinically relevant AML variables, to study the association of interval CVE development with all-cause mortality. The proportional hazards assumption was tested for each variable (except for time-varying covariates) by adding the cross-product of each variable with the natural logarithm of the time variable to the models.

SPSS version 25 (IBM) and SAS Studio version 3.8 (SAS Institute) were used for statistical analyses. Two-tailed $P$ values <0.05 were considered to indicate statistical significance.

### RESULTS

#### PATIENTS AND BASELINE CHARACTERISTICS

Of 646 consecutive patients with AML, 23 were excluded because of incomplete follow-up data, leaving 623 patients in the study. The mean age was 64.6 ± 15.3 years, and 265 (42.5%) were women. Overall, 393 patients (63.1%) had at least 1 CHIP-related mutation. A total of 613 CHIP-related mutations, 189 variants of
uncertain significance in CHIP-related genes, 1,111 non-CHIP-related mutations, and 316 variants of uncertain significance in non-CHIP-related genes were identified among the cohort. In total, 174 patients (27.9%) had only non-CHIP-related mutations, while 312 (50.1%) had CHIP-related and non-CHIP-related mutations. Only 81 (13%) and 56 (9%) patients had lone CHIP-related mutations and no mutations identified in our panel, respectively. The median number of mutated genes (CHIP-related and non-CHIP-related) per patient was 2 (IQR: 1-4). The distribution of CHIP-related and non-CHIP-related mutations is summarized in Supplemental Figures 1 and 2. Supplemental Table 2 shows the list of annotated variants in our patients.

**FIGURE 2** Incidence of Cardiovascular Events and Death as a Competing Risk

Cumulative incidence function (CIF) curves illustrating the risk for cardiovascular events (CVEs) and competing risk (CR) stratified by clonal hematopoiesis of indeterminate potential (CHIP)–related mutation status among the complete cohort (left), among patients treated with intensive chemotherapy (right), and among patients not treated with intensive chemotherapy (bottom). In the complete cohort, the 3-year cumulative incidence of CVEs (solid lines) among patients with and without CHIP-related mutations was 22.3% (95% CI: 17.6%-27.3%) vs 15.7% (95% CI: 11.1%-21.1%) (P = 0.17, Gray test). Dotted lines show comparison of CIF of death as a CR between both groups (51.1% [95% CI: 45.1%-56.8%] vs 26.9% [95% CI: 20.7%-33.5%]; P < 0.001). In the intensive chemotherapy group, the 3-year cumulative incidence of CVEs (solid lines) among patients with and without CHIP-related mutations was 25.8% (95% CI: 19.6%-32.4%) vs 15.4% (95% CI: 10.4%-21.4%) (P = 0.044, Gray test). Dotted lines show comparison of CIF of death as a CR between both groups (41.5% [95% CI: 34.3%-48.5%] vs 23.1% [95% CI: 16.7%-30.1%]; P < 0.001). In the nonintensive treatment group, the 3-year cumulative incidence of CVEs (solid lines) among patients with and without CHIP-related mutations was 15.3% (95% CI: 9.4%-22.5%) vs 14.2% (95% CI: 5.4%-27.1%) (P = 0.61, Gray test). Dotted lines show comparison of CIF of death as a CR between both groups (70.5% [95% CI: 59.3%-79.1%] vs 47.2% [95% CI: 27.5%-64.6%]; P = 0.14).
number of patients with each individual mutation and median VAF are summarized in Supplemental Table 3. Overall, median VAF of CHIP-related mutations ranged from 34% to 48%.

The baseline characteristics of all patients with AML and stratified by the presence of CHIP-related mutations are shown in Table 1. Patients with CHIP-related mutations were older (P < 0.001), with 78% being >60 years of age (Figure 1). These patients were more likely to have CVD risk factors and to have been treated with aspirin, statins, or beta-blockers. Ninety-eight patients (24.9%) with ≥1 CHIP-related mutation had at least 1 prior CVE event (HF, CAD, stroke, peripheral artery disease, or atrial fibrillation) compared with 38 (16.5%) without any CHIP-related mutations (P = 0.014).

CVEs AFTER AML DIAGNOSIS. During a median follow-up time of 13.3 months (IQR: 3.2–30.5 months), 101 patients (16.2%) developed at least 1 CVE, including 37 HF hospitalizations, 15 ACS, 7 coronary revascularizations, 12 ischemic strokes, 49 venous thromboembolism events, and 17 cardiac deaths. At 3 years, the cumulative incidence of CVEs was 19.5% (95% CI: 16.1%-23.2%), while the incidence of death before a CVE was 41.5% (95% CI: 37.1%-46.0%). The 3-year risk for CVEs in patients with CHIP-related mutations was 22.3% (95% CI 17.6%-27.3%) compared with 15.7% (95% CI: 11.1%-21.1%) in those without these mutations (P = 0.17) (Figure 2).

A summary of patient characteristics according to incident CVEs and death before CVEs is provided in Supplemental Table 4. On univariable analysis, the risk for CVEs was associated with age, presence of CVRFs, prior CVD, baseline LVEF, allo-HCT, and CHIP-related mutations (Supplemental Table 5). After multivariable adjustment, dyslipidemia (HR: 1.65; 95% CI: 1.00-2.67; P = 0.043), previous CVD (HR: 1.93; 95% CI: 1.19-3.13; P = 0.008), and allo-HCT (HR: 2.35; 95% CI: 1.30-4.26; P = 0.005), but not the presence of any CHIP-related mutation (HR: 1.26; 95% CI: 0.81-1.97; P = 0.31), were significantly associated with incident CVEs (Table 2). However, among specific CHIP-related mutations, TP53 was associated with an increased rate of CVEs after multivariable adjustment (HR: 1.90; 95% CI: 1.06-3.39; P = 0.031) (Table 3).

There were 393 anthracycline-treated patients, among whom 74 (18.8%) developed at least 1 CVE. The 3-year risk for CVEs in patients with CHIP-related mutations was 25.8% (95% CI: 19.6%-32.4%) compared with 15.4% (95% CI: 10.4%-21.4%) in those without (P = 0.004) (Figure 2). Multivariable analysis demonstrated that the rate of CVEs was significantly associated with baseline LVEF, prior CVD, diabetes, allo-HCT, and CHIP-related mutations (HR: 1.74; 95% CI: 1.03-2.93; P = 0.037) (Table 2). Both TP53 (HR: 4.18; 95% CI: 2.07-8.47; P < 0.001) and ASXL1 (HR: 2.37; 95% CI: 1.21-4.63; P = 0.012) were positively associated with the occurrence of CVEs (Table 3).

In total, 147 patients (23.6%) had non-CHIP-related mutations in IDH1 and/or IDH2. In exploratory analysis, adjusting for these mutations in the regression models did not change the association between CHIP-related mutations and CVEs (Supplemental Table 6). When restricting the analysis to patients in our cohort without preexisting CVD at AML diagnosis, the association between CHIP-related mutations and CVEs was stronger in the intensively treated group (HR: 2.37; 95% CI: 1.27-4.43; P = 0.007) but remained nonsignificant in the whole cohort (HR: 1.60; 95% CI: 0.93-2.75; P = 0.092) (Supplemental Table 7). Finally, the unadjusted HRs for CHIP-related mutations for MACCE and venous thromboembolism were 1.45 (95% CI: 0.85-2.47; P = 0.17) and 1.75 (95% CI: 0.95-3.23; P = 0.074), respectively, for the whole cohort and 1.64 (95% CI: 0.90-2.98; P = 0.10) and 1.93 (95% CI: 0.99-3.78; P = 0.054), respectively, for intensively treated patients.

The distribution of specific CHIP-related and non-CHIP-related mutations among patients who developed CVEs is shown in Supplemental Figure 3. The number of CHIP-related mutations and VAF were not associated with CVEs in the whole cohort (Supplemental Table 8). However, among intensively treated patients, defined as at least 1 induction with anthracycline-based chemotherapy, the presence of 2

| Table 2 Multivariable Cause-Specific Hazard Regression Analyses for the Outcome of Cardiovascular Events |
|-------------------------------------------------|---------------------------------|---------------------------------|---------------------------------|---------------------------------|
| Function Manner Hazard Regression Analyses for the Outcome of Cardiovascular Events                     |
| Complete Cohort | Intensive Chemotherapy Cohort |
| Value HR (95% CI) | Value HR (95% CI) |
| Age | 1.01 (0.99-1.03) | 1.01 (0.99-1.04) |
| Female | 1.21 (0.79-1.88) | 1.17 (0.68-2.02) |
| Diabetes | 1.26 (0.77-2.06) | 1.76 (1.00-3.11) |
| Dyslipidemia | 1.65 (1.0-2.67) | 1.37 (0.77-2.45) |
| Obesity | 1.32 (0.86-2.02) | 1.10 (0.66-1.86) |
| Hypertension | 1.19 (0.73-1.92) | 0.49 (0.65-2.49) |
| Smoking | 1.35 (0.88-2.06) | 0.98 (0.59-1.64) |
| Allo-HSCTa | 2.35 (1.30-4.26) | 2.41 (1.25-4.65) |
| Prior CVDb | 1.93 (1.9-3.13) | 2.43 (1.35-4.38) |
| Any CHIP-related mutation | 1.26 (0.81-1.97) | 1.74 (1.03-2.93) |
| Baseline LVEF (per 5%) | – | 0.72 (0.59-0.87) |
| Cumulative anthracycline dose (per 50 mg/m² of doxorubicin equivalent) | – | 1.08 (0.99-1.19) |

*Modeled as a time-varying covariate. Heart failure, coronary artery disease, stroke, peripheral artery disease, or atrial fibrillation. HR = hazard ratio; other abbreviations as in Table 1.
or more CHIP-related mutations was associated with a higher rate of CVEs compared with those without CHIP-related mutations (HR: 2.54; 95% CI: 1.27-5.05; \( P = 0.008 \)). Similarly, the HR for a CVE for each 5% increase in TP53 VAF was 1.10 (95% CI: 1.04-1.17; \( P = 0.002 \)). There were no associations between other individual mutation VAFs and CVEs in the full cohort or in the intensively treated patients (Supplemental Table 8).

**CHIP-RELATED MUTATIONS AND AML CHARACTERISTICS.** Patients with CHIP-related mutations tended to have higher risk AML. Such patients were more likely to be classified within the European Leukemia Network adverse risk category (62.6% vs 26.1%; \( P < 0.001 \)), to have secondary AML (15.3% vs 4.8%; \( P = 0.001 \)), and to have a shorter median first complete remission duration (14 months [IQR: 7-30 months] vs 24 months [IQR: 10-40 months]; \( P < 0.001 \)) if treated with intensive chemotherapy. Despite these higher risk AML features, and consistent with older age and comorbidities, patients with CHIP-related mutations were less likely to receive intensive chemotherapy and undergo allo-HCT (Table 1). Among patients who received anthracycline induction, those with CHIP-related mutations received lower cumulative anthracycline doses.

**DEATH.** Overall, 277 patients (44.5%) died during follow-up (median time to death 9.5 months; IQR: 3.2-18.5 months). In adjusted analysis, the occurrence of a new CVE after AML diagnosis (HR: 1.99; 95% CI: 1.45-2.73; \( P < 0.001 \)) and the presence of CHIP-related mutations (HR: 1.54; 95% CI: 1.14-2.08; \( P = 0.005 \)) were independently associated with an increased rate of all-cause death (Table 4). Other variables associated with all-cause death included age, diabetes, European Leukemia Network adverse risk category, and secondary AML (Table 4). Patients harboring 2 or more CHIP-related mutations were at the highest risk for death (Supplemental Table 9).

**DISCUSSION**

We conducted a retrospective cohort study of 623 patients with AML, of whom 63% had CHIP-related mutations. Individuals with CHIP-related mutations were older and had a higher prevalence of CVRFs and CVD at time of AML diagnosis (Central Illustration). Incident CVEs were more frequent in patients with CHIP-related mutations compared with those without (3-year cumulative incidence 22.3% vs 15.7%). The presence of 1 or more CHIP-related mutations was independently associated with an increased risk for CVEs (HR: 1.74) in patients treated with intensive chemotherapy (defined as at least 1 induction with anthracycline-based chemotherapy) but not in the whole cohort. The interval development of CVEs after the diagnosis of AML was independently associated with an HR of about 2 for all cause-death.

Consistent with prior studies, we observed a high incidence of CVEs (\( > 20% \) at 3 years) after AML diagnosis in our cohort.\(^{16,19}\) This can be attributed, at least in part, to the high prevalence of CVRFs and preexisting CVD. Approximately 70% of our patients had at least 1 CVRF, and the prevalence of individual CVRFs ranged from 20% to 40%. This high prevalence may reflect advanced age or shared risk factors for AML and CVD.\(^{16,20}\) In patients without hematologic malignancies, CHIP is now recognized as a risk factor for myocardial infarction, stroke, HF, thromboembolic disease, and poor prognosis in aortic stenosis, independent of traditional CVRFs.\(^{7,21-23}\) These associations are supported by robust evidence of causality from animal models and translational experiments.\(^{7,24}\) Although it is likely that the presence of somatic mutations described in CHIP (ie, CHIP-related mutations) when also identified in patients with AML may similarly increase the incidence of CVD, this association has not been previously examined. It is important to highlight that the use of the term “CHIP-related mutations” in our work does not imply that patients with AML have CHIP, as these entities do not coexist. It is rather used to differentiate mutations described in CHIP but also identified

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**TABLE 3 Multivariable Cause-Specific Hazard Regression Analyses for Cardiovascular Events Including Individual CHIP-Related Mutations as Covariates**

| Covariate | Complete Cohort | Intensive Chemotherapy Cohort |
|-----------|----------------|------------------------------|
|           | HR (95% CI)    | P Value | HR (95% CI) | P Value |
| Age       | 1.01 (0.99-1.03) | 0.14 | 1.03 (0.99-1.05) | 0.059 |
| Female    | 1.25 (0.82-1.89) | 0.30 | 1.50 (0.90-2.50) | 0.12 |
| At least 1 CVRF | 1.40 (0.82-2.39) | 0.22 | 1.06 (0.59-1.90) | 0.86 |
| Allo-HSC \(^a\) | 2.36 (1.30-4.29) | 0.005 | 2.33 (1.20-4.55) | 0.013 |
| Prior CVD \(^b\) | 2.24 (1.39-3.61) | <0.001 | 2.94 (1.64-5.27) | <0.001 |
| DNMT3A    | 0.86 (0.52-1.42) | 0.56 | 0.99 (0.56-1.76) | 0.97 |
| TET2      | 1.16 (0.69-1.95) | 0.57 | 1.69 (0.91-3.16) | 0.098 |
| ASXL1     | 1.40 (0.80-2.46) | 0.24 | 2.37 (1.21-4.63) | 0.012 |
| TP53      | 1.90 (1.06-3.39) | 0.031 | 4.18 (2.07-8.47) | <0.001 |
| JAK2 \(^c\) | 0.32 (0.04-2.33) | 0.26 | – | – |
| SF3BI     | 0.71 (0.25-1.98) | 0.51 | 0.75 (0.17-3.25) | 0.70 |
| SRSF2     | 0.83 (0.45-1.52) | 0.55 | 1.02 (0.49-2.09) | 0.97 |
| Baseline LVEF (per 5%) | – | – | 0.72 (0.59-0.86) | <0.001 |
| Cumulative anthracycline dose (per 50 mg/m\(^2\) of doxorubicin equivalent) \(^d\) | – | – | 1.12 (1.02-1.23) | 0.019 |

\(^a\)Modeled as a time-varying covariate. \(^b\)Heart failure, coronary artery disease, stroke, peripheral artery disease, or atrial fibrillation. \(^c\)No patients in the anthracycline-treated cohort had cardiovascular events. \(^d\)Abbreviations as in Tables 1 and 2.
in patients with AML vs other somatic mutations seen in AML.

In our AML cohort that received intensive chemotherapy, the presence of any CHIP-related mutation was associated with an increased risk for incident CVEs of about 75%, independent of age, sex, traditional CVRFs, preexisting CVD, LVEF, cumulative anthracycline dose, and IDH1/2 mutations. However, this was not seen when patients who were not treated intensively were also included in the analysis. This is likely driven by the higher competing risk for AML-related death, especially among those with CHIP-related mutations, who were likely considered unfit for intensive therapy despite having more adverse AML features, as seen in our study. This observation relates to the association of CHIP-related mutations with older age, which in turn is associated with more comorbidities (eg, preexisting CVD, CVRFs) and frailty, increasing the likelihood of non-intensive management approaches and inflating the competing risk for early death before developing CVEs. However, we were unable to assess this hypothesis given the lack of follow-up blood and bone marrow samples. Given the known poor prognosis among patients with AML with mutated TP53, it is more likely that our findings are explained by blunting of the competing risk for AML-related death in the intensively treated patients.26 Loss of TP53 in mice has been associated with atherosclerosis progression.27 However clinical data specific to mutated TP53-related CVD is not available given the low frequency of these mutations outside the malignancy setting. In contrast, among individuals with CHIP (ie, in the absence of hematologic malignancies), the presence of mutated ASXL1 is associated with a 2-fold higher risk for incident CAD compared with absence of this mutation.28 Nevertheless, mechanisms for atherosclerosis and HF specific to ASXL1 mutations remain to be elucidated.

In our cohort, DNMT3A, TET2, and JAK2 mutations were not independently associated with CVEs. It is possible that some individuals with AML and these specific mutations may represent a different phenotype in which the risk for early AML-related death maybe greater than the CVD risk. Conversely, patients with these mutations in the setting of CHIP (ie, stage prior to AML) may have developed CVD and died before the development of hematologic malignancies, leading to selection bias toward lower cardiovascular risk CHIP-related mutations in subjects who subsequently developed AML.26 Hence, CVD and AML may function as mutual competing risks for death across the clonal hematopoiesis spectrum with variations within the mutational landscape. In support of our hypothesis, 2 recent studies including individuals >80 years of age showed that participants harboring DNMT3A and TET2 mutations were not at higher risk for all-cause death or cardiovascular mortality than patients without such mutations.29 This suggests a survivorship bias among highly aged individuals who have an expected high mortality rate from various other causes. Alternatively, the risk for CVEs attributed to CHIP-related mutations may be more complex than attributing risk to individual genes only. Our exploratory analysis suggests that the total clonal burden attributed to CHIP-related mutations (presence of 2 or more CHIP-related mutations) may play a role in the development of CVEs.

Approximately 50% of our cohort had CHIP-related mutations but no prior CVD at AML.
diagnosis. Among these patients, those who received intensive chemotherapy and harbored CHIP-related mutations had a >2-fold risk for developing CVEs. It is possible that these patients could have had subclinical CVD that became clinically overt after the onset of AML. Consistent with prior research, among patients treated with anthracyclines in our cohort, LVEF was a strong independent predictor of subsequent CVEs. LVEF was not associated with CHIP-related mutations. Overall, this highlights the potential clinical importance of incorporating the presence of CHIP-related mutations into cardiovascular risk stratification strategies in addition to CVD history, CVRFs, full cohort model and

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**CENTRAL ILLUSTRATION**

CHIP-Related Mutations, CV Events, and Mortality

**Prevalence of CHIP-related mutations in study sample (n=623)**
- No CHIP-related mutation (n=230; 37%)
- ≥1 CHIP-related mutation (n=393; 63%)

**3-year Cumulative Incidence of CV Events & Mortality**

| CHIP-Related Mutation | CV Events | Mortality |
|-----------------------|-----------|-----------|
| None                  | 0%        | 0%        |
| ≥1                    | 16%       | 22%       |

**Adjusted HR (95% CI) for CV Events in Patients with Any CHIP-Related Mutation**

| Mutation | HR (95% CI) |
|----------|-------------|
| TP53     | 1.90 (1.06-3.39) |
| ASXL1    | 1.40 (0.80-2.46) |

**Adjusted HR (95% CI) for All-Cause Mortality**

| Patients Experiencing Any CV Event | Patients with Any CHIP-Related Mutation |
|------------------------------------|----------------------------------------|
| 1.99 (1.45-2.73)                   | 1.54 (1.14-2.08) |

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Clonal hematopoiesis of indeterminate potential-related mutations (DNMT3A, TET2, ASXL1, TP53, JAK2, SF3B1, and SRSF2) are commonly present among patients with acute myeloid leukemia (AML) and are associated with higher risk for cardiovascular events (CVEs) in those receiving intensive chemotherapy. The following variables were included in CVE models of the full cohort: age, sex, cardiovascular risk factors (diabetes, dyslipidemia, hypertension, obesity, smoking), cardiovascular disease (heart failure, coronary artery disease, peripheral vascular disease, atrial fibrillation, stroke), and allogeneic hematopoietic cell transplantation. The following variables were included in CVE models of patients treated with intensive chemotherapy: all variables included for the full cohort, baseline left ventricular ejection fraction, and cumulative anthracycline dose. The following variables were included in the all-cause mortality model: all variables included in the full cohort model, European Leukemia Network adverse risk category, secondary AML, and therapy-related AML. Noncardiovascular mortality was used as competing risk for CV events. CHIP = clonal hematopoiesis of indeterminate potential; CV = cardiovascular; CVD = cardiovascular disease.
baseline left ventricular systolic function in patients with AML.10

The clinical relevance of our findings is highlighted by the fact that incident CVEs were associated with higher mortality among patients with AML. To put this into context, the risk for dying once CVEs occurred was comparable with that attributed to adverse-risk AML by European Leukemia Network classification. As such, the multidisciplinary management of these patients by involving primary practice and cardio-oncology services should be considered to mitigate cardiovascular risk. This may be most relevant for patients who are considered candidates for intensive chemotherapy regimens, in whom the survival benefit from treatment could be blunted by the development of CVEs. In addition, current management of AML is undergoing a practice shift toward targeted therapies according to specific genomic profiling.31 The preliminary results of the BEAT AML (A Master Protocol for Biomarker-Based Treatment of AML) trial have shown that a genetically targeted therapy-based approach according to mutation profile among individuals with AML older than 60 years resulted in an increased median survival (12.8 vs 3.9 months) compared with standard of care (intensive therapy or nonintensive hypomethylating treatment).32 Hence, in the future, prevention of the development of CVEs would become relevant in patients with high-risk AML even in the context of nonintensive therapy. As NGS becomes widely available and part of the standard of care for patients with cancer, the identification of patients at high risk for CVEs on the basis of the presence of CHIP-related mutations as one method represents a unique opportunity to explore and incorporate precision medicine-based interventions in collaboration with cardio-oncology programs to mitigate CVD risk.

STUDY LIMITATIONS. Our study was retrospective in nature. We considered any oncogenic mutation rather than specific variants to establish a more generalizable approach. As different mutations within the same gene (eg, DNMT3A R882) may be associated with different AML or cardiovascular profiles, the effect of specific variants on cardiovascular risk may have been underestimated.32 Our study was not powered to assess the association between CHIP-related mutations and individual CVEs. However, we show in unadjusted analysis that CHIP-related mutations have similar direction of association with MACCE and VTEs separately. This association warrants further exploration in larger cohorts. Our gene panel did not include some mutations associated with CHIP, such as PPM1D and GNB1, which may be a potential source of residual confounding. In our multivariable analyses, given the number of outcomes, some of our models may be overfit. However, we chose to adjust for clinically relevant confounders. Also the ratio of predictors to outcomes was still within the acceptable range.33 We also show that most patients with CHIP-related mutations had concomitant non-CHIP-related mutations. However, given our sample size, we were unable to explore whether interactions regarding the occurrence of CVEs exist between specific CHIP-related and non-CHIP-related mutations or between specific CHIP-related mutation combinations in a similar way as seen with AML prognosis.14

CONCLUSIONS

In patients with AML, those treated with intensive chemotherapy who harbor somatic mutations that have been previously described in CHIP (ie, in the absence of malignancy) were at a higher risk for developing CVEs after AML diagnosis. Individual CHIP-related mutations in TP53 and ASXL1 are associated with a 2- to 4-fold increased risk for CVEs. The development of CVEs was associated with higher risk for subsequent overall death. These observations suggest that patients with AML and CHIP-related mutations may benefit from cardio-oncology consultation for consideration of prevention strategies, aggressive cardiovascular risk management, or more intensive cardiovascular monitoring. This is particularly relevant for those patients who will receive intensive chemotherapy for their AML.

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ADDRESS FOR CORRESPONDENCE: Dr Paaladinesh Thavendiranathan, Ted Rogers Program in Cardiotoxicity Prevention, Peter Munk Cardiac Center, Toronto General Hospital, University of Toronto, 585 University Avenue, Toronto, Ontario M5G2 N2, Canada. E-mail: dinesh.thavendiranathan@uhn.ca. Twitter: @dineshpmcct, @oscaar84, @drmarcarillos, @husam247, @pnatarajanmd.

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APPENDIX For outcome definitions, next generation sequencing as well as supplemental tables, figures and references, please see the online version of this paper.