# Clonal Hematopoiesis of Indeterminate Potential in Patients with Immunoglobulin Light Chain AL Amyloidosis

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Paolo Lopedote (St Elizabeth's Medical Center, United States) Benjamin Evans (Brigham and Women's Hospital, United States) Alfredo Marchetti (University of Milan, Italy) Tianzeng Chen (Brigham and Women's Hospital, United States) Maria Moscvin (Brigham and Women's Hospital, United States) Samuel Boullt (Brigham and Women's Hospital, United States) Niccolo Bolli (University of Milan, Italy) Giada Bianchi (Brigham and Women's Hospital, United States)

**Abstract:**
Immunoglobulin light chain (AL) amyloidosis is characterized by the deposition of misfolded monoclonal free light chains, with cardiac complications accounting for patients' mortality. Clonal hematopoiesis of indeterminate potential (CHIP) has been associated with worse cardiovascular outcomes in the general population. Its significance in AL amyloidosis remains unclear. We collected clinical information and outcome data on 76 patients with a diagnosis of AL amyloidosis who underwent deep-targeted sequencing for myeloid neoplasia-associated mutations between April 2018 and August 2023. Variant allele fraction was set at 2% to call CHIP-associated mutations. CHIP mutations were present in AL amyloidosis patients at a higher frequency than age-matched control individuals. Sixteen patients (21%) had at least 1 CHIP mutation. DNMT3A was the most frequent mutation (7/16, 44%). Compared to patients without CHIP, patients with CHIP were enriched for the presence of t(11;14) (69% vs 25%, respectively, p = 0.004) and, for patients with renal involvement, a lower Palladini renal stage (p = 0.001). At a median follow-up of 32.5 months, the presence of CHIP was not associated with worse overall survival or major organ dysfunction progression-free survival. Larger studies and longer follow-up are needed to better define the impact of CHIP in patients with AL amyloidosis.

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1   **Clonal Hematopoiesis of Indeterminate Potential in Patients with Immunoglobulin Light**

2   **Chain AL Amyloidosis**

3   **CHIP in AL Amyloidosis**

4   Paolo Lopedote[1], Benjamin Evans[2,3*], Alfredo Marchetti[4*], Tianzeng Chen[2,3], Maria Moscvin[2,3], Samuel

5   Boullt[2,3], Niccolo' Bolli[4,5], and Giada Bianchi[2,3,6]

6   [1]Department of Medicine, St. Elizabeth's Medical Center, Boston University, Boston, MA; [2]Amyloidosis

7   Program, Brigham and Women's Hospital, Dana Farber Cancer Institute, Boston, MA; [3]Division of

8   Hematology, Brigham and Women's Hospital, Boston, MA; [4]Department of Oncology and Onco-

9   Hematology, University of Milan, Milan, Italy; [5]Hematology Section, Fondazione IRCCS Ca' Granda

10  Ospedale Maggiore Policlinico, Milan, Italy; [6]Harvard Medical School, Boston, MA

11

12  B.E. and A.M. contributed equally to this study.

13

14  Corresponding author:   Giada Bianchi, MD

15                          Harvard Institute of Medicine

16                          4 Blackfan Circle

17                          Boston, MA

18                          02115

19                          E-mail: gbianchi1@bwh.harvard.edu

20                          Phone: 617-525-4953

21                          Fax: 617-525-4986

22

23  For original data, please contact gbianchi1@bwh.harvard.edu

24

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30  **Key Points**

31  • CHIP is more prevalent in AL amyloidosis patients than in the general population

32  • CHIP presence was associated with the presence of t(11;14), and a lower Palladini renal

33  stage in patients with renal involvement

34

35  **Abstract**

36  Immunoglobulin light chain (AL) amyloidosis is characterized by the deposition of misfolded

37  monoclonal free light chains, with cardiac complications accounting for patients' mortality.

38  Clonal hematopoiesis of indeterminate potential (CHIP) has been associated with worse

39  cardiovascular outcomes in the general population. Its significance in AL amyloidosis remains

40  unclear. We collected clinical information and outcome data on 76 patients with a diagnosis of

41  AL amyloidosis who underwent deep-targeted sequencing for myeloid neoplasia-associated

42  mutations between April 2018 and August 2023. Variant allele fraction was set at 2% to call

43  CHIP-associated mutations. CHIP mutations were present in AL amyloidosis patients at a higher

44  frequency than age-matched control individuals. Sixteen patients (21%) had at least 1 CHIP

45  mutation. DNMT3A was the most frequent mutation (7/16, 44%). Compared to patients without

46  CHIP, patients with CHIP were enriched for the presence of t(11;14) (69% vs 25%, respectively,

47  p = 0.004) and, for patients with renal involvement, a lower Palladini renal stage (p = 0.001). At

48  a median follow-up of 32.5 months, the presence of CHIP was not associated with worse overall

49 survival or major organ dysfunction progression-free survival. Larger studies and longer follow-

50 up are needed to better define the impact of CHIP in patients with AL amyloidosis.

## Introduction

Immunoglobulin light chain (AL) amyloidosis is a plasma cell (PC) dyscrasia whose hallmark is the production and secretion of misfolded, monoclonal immunoglobulin free light chains (FLC).[1,2] The circulating FLC drives pathogenesis causing direct cytotoxicity in its soluble form and disruption of target organ architecture upon deposition as insoluble fibrillary aggregates. It is thought that any organ/system in the body can be involved by AL amyloidosis deposition with the heart and kidneys being the most commonly affected organs.[3,4] The severity of cardiac involvement is the single most important prognostic factor in AL amyloidosis as cardiac events represent the major cause of mortality.[5,6] Renal involvement occurs in up to 70% of cases and is a key determinant of patients morbidity, decreased quality of life, and ineligibility for clinical trials.[7,8] To aid in patients' prognostication and management, several staging systems have been developed to evaluate cardiac involvement [9–12], while Palladini and colleagues have introduced a score predicting the risk of progression to renal replacement therapy.[7] Cytogenetic alterations have also prognostic implications and are an important predictor of response to anti-neoplastic therapies.[13–16] Among those, t(11;14) is the most prevalent.[16] It portends a lower response rate to bortezomib-based therapies[13] and is a negative prognostic factor, being associated with decreased progression-free survival (PFS) compared to patients without any alteration.[15] Based on the recently published Andromeda study, daratumumab appears to overcome the negative prognostic impact of t(11;14).[8] Further, extrapolating from multiple myeloma, the presence of t(11;14) is a biomarker for response to Bcl2 inhibitor venetoclax.[4,8,17]

Clonal hematopoiesis of indeterminate potential (CHIP) refers to the presence of clonal, somatic mutations of myeloid-related genes in the absence of overt myeloid neoplasia or cytopenia. The most commonly CHIP-associated mutations involve DNMT3A, TET2, and/or ASXL1 genes,

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74    commonly known under the acronym DTA.[18] CHIP incidence increases with age, being most

75    frequent in the elderly population and nearly absent before the age of 40.[19–23] Its occurrence has

76    been associated with a higher incidence of hematological malignancies[19,20,22] and cardiovascular

77    disease, as well as worse cardiovascular outcomes[22,24–27]. In the context of hematological

78    neoplasms, CHIP has been detected in 9.7 – 21.6% of patients with PC dyscrasia and 14 – 29%

79    with lymphoma.[23,28–32] In patients with multiple myeloma (MM) and lymphoma receiving

80    autologous stem cell transplant (ASCT), CHIP presence was identified as an adverse prognostic

81    factor.[28,31] Thari and colleagues also noted a higher risk of progression to Waldenström

82    macroglobulinemia (WM) in patients with IgM monoclonal gammopathy of undeterminate

83    significance or smoldering WM carrying DTA mutations.[29] Two studies previously reported on

84    the incidence of CHIP in patients with AL amyloidosis. The incidence of CHIP was 15% (4 out

85    of 27 patients) in one study and 21% (10 out of 47 patients) in the other and did not correlate

86    with any specific clinical features. It is important to note that while the presence of CHIP was not

87    found to have prognostic significance in these studies, the association between CHIP and

88    cytogenetic alterations or cardiac involvement was not investigated.[32,33]

89    We were interested in exploring whether an association exists between CHIP and cardiac

90    outcome, based on prior studies. We were also interested in understanding the co-existence of

91    relevant disease characteristics with CHIP.

92    Hence, we performed a single-center, retrospective cohort study including 76 consecutive

93    patients with AL amyloidosis who were seen at BWH/DFCI between April 2018 and August

94    2023 and had a bone marrow biopsy performed with a targeted myeloid mutation panel assessed.

95    This is the largest study to date looking broadly at the prevalence and impact of the presence of

96    CHIP in AL amyloidosis patients.

97

## Materials and Methods

<u>Patients</u>

We identified patients seen at BWH/DFCI for a diagnosis of AL amyloidosis who underwent deep-targeted sequencing for myeloid neoplasia-associated mutations between April 2018 and August 2023. We retrospectively collected clinical information, including age, gender, ethnicity, smoking status, FLC subtype, European modification of 2004 Mayo stage, organ involvement, cytogenetics alterations detected by fluorescent in-situ hybridization (FISH), left ventricular ejection fraction, Palladini Renal stage for patients with renal involvement.[7,9] Additionally, we looked at the type of anti-neoplastic treatment, depth of hematological response, and whether or not patients received an ASCT. We chose as a primary outcome the major-organ dysfunction event-free survival (MOD-PFS) as defined by Kastritis and colleagues[8] and the overall survival (OS). We selected as a secondary outcome the cardiac-specific disease response and PFS (assessed at 6 months and 12 months post-commencement of therapy), as defined by Palladini and colleagues.[7] Next-generation sequencing on bone marrow samples was performed using our custom-validated assay, Rapid Heme Panel (RHP).[34] Genes assessed for CHIP attribution included: JAK1, JAK3, PDGFRA, SFA3A1, DNMT3A, GNB1, CEBPA, SBDS, FLT3, KRAS, BCORL1, PIGA, SF3B1, ASXL1, CTCF, CSF3R, CUX1, NOTCH3, PPM1D, ZRSR2, ATM, CCND1, KMT2A, EP300, EZH2, SETD2, SH2B3, GNAS, GATA1, IKZF3, PRPF8, KIT, NOTCH2, WT1, TET2, PIK3CA, PTPN11, CREBBP, NOTCH1, BRCC3, DDX41, TP53, CALR, LUC7L2. The median coverage obtained was 759 reads per base (IQR 1131).

118 CHIP status was assigned to patient samples when putative driver lesions in genes associated

119 with myeloid neoplasms were observed at Variant Allele Frequency (VAF) higher than 2%.

120 Reported variants were then analyzed and filtered according to common practice standards

121 through a semi-automatic pipeline.[35–37] The age-specific CHIP rates reported by Jaiswal et al.

122 have been used to calculate the standardized incidence rate.[19]

123 This study was approved by the BWH/DFCI Institutional Review Board with the approval

124 number 2023P001501 and was conducted in accordance with the Declaration of Helsinki.

125

126 <u>Statistical Analysis</u>

127 Statistical analyses were performed using Stata statistical software release 17 (StataCorp LLC,

128 College Station, TX) and R version 4.2.3 (*Shortstop Beagle*). Normal distribution was visually

129 assessed for all continuous variables. Data dispersion was assessed with standard deviation (SD)

130 for normally distributed variables and with interquartile range (IQR) for non-normally

131 distributed variables. Baseline demographics and disease characteristics were compared between

132 patients with and without CHIP. Comparison for normally-distributed variables was performed

133 with the independent samples t-test, while, for non-normally distributed, with the Wilcoxon rank

134 sum (Mann Whitney U) test. Comparison for categorical variables was performed with the Chi-

135 square test and the Fisher exact test. MOD-PFS and cardiac-specific PFS were measured from

136 the time of diagnosis to the time of the first MOD-PFS/cardiac-progression defining event or

137 were censored at the last follow-up. All reported p values were two-sided, with a statistical

138 significance set at <0.05. We used the Kaplan-Meier method to estimate the survival curves for

139 the OS and MOD-PFS, and the Log-rank test to assess the difference between survival curves.

140 We used a Cox regression and a stratified Cox regression model to assess the time-to-event

141 outcome and calculate hazard ratios (HR) with 95% confidence intervals (CI). The stratified

142 multivariate Cox regression model was built using a forward selection principle and following a

143 10:1 events to covariate ratio.

144 This study was approved by the BWH/DFCI Institutional Review Board with the approval

145 number 2023P001501.

146

147 **Results**

148 <u>CHIP is present at a higher prevalence in AL amyloidosis patients as compared to a healthy</u>

149 <u>population</u>

150 We identified a total of 76 patients. Sixteen patients (21%) had at least 1 CHIP mutation. Figure

151 1 shows the detected mutational profile. DNMT3A was the most frequently involved gene (7/16,

152 44%), followed by TET2, GNB1, ATM (each 2/16, 12.5%), and SF3B1, TP53, ZRSR2, EZH2,

153 BRCC3, PPM1D, ASXL1 (6%). DNMT3A variants included 6 missense (2 of which were in the

154 same patient) at the R882, R736, Y735, I780, and F755 residues, and 3 nonsense lesions. TET2

155 lesions included 3 stop codons and 1 variant in the catalytic domain (residues 1843-2002).

156 ASXL1, GNB1, and ATM-reported variants included known missense hotspots. Of note, 5

157 samples carried more than one CHIP-defining lesion. The median VAF of CHIP-associated

158 mutations was 0.036. A subset of 4 cases carried CHIP variants with an allele frequency equal to

159 or higher than 10%, suggesting the presence of a larger clone. Based on the age distribution of

160 our patients, the age-standardized incidence rate of CHIP would be 6%.

161 Ten patients (13%) had more than one sample available. Eight out of ten patients with more than

162 one bone marrow biopsy available had no evidence of CHIP on either biopsy. In contrast, the

163 other 2 patients (20%) were initially negative and subsequently had a biopsy positive for CHIP

164 (DNMT3A, ZRSR2). There were no patients with CHIP on a first biopsy who were CHIP

165 negative on a subsequent biopsy (Figure 2).

166

167 <u>Clinical characteristics</u>

168 Baseline demographics are shown in Table 1. The mean age of our cohort was 63 years (range 44

169 – 85). Thirty-two patients were females (42%), 6 patients (8%) were black, 2 (3%) were Asian, 1

170 was Middle-Eastern (1%), 2 (3%) self-reported as other, and 65 (85%) were white. No

171 significant difference was noted in epidemiologic characteristics between patients harboring a

172 CHIP mutation and those without (p>0.05). Sixty-nine percent of patients with CHIP and 67% of

173 patients without CHIP (p = 1) were treatment naïve at the time the RHP was obtained. Among

174 patients with CHIP, 2 (12.5%), 5 (31%), 7 (44%), and 2 (12.5%) had a Mayo stage of I, II, IIIA,

175 and IIIB, respectively. Among those without CHIP, 19 (32%), 13 (22%), 14 (24%), and 13

176 (24%), had a Mayo stage of I, II, IIIA, and IIIB, respectively. There was no statistically

177 significant difference in the partition of the Mayo stage between the two groups. When focusing

178 on patients with histopathology-proven or clinically determined renal involvement, patients with

179 CHIP were more likely to have a lower Palladini renal stage[7] (p = 0.001). No significant

180 difference was noted regarding the frequency of organ involvement (i.e., heart, kidneys, liver,

181 lung, autonomic nervous system, peripheral nervous system, gastrointestinal tract, and soft

182 tissue) and the total number of organs affected. The left ventricular ejection fraction (LVEF) and

183 the presence of anginal symptoms were also evaluated: the mean LVEF was 55% (SD 6.4%) for

9

184  patients with CHIP and 53% (SD 10.2%) for those without. None of the CHIP patients had an

185  LVEF ≤40% as opposed to 7 (12%) non-CHIP patients, but this difference was not statistically

186  significant (p = 0.34). Two patients with CHIP (13%) reported anginal symptoms, as opposed to

187  4 patients without CHIP (7%) but this difference was not statistically significant (p = 0.60). We

188  then assessed the cytogenetic profile of our cohort (Table 2). A total of 54 patients could be

189  evaluated with FISH, 13 with CHIP (81%), and 41 without CHIP (68%). Among those with

190  CHIP, 11/13 (85%) were found to harbor the t(11;14) as opposed to 15/41 (37%) without CHIP

191  (p = 0.004). To exclude a possible confounding effect of age on the association between CHIP

192  and the t(11;14) we performed a multivariate logistic regression to assess this association when

193  keeping age constant. Even after adjusting for age, the association between CHIP and t(11;14)

194  remained statistically significant (adjusted odds ratio 10.92, CI 1.91 – 62.31, p = 0.007). No

195  other cytogenic abnormality was found to be significantly associated with CHIP. In regards to the

196  treatment received, 35 (46%) patients received the combination cyclophosphamide, bortezomib,

197  dexamethasone (CyBorD), 28 (37%) CyBorD in association with Daratumumab (Dara-CyBorD),

198  while 12 (16%) other regimens (bortezomib – dexamethasone [VD], lenalidomide – bortezomib

199  – dexamethasone [RVD], Dara-VD, and melphalan - dexamethasone). Ten (13%) patients

200  received ASCT during their disease course, including 2 (12.5%) patients with CHIP and 8 (13%)

201  without.

202

203  The presence of CHIP does not impact MOD-PFS or OS

204  After a median follow-up from diagnosis of 32.5 months (range 0.5 – 168), 11 patients (14%)

205  died and 30 (39%) had a MOD-PFS defining event including death. The presence of CHIP was

206  not associated with lower OS (p = 0.483) (Figure 2A) or with lower MOD-PFS (p = 0.815)

10

207 (Figure 2B). In the univariate Cox proportional hazard model, variables associated with an

208 increased hazard of mortality were age (HR 1.12, 95% CI, 1.04 – 1.21, p = 0.003), a Mayo stage

209 >2 (HR 5.74, 95% CI 1.22 – 26.93, p= 0.027), LVEF ≤40% (4.11, 95% CI 1.09 – 15.53, p =

210 0.037) and a co-occurring diagnosis of coronary artery disease (CAD) (HR 4.94, 95% CI 1.31 –

211 18.63, p = 0.018). In this cohort, CHIP was not associated with an increased hazard for mortality.

212 We then looked at MOD-PFS, and the variables associated with an increased hazard for MOD-

213 defining events were Mayo stage 3a or 3b (HR 4.68, 95% CI 1.94 – 11.29, p = 0.001), an LVEF

214 ≤40% (HR 3.09, 95% CI 1.15 – 8.34, p = 0.026), a diagnosis of CAD (HR 5.43, 95% CI 2.13 –

215 13.88, p < 0.001), the presence of orthostatic hypotension requiring treatment (HR 2.35, 95% CI

216 1.08 – 5.10, p = 0.030). CHIP was not associated with an increased hazard for MOD events.

217 A multivariate Cox regression model stratified for age (44-54, 55-64, 65-71, >71) for MOD-PFS

218 was constructed, which included CAD and Mayo stage 3a or 3b, in addition to CHIP status.

219 Mayo stage 3a or 3b (HR 4.10, 95% CI 1.55 – 10.81, p = 0.004) and the presence of CAD (HR

220 4.89, 95% CI 1.58 – 15.16, p = 0.006) were associated with an increased hazard for MOD-

221 defining event (Figure 3). CHIP was not associated with an increased hazard for MOD-defining

222 events in the multivariate models.

223 We then focused solely on cardiac-specific outcomes assessed at 6-months and 12-months. In

224 patients with cardiac involvement at diagnosis (n = 58), patients harboring CHIP had a lower rate

225 of cardiac organ response compared to those without CHIP, but this difference was not

226 statistically significant (7/14, 50%, versus 28/44, 64%, respectively, p = 0.532). Cardiac-specific

227 disease progression was observed in a total of 29 patients (38%), including 8 patients with CHIP

228 (50%) and 21 patients without CHIP (35%). CHIP presence was not associated with a higher

229   hazard for cardiac-specific disease progression in a univariate Cox regression analysis (HR 1.66,

230   95% CI 0.70 – 3.94, p = 0.250).

231

**Discussion**

233   Hereby, we describe the prevalence, clinical characteristics, and outcome implications of CHIP

234   presence in a cohort of 76 consecutive AL amyloidosis patients seen at our center. We noted that

235   CHIP occurs at a higher frequency (21%) than expected for an age-matched healthy population

236   (5-10% depending on the studies).[19,20] The median VAF of CHIP-associated mutations was

237   0.036, lower than what was previously reported in AL amyloidosis patients, but comparable to

238   MM patients.[31,32] We also report on an association between CHIP and the presence of

239   prognostically adverse, t(11;14). Harboring CHIP was not associated with a decreased OS,

240   MOD-PFS, or cardiac-PFS, and, although a lower rate of cardiac response was observed, this

241   difference was not statistically significant. The presence of orthostatic hypotension requiring

242   treatment with midodrine was found to be associated with worse MOD-PFS in a univariate

243   analysis but was not included in the multivariate model. Conversely, an association between an

244   underlying diagnosis of CAD and a Mayo stage 3a or 3b and decreased MOD-PFS was detected

245   in a multivariate model.

246   The prevalence of CHIP we reported (21%) is consistent with the available literature for patients

247   with hematological malignancies, where CHIP driven by DTA genes was identified in 15 – 21%

248   of patients.[32,33] In our cohort, DNMT3A was the most frequently mutated gene, followed by

249   TET2, which appears in line with previous studies on patients with plasma cell dyscrasia.[32,33]

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250 Consistent with prior data in AL patients, CHIP status did not correlate with age or smoking

251 status.[32] This is different from what others have observed in the general population and in the

252 context of MM.[20,31,38]

253 Furthermore, our study included longitudinal data for a subset of patients. Interestingly, two

254 patients (59 and 80 years old at the time of diagnosis) with no CHIP detected at the initial RHP

255 evaluation were subsequently found to have a CHIP (DNMT3A and ZRSR2, respectively) after

256 only a few cycles of therapy.

257 The association between lower Palladini renal stage[7] and the presence of CHIP was unexpected,

258 as it was the association between CHIP and the presence of the t(11;14). Age was found not to be

259 a significant confounder in the association between t(11;14) and CHIP, making this association

260 even more intriguing. However, in this cohort of patients, it is impossible to ascertain whether

261 CHIP followed or preceded a diagnosis of t(11;14) plasma cell disorder, and the biological

262 significance of this association still needs to be elucidated. No difference in regards to the

263 kidney-specific outcome was noted between patients with or without CHIP (data not shown), but

264 given the low number of patients, no adjustment could be performed for the renal involvement at

265 diagnosis or the Palladini renal stage[7] in patients with known renal involvement. Larger

266 prospective studies are needed to clarify the significance of these preliminary findings and

267 potential cause-effect relationship.

268 In our study, the presence of CHIP was not associated with all-cause mortality or MOD-PFS.

269 Although this finding appears in contrast with previous studies on MM and lymphomas

270 undergoing ASCT[28,31], they are consistent with what was observed in AL amyloidosis patients in

271 other studies.[32,33] However, other possible explanations for the absence of an association between

272 CHIP and worse cardiac outcomes may be the small sample size and the limited follow-up of our

13

273    series. Furthermore, there may have been a bias in patients enrolled in our study as critically ill

274    patients who could not travel to our center for care or who died prior to commencing plasma

275    cell-directed therapy were not included, potentially confounding the association with CHIP.

276    Interestingly, we identified an association between the presence of orthostatic hypotension

277    requiring treatment and reduced MOD-PFS. Although we chose not to include this variable in

278    the final multivariate analysis to avoid overfitting the model, the impact between orthostatic

279    hypotension and lower outcome persisted when included in the multivariate analysis (data not

280    shown). This has not been previously reported and warrants further evaluation.

281    There are several limitations to our study. First, given the limited sample size, the power of our

282    analysis is significantly reduced, with wide confidence intervals. Second, because of the short

283    duration of follow-up, a low number of events was detected, reducing our capacity to fit more

284    variables into our regression model. Lastly, in our series, t(11;14) did not emerge as a negative

285    prognostic factor and was therefore not included in the multivariate model. This could be due to

286    the use of DaraCyBorD in over a third of the patients or possibly to the short duration of follow-

287    up and low incidence of events.

288    In conclusion, we showed that CHIP mutations are frequent in the largest cohort of patients with

289    AL amyloidosis analyzed to date. We demonstrated an association between CHIP and a lower

290    Palladini renal stage[7] at diagnosis and the presence of t(11;14). No impact on OS or MOD-PFS

291    was observed for patients with CHIP as compared to patients without CHIP. Larger prospective

292    studies and a more prolonged follow-up are needed to better elucidate the impact of CHIP in

293    patients with AL amyloidosis and to establish a cause-effect relationship with the observed

294    associations.

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295

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**Authorship**

Contribution: G.B. and N.B. designed the study. P.L. and G.B. wrote the manuscript. P.L. and

A.M. performed the analysis. E.B., T.C., M.M., and S.B. collected the data. G.B. and N.B.

secured funding for research. All the authors critically revised the manuscript and agreed on the

final version.

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Correspondence: Giada Bianchi, Harvard Institute of Medicine, 4 Blackfan Circle, Boston, MA

02115; e-mail: gbianchi1@bwh.harvard.edu

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420

421    **Table 1.** Demographics of AL amyloidosis patients with and without CHIP.

| Demographics | Total (n=76) | With CHIP (n=16) | Without CHIP (n=60) | P (CHIP *versus* no CHIP) |
|---|---|---|---|---|
| Age (mean ±SD) | 63 ±9.9 | 66 ±10.1 | 63 ±9.8 | 0.28* |
| **Gender** | | | | |
|     Male | 44 (57.9%) | 7 (43.7%) | 37 (61.7%) | 0.26† |
|     Female | 32 (42.1%) | 9 (56.3%) | 23 (38.3) | |
| **Ethnicity** | | | | |
|     White | 65 (85%) | 15 (94%) | 50 (84%) | 0.83† |
|     Black | 6 (8%) | 1 (6%) | 5 (8%) | |
|     Asian | 2 (3%) | 0 | 2 (3%) | |
|     Middle-Eastern | 1 (1%) | 0 | 1 (2%) | |
|     Other | 2 (3%) | 0 | 2 (3%) | |
| **Smoking status** | | | | |
|     Smoker | 43 (58%) | 7 (44%) | 24 (41%) | 0.54† |
|     Non-Smoker | 31 (42%) | 9 (56%) | 34 (59%) | |
| **Other Neoplasm** | | | | |
|     Yes | 13 (17%) | 3 (19%) | 10 (17%) | 0.55† |
|         MM | 9 (12%) | 3 (19%) | 6 (10%) | |
|         SMM | 2 (3%) | 0 | 2 (3%) | |
|         WM | 1 (1%) | 0 | 1 (2%) | |
|         CML | 1 (1%) | 0 | 1 (2%) | |
|     No | 63 (83%) | 13 (81%) | 50 (83%) | |
| **Type of FLC** | | | | |
|     Lambda | 63 (82.9%) | 12 (75%) | 51 (85%) | 0.45† |
|     Kappa | 13 (17.1%) | 4 (25%) | 9 (15%) | |
| **Treatment status at CHIP evaluation** | | | | |
|     Newly Diagnosed | 51 (67%) | 11 (69%) | 40 (67%) | 1† |

|  |  |  |  |  |
|---|---|---|---|---|
| Rel/Ref | 25 (33%) | 5 (31%) | 20 (33%) |  |
| Median time in months from diagnosis | 1 (0 – 159) | 1 (0 – 41) | 1 (0 – 159) | 0.73‡ |
| Median number of lines of therapy (range) | 1 (1 – 3) | 1 (1 – 3) | 1.5 (1 – 2) | 0.64‡ |
| Mayo Stage |  |  |  |  |
| I | 21 (28%) | 2 (12.5%) | 19 (32%) | 0.44‡ |
| II | 18 (24%) | 5 (31%) | 13 (22%) |  |
| IIIA | 21 (28%) | 7 (44%) | 14 (24%) |  |
| IIIB | 15 (20%) | 2 (12.5%) | 13 (22%) |  |
| Mayo stage IIIA - IIIB | 36 (48%) | 9 (56%) | 27 (46%) | 0.58 |
| Palladini Kidney Stage (if kidney involvement) |  |  |  | **0.001‡** |
| I | 9 (28%) | 5 (100%) | 4 (15%) |  |
| II | 14 (44%) | 0 | 14 (52%) |  |
| III | 9 (28%) | 0 | 9 (33%) |  |
| Number of Organs involved (median, range) | 3 (1 – 7) | 3 (1 – 5) | 3 (1 – 7) | 0.43‡ |
| Sites |  |  |  |  |
| Heart | 60 (79%) | 15 (94%) | 45 (75%) | N.S. |
| Renal | 32 (42%) | 5 (31%) | 27 (45%) |  |
| ANS | 23 (30%) | 8 (50%) | 15 (25%) |  |
| PNS | 19 (25%) | 3 (19%) | 16 (27%) |  |
| GI | 36 (47%) | 8 (50%) | 28 (47%) |  |
| Soft tissue/Skin | 32 (42%) | 7 (44%) | 25 (42%) |  |
| Liver | 3 (4%) | 1 (6%) | 2 (3%) | N.As. |
| Lungs/Pleura | 4 (5%) | 0 | 4 (7%) |  |
| Lymph node | 2 (3%) | 0 | 2 (3%) |  |
| LVEF (mean ±SD) | 53 ±9.5 | 55 ±6.4 | 53 ±10.2 | 0.28* |

| | | | | |
|---|---|---|---|---|
| ≤40% | 9.5% | 0 | 12% | 0.34† |
| ≤50% | 28% | 13% | 33% | 0.13† |
| **Coronary artery disease** | | | | |
| Yes | 6 (8%) | 2 (12%) | 4 (7%) | 0.6† |
| No | 70 (92%) | 14 (88%) | 56 (93%) | |
| **Del 17p** | | | | |
| Yes | 36 (13%) | 0 (13%) | 1 (13%) | 1† |
| No | 18 (58%) | 13 (69%) | 40 (55%) | |
| N.A. | 22 (29%) | 3 (19%) | 19 (32%) | |
| **t(4;14)** | | | | |
| Yes | 1 (1%) | 0 | 1 (2%) | 1† |
| No | 53 (70%) | 13 (81%) | 40 (67%) | |
| N.A. | 22 (29%) | 3 (19%) | 19 (32%) | |
| **Monosomy 13** | | | | |
| Yes | 5 (7%) | 0 | 5 (8%) | 0.32† |
| No | 49 (64%) | 13 (81%) | 36 (60%) | |
| N.A. | 22 (29%) | 3 (19%) | 19 (32%) | |
| **Gain 1q** | | | | |
| Yes | 6 (8%) | 2 (12%) | 4 (7%) | 0.62† |
| No | 48 (63%) | 11 (69%) | 37 (61%) | |
| N.A. | 22 (29%) | 3 (19%) | 19 (32%) | |
| **≥2 trisomies** | | | | |
| Yes | 3 (4%) | 0 | 3 (5%) | 1† |
| No | 51 (67%) | 13 (81%) | 38 (63%) | |
| N.A. | 22 (29%) | 3 (19%) | 19 (32%) | |
| **t(11;14)** | | | | |
| Yes | 26 (34%) | 11 (69%) | 15 (25%) | **0.004†** |
| No | 28 (37%) | 2 (12%) | 26 (43%) | |

| | | | | |
|---|---|---|---|---|
| N.A. | 22 (29%) | 3 (19%) | 19 (32%) | |
| BM PC percentage (median, IQR) | 15% (7% – 20%) | 17% (10% – 22%) | 15% (7% – 20%) | 0.08‡ |
| First line treatment | | | | 0.33† |
|     CyBorD | 35 (46%) | 5 (31%) | 30 (50%) | |
|     Dara-CyBorD | 28 (37%) | 8 (50%) | 20 (33%) | |
|     Other§ | 12 (16%) | 3 (19%) | 9 (15%) | |
|     None | 1 (1%) | 0 | 1 (2%) | |
| ASCT | | | | 1† |
|     Yes | 10 (13%) | 2 (12.5%) | 8 (13%) | |
|     No | 66 (87%) | 14 (87.5%) | 52 (87%) | |

422

423  SD, standard deviation; rel/ref, relapsed/refractory; SMM, smoldering multiple myeloma; CML, chronic

424  myeloid leukemia; N.S., non-statistically significant; N.As., statistical significance not assessed; N.A., not

425  available; BM, bone marrow; PC, plasma cells; IQR, interquartile range.

426  * Using two-samples independent t-test.

427  † Using Fisher's exact test.

428  ‡ Using Wilcoxon rank-sum test.

429  §Other regimens were bortezomib – dexamethasone, lenalidomide – bortezomib – dexamethasone,

430  daratumumab – bortezomib – dexamethasone, and melphalan – dexamethasone.

431

**Figure 1. Oncoplot displaying CHIP results.** Oncoplot showing the relative genetic contribution to CHIP in the cohort (76 patients, of which 16 carrying CHIP-defining lesions). Mutation types are color-coded according to legend. The right barchart shows the number of samples carrying a specific genetic variant while the top barchart recapitulates the number of variants per sample. DTA lesions are shown in the first three lines followed by other genes involved in hematologic neoplasms**.**

**Figure 2. Longitudinal data.** Plot showing patients with longitudinal samples available. White circles represent RHP not showing any CHIP, while black squares represent RHP where CHIP was detected. Two patients (PT 14 and PT16) who had no CHIP at the initial RHP, were later found to harbor one CHIP (DNMT3A and ZRSR2, respectively). In contrast, no CHIP was found in the repeated RHP of the remaining eight patients.

**Figure 3. Overall survival and MOD-PFS.** Kaplan-Mayer curves showing overall survival (A) and MOD-PFS (B) expressed in months among AL amyloidosis patients with (red line) and without (black line) CHIP.

**Figure 4. Multivariate Cox regression model.** Forest plot recapitulating MOD-PFS determinants in terms of Hazard Ratios. Mayo stage >2, and the presence of CAD were found to be statistically significant negative MOD-PFS predictors (p=0.004 and p=0.006, respectively).
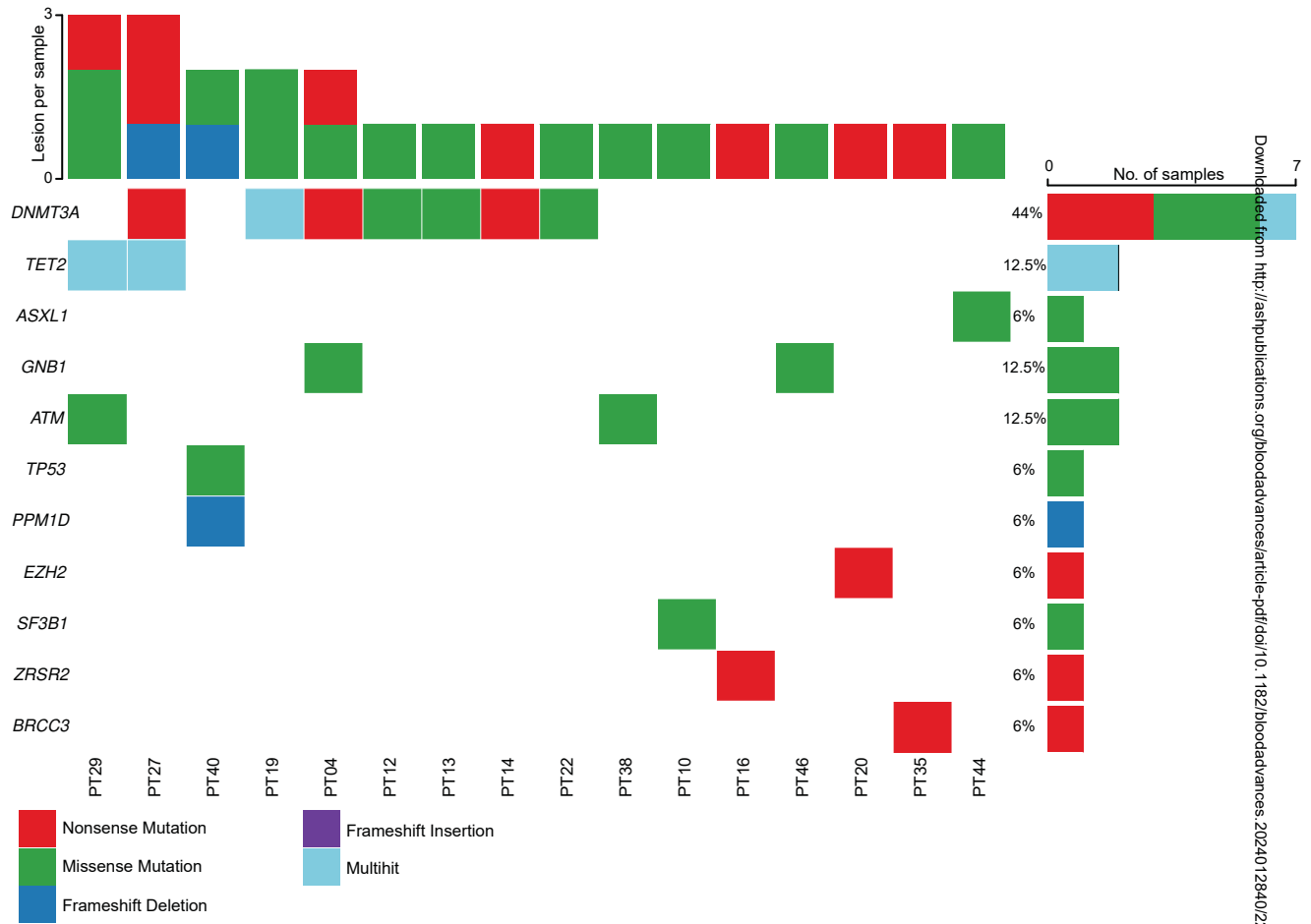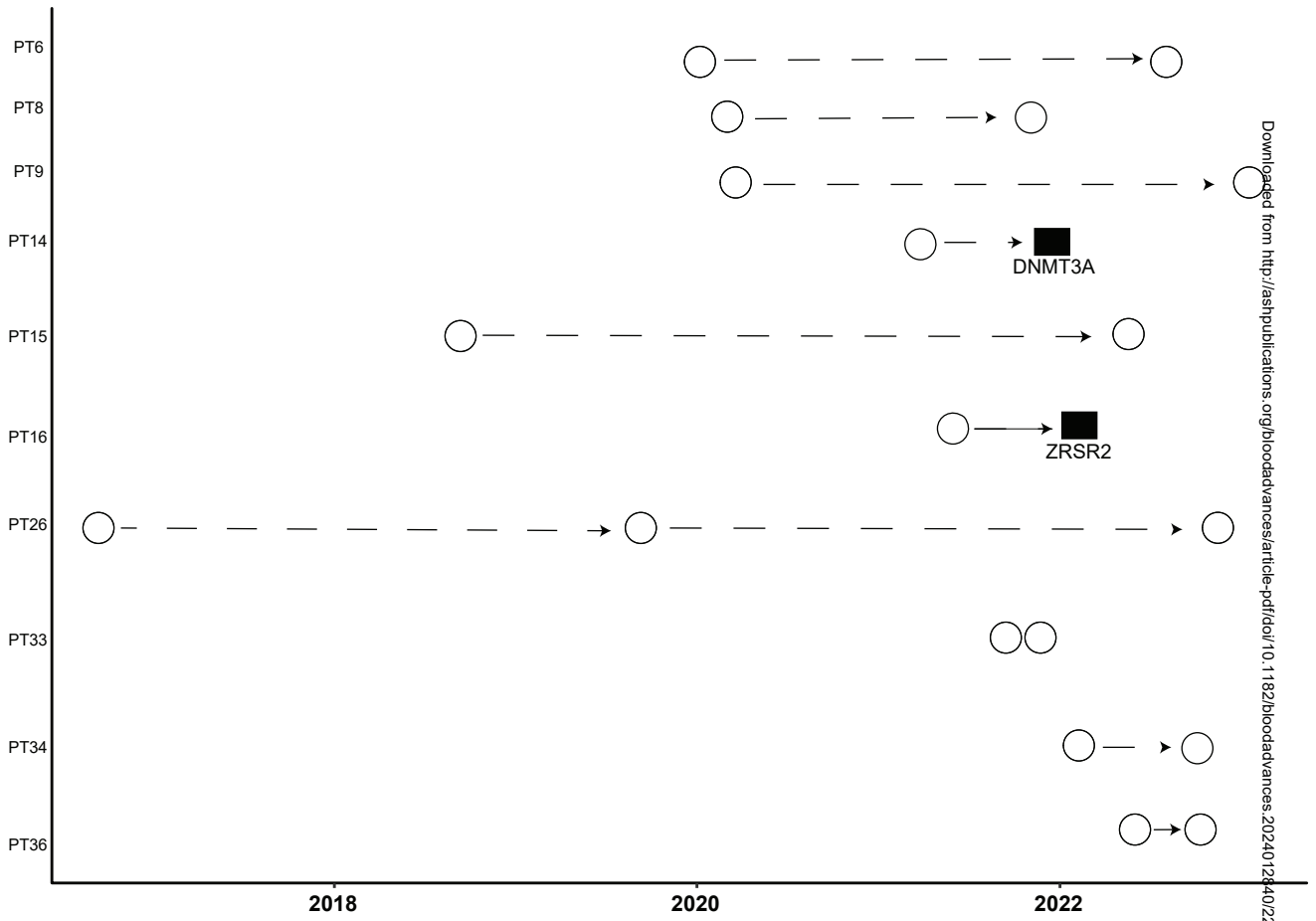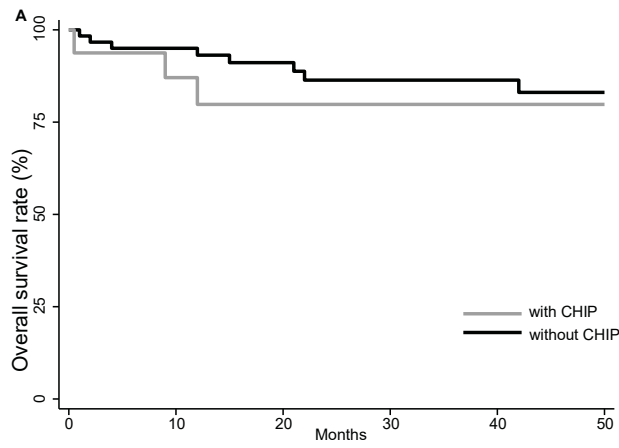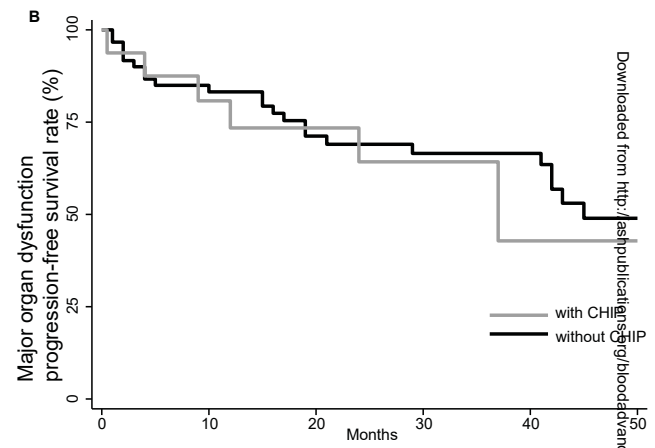
24

# Figure 1

# Figure 2

# Figure 3



A

Overall survival rate (%)

Months

— with CHIP
— without CHIP

| | Number at risk | | | | | |
|---|---|---|---|---|---|---|
| With CHIP | 16 | 13 | 9 | 7 | 3 | 3 |
| Without CHIP | 60 | 53 | 41 | 34 | 28 | 12 |
| Months | 0 | 10 | 20 | 30 | 40 | 50 |

B

Major organ dysfunction progression-free survival rate (%)

Months

— with CHIP
— without CHIP

| | Number at risk | | | | | |
|---|---|---|---|---|---|---|
| With CHIP | 16 | 12 | 8 | 7 | 2 | 2 |
| Without CHIP | 60 | 48 | 34 | 27 | 22 | 8 |
| Months | 0 | 10 | 20 | 30 | 40 | 50 |

# Figure 4

| | Hazard Ratio | p value | 95% CI |
|---|---|---|---|
| CHIP | 0.93 | 0.880 | 0.36–2.40 |
| Mayo stage >2 | 4.10 | 0.004 | 1.55–10.81 |
| CAD | 4.89 | 0.006 | 1.58–15.16 |