

## Telomere length and clonal hematopoiesis interact to influence outcomes in hematopoietic stem cell transplantation.

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### Abstract:

Clonal hematopoiesis (CH), the clonal expansion of a hematopoietic stem cell (HSC) and its progeny driven by somatic mutations, has been associated with inferior survival outcomes amongst recipients of autologous stem cell transplants (ASCT). Leukocyte telomere length (LTL) has a complex but well-documented interaction with CH, but the impact of this interaction on stem cell transplantation (SCT) has not been adequately examined. We measured LTL in graft cell DNA from 452 patients undergoing ASCT for myeloma, for whom targeted DNA sequencing for CH driver gene mutations was available. We interrogated clinical and longitudinal large-scale laboratory data for these patients to understand the impact of graft LTL on progression-free (PFS) and overall survival (OS) post-transplantation, as well as blood count indices and their trajectories. In multivariate analyses, longer LTL was associated with increased PFS amongst patients without CH. However, this protective association was not seen in patients with CH. We also report that amongst patients with CH, longer LTL was associated with an increased red cell distribution width (RDW) prior to myeloablative chemotherapy and after ASCT. Collectively, these data reveal hitherto undescribed interactions between LTL, CH and ASCT outcomes.

**Conflict of interest:** COI declared - see note

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2 *hematopoietic stem cell transplantation.*

3  
4 Short title:

5 Telomere lengths in autologous SCT outcomes.

6  
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33 Data Sharing Statement: For original data, please contact [gsv20@cam.ac.uk](mailto:gsv20@cam.ac.uk)

34

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36

37 Abstract:

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39 and its progeny driven by somatic mutations, has been associated with inferior  
40 survival outcomes amongst recipients of autologous stem cell transplants (ASCT).  
41 Leukocyte telomere length (LTL) has a complex but well-documented interaction with  
42 CH, but the impact of this interaction on stem cell transplantation (SCT) has not been  
43 adequately examined. We measured LTL in graft cell DNA from 452 patients  
44 undergoing ASCT for myeloma, for whom targeted DNA sequencing for CH driver  
45 gene mutations was available. We interrogated clinical and longitudinal large-scale  
46 laboratory data for these patients to understand the impact of graft LTL on  
47 progression-free (PFS) and overall survival (OS) post-transplantation, as well as  
48 blood count indices and their trajectories. In multivariate analyses, longer LTL was  
49 associated with increased PFS amongst patients without CH. However, this  
50 protective association was not seen in patients with CH. We also report that amongst  
51 patients with CH, longer LTL was associated with an increased red cell distribution  
52 width (RDW) prior to myeloablative chemotherapy and after ASCT. Collectively, these  
53 data reveal hitherto undescribed interactions between LTL, CH and ASCT outcomes.

54

55 Key points:

- 56 • Longer telomeres are associated with a progression-free survival benefit in  
57 patients without clonal hematopoiesis.
- 58 • Longer telomeres are associated with an increase in red cell distribution width  
59 among patients with clonal hematopoiesis.

60

61

62 Introduction:

63 Clonal hematopoiesis (CH), the clonal expansion of a hematopoietic stem cell (HSC)  
64 and its progeny driven by somatic driver mutations, becomes increasingly prevalent  
65 with advancing age to affect more than 50% of those aged 70 years or older.<sup>1-3</sup>  
66 Most cases of CH are driven by mutations in genes involved in DNA methylation  
67 (*DNMT3A* and *TET2*), chromatin modification (*ASXL1*), splicing (*SF3B1*, *SRSF2* and  
68 *U2AF1*), cytokine signalling (*JAK2*) or the DNA damage response (*TP53* and  
69 *PPM1D*).<sup>2</sup> CH carriers have an increased risk of developing myeloid malignancy,  
70 non-hematological disorders including cardiovascular and renal disease, and certain  
71 solid cancers.<sup>4-6</sup> The presence of CH was found to influence clinical outcomes after  
72 autologous stem cell transplantation (ASCT), with CH carriers exhibiting higher rates  
73 of treatment-related myeloid neoplasia and inferior overall survival relative to non-CH  
74 controls.<sup>7,8</sup>

75  
76 Studies evaluating the impact of leukocyte telomere length (LTL) on stem cell  
77 transplantation (SCT) outcomes report mixed conclusions: although there is  
78 consensus that SCT leads to accelerated telomere shortening, different studies  
79 report conflicting results on the impact of graft cell LTL and hematopoietic  
80 reconstitution post-transplantation.<sup>9-11</sup> Longer LTL has been associated with  
81 enhanced granulocyte recovery in some studies, whilst others report no impact of  
82 donor LTL on trilineage hematopoietic reconstitution.<sup>10-12</sup> This may reflect variation in  
83 the clinical indications for SCT; differences in LTL between autologous and  
84 allogeneic SCT; and variation in the many other variables affecting hematopoietic  
85 recovery post-SCT.

86  
87 Recent studies have revealed that most forms of CH arise preferentially in people  
88 inheriting longer LTL, whilst CH itself causes LTL attrition.<sup>5,13</sup> However, we recently  
89 found that the association between CH and long telomeres is not uniform, and that  
90 CH driven by mutations in *PPM1D* and splicing factor genes *SF3B1*, *SRSF2* and  
91 *U2AF1* arises more frequently in individuals inheriting shorter telomeres.<sup>14</sup> Prompted  
92 by these observations, we sought to examine the importance of graft LTL on  
93 survivorship and blood count indices in the peri-transplant period, and establish  
94 whether outcomes of ASCT for myeloma were influenced by LTL and CH status of  
95 the graft cells.

96

97 Methods:98 **Selection of patients and stem cell grafts**

99 Mobilized stem cell products (peripheral blood with CD34+ cells) from 452 myeloma  
100 patients were harvested by leukapheresis at the University Hospital Heidelberg  
101 between 2004 and 2016. Clinical data was analyzed in May 2022. Stem cell  
102 mobilization was performed with G-CSF (filgrastim) combined with either  
103 cyclophosphamide monotherapy (2 g/m<sup>2</sup>) or a combination of cyclophosphamide (2  
104 g/m<sup>2</sup>), doxorubicin (60 mg/m<sup>2</sup>) and dexamethasone (80 mg). All patients provided  
105 written informed consent to the use of their biomaterial and clinical data. The project  
106 was approved by the ethics committee of the University of Heidelberg (Reference no.  
107 S-850/2021).

108

109 **Annotation of myeloma disease status and cytogenetic risk**

110 Response to therapy was assessed retrospectively from medical records and  
111 response categories were documented according to EBMT and IMWG uniform  
112 response criteria.<sup>15,16</sup> Cytogenetic high-risk disease was defined by the detection of  
113 one of the following genetic aberrations by fluorescence *in situ* hybridization (FISH):  
114 t(4;14), t(14;16) or del(17p) or chromosome 1q21 gain/amplification.

115

116 **Isolation of genomic bulk DNA and targeted bulk DNA sequencing**

117 Genomic DNA was isolated from patient stem cell grafts before undergoing targeted  
118 DNA sequencing to detect CH driver mutations. Stem cell grafts underwent  
119 quantification of CD34<sup>+</sup> cell yield, but not immunophenotypic sorting, ensuring that  
120 cellular material taken forward into these analyses comprised an admixture of both  
121 CD34<sup>+</sup> and CD34<sup>-</sup> mononuclear cells, as well as granulocytes. DNA extraction  
122 techniques were as previously described in Stelmach *et al.* (2023).<sup>17</sup>

123

124 **Mutation analysis and CH variant calling**

125 We analysed blood DNA from the mobilized stem cell products from 452 myeloma  
126 patients. Targeted enrichment was performed for 56 genes implicated in CH and  
127 myeloid malignancies (Agilent SureSelect ELID 3156971, Supplementary table 1).  
128 Libraries were sequenced on Illumina Novaseq to a median depth of 1062 reads,  
129 with a 5<sup>th</sup>-95<sup>th</sup> percentile interval of 492-1401 reads. Variant calling was performed

130 using GATK Mutect2 version 4.1.8.1 using the ‘tumor-only’ mode.<sup>18</sup> Variants with  
131 fewer than two reads on either the forward or reverse strands of DNA, or with VAF <  
132 0.05 were discarded. To recover falsely discarded calls, we first calculated the  
133 recurrences of each variant in the cohort with VAF > 0.005 ( $N_{0.005}$ ), VAF > 0.01 ( $N_{0.01}$ )  
134 and the recurrence in the COSMIC database ( $N_{cosmic}$ ).<sup>19</sup> Discarded substitution  
135 variants were rescued if they satisfied either of these criteria: a)  $N_{0.005} \geq 100$  and  
136  $N_{0.01} < 5$ ; b)  $N_{0.005} < 100$  and  $N_{cosmic} \geq 30$ ; c)  $N_{cosmic} \geq 10$ . Insertion/deletion variants  
137 with  $N_{0.01} > 2$  were discarded except for *NPM1* p.W288fs and *ASXL1* p.G646fs.  
138 Driver mutations were defined according to evidence for functional relevance in CH  
139 and haematological malignancy (Supplementary table 2).

140

### 141 **Data warehouse**

142 The data warehouse (Department of Medicine V, Heidelberg University Hospital)  
143 centralizes department-specific data from the hospital information system and  
144 peripheral systems in an automated way. To date, it contains clinical data of  
145 approximately 120,000 patients dating back to 2004. In addition to basic data, it also  
146 includes diagnostic results and treatment-related data. Since every patient contact is  
147 stored together with the collected information, detailed and dense longitudinal follow-  
148 up data are included.

149

### 150 **Telomere length measurement**

151 Relative leukocyte telomere lengths (rLTL) were measured for each patient sample  
152 using a quantitative polymerase chain reaction (qPCR) protocol modified from  
153 Cawthon (2002).<sup>20</sup> DNA samples were quantified using the Qubit dsDNA HS Kit  
154 (Invitrogen Q32851). Each PCR reaction contained 0.5ng of genomic DNA and 5 $\mu$ L  
155 of QuantiTect SYBR Green PCR Mastermix (Qiagen 204143). Telomere reactions  
156 contained 270nM tel1 and 900nM tel2 primers (Supplementary table 3) and were run  
157 on an Applied Biosystems QuantStudio<sup>TM</sup> 5 Real-Time PCR system using the  
158 following conditions: 95°C for 15 minutes, 40 cycles of (95°C for 15 seconds, 54°C  
159 for 2 minutes). *36B4* reactions contained 300nM 36B4u and 500nM 36B4d primers  
160 and were run using the following conditions: 95°C for 15 minutes, 40 cycles of (95°C  
161 for 15 seconds, 58°C for 1 minute).

162

163 All samples were analysed in triplicate. For inclusion in analyses, samples were  
164 required to have a mean  $C_t$  value  $> 1.0$  outside the mean for the negative control  
165 wells (DNA replaced with  $H_2O$ ) for both primer pairs. In addition, samples were  
166 excluded if the standard deviation of  $C_t$  values for either primer pair was  $> 1.0$ . To  
167 account for variation in qPCR analyser performance between runs, serial dilutions  
168 (0.01ng – 2.5ng) derived from the same aliquot of K562 cell line DNA were included  
169 on each PCR plate for a standard curve. rTLs for each sample were inferred from  
170 the ratio of mean  $C_t$  values for telomere (T) and *36B4* (S) primer pairs using the  
171 formula:  $T/S \text{ ratio} = 2^{(C_t \text{ 36B4} - C_t \text{ telomere})}$ . This T/S ratio was in turn normalised to the T/S  
172 ratio for 0.5ng of K562 DNA on the same PCR plate.

173

174 To ensure that our conclusions were not affected by the disproportionate impact of a  
175 small number of more extreme rTL values, we repeated analyses excluding a small  
176 number ( $n = 16$ ) of samples for which telomere qPCR had generated  $C_t$  values  
177 passing QC criteria, but which lay outside of 1.5x the interquartile range (IQR) of the  
178 cohort.

179

## 180 **Statistical analyses**

181 Univariate and multivariable analyses of time-to-event endpoints were performed  
182 using Cox regression. OS was defined as time from ASCT. Subjects not confirmed  
183 dead were censored at the time last known to be alive. PFS was defined as time  
184 from ASCT until the earliest time of progression or death from any cause and  
185 censored at time last known to be alive and free of progression. HRs with 95%  
186 confidence intervals (CI) and Wald p-values were reported for model covariates, and  
187 likelihood-ratio tests and p-values were reported for multivariable models. Median  
188 event times were estimated using the method of Kaplan and Meier (KM) and  
189 reported with 95% CIs. Greenwood's formula was used to approximate the variance  
190 of KM estimates. Differences in survival curves were assessed using log-rank tests.

191

192 To assess the trajectories with multiple measurements per patient, mixed-effects  
193 models were used to investigate the pattern in RDW after ASCT. To satisfy the model  
194 assumptions, the outcome variable, RDW, was transformed with the natural  
195 logarithm. As an independent variable, time after ASCT was included and modeled  
196 with a natural cubic spline, to allow a non-linear effect and to capture the trend in the

197 data with more precision. We modeled outcome variables up to day 20 after ASCT.  
198 For models to day 20 one spline knot was set on day 9. Residual plots were used to  
199 validate the models' assumptions.

200

201 LASSO regression (L1 penalty) with  $\lambda$  tuned by cross-validation was performed  
202 using the glmnet R package to model log-transformed rLTL. LASSO regression  
203 identified *SF3B1* and *JAK2* mutations as the strongest negative predictors of rLTL.

204

205 Continuous and categorical data are reported as mean (standard deviation) and  
206 count (percent), respectively. Fisher's exact test was used to test for associations  
207 between categorical variables. The Mann-Whitney-U or Wilcoxon rank-sum test were  
208 used to assess a location shift in the distribution of continuous variables between  
209 two, or more than two groups, respectively.

210

211 All p-values were two-sided, and significance levels set at  $p < 0.05$ . Calculations  
212 were done using R version 4.4.2 R (Foundation for Statistical Computing, Vienna,  
213 Austria).

214

### 215 **UK Biobank analyses**

216 The UK Biobank is a large-scale biomedical database and research resource  
217 containing genetic, lifestyle and health information from half a million UK participants.  
218 The UK Biobank has approval from the North West Multicentre Research Ethics  
219 Committee (11/NW/0382) and all participants provided written informed consent. The  
220 present study has been conducted under approved UK Biobank application number  
221 56844.

222

### 223 Results:

#### 224 **Cohort characteristics and LTL measurement**

225 Graft cell telomere lengths were measured in 452 samples from patients undergoing  
226 first ASCT. Quality control criteria for the qPCR reaction, as described previously,  
227 were satisfied for 422 (93.4%) patients, who were included in analyses. A summary  
228 of demographic and clinical features of these patients, stratified by CH status and  
229 relative telomere length, is provided in Table 1. The distribution of patient telomere

230 lengths relative to control DNA was positively skewed (range 0.06 – 13.91 arbitrary  
231 units; median 1.41; Figure 1A).

232

### 233 **Prevalence of CH driver mutations**

234 Of the 422 patients for whom valid rLTL measurements could be obtained, 141  
235 (33.4%) harbored one or more CH driver mutations (Figure 1A). The full spectrum of  
236 called CH mutations is provided in Supplementary table 4, as well as summaries of  
237 mutational burden by driver gene (Supplementary figure 1A) and an overview of  
238 single- and multi-mutant cases (Supplementary figure 1B). Within the subsets of  
239 patients for whom information on ASCT mobilization chemotherapy (n = 273; 64.7%)  
240 or prior induction chemotherapy (n = 418; 99.1%) was available, there were no  
241 differences in the incidence of CH according to mobilization or induction regimen (p =  
242 0.0723 and p = 0.158 respectively, Fisher's exact test; Supplementary figure 1C-D).

243

### 244 **LTL and CH mutational profile**

245 LTLs (rLTLs) were shorter in patients with CH driver mutations compared to those  
246 without (median 0.99 vs 1.74 arbitrary units, respectively; p < 0.001, Wilcoxon rank-  
247 sum test; Figure 1B). This finding withstood linear regression-based adjustment for  
248 age, sex, myeloma cytogenetic risk status and time until ASCT (Supplementary  
249 figure 1E). In keeping with recent evidence suggesting that CH driven by splicing  
250 factor gene mutations arises preferentially in patients with shorter LTL, rLTLs were  
251 shorter in patients with *SF3B1* mutations (n=6) compared to the remaining 135  
252 patients with CH driven by other mutations (0.47 vs 1.06; p = 0.014, Wilcoxon rank-  
253 sum test).<sup>14,21</sup> As splicing factor mutations occurred alongside other CH driver  
254 mutations in all but one patient, we performed LASSO regression analysis to  
255 disentangle the contribution of specific CH mutations on telomere length, which  
256 confirmed that *SF3B1* mutations were independently associated with short telomeres  
257 (regression coefficient: -0.84; Figure 1C). As previous studies highlighted *PPM1D*  
258 mutations as being of particular prognostic significance in the context of ASCT, rLTLs  
259 were compared between CH-carriers with and without *PPM1D* mutations, but no  
260 statistically significant difference was identified.<sup>8</sup>

261

### 262 **LTL and survival post-ASCT**

263 Next, we investigated whether telomere length was of prognostic relevance in the  
264 context of ASCT. To account for the non-Gaussian distribution of rLTLs and moderate  
265 the influence of a small number of patients with particularly long or short TL, we  
266 stratified patients according to whether their rLTL was above or below the cohort  
267 median. Considering the cohort as a whole, there was no difference in PFS or OS  
268 between patients with rLTL above vs below the cohort median. When patients were  
269 stratified according to the presence or absence of a CH driver mutation, we found  
270 that longer telomere lengths were associated with improved PFS ( $p = 0.017$ , log-rank  
271 test) and OS ( $p = 0.036$ , log-rank test) for patients without a CH driver mutation  
272 (Figure 1D-E).

273

274 As the relationship between LTL and survival post-ASCT might be confounded by  
275 variables such as age and sex, we confirmed that there were no significant  
276 differences in age, sex or myeloma cytogenetic risk stratification between the above-  
277 median and below-median LTL cohorts (Table 1). A Cox proportional hazards model  
278 revealed that after adjusting for age, sex and disease status prior to transplantation,  
279 above-median rLTL in patients without CH was associated with a 26% improvement  
280 in PFS (HR 0.74; 95% CI 0.56-0.98;  $p = 0.035$ ), whilst the corresponding effect on  
281 OS did not reach statistical significance (HR 0.71; 95% CI 0.49-1.02;  $p = 0.064$ ;  
282 Figure 1F-G). In contrast, among patients with CH, above-median rLTL was not  
283 significantly associated with PFS (HR 1.21; 95% CI 0.82-1.80;  $p = 0.335$ ;  
284 Supplementary figure 2A) or OS (Supplementary figure 2B). A significant interaction  
285 was observed between rLTL and CH status for both endpoints (PFS: HR for  
286 interaction = 1.64, 95% CI 1.01–2.66,  $p = 0.045$ ; OS: HR for interaction = 1.93, 95%  
287 CI 1.03–3.63,  $p = 0.041$ ), ensuring that the survival benefits of longer rLTL were not  
288 seen amongst patients with a CH mutation (Supplementary figure 2A-E).

289

290 Associations between CH status, rLTL and survivorship were validated after exclusion of  
291 outlier rLTL values as defined in Methods. Of note, patients from our cohort who  
292 underwent ASCT at later time periods (i.e. post-2009 compared to pre-2009) showed  
293 shortened PFS, but non-inferior overall survival (Supplementary figure 3A-B). In an  
294 attempt to reflect the heterogeneity of clone sizes found within our CH cohort, variant  
295 allele fraction (VAF) of the patient's largest CH driver mutation was integrated as an

296 additional variable into our regression analyses, and was not found to influence survival  
297 (Supplementary figure 3C-D).

298

### 299 **LTL, CH mutations and complete blood count parameters**

300 Next, we sought to examine the impact of LTL and CH mutational status on  
301 hematopoiesis around the time of ASCT, by analyzing their relationship with different  
302 hematological parameters.

303

304 We assessed the trajectories of red cell distribution width (RDW) between days 0-20  
305 post-ASCT with respect to rLTL (Figure 2A-B) and developed a time-dependent  
306 linear mixed-effects model. This model confirmed an association between increased  
307 RDW and above-median rLTL that was specific to CH carriers ( $p = 0.003$ ; Figure 2C-  
308 D), and withstood regression-based adjustment for age, sex, serum creatinine and  
309 red blood cell (RBC) transfusional burden (Figure 2E). Patients with CH and above-  
310 median rLTL also showed increased RDW prior to conditioning chemotherapy  
311 (16.1% vs 15.2%,  $p = 0.0022$ , Wilcoxon rank sum test; Figure 2F). The association  
312 between above-median rLTL and increased RDW amongst CH patients was not  
313 significantly affected by clone size (Figure 2G).

314

315 To investigate whether these associations were specific to this cohort of myeloma  
316 patients undergoing ASCT, we examined the relationship between telomere length  
317 and RDW within a cohort of 431,531 UK Biobank participants who had no history of  
318 hematological malignancy. No consistent associations were found between either  
319 measured or genetically-predicted telomere length and RDW for the most frequently  
320 mutated CH driver genes (Supplementary figure 4A-B).

321

322 Amongst individuals with CH due to mutations in one of the three most commonly  
323 mutated genes (*DNMT3A*,  $n = 55$ ; *TET2*,  $n = 33$ ; and *PPM1D*,  $n = 11$ ), an association  
324 with increased RDW was found in patients with *PPM1D* single- or co-mutated CH.  
325 Median RDW prior to conditioning chemotherapy did not differ between patients with  
326 a *PPM1D* mutation compared to other CH carriers (16.2% vs 15.5%,  $p = 0.081$ ,  
327 Wilcoxon rank sum test; Figure 3A). However, visualisation (Figure 3B) and mixed-  
328 effects modelling ( $p = 0.027$ , Figure 3C-D) of RDW trajectories between days 0-20  
329 post-ASCT revealed an increased RDW in *PPM1D*-mutant CH compared to *PPM1D*-

330 wild-type CH. This association between *PPM1D*-mutant CH and increased RDW was  
331 not evident in a comparison of *PPM1D*-mutant cases and age- and sex-matched  
332 controls in the UK Biobank (Supplementary figure 4C).

333

334 Relative telomere length was not associated with leukapheresis harvest yields,  
335 leukocyte counts, or platelet counts, either at harvest or prior to conditioning  
336 chemotherapy, in this patient cohort.

337

338 With respect to hemoglobin (Hb), concentration prior to conditioning was higher in  
339 patients without CH with above-median rLTL compared to those with below-median  
340 rLTL (median 119g/L vs 115g/L;  $p = 0.01$ , Wilcoxon rank-sum test), whereas no  
341 difference was observed in patients with CH (115g/L vs 118g/L,  $p = 0.12$ ;  
342 Supplementary figure 5A). However, a linear mixed-effects model revealed that rLTL  
343 was not an independent predictor of Hb concentration after adjustment for age, sex  
344 and CH mutation status (Supplementary figure 5B). Hemoglobin concentrations did  
345 not differ between *PPM1D*-mutant and *PPM1D*-wild-type CH carriers, either prior to  
346 conditioning chemotherapy (median 109g/L vs 118g/L,  $p = 0.05$ , Wilcoxon rank sum  
347 test; Supplementary figure 5C), or between days 0-20 post-ASCT (Supplementary  
348 figure 5D).

349

#### 350 Discussion:

351 In this paper, we investigated the impact of stem cell graft LTL on different clinical  
352 and laboratory parameters at the time of ASCT for myeloma. We report previously  
353 undocumented associations between survivorship and LTL which vary according to  
354 the presence of CH driver mutations. We also show a consistent, small magnitude  
355 correlation between longer LTL and increased RDW in patients with CH.

356

357 Attempts to understand the relevance of telomere lengths to clinical outcomes are  
358 inevitably complicated by demographic variables understood to influence both  
359 telomere length and all-cause morbidity and mortality. To account for this, we have  
360 ensured that only associations between LTL and clinical or laboratory variables that  
361 withstand regression-based adjustment for age and sex are reported in this study.  
362 However, we did not have access to some potentially relevant data, such as smoking  
363 status and medical comorbidities, which could affect both rLTL and survivorship.<sup>22</sup>

364 Moreover, factors such as patient age, fitness, and choice of chemotherapy regimen  
365 may affect both rLTL and post-ASCT survival, with biological and clinical interactions  
366 that are hard to account for fully with standard regression analyses.<sup>23</sup> A more  
367 detailed interrogation of potential mechanisms explaining the apparent survival  
368 benefit of longer graft LTL is hampered by a lack of comprehensive cause of death  
369 data for our cohort.

370

371 The fact that PFS, more than OS, was influenced by LTL in patients without CH may  
372 suggest that this protective effect of long telomeres is related to transplant efficacy  
373 and disease course, rather than broader determinants of survivorship. Whilst our  
374 study was only powered to analyze outcomes post-ASCT, previous studies have  
375 reported a positive association between germline polymorphisms associated with  
376 longer LTL and improved overall survival in myeloma.<sup>24</sup> As such, it is possible that  
377 telomere length has broader relevance to myeloma disease trajectories outside of  
378 the context of ASCT – this is especially important given the evolving landscape of  
379 myeloma treatment, and the increasing role for immunomodulatory and cellular  
380 therapies. Our data show that PFS appeared worse among patients recruited later  
381 within the study, whilst the trend for OS was reversed, but non-significant. As the age  
382 profile of patients did not differ between calendar periods, this raises the possibility  
383 that improvements in surveillance and detection of myeloma relapse could have  
384 affected documentation of progression, and hence PFS in this cohort. Accordingly, it  
385 is noteworthy that the PFS benefit seen with longer LTL among CH patient withstood  
386 formal adjustment for transplantation date.

387

388 The mechanisms underpinning apparent interactions between LTL and CH driver  
389 mutations in determining post-ASCT clinical outcomes are not immediately obvious.  
390 Considering progression-free survival, an above-median graft LTL was independently  
391 associated with improved outcomes for patients without CH. This is potentially a  
392 reflection of improved stem cell reserve, with implications for haematopoietic  
393 reconstitution, and immunological surveillance of residual myeloma. As we and  
394 others have shown that telomere attrition naturally constrains clonal expansion, it is  
395 conceivable that this explains the loss of benefit associated with increased rLTL  
396 among the subset of patients with clonal hematopoiesis, where longer LTL could  
397 facilitate the expansion of a dysfunctional hematopoietic clone.<sup>13,14</sup> In addition, it is

398 possible that the diversity of CH driver mutations and their associations with other  
399 variables relevant to survivorship (including age and prior cytotoxic therapy)  
400 complicates analyses and confounds any impact of LTL in this subgroup.

401

402 Considering data for laboratory parameters, an association was noted between  
403 above-median LTL and increased red cell distribution width (RDW) for patients with  
404 CH. This difference was apparent when tracing RDW values for up to 20 days post-  
405 ASCT, and withstood adjustment for renal function and the number of transfused red  
406 cell units. Our analyses of UK Biobank data did not reveal meaningful associations  
407 between LTL, CH and RDW in this larger, unselected cohort, suggesting that any  
408 interactions noted in our study may be specific to this particular clinical context. The  
409 precise mechanisms underlying the association between longer telomeres, CH  
410 mutations and increased RDW in this cohort are not apparent. As we did not have  
411 exhaustive data available for all laboratory parameters capable of affecting RDW, the  
412 lack of an association between clone size (VAF), rLTL and RDW seen in our data  
413 raise the possibility that the increased RDW among CH carriers with longer  
414 telomeres is mediated by non-cell autonomous processes, and subject to residual  
415 confounding.

416

417 *PPM1D* mutations were most strongly associated with increased RDW in our study,  
418 and were likely to have expanded in response to cytotoxic therapy not received by  
419 the majority of UK Biobank participants. This difference in context for the expansion  
420 of *PPM1D* mutations between our cohort, where *PPM1D* was often multiply mutated  
421 within the same patient, may explain why these findings were not corroborated in this  
422 larger, undifferentiated cohort. It is also important to acknowledge the small number  
423 of *PPM1D*-mutant patients in our cohort before over-interpreting the significance and  
424 generalizability of this finding.

425

426 Although statistically significant, the absolute magnitude of differences in RDW  
427 between the longer and shorter LTL subgroups of CH patients were still relatively  
428 small (< 1%), suggesting that RDW is unlikely to be useful as a predictive tool for LTL  
429 and/or CH status. Consistent with numerous previous studies, no clear associations  
430 were seen between LTL and blood counts in the peri-transplant period.

431

432 Taken together, our data suggest an interaction between LTL and CH that may  
433 influence clinical and laboratory outcomes post-ASCT. Although the effect sizes are  
434 small and unlikely to be clinically predictive when considered in isolation, these  
435 findings align with our current understanding of CH and underscore the need for  
436 larger, longitudinal studies to clarify their clinical relevance.

437  
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453 Author contributions:

454 J.T. performed experimental work, conducted statistical analyses and wrote the  
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456 W.G.D. performed UK Biobank analyses. D.X. performed experimental work. M.G.  
457 and M.A.F. performed clonal hematopoiesis variant calling. S.S. and M. R. provided  
458 patient samples and patient data. C. M-T. and G.S.V. supervised the study. All  
459 authors reviewed and approved the final manuscript.

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466

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468

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534

535 Tables:

536

|   | CH-<br>negative<br>rLTL ≤<br>median<br>(n =<br>123) | CH-<br>negative<br>rLTL ><br>median<br>(n =<br>158) | CH-<br>positive<br>rLTL ≤<br>median<br>(n = 88) | CH-<br>positive<br>rLTL ><br>median<br>(n = 53) | P<br>(wit<br>hin<br>CH-<br>neg<br>ativ<br>e) | P<br>(wit<br>hin<br>CH<br>-<br>pos<br>itiv<br>e) |
|---|---|---|---|---|--|--|
| <b>Age in years at apheresis</b>        |   |   |   |   |  |  |
| Mean (SD)                               | 57.3<br>(8.21)                                      | 56.3<br>(9.24)                                      | 59.7<br>(8.12)                                  | 61.4<br>(7.19)                                  | 0.4<br>73                                    | 0.2<br>48  |
| Median [min, max]                       | 58.0<br>[37.0,<br>72.0]                             | 57.0<br>[28.0,<br>72.0]                             | 62.0<br>[39.0,<br>74.0]                         | 62.0<br>[45.0,<br>72.0]                         |  |  |
| <b>Gender, N (%)</b>                    |   |   |   |   |  |  |
| Male                                    | 75<br>(61.0%)                                       | 105<br>(66.5%)                                      | 48<br>(54.5%)                                   | 34<br>(64.2%)                                   | 0.3<br>81                                    | 0.2<br>94  |
| Female                                  | 48<br>(39.0%)                                       | 53<br>(33.5%)                                       | 40<br>(45.5%)                                   | 19<br>(35.8%)                                   |  |  |
| <b>MM cytogenetic risk, N (%)</b>       |   |   |   |   |  |  |
| Standard risk                           | 84<br>(68.3%)                                       | 115<br>(72.8%)                                      | 62<br>(70.5%)                                   | 35<br>(66.0%)                                   | 0.4<br>3                                     | 0.5<br>8   |
| High risk                               | 39<br>(31.7%)                                       | 43<br>(27.2%)                                       | 26<br>(29.5%)                                   | 18<br>(34.0%)                                   |  |  |
| <b>Mobilization chemotherapy, N (%)</b> |   |   |   |   |  |  |
| CAD                                     | 72<br>(58.5%)                                       | 93<br>(58.9%)                                       | 56<br>(63.6%)                                   | 28<br>(52.8%)                                   | 0.5<br>24                                    | 0.2<br>86  |

|   | CH-<br>negative<br>rLTL ≤<br>median<br>(n =<br>123) | CH-<br>negative<br>rLTL ><br>median<br>(n =<br>158) | CH-<br>positive<br>rLTL ≤<br>median<br>(n = 88) | CH-<br>positive<br>rLTL ><br>median<br>(n = 53) | P<br>(wit<br>hin<br>CH-<br>neg<br>ativ<br>e) | P<br>(wit<br>hin<br>CH<br>-<br>pos<br>itiv<br>e) |
|---|---|---|---|---|--|--|
| Cyclophosphamide<br>mono                            | 5 (4.1%)  | 7<br>(4.4%)   | 1 (1.1%)  | 1<br>(1.9%)                                     |  |  |
| Other   | 3 (2.4%)  | 1<br>(0.6%)   | 2 (2.3%)  | 4<br>(7.5%)                                     |  |  |
| NA  | 43<br>(35.0%)                                       | 57<br>(36.1%)                                       | 29<br>(33.0%)                                   | 20<br>(37.7%)                                   |  |  |
| <b>Harvested CD34+<br/>cells x10<sup>6</sup>/kg</b> |   |   |   |   |  |  |
| Mean (SD)   | 7.93<br>(5.85)                                      | 9.26<br>(6.44)                                      | 8.31<br>(5.84)                                  | 7.49<br>(6.20)                                  | 0.0<br>425                                   | 0.3<br>05  |
| Median [min, max]                                   | 7.40<br>[0.590,<br>40.4]                            | 8.51<br>[0.600,<br>44.9]                            | 7.36<br>[0.530,<br>23.0]                        | 5.59<br>[0.650,<br>32.1]                        |  |  |
| <b>MM remission<br/>before ASCT</b>                 |   |   |   |   |  |  |
| CR  | 37<br>(30.1%)                                       | 58<br>(36.7%)                                       | 28<br>(31.8%)                                   | 11<br>(20.8%)                                   | 0.3<br>8                                     | 0.5<br>66  |
| SD  | 29<br>(23.6%)                                       | 25<br>(15.8%)                                       | 15<br>(17.0%)                                   | 11<br>(20.8%)                                   |  |  |
| PD  | 6 (4.9%)  | 8<br>(5.1%)   | 5 (5.7%)  | 3<br>(5.7%)                                     |  |  |
| PR  | 51  | 67  | 40  | 28  |  |  |

|  | CH-<br>negative<br>rLTL ≤<br>median<br>(n =<br>123) | CH-<br>negative<br>rLTL ><br>median<br>(n =<br>158) | CH-<br>positive<br>rLTL ≤<br>median<br>(n = 88) | CH-<br>positive<br>rLTL ><br>median<br>(n = 53) | P<br>(with<br>in<br>CH-<br>neg<br>ative) | P<br>(with<br>in<br>CH-<br>pos<br>itive) |
|--|---|---|---|---|--|--|
|  | (41.5%)   | (42.4%)   | (45.5%)   | (52.8%)   |  |  |
| <b>Platelet count before ASCT /nL</b>  |   |   |   |   |  |  |
| Mean (SD)                              | 267<br>(93.0)                                       | 268<br>(67.2)                                       | 251<br>(82.7)                                   | 249<br>(73.2)                                   | 0.3<br>14                                | 0.9<br>25                                |
| Median [min, max]                      | 249<br>[82.0,<br>785]                               | 261<br>[127,<br>490]                                | 240<br>[99.0,<br>498]                           | 231<br>[125,<br>414]                            |  |  |
| NA, N (%)                              | 1 (0.8%)  | 1<br>(0.6%)   | 0 (0%)  | 0 (0%)  |  |  |
| <b>Leukocyte count before ASCT G/L</b> |   |   |   |   |  |  |
| Mean (SD)                              | 5.31<br>(2.00)                                      | 5.17<br>(1.71)                                      | 5.12<br>(1.57)                                  | 4.76<br>(1.73)                                  | 0.7<br>83                                | 0.2<br>07                                |
| Median [min, max]                      | 5.05<br>[2.02,<br>13.2]                             | 4.98<br>[1.70,<br>10.3]                             | 4.82<br>[1.53,<br>9.67]                         | 4.61<br>[1.14,<br>9.97]                         |  |  |
| NA, N (%)                              | 1 (0.8%)  | 1<br>(0.6%)   | 0 (0%)  | 0 (0%)  |  |  |
| <b>RDW before ASCT %</b>               |   |   |   |   |  |  |
| Mean (SD)                              | 15.8<br>(1.57)                                      | 15.5<br>(1.32)                                      | 15.5<br>(1.44)                                  | 16.2<br>(1.59)                                  | 0.2<br>79                                | 0.0<br>022                               |

|  | CH-<br>negative<br>rLTL ≤<br>median<br>(n =<br>123) | CH-<br>negative<br>rLTL ><br>median<br>(n =<br>158) | CH-<br>positive<br>rLTL ≤<br>median<br>(n = 88) | CH-<br>positive<br>rLTL ><br>median<br>(n = 53) | P<br>(with<br>in<br>CH-<br>neg<br>ative) | P<br>(with<br>in<br>CH-<br>pos<br>itive) |
|--|---|---|---|---|--|--|
| Median [min, max]                                | 15.5<br>[12.5,<br>21.4]                             | 15.3<br>[13.1,<br>20.5]                             | 15.2<br>[12.5,<br>20.9]                         | 16.1<br>[12.7,<br>20.4]                         |  |  |
| NA, N (%)  | 13<br>(10.6%)                                       | 17<br>(10.8%)                                       | 7 (8.0%)  | 5<br>(9.4%)                                     |  |  |
| <b>Hemoglobin before ASCT g/dL</b>               |   |   |   |   |  |  |
| Mean (SD)  | 11.4<br>(1.48)                                      | 11.8<br>(1.41)                                      | 11.8<br>(1.63)                                  | 11.3<br>(1.66)                                  | 0.0<br>103                               | 0.1<br>16                                |
| Median [min, max]                                | 11.5<br>[7.50,<br>15.2]                             | 11.9<br>[7.70,<br>14.8]                             | 11.8<br>[8.00,<br>14.9]                         | 11.5<br>[7.70,<br>14.4]                         |  |  |
| NA, N (%)  | 1 (0.8%)  | 1<br>(0.6%)   | 0 (0%)  | 0 (0%)  |  |  |
| <b>Platelet count (before leukapheresis) /nL</b> |   |   |   |   |  |  |
| Mean (SD)  | 166<br>(64.1)                                       | 178<br>(74.8)                                       | 167<br>(73.6)                                   | 166<br>(80.9)                                   | 0.4<br>6                                 | 0.7<br>3                                 |
| Median [min, max]                                | 168<br>[34.0,<br>356]                               | 168<br>[41.0,<br>528]                               | 158<br>[41.0,<br>452]                           | 149<br>[51.0,<br>494]                           |  |  |

|   | CH-<br>negative<br>rLTL ≤<br>median<br>(n =<br>123) | CH-<br>negative<br>rLTL ><br>median<br>(n =<br>158) | CH-<br>positive<br>rLTL ≤<br>median<br>(n = 88) | CH-<br>positive<br>rLTL ><br>median<br>(n = 53) | P<br>(with<br>in<br>CH-<br>neg<br>ative) | P<br>(with<br>in<br>CH-<br>pos<br>itive) |
|---|---|---|---|---|--|--|
| <b>Leukocyte count (before leukapheresis) G/L</b> |   |   |   |   |  |  |
| Mean (SD)   | 23.1<br>(15.0)                                      | 20.0<br>(10.6)                                      | 17.8<br>(11.0)                                  | 23.4<br>(17.7)                                  | 0.1<br>51                                | 0.0<br>436                               |
| Median [min, max]                                 | 19.6<br>[3.50,<br>92.7]                             | 17.7<br>[3.35,<br>58.6]                             | 15.8<br>[2.40,<br>51.3]                         | 20.6<br>[4.59,<br>97.4]                         |  |  |
| <b>RDW (before leukapheresis) %</b>               |   |   |   |   |  |  |
| Mean (SD)   | 15.9<br>(1.34)                                      | 15.8<br>(1.50)                                      | 15.9<br>(1.53)                                  | 16.4<br>(1.54)                                  | 0.4<br>58                                | 0.0<br>351                               |
| Median [min, max]                                 | 15.8<br>[13.4,<br>19.8]                             | 15.6<br>[12.7,<br>20.6]                             | 15.6<br>[13.1,<br>19.3]                         | 16.4<br>[13.2,<br>20.2]                         |  |  |
| NA, N (%)   | 17<br>(13.8%)                                       | 17<br>(10.8%)                                       | 7 (8.0%)  | 7<br>(13.2%)                                    |  |  |
| <b>Hemoglobin (before leukapheresis) g/dL</b>     |   |   |   |   |  |  |
| Mean (SD)   | 10.6<br>(1.31)                                      | 10.9<br>(1.40)                                      | 10.8<br>(1.40)                                  | 10.7<br>(1.41)                                  | 0.1<br>25                                | 0.6<br>06                                |
| Median (min, max)                                 | 10.6  | 10.9  | 10.9  | 11.0  |  |  |

|   | CH-<br>negative<br>rLTL ≤<br>median<br>(n =<br>123) | CH-<br>negative<br>rLTL ><br>median<br>(n =<br>158) | CH-<br>positive<br>rLTL ≤<br>median<br>(n = 88) | CH-<br>positive<br>rLTL ><br>median<br>(n = 53) | P<br>(wit<br>hin<br>CH-<br>neg<br>ativ<br>e) | P<br>(wit<br>hin<br>CH<br>-<br>pos<br>itiv<br>e) |
|---|---|---|---|---|--|--|
|   | [7.90,<br>14.5]                                     | [7.80,<br>16.3]                                     | [7.20,<br>14.3]                                 | [7.00,<br>13.1]                                 |  |  |
| <b>rLTL (derived from normalized T/S ratio)</b> |   |   |   |   |  |  |
| Mean (SD)                                       | 0.720<br>(0.347)                                    | 3.20<br>(1.94)                                      | 0.669<br>(0.330)                                | 2.95<br>(1.90)                                  | <0.<br>001                                   | <0.<br>001                                       |
| Median [min, max]                               | 0.651<br>[0.0553,<br>1.39]                          | 2.55<br>[1.43,<br>13.9]                             | 0.572<br>[0.0597,<br>1.38]                      | 2.38<br>[1.43,<br>11.8]                         |  |  |
| <b>Single-mutant CH</b>                         |   |   |   |   |  |  |
| CH-negative                                     | 123<br>(100%)                                       | 158<br>(100%)                                       | 0 (0%)  | 0 (0%)  | 1  | 0.2<br>95  |
| <i>DNMT3A</i> -mutated                          | 0 (0%)  | 0 (0%)  | 21<br>(23.9%)                                   | 20<br>(37.7%)                                   |  |  |
| <i>PPM1D</i> -mutated                           | 0 (0%)  | 0 (0%)  | 4 (4.5%)  | 3<br>(5.7%)                                     |  |  |
| <i>TET2</i> -mutated                            | 0 (0%)  | 0 (0%)  | 14<br>(15.9%)                                   | 8<br>(15.1%)                                    |  |  |
| Other   | 0 (0%)  | 0 (0%)  | 49<br>(55.7%)                                   | 22<br>(41.5%)                                   |  |  |
| <b>Year of ASCT</b>                             |   |   |   |   |  |  |

|           | CH-<br>negative<br>rLTL $\leq$<br>median<br>(n =<br>123) | CH-<br>negative<br>rLTL $>$<br>median<br>(n =<br>158) | CH-<br>positive<br>rLTL $\leq$<br>median<br>(n = 88) | CH-<br>positive<br>rLTL $>$<br>median<br>(n = 53) | P<br>(with<br>CH-<br>negative) | P<br>(with<br>CH-<br>positive) |
|-----------|--|---|--|---|--------------------------------|--------------------------------|
| 2004-2008 | 45<br>(36.6%)  | 50<br>(31.6%)   | 17<br>(19.3%)  | 15<br>(28.3%)                                     | 0.6<br>89                      | 0.4<br>37                      |
| 2009-2012 | 34<br>(27.6%)  | 45<br>(28.5%)   | 27<br>(30.7%)  | 13<br>(24.5%)                                     |                                |                                |
| 2013-2016 | 44<br>(35.8%)  | 63<br>(39.9%)   | 44<br>(50.0%)  | 25<br>(47.2%)                                     |                                |                                |

537

538 **Table 1.** Baseline cohort characteristics stratified by clonal hematopoiesis (CH)  
539 status and by relative leukocyte telomere length (rLTL)  $\leq$  median or  $>$  median within  
540 each CH group. Comparisons between rLTL strata within CH-positive and CH-  
541 negative groups were performed using Student's t-test or one-way ANOVA for  
542 normally distributed continuous variables, Wilcoxon rank-sum or Kruskal–Wallis tests  
543 for non-normally distributed variables, and  $\chi^2$  or Fisher's exact test for categorical  
544 variables.

545

546

547 Figure legends:

548 **Figure 1. Association of relative telomere length (rLTL) with clonal hematopoiesis (CH)**  
549 **and outcomes.**

550

551 **(A)** Study cohort schematic and distribution of graft rLTL (median 1.41 arbitrary units; left-  
552 skewed distribution).

553

554 **(B)** Boxplot of telomere length in patients with (red) versus without (gray) CH, demonstrating  
555 shorter telomeres in patients with CH. Statistical comparisons were performed using the  
556 Wilcoxon test; p-values are shown.

557

558 **(C)** LASSO regression of CH driver mutations on telomere length identifying *SF3B1*  
559 mutations as associated with shorter telomeres.

560  
 561 **(D–E)** Kaplan–Meier estimates of progression-free survival (PFS) **(D)** and overall survival  
 562 (OS) **(E)** in CH-negative, stratified by rLTL > median (range, 1.41–13.91) vs. ≤ median  
 563 (range, 0.06–1.41). Longer telomeres were associated with improved PFS and OS. Log-rank  
 564 test p-values are indicated.

565  
 566 **(F–G)** Multivariate Cox proportional hazards models for PFS **(F)** and OS **(G)** including an  
 567 interaction term between rLTL above the median and CH presence ( $n = 141$  CH-positive  
 568 patients). In these models, the interaction between rLTL above the median and CH presence  
 569 was independently associated with increased risk of myeloma progression, as were older  
 570 age and progressive disease (PD) before autologous stem cell transplantation (ASCT).  
 571 Patients harboring both factors had a significantly higher risk of myeloma progression than  
 572 those with either factor alone. **Abbreviations:** PD = progressive disease, PR = partial  
 573 response, SD = stable disease.

574  
 575  
 576 **Figure 2. Longitudinal modeling of post-transplant red cell distribution width (RDW) in**  
 577 **relation to CH and relative leukocyte telomere length (rLTL).**

578  
 579 **(A–B)** RDW trajectories from day 0 to day 20 post-transplant in patients without CH **(A)** and  
 580 with CH **(B)**. Bold colored lines represent the mean values in patients with rLTL > median  
 581 (1.41) vs. ≤ median, showing higher RDW values over time in CH patients with longer  
 582 telomeres.

583  
 584 **(C–E)** Mixed-effects model of RDW over time stratified by CH status **(C, No CH; D, CH)**. After  
 585 adjusting for the covariates shown **(E)**, rLTL above the median remained significantly  
 586 associated with higher RDW in CH patients, but not in patients without CH. The interaction  
 587 between CH status and rLTL group demonstrated that the effect of longer telomeres on  
 588 RDW over time was restricted to CH-positive patients.

589  
 590 **(F)** Boxplot of pre-conditioning RDW (in %) before ASCT by rLTL group within CH and non-  
 591 CH patients. Statistical comparisons were performed using the Wilcoxon test; p-values are  
 592 shown.

593  
 594 **(G)** Mixed-effects model of RDW over time for CH patients, adjusting for the covariates  
 595 shown. Variant allele frequency (VAF), modeled as the VAF of the largest clone identified in  
 596 a patient, did not affect the relationship between RDW and LTL shown in **(E)**.

597  
 598  
 599 **Figure 3. Longitudinal modeling of post-transplant red cell distribution width (RDW) in**  
 600 **relation to *PPM1D* mutations and relative leukocyte telomere length (rLTL).**

601  
 602 **(A)** Boxplot of pre-conditioning RDW (%) before ASCT in *PPM1D*-mutant versus *PPM1D*-  
 603 wild-type CH patients. Statistical comparisons were performed using the Wilcoxon test; p-  
 604 values are shown.

605  
 606 **(B)** RDW trajectories from day 0 to day 20 post-transplant in CH patients according to  
 607 *PPM1D* mutation status. Bold colored lines represent mean RDW values in patients with ( $n$   
 608 = 11) or without ( $n = 130$ ) *PPM1D* mutations, showing higher RDW over time in *PPM1D*-  
 609 mutant patients.

610  
 611 **(C–D)** Mixed-effects model of RDW over time, stratified by *PPM1D* mutation status. After  
 612 adjusting for the covariates shown **(D)**, *PPM1D* mutations remained significantly associated  
 613 with higher RDW in CH patients.

# Figure 1

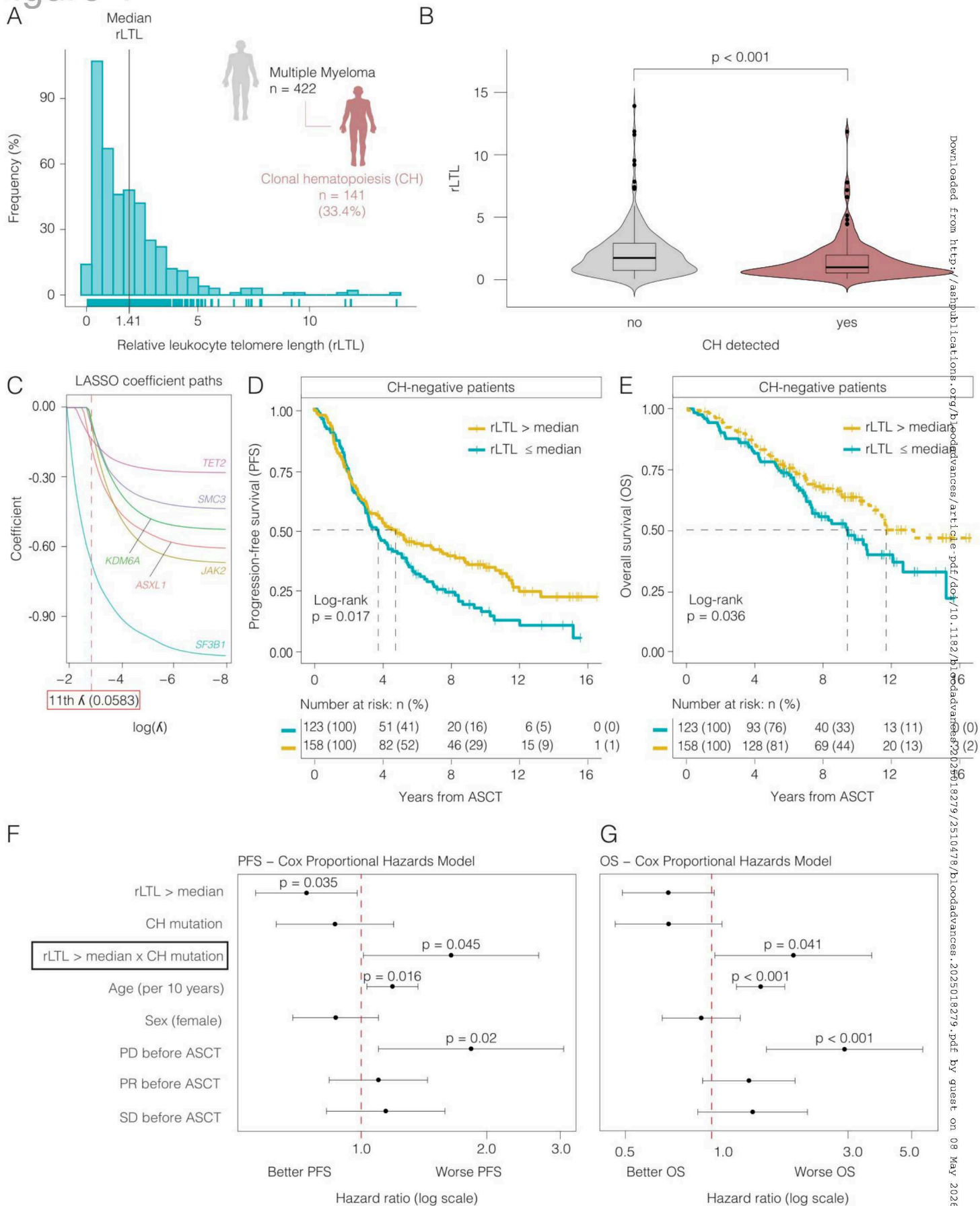


Figure 2

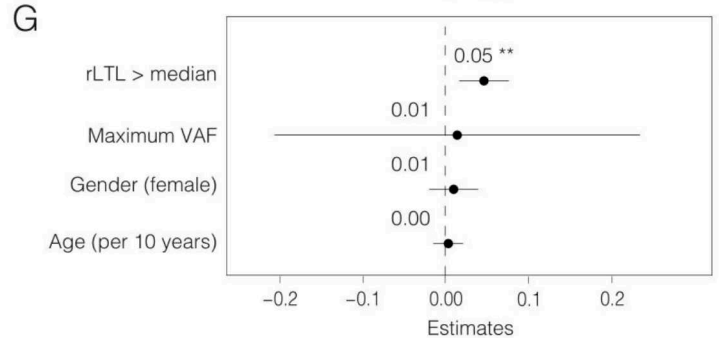
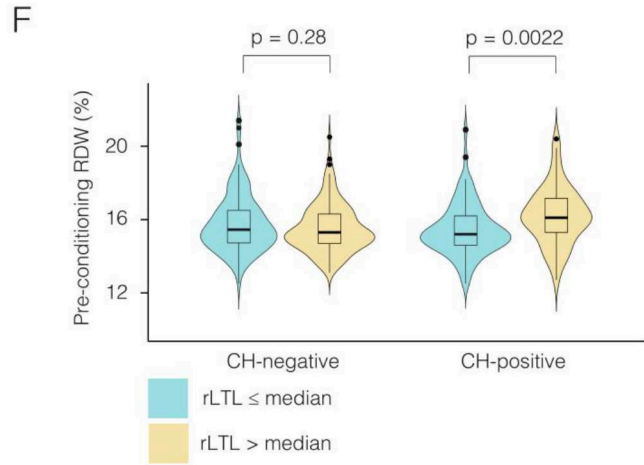
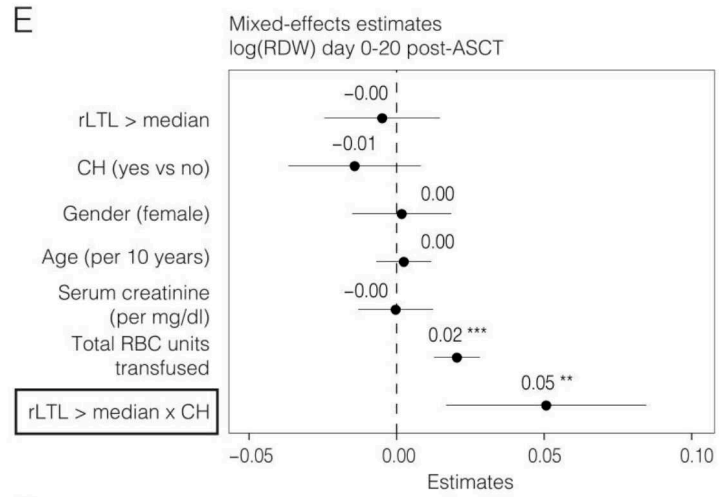
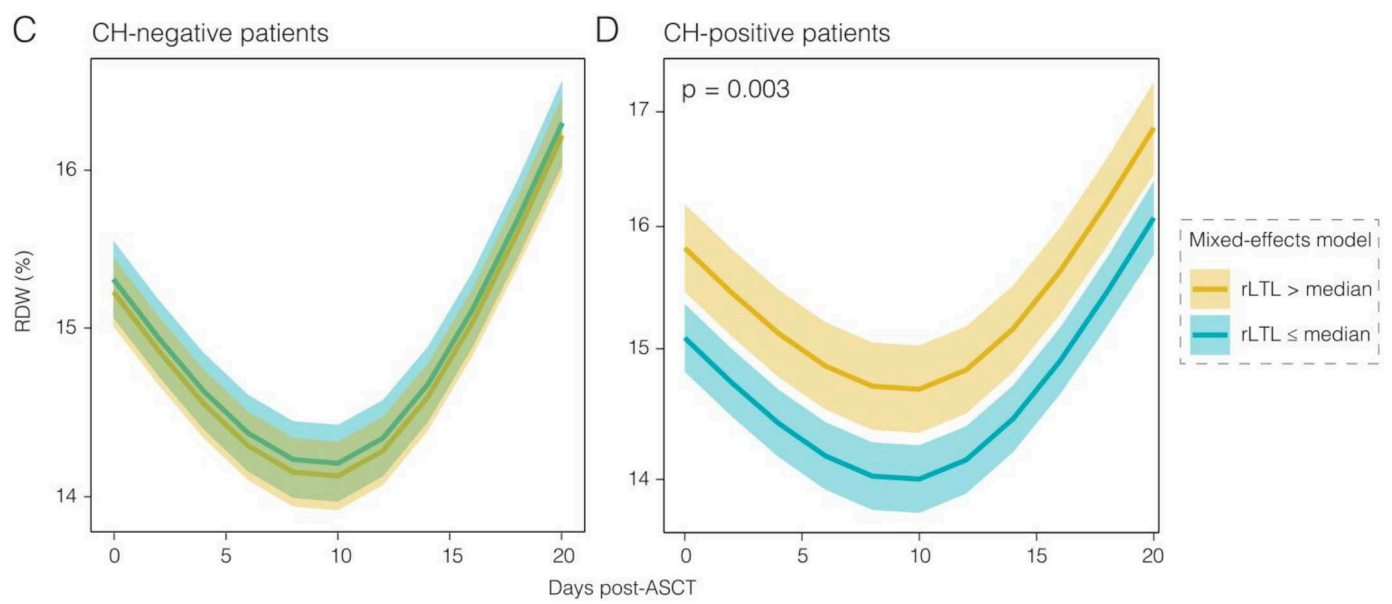
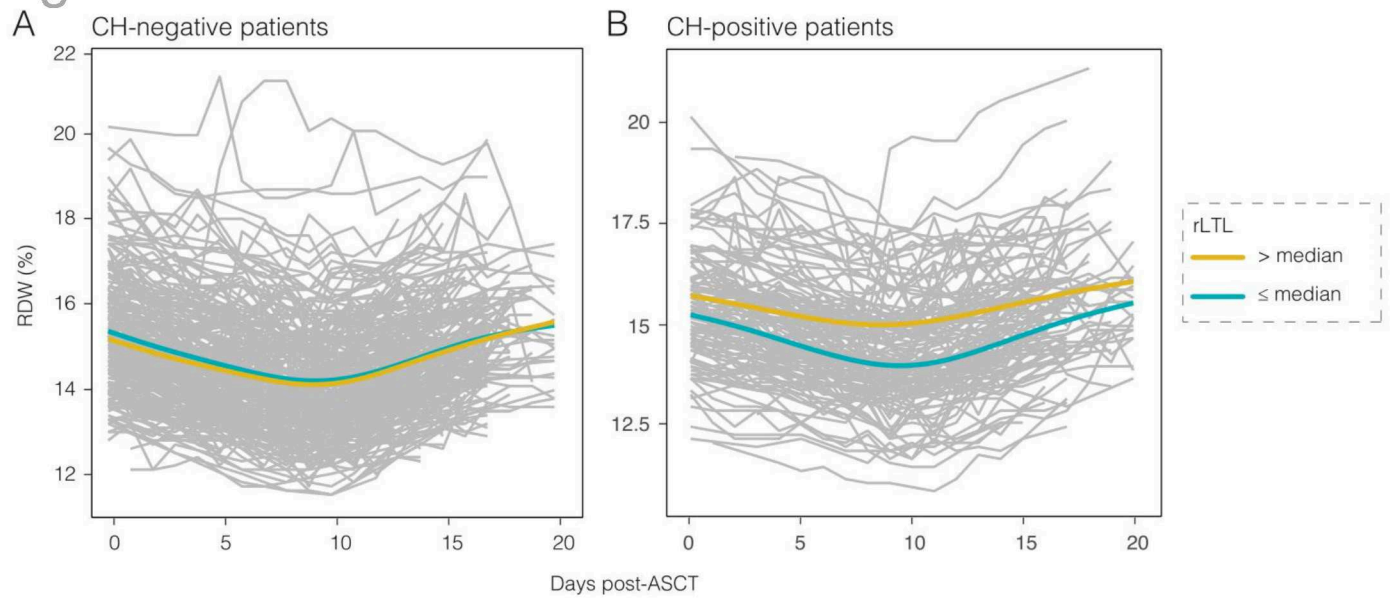
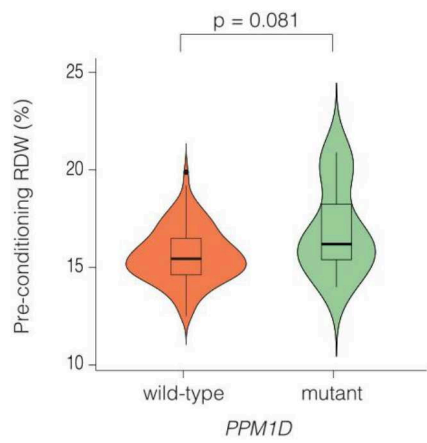
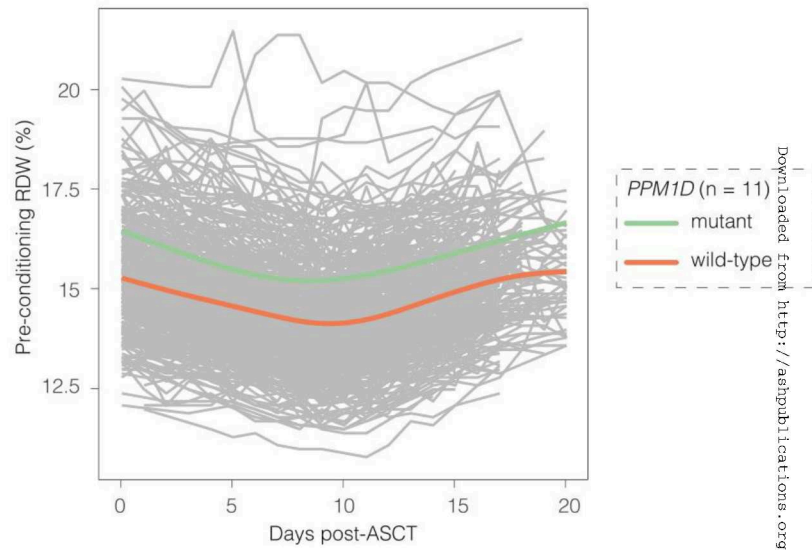


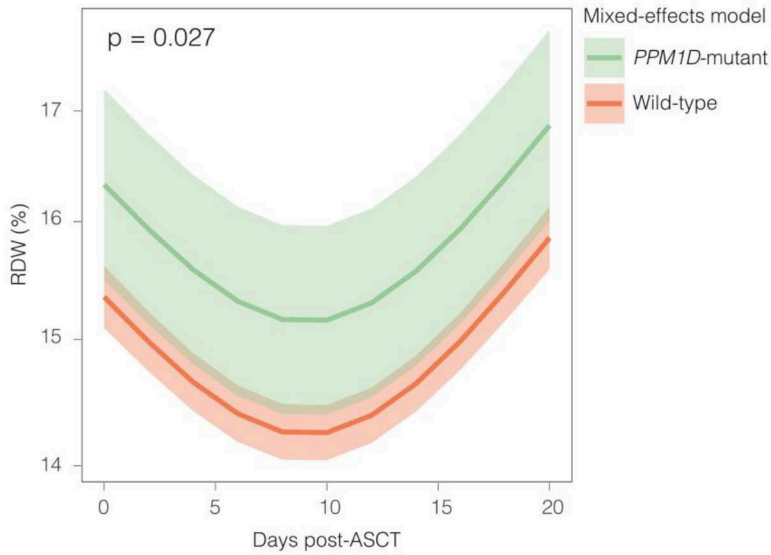
Figure 3  
A



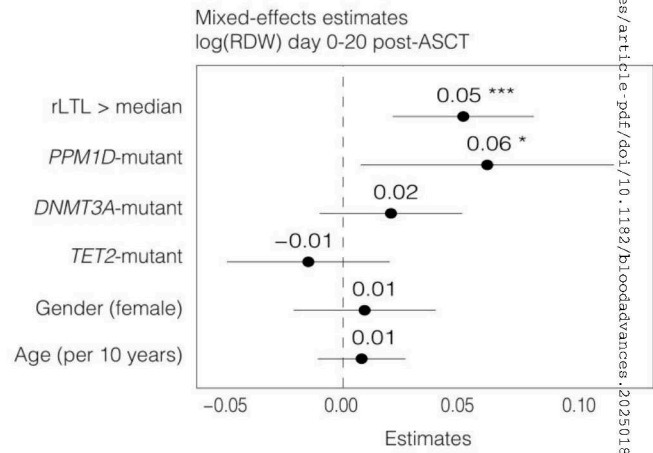
B  
CH-positive patients



C  
CH-positive patients



D



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