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

Short title:

Telomere lengths in autologous SCT outcomes.

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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.

Key points:

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

Introduction:

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

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

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

Methods:

Selection of patients and stem cell grafts

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

Annotation of myeloma disease status and cytogenetic risk

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

Isolation of genomic bulk DNA and targeted bulk DNA sequencing

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

Mutation analysis and CH variant calling

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

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

Data warehouse

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

Telomere length measurement

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

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

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

Statistical analyses

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

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

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

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

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

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

UK Biobank analyses

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

Results:

Cohort characteristics and LTL measurement

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

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

Prevalence of CH driver mutations

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

LTL and CH mutational profile

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

LTL and survival post-ASCT

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

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

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

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

LTL, CH mutations and complete blood count parameters

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

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

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

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

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

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

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

Discussion:

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

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

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

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

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

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

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

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

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

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

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Author contributions:

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

Disclosure of conflicts of interest:

G.S.V. is a consultant to STRM.BIO and Athernal Bio and holds a research grant from AstraZeneca for research unrelated to that presented here. J.T. receives research funding from AstraZeneca. M.A.F. is an employee and stockholder of AstraZeneca. The other authors declare no competing interests.

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Tables:

	CH-negative rLTL ≤ median (n = 123)	CH-negative rLTL > median (n = 158)	CH-positive rLTL ≤ median (n = 88)	CH-positive rLTL > median (n = 53)	P (within CH-negative)	P (within CH-positive)
Age in years at apheresis						
Mean (SD)	57.3 (8.21)	56.3 (9.24)	59.7 (8.12)	61.4 (7.19)	0.473	0.248
Median [min, max]	58.0 [37.0, 72.0]	57.0 [28.0, 72.0]	62.0 [39.0, 74.0]	62.0 [45.0, 72.0]		
Gender, N (%)						
Male	75 (61.0%)	105 (66.5%)	48 (54.5%)	34 (64.2%)	0.381	0.294
Female	48 (39.0%)	53 (33.5%)	40 (45.5%)	19 (35.8%)		
MM cytogenetic risk, N (%)						
Standard risk	84 (68.3%)	115 (72.8%)	62 (70.5%)	35 (66.0%)	0.43	0.58
High risk	39 (31.7%)	43 (27.2%)	26 (29.5%)	18 (34.0%)		
Mobilization chemotherapy, N (%)						
CAD	72 (58.5%)	93 (58.9%)	56 (63.6%)	28 (52.8%)	0.524	0.286

	CH-negative rLTL ≤ median (n = 123)	CH-negative rLTL > median (n = 158)	CH-positive rLTL ≤ median (n = 88)	CH-positive rLTL > median (n = 53)	P (within CH-negative)	P (within CH-positive)
Cyclophosphamide mono	5 (4.1%)	7 (4.4%)	1 (1.1%)	1 (1.9%)		
Other	3 (2.4%)	1 (0.6%)	2 (2.3%)	4 (7.5%)		
NA	43 (35.0%)	57 (36.1%)	29 (33.0%)	20 (37.7%)		
Harvested CD34+ cells x10^6/kg						
Mean (SD)	7.93 (5.85)	9.26 (6.44)	8.31 (5.84)	7.49 (6.20)	0.0425	0.305
Median [min, max]	7.40 [0.590, 40.4]	8.51 [0.600, 44.9]	7.36 [0.530, 23.0]	5.59 [0.650, 32.1]		
MM remission before ASCT						
CR	37 (30.1%)	58 (36.7%)	28 (31.8%)	11 (20.8%)	0.38	0.566
SD	29 (23.6%)	25 (15.8%)	15 (17.0%)	11 (20.8%)		
PD	6 (4.9%)	8 (5.1%)	5 (5.7%)	3 (5.7%)		
PR	51	67	40	28		

	CH-negative rLTL ≤ median (n = 123)	CH-negative rLTL > median (n = 158)	CH-positive rLTL ≤ median (n = 88)	CH-positive rLTL > median (n = 53)	P (within CH-negative)	P (within CH-positive)
	(41.5%)	(42.4%)	(45.5%)	(52.8%)		
Platelet count before ASCT /nL						
Mean (SD)	267 (93.0)	268 (67.2)	251 (82.7)	249 (73.2)	0.314	0.925
Median [min, max]	249 [82.0, 785]	261 [127, 490]	240 [99.0, 498]	231 [125, 414]		
NA, N (%)	1 (0.8%)	1 (0.6%)	0 (0%)	0 (0%)		
Leukocyte count before ASCT G/L						
Mean (SD)	5.31 (2.00)	5.17 (1.71)	5.12 (1.57)	4.76 (1.73)	0.783	0.207
Median [min, max]	5.05 [2.02, 13.2]	4.98 [1.70, 10.3]	4.82 [1.53, 9.67]	4.61 [1.14, 9.97]		
NA, N (%)	1 (0.8%)	1 (0.6%)	0 (0%)	0 (0%)		
RDW before ASCT %						
Mean (SD)	15.8 (1.57)	15.5 (1.32)	15.5 (1.44)	16.2 (1.59)	0.279	0.0022

	CH-negative rLTL ≤ median (n = 123)	CH-negative rLTL > median (n = 158)	CH-positive rLTL ≤ median (n = 88)	CH-positive rLTL > median (n = 53)	P (within CH-negative)	P (within CH-positive)
Median [min, max]	15.5 [12.5, 21.4]	15.3 [13.1, 20.5]	15.2 [12.5, 20.9]	16.1 [12.7, 20.4]		
NA, N (%)	13 (10.6%)	17 (10.8%)	7 (8.0%)	5 (9.4%)		
Hemoglobin before ASCT g/dL						
Mean (SD)	11.4 (1.48)	11.8 (1.41)	11.8 (1.63)	11.3 (1.66)	0.0103	0.116
Median [min, max]	11.5 [7.50, 15.2]	11.9 [7.70, 14.8]	11.8 [8.00, 14.9]	11.5 [7.70, 14.4]		
NA, N (%)	1 (0.8%)	1 (0.6%)	0 (0%)	0 (0%)		
Platelet count (before leukapheresis) /nL						
Mean (SD)	166 (64.1)	178 (74.8)	167 (73.6)	166 (80.9)	0.46	0.73
Median [min, max]	168 [34.0, 356]	168 [41.0, 528]	158 [41.0, 452]	149 [51.0, 494]		

	CH-negative rLTL ≤ median (n = 123)	CH-negative rLTL > median (n = 158)	CH-positive rLTL ≤ median (n = 88)	CH-positive rLTL > median (n = 53)	P (within CH-negative)	P (within CH-positive)
Leukocyte count (before leukapheresis) G/L						
Mean (SD)	23.1 (15.0)	20.0 (10.6)	17.8 (11.0)	23.4 (17.7)	0.151	0.0436
Median [min, max]	19.6 [3.50, 92.7]	17.7 [3.35, 58.6]	15.8 [2.40, 51.3]	20.6 [4.59, 97.4]		
RDW (before leukapheresis) %						
Mean (SD)	15.9 (1.34)	15.8 (1.50)	15.9 (1.53)	16.4 (1.54)	0.458	0.0351
Median [min, max]	15.8 [13.4, 19.8]	15.6 [12.7, 20.6]	15.6 [13.1, 19.3]	16.4 [13.2, 20.2]		
NA, N (%)	17 (13.8%)	17 (10.8%)	7 (8.0%)	7 (13.2%)		
Hemoglobin (before leukapheresis) g/dL						
Mean (SD)	10.6 (1.31)	10.9 (1.40)	10.8 (1.40)	10.7 (1.41)	0.125	0.606
Median (min, max)	10.6	10.9	10.9	11.0		

	CH-negative rLTL ≤ median (n = 123)	CH-negative rLTL > median (n = 158)	CH-positive rLTL ≤ median (n = 88)	CH-positive rLTL > median (n = 53)	P (within CH-negative)	P (within CH-positive)
	[7.90, 14.5]	[7.80, 16.3]	[7.20, 14.3]	[7.00, 13.1]		
rLTL (derived from normalized T/S ratio)						
Mean (SD)	0.720 (0.347)	3.20 (1.94)	0.669 (0.330)	2.95 (1.90)	<0.001	<0.001
Median [min, max]	0.651 [0.0553, 1.39]	2.55 [1.43, 13.9]	0.572 [0.0597, 1.38]	2.38 [1.43, 11.8]		
Single-mutant CH						
CH-negative	123 (100%)	158 (100%)	0 (0%)	0 (0%)	1	0.295
DNMT3A-mutated	0 (0%)	0 (0%)	21 (23.9%)	20 (37.7%)		
PPM1D-mutated	0 (0%)	0 (0%)	4 (4.5%)	3 (5.7%)		
TET2-mutated	0 (0%)	0 (0%)	14 (15.9%)	8 (15.1%)		
Other	0 (0%)	0 (0%)	49 (55.7%)	22 (41.5%)		
Year of ASCT						

	CH-negative rLTL ≤ median (n = 123)	CH-negative rLTL > median (n = 158)	CH-positive rLTL ≤ median (n = 88)	CH-positive rLTL > median (n = 53)	P (within CH-negative)	P (within CH-positive)
2004-2008	45 (36.6%)	50 (31.6%)	17 (19.3%)	15 (28.3%)	0.689	0.437
2009-2012	34 (27.6%)	45 (28.5%)	27 (30.7%)	13 (24.5%)		
2013-2016	44 (35.8%)	63 (39.9%)	44 (50.0%)	25 (47.2%)		

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

Figure legends:

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

Figure 1

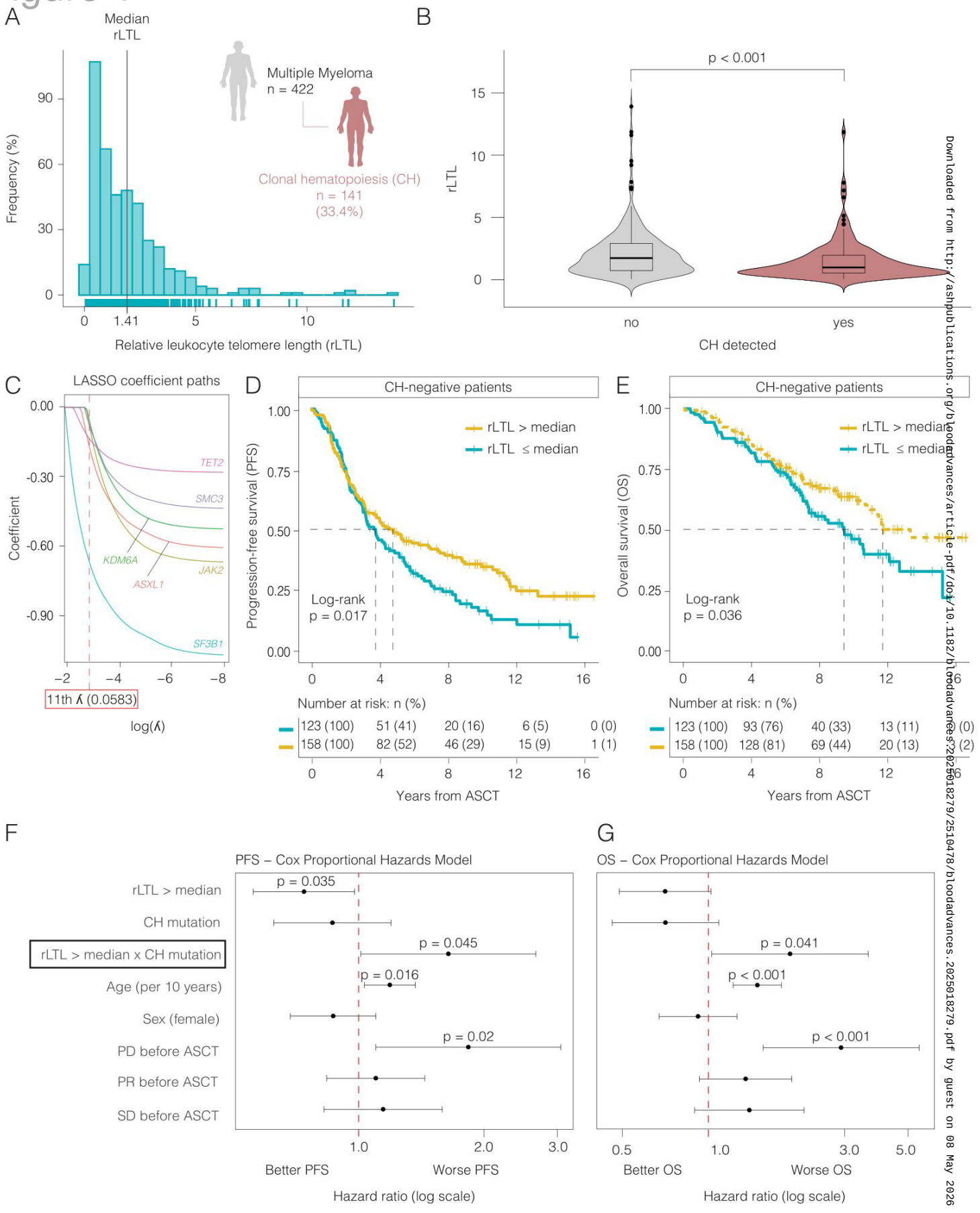
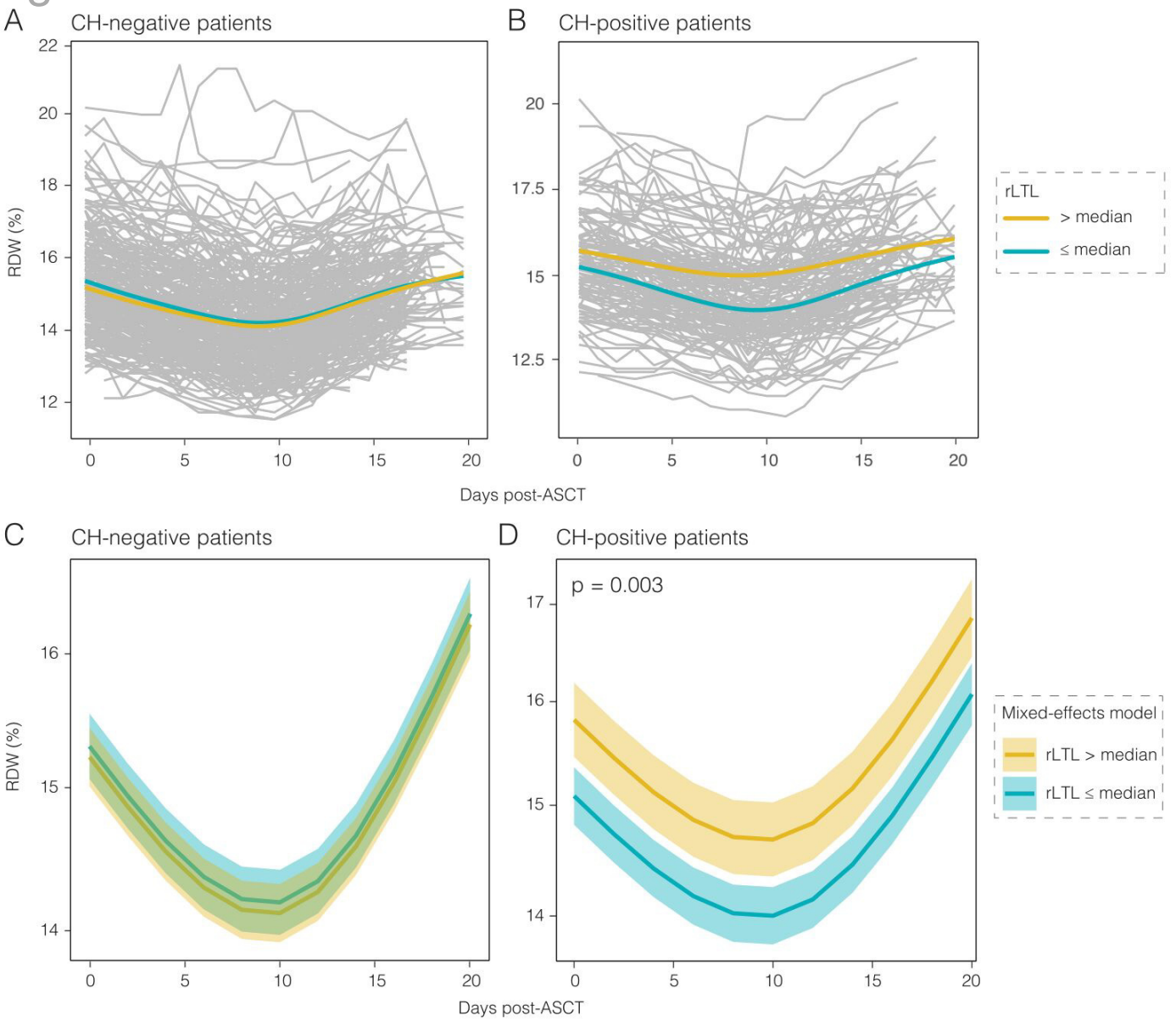
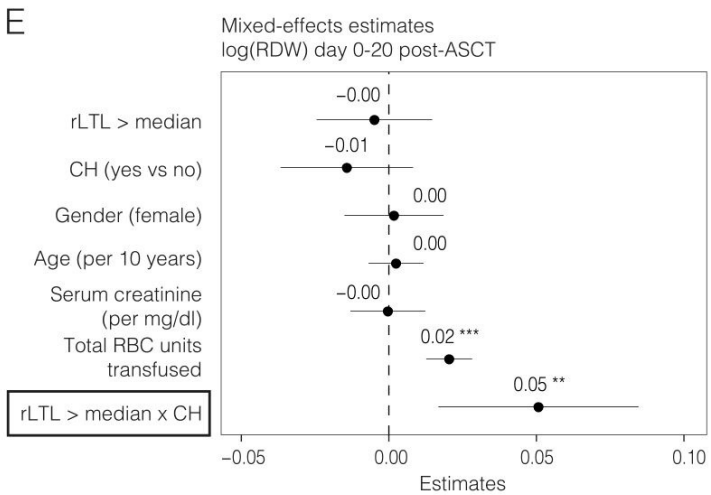
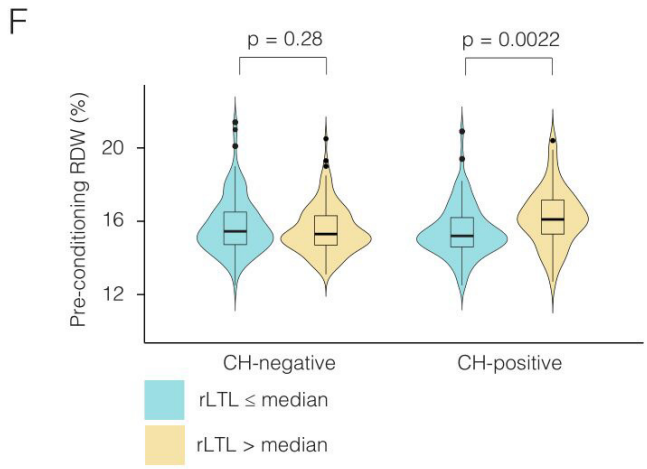


Figure 2







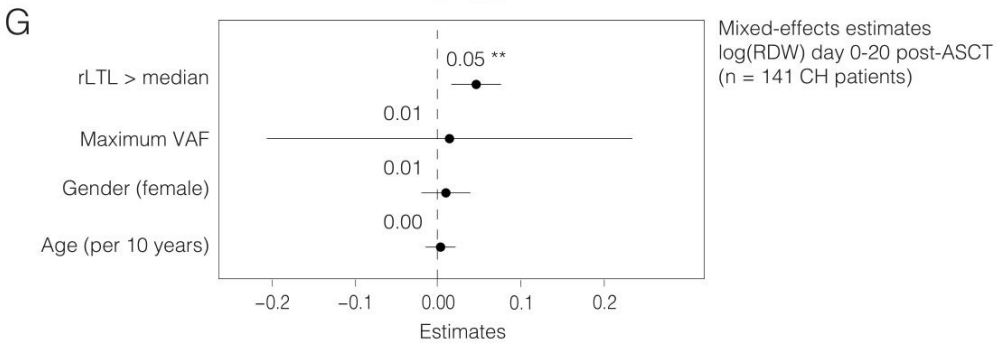


Figure 3