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Clonal Hematopoiesis Is Not Significantly Associated with Covid-19 Disease Severity

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Abstract:

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Clonal Hematopoiesis Is Not Significantly Associated with Covid-19 Disease Severity

Short title for running head

CH Is Not Associated with COVID-19 Severity

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Key points

- CH is not associated with COVID-19 disease severity

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The severity of Covid-19 disease, caused by the SARS-CoV-2 virus, is highly variable ranging from asymptomatic to a self-limited flu-like illness to severe respiratory failure, often accompanied by cardiovascular events, coagulopathy, thrombosis and high mortality.[1-3] While several risk factors for severe disease have been identified, including age, sex, ethnicity, genetic variation and a range of comorbidities, these only partially predict disease severity and additional determinants remain to be identified.[3-7]

Clonal hematopoiesis (CH) describes the disproportionate expansion of a hematopoietic stem cell (HSC) and its progeny, in association with leukemia-associated somatic mutations, most commonly affecting the genes for epigenetic regulators *DNMT3A*, *TET2* and *ASXL1*.[8,9] The prevalence and size of such clones rise with age, in association with changes in the driver gene landscape.[10] CH is associated with an increased risk of hematologic malignancies, but also of cardiovascular disease (CVD), independently of other known CVD risk factors.[11,12] The basis for this increased CVD risk has been linked to hyperinflammatory positive feedback loops driven by increased cytokine release from clonal myeloid cells, particularly interleukin IL-6 and IL-1β.[13-17]

The close association of CH with advancing age and chronic inflammation led us to hypothesize that it may be another factor associated with increased risk of severe Covid-19 disease, through hyperactivation of abnormal, clonally-derived, myeloid cells, including monocytes and macrophages, following SARS-CoV-2 infection.

To investigate a possible association between CH and Covid-19 disease severity, we studied 568 patients aged 50-90 years old (median age 64), including 120 non-hospitalized individuals with

asymptomatic or mild disease, 241 hospitalized patients not requiring intensive care unit (ICU) support, and 207 critically ill patients who required ICU admission, or mechanical ventilation or went on to die (Table 1). All patients had laboratory-confirmed SARS-CoV-2 infection during the first 6 months of 2020. All participants provided written informed consent as part of ethics committee-approved studies (Supplementary Note).

To identify individuals with CH, we performed error-corrected targeted sequencing of blood DNA for 56 genes implicated in CH using a custom set of RNA baits (Twist Bioscience design ID TE-99420296, Supplementary Table S1). Sequences were mapped to human reference genome GRCh38 and CH somatic driver mutations with a variant allele fraction (VAF) of 1-40 % were identified using Shearwater (SNV)[18] and Mutect2 (Indels).[19] With median sequencing coverage of 2,000X, we detected 266 CH driver mutations within 22 genes (Supplementary Tables S2 and S3), with 188/568 (33%) patients having at least one mutation (Figure 1A). *DNMT3A* and *TET2* mutations were most common in all three groups, with no significant enrichment for particular genes in any group (Figure 1B).

CH mutations in at least one gene were identified in 37(31%) non-hospitalized, 74(31%) hospitalized and 77(37%) critically ill patients (Figure 1C). There was no significant difference in the prevalence of CH between groups (p=0.35, Chi-Squared test). We next examined CH prevalence by age and found that, whilst this increased with advancing age in all three groups, the groups did not differ significantly when comparing individuals in the same age ranges (Figure 1D). In addition, with most CH carriers harboring one or two mutations, there were no differences between the three groups with regards to the mean number of mutations per patient

(p = 0.80, One-Way ANOVA test, Figure 1E) or the average clone size as measured by variant allele fraction (VAF, p = 0.23, One-Way ANOVA test, Fig 1F). To investigate whether mutation-bearing myeloid cells were preferentially mobilized or expanded during the acute clinical course of Covid-19, we studied available paired samples, taken 8 days apart, from 54 critically ill patients. CH was identified in 16 (32%). Comparison of VAFs between day 1 and day 9 samples did not differ significantly (p = 0.27, pairwise T-test) (Figure 1G), indicating that there was no preferential expansion of myeloid progeny arising from the CH clone.

To take account for covariates previously implicated in Covid-19 disease severity including age, sex, ethnicity, diabetes, COPD/Asthma, CVD, cancer/neoplasm, immunodeficiencies and smoking status, we applied a multivariable proportional odds model to re-test for a possible association between Covid-19 disease severity and CH (Supplementary Table S4). We found that male sex (adjusted OR=2.84, 95% CI 1.91-4.24, $p < 0.01$), diabetes (adjusted OR=1.56, 95% CI 1.05-2.44, p = 0.044), cardiovascular disease (adjusted OR=1.64, 95% CI 1.01-2.44, p = 0.046) and immunodeficiency (adjusted OR=2.10, 95% CI 1.11-4.07, p = 0.024) were all significantly associated with Covid-19 hospitalization and ICU admission, consistent with previous findings.[5] However, even after adjusting for these factors, the presence of CH was not associated with an increased risk of severe Covid-19 (OR=1.24, 95% CI 0.82-1.89, p = 0.31, Figure 1H). Similarly, neither were the number of mutations per patient (adjusted OR=1.09, 95% CI 0.88-1.36, p = 0.43) nor the CH clone size (adjusted OR=1.63, 95% CI 0.05-53.88, p = 0.78) (Figure 1H).

Given the reported links between *TET2* and *DNMT3A* mutations and hyperinflammation or response to infection,[16,17,20] these two gene mutations were interrogated individually for a

possible association with Covid-19 severity using a proportional odds model. Mutations in *TET2* and *DNMT3A* were also not associated with Covid-19 disease severity (Figure 1H). Finally, given that large clone size is more strongly associated with CVD,[11] we analyzed the risk associated with large CH clones (VAF≥5%), and again found no association with Covid-19 disease severity (OR =1.29, 95% CI 0.69-2.42, p = 0.42, Figure 1H). Similarly, we found no association between CH with VAF≥10% and Covid-19 disease severity (OR = 1.06, 95% CI 0.50-2.28, p = 0.88).

In summary, our study found no evidence that CH is associated with Covid-19 disease severity, even after adjusting for covariates known to affect the risk of severe disease. Previous studies examining the association between Covid-19 disease severity and clonal hematopoiesis CH have produced conflicting results. Three smaller studies concluded that CH is not associated with Covid-19 disease severity.[21-23] However, conclusions were less definitive due to comparisons only to historical non-Covid19 controls,[21] small sample size/ power to detect potentially-relevant associations,[22] different sequencing platforms utilized for cases and controls,[21] and/or limited availability of additional comorbidity/risk factor data.[22] A larger study (n=413) examined the relationship amongst patients with solid cancers at various stages during treatment (MSK-IMPACT cohort), and reported that non-putative driver (non-PD) clonal hematopoiesis mutations were associated with Covid-19 disease severity.[24] This finding may have reflected the impact of prior cancer treatment on patients with reduced HSC numbers/reserve. The same study used an independent non-cancer cohort (n=112) for validation, and found no significant associations within this smaller cohort, although fixed-effects meta-analysis of the combined two cohorts remained positive, likely driven by the larger MSC-IMPACT cohort.[24] Also, a recent

study reported an association between mosaic chromosomal alterations, a distinct form of CH, and risk of Covid-19 hospitalization.[25] Our current study attempted to mitigate these prior limitations by studying the largest number of patients to date, directly comparing relevant patient groups (asymptomatic/mild, hospitalized, critically ill), incorporating covariates from well-characterized additional risk factors, and performing sequencing and mutation calling using the same platforms. Overall, we found no evidence of an association between CH and Covid-19 severity, resolving much of the uncertainty surrounding this question. Whilst it is never possible to rule out an association with absolute certainty, our study indicates that the clinical impact of any theoretical association is unlikely to be substantial (Figure 1H).

Data Sharing

For access to the original sequencing data, please contact: cmdl_ngs@medschl.cam.ac.uk.

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Authorship

G.S.V., CE.D., and Z.M. conceived the study. G.S.V., C.E.D., J.K.B., Z.M., and Y.Z. designed and supervised the study. Y.Z., R.S., C.W., M.G., M.A.F., and P.M.Q. performed and advised on the bioinformatic and statistical analysis. S.N.R., S.Y., A.D.,W.D.,S.A.,W.L.,and M.C., prepared library and sequenced samples. A.G., J.P., J.H., E.J., A.C., L.A.,F.R.-L., B.T., A.F., I.G., L.N., L.S., M.R.G., A.L., I.S., T.J.G., A.B., P.B., L.I., C.D., Y.Z., K.D., H.C.S., L.D.N., P.J.M.O., and

M.G.S. provided samples and clinical information. P.J.M.O., M.G.S., and J.K.B. set up the ISARIC4C cohort. G.S.V., C.E.D., and Y.Z., wrote the manuscript with input from all co-authors.

Conflict of Interest

G.S.V. is a consultant with STRM.BIO and receives research grant from AstraZeneca. During 2021, T Gniadek was a paid consultant for Frenwal / Fresenius Kabi and became a full-time employee of the company in March 2022. Other authors have no relevant conflicts of interest to disclose.

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Table 1: Covid-19 patients characteristics

	Non-hospitalized	Hospitalized	ICU	Total
Total N	120	241	207	568
Age				
50-59	6 (5%)	90 (37%)	61 (29%)	157 (28%)
60-69	104 (86%)	50 (21%)	77 (37%)	231 (41%)
70-79	8 (7%)	67 (28%)	53 (26%)	128 (23%)
80+	2 (2%)	34 (14%)	16 (8%)	52 (9%)
Gender				
Female	71 (59%)	107 (45%)	48 (23%)	226 (40%)
Male	49 (41%)	134 (55%)	159 (77%)	342 (60%)
Ethnicity				
White	114 (95%)	196 (81%)	143 (69%)	453 (80%)
Non-White	6 (5%)	27 (11%)	35 (17%)	68 (12%)
Other	0 (0%)	8 (3%)	21 (10%)	29 (5%)
NA	0 (0%)	10 (4%)	8 (4%)	18 (3%)
Smoking				
Never	40 (33%)	134 (56%)	107 (52%)	281 (49%)
Former	26 (22%)	69 (29%)	62 (30%)	157 28%)
Current	0 (0%)	8 (3%)	7 (3%)	15 (3%)
Missing	54 (45%)	30 (12%)	31 (15%)	115 (20%)
Hypertension				
NO	38 (32%)	24 (10%)	21 (10%)	83 (15%)
YES	31 (26%)	24 (10%)	33 (16%)	88 (15%)
Missing	51 (41%)	193 (80%)	153 (74%)	397 (70%)
CVD				
NO	61 (51%)	172 (71%)	154 (74%)	387 (68%)
YES	8 (6%)	64 (27%)	47 (23%)	119 (21%)
Missing	51 (43%)	5 (2%)	6 (3%)	62 (11%)
COPD/Asthma				
NO	56 (47%)	194 (80%)	174 (84%)	424 (75%)
YES	14 (12%)	41 (17%)	27 (13%)	82 (14%)
Missing	50 (42%)	6 (3%)	6 (3%)	62 (11%)
Diabetes				
NO	56 (47%)	176 (73%)	138 (67%)	370 (65%)
YES	9 (7%)	59 (24%)	62 (30%)	130 (23%)
Missing	55 (46%)	6 (3%)	7 (3%)	68 (12%)
Cancer (Neoplasm & hematological)				
NO	66 (55%)	208 (86%)	190 (92%)	464 (82%)
YES	4 (3%)	22 (9%)	11 (6%)	38 (7%)
Missing	50 (42%)	11 (5%)	5 (2%)	66 (12%)
Immunodeficiency				
NO	71 (59%)	204 (85%)	181 (88%)	456 (80%)
YES	0	30 (12%)	17 (8%)	47 (8%)
Missing	49 (41%)	7 (3%)	9 (4%)	65 (11%)

FIGURE LEGEND

Figure 1. Investigation of the impact of clonal hematopoiesis on Covid-19 disease severity

(A) Proportion of patients carrying at least one driver CH mutation in all three Covid-19 patient cohorts combined. (B) Distribution of CH driver mutations by gene in non-hospitalized, hospitalized, and ICU patients. (C) Proportion of patients carrying at least one driver mutation in non-hospitalized, hospitalized, and ICU Covid-19 patients. p value was calculated using Chi-Square test. (D) Proportion of patients at least one CH driver mutation in non-hospitalized, hospitalized, and ICU Covid-19 patients across different age groups. (E) Number of CH driver mutations per patient in non-hospitalized, hospitalized, and ICU Covid-19 patients. (F) VAF distribution of CH driver mutations in non-hospitalized, hospitalized, and ICU Covid-19 patients. p value is calculated using One Way ANOVA. (F) Driver CH mutation gene distribution in non-hospitalized, hospitalized, and ICU Covid-19 patients. (G) VAF of driver CH mutations on day 1 and day 9 of ICU admission in individual patients. p value is calculated using T-test. (H) Multivariate proportional pdds model shows that the presence of CH, number of mutations per patient, VAF, CH mutation of ≥ 5% VAF, CH mutation of ≥ 10% VAF, *DNMT3A* mutation and *TET2* mutation are not associated with an increased risk of Covid-19 hospitalization and ICU admission. Age, sex, ethnicity, diabetes, COPD/Asthma, CV disease, cancer/neoplasm, immunodeficiency, and smoking status were adjusted for.

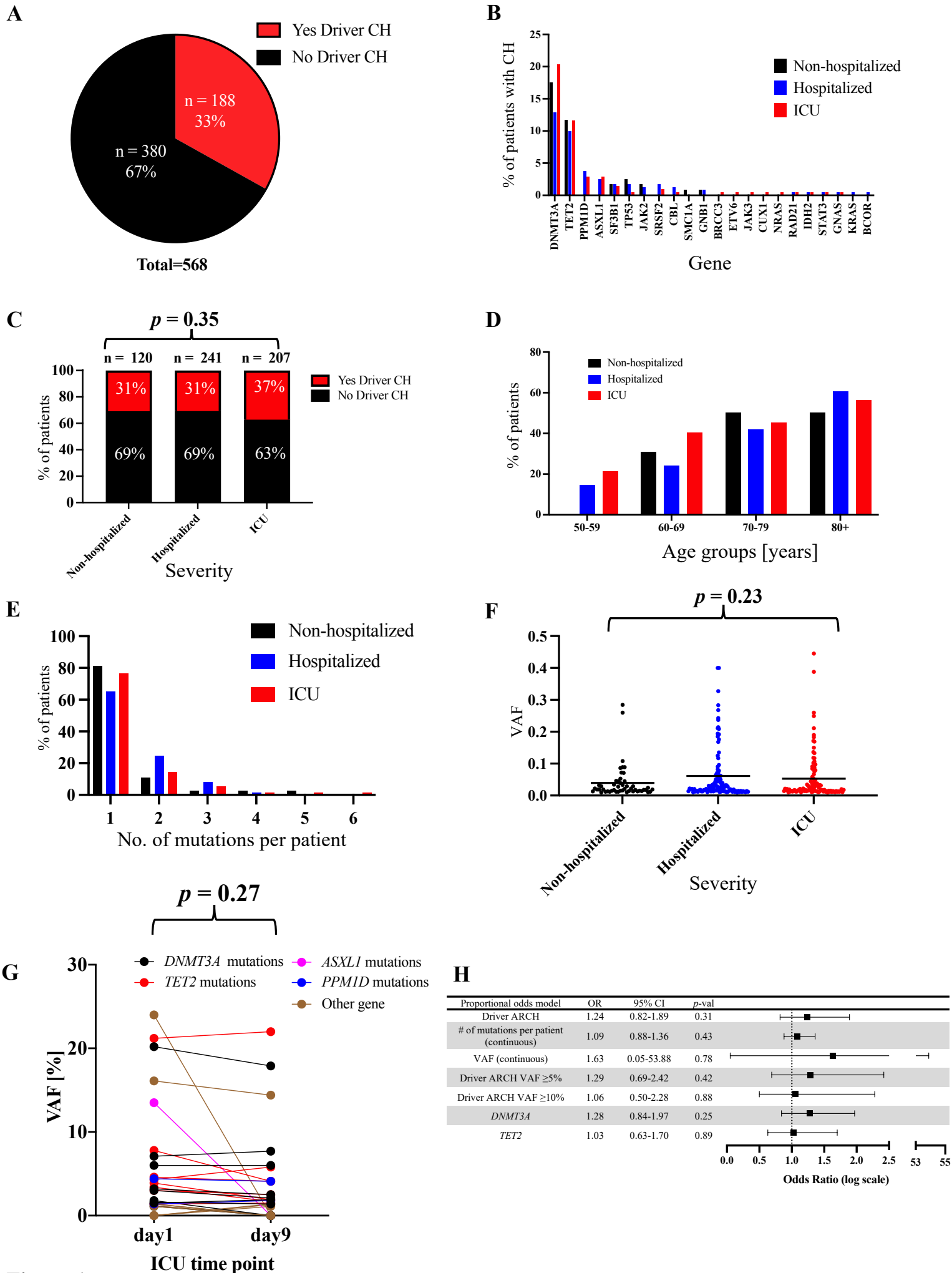
A
Yes Driver CH
No Driver CH
n = 188
33%
n = 380
67%
Total=568
B
% of patients with CH
Non-hospitalized
Hospitalized
ICU
DNMT3A
TET2
PPM1D
ASXL1
SF3B1
TP53
JAK2
SRSF2
CBL
SMC1A
GNB1
BRCC3
ETV6
JAK3
CUX1
NRAS
RAD21
IDH2
STAT3
GNAS
KRAS
BCOR
Gene
C
p = 0.35
n = 120
n = 241
n = 207
31%
31%
37%
69%
69%
63%
% of patients
Yes Driver CH
No Driver CH
Non-hospitalized
Hospitalized
ICU
Severity
D
% of patients
Non-hospitalized
Hospitalized
ICU
50-59
60-69
70-79
80+
Age groups [years]
E
% of patients
Non-hospitalized
Hospitalized
ICU
No. of mutations per patient
F
p = 0.23
VAF
Non-hospitalized
Hospitalized
ICU
Severity
p = 0.27
G
DNMT3A mutations
TET2 mutations
ASXL1 mutations
PPM1D mutations
Other gene
VAF [%]
day1
day9
ICU time point
H
Proportional odds model
OR
95% CI
p-val
Driver ARCH
1.24
0.82-1.89
0.31
# of mutations per patient (continuous)
1.09
0.88-1.36
0.43
VAF (continuous)
1.63
0.05-53.88
0.78
Driver ARCH VAF ≥5%
1.29
0.69-2.42
0.42
Driver ARCH VAF ≥10%
1.06
0.50-2.28
0.88
DNMT3A
1.28
0.84-1.97
0.25
TET2
1.03
0.63-1.70
0.89
Odds Ratio (log scale)

Figure 1.