nature genetics

Supplementary information

https://doi.org/10.1038/s41588-026-02526-w

CDK4/6 inhibition mitigates chemotherapy-induced expansion of *TP53*-mutant clonal hematopoiesis

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SUPPLEMENTARY METHODS

Raw sequence processing, variant calling and annotation

Following consensus sequence building, individual base quality scores were readjusted using GATK[1] v4.1.8.1 Base Quality Score Recalibration Tool. Variant calling was performed using three separate variant callers: Mutect2[2] v4.2.0.0, VarDictJava[3] v1.8.2, and LoFreq2[4] v2.1.5. Variants that passed one or more mutation callers were retained. Variants were annotated using Variant Effect Predictor[5] (VEP) version 104 using the canonical transcript.

Clonal hematopoiesis variant call filtering

Sequencing data from 20 young adults (<40 years old) and 7 children (<15 years old) was used internal panel of normal (PoN). For every variant, we interrogated the PoN for supporting reads. The proportion of alleles was compared between the PoN and the sample using a Fisher's exact test. Variants that were not significantly enriched within the sample compared to the PoN were excluded. Statistical significance was defined as lower than an adjusted p-value using a Bonferroni correction using the number of base pairs within the panel capture region (23,650 bp). Additionally, after re-analyzing data from Figure 4 of Chan *et al.* 2024 (PMID 38485690)[6], we determined that utilizing a read depth cut-off of 17,000 increased the precision for variants under 0.2% VAF (Supplemental Figure 3 and 4a) while still allowing us to explore biologically relevant signals separate from measurement error (Supplemental Figure 4b).

We also removed the following variants if they met one or more of following criteria:

1) VAF >2% in two or more PoN samples
2) Failed varscan2[7] false positive filter
3) INDELs greater than 20 bp called by only one variant caller
4) Evidence from only the forward or reverse strand
5) Alternate allelic depth of less than 5
6) Recurrent in >6% of all samples
7) Recurrent in >3% of samples and not previously identified in two previously large CH publications analyzing healthy individuals[8] and solid tumor patients[9].
8) Present in gnomAD[10] v2.1.1 exome dataset with $>5x10^{-4}$ population allele frequency or a maximum sub-population allele frequency $>5x10^{-3}$.
9) VAF >=35%
10) Recurrent variants with a VAF >=25% and set median VAF >=35%. were exempt from these germline mutations as loss of function variants in these genes have not been previously reported as germline.

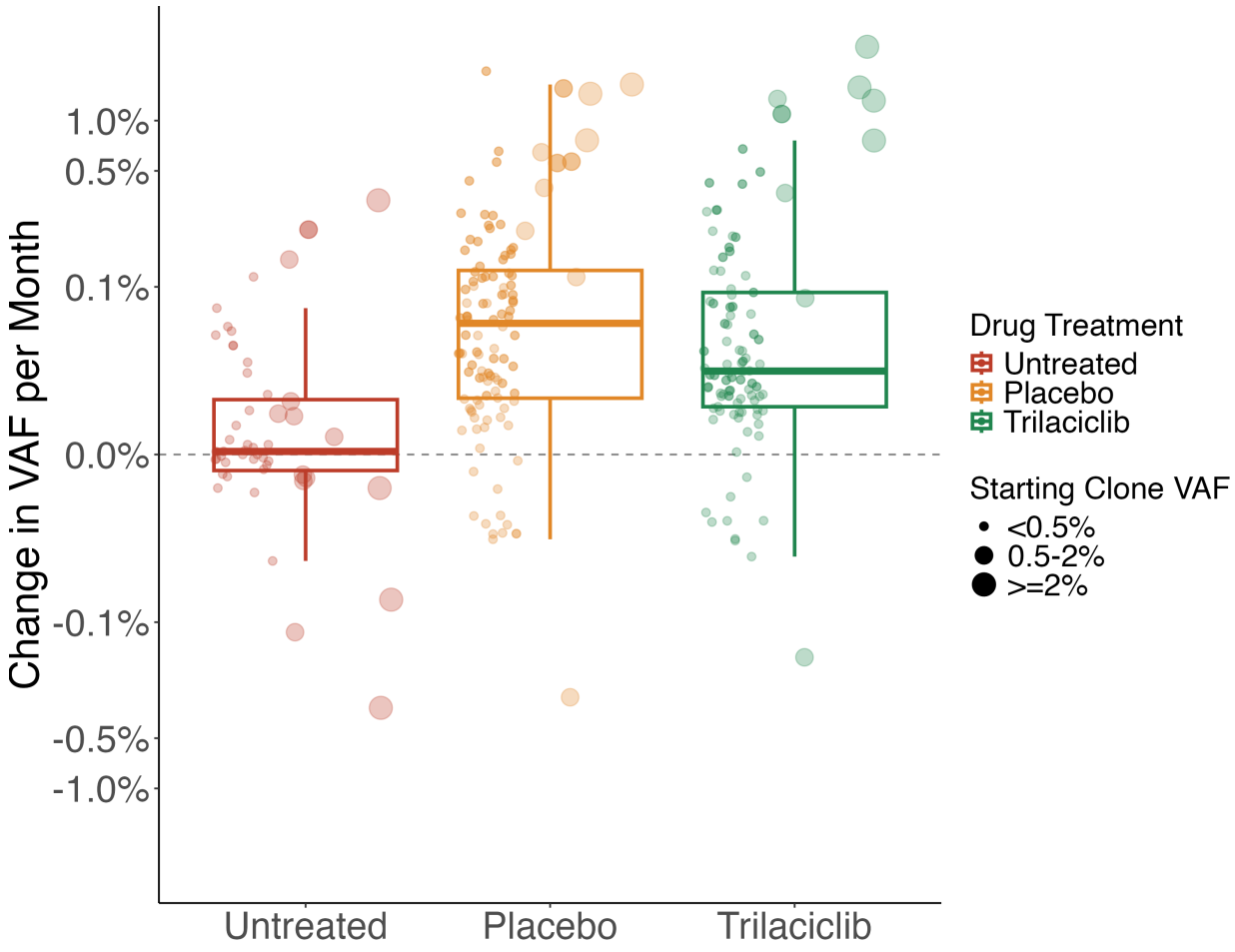
Of note, we excluded known somatic hotspot mutations in hematologic malignancies based on COSMIC[11] and loss of function mutations in *DNMT3A, TET2, ASXL1,* and *PPM1D* from 9 and 10.

Clonal hematopoiesis putative driver classification

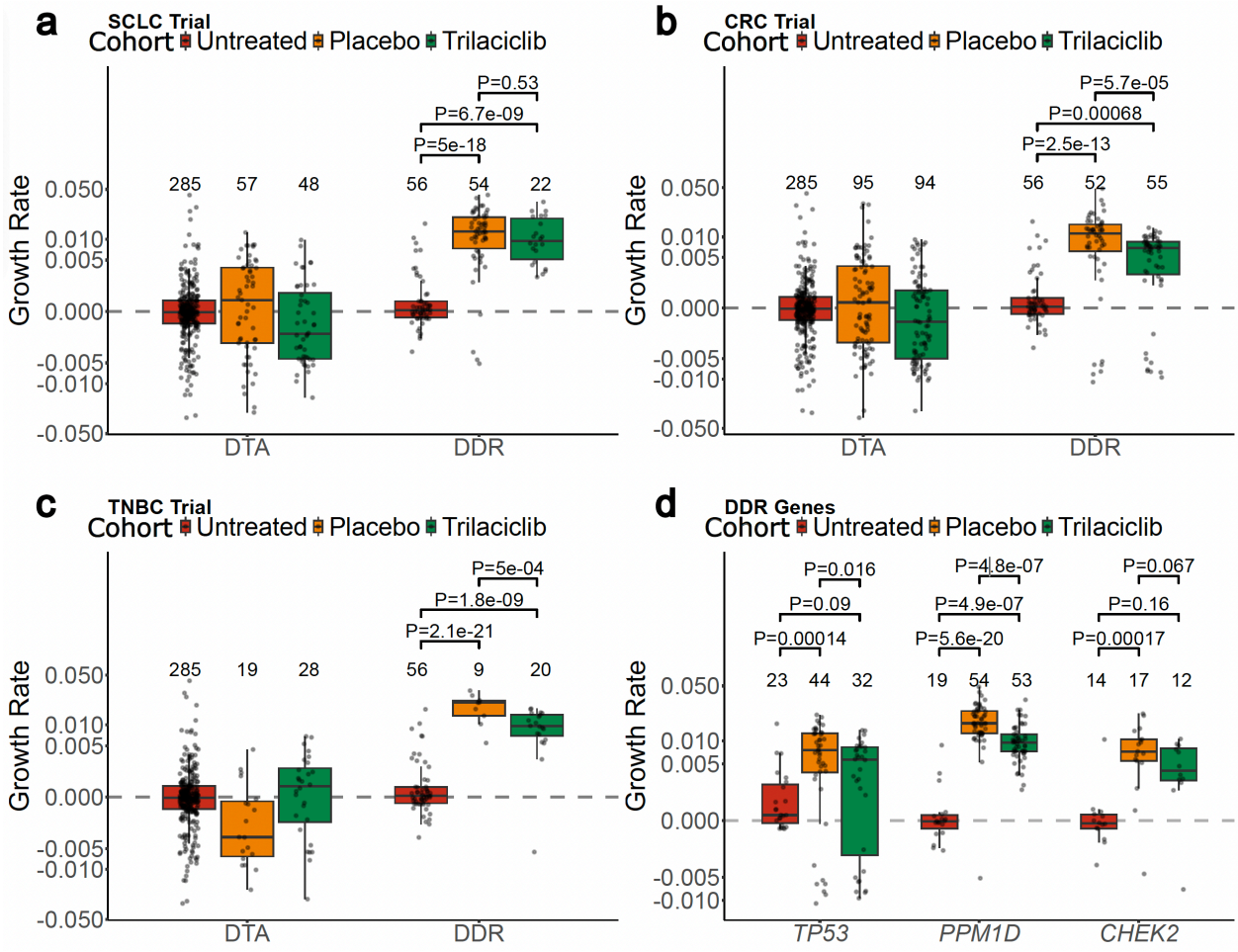
Mutations were considered to be possible putative drivers if they were:

1) Truncating mutations within *DNMT3A, TET2, TP53, ASXL1, and CHEK2*
2) Truncating mutations within exon 6 of *PPM1D*
3) In-frame INDELs within *CHEK2*

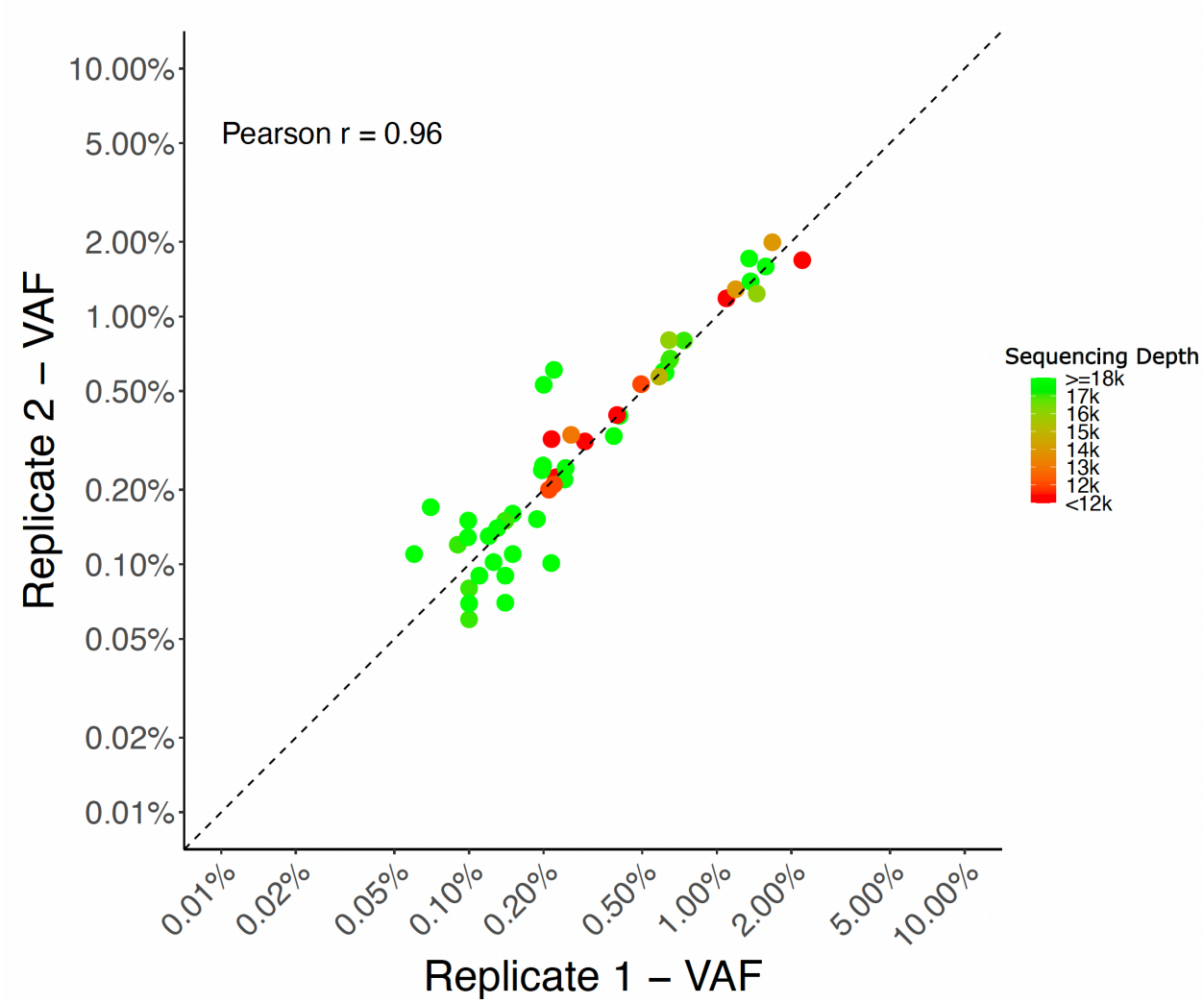
4) Missense mutations which presented in COSMIC[11] v95 at least 10x or at least 5x within the “hematopoietic and lymphoid” category
5) Previously reported as pathogenic by two previously large CH publications analyzing healthy individuals[8] and solid tumor patients[9].
6) Classified as either oncogenic or likely oncogenic within the OncoKB[12] v3.10 database
7) Missense mutations within the same amino acid loci as a previously reported CH mutation[8,9] with computational evidence of being potentially damaging by the SIFT[13] or PolyPhen[14] algorithms
8) Missense mutations within 3 amino acid residues or within 9 nucleotides of previously reported CH hotspot mutations[8,9]
9) Missense mutations within established regions for *SRSF2* (amino acid position 95) and *SF3B1* (amino acid positions 622-626, 662-666, 700-704, 740-742).
10) Variants reported as likely pathogenic or pathogenic in ClinVar[15]



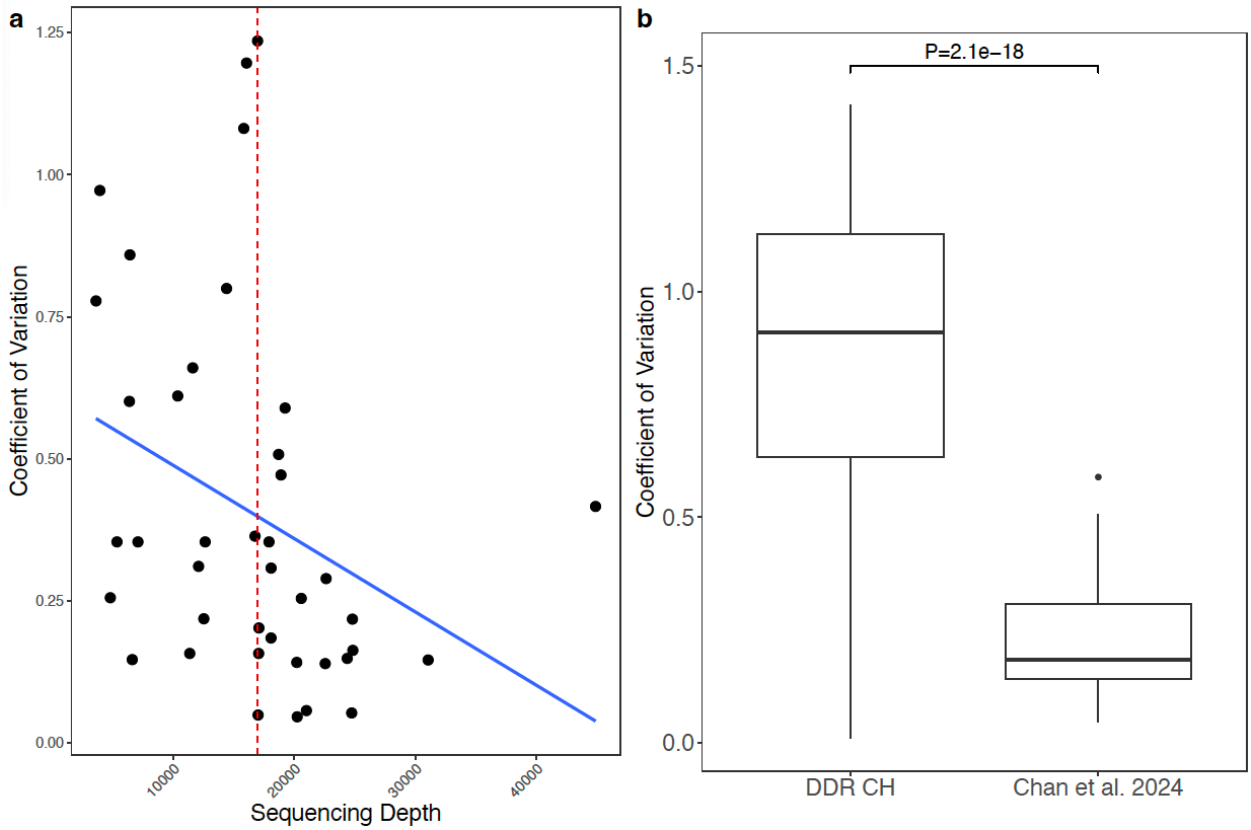
Supplementary Figure 1. Impact of trilaciclib on the change in variant allele fraction per month for DNA damage response clonal hematopoiesis within each patient during cytotoxic therapy. The relative change in variant allele fraction (VAF) over the duration of the clinical trial in months for patients harboring a DNA damage response (DDR) clonal hematopoiesis (CH) mutation (*TP53, PPM1D, CHEK2*) in the trilaciclib (green) and placebo (orange) arm compared to untreated healthy individuals (red) for all clinical trials of trilaciclib. Boxplots were drawn using the Tukey method, with the bar indicating the median, interquartile range shown as the box edges and whiskers extending to 1.5x the interquartile range. Each point represents CH mutational growth rates used to derive the boxplot.



Supplementary Figure 2. Sensitivity analysis exploring the impact of trilaciclib on DNMT3A, TET2, ASXL1 and DNA damage response clonal hematopoiesis limited to mutations present ≥0.2% VAF in either pre- or post-treatment samples. Growth rate of *DNMT3A, TET2, ASXL1* (DTA) clonal hematopoiesis (CH) and DNA damage response (DDR) CH mutations in trilaciclib (green) and placebo arm (orange) compared to untreated healthy individuals (red) after removing variants that were below 0.2% VAF in both timepoints in **(a)** the small cell lung cancer (SCLC) trials, **(b)** the colorectal cancer (CRC) trial, **(c)** the triple negative breast cancer (TNBC) trial, and **(d)** pooled data across trials for individual DNA Damage response (DDR) genes. Boxplots were drawn using the Tukey method, with the bar indicating the median, interquartile range shown as the box edges and whiskers extending to 1.5x the interquartile range. Each point represents CH mutational growth rates used to derive the boxplot. Differences in the growth rate of CH was tested using linear regression adjusted for age. *P <0.05, **P <0.01, ***P <0.001.



Supplementary Figure 3. Accuracy of clonal hematopoiesis variant allele frequency for mutations present at a variant allele fraction under 10% using the ArCH variant calling pipeline. Data from Chan *et al.* 2024 (PMID 38485690) after removing variants with a variant allele fraction (VAF) below 0.2% and a total read depth below 17,000x. Shown is the comparison of VAF between two technical replicates for clonal hematopoiesis mutations that were identified as putative drivers. Correlation between VAF estimates between paired replicates was determined using the Pearson correlation coefficient (r) where a value of 1 indicates perfect positive correlation.



Supplementary Figure 4. Coefficient of variation among clonal hematopoiesis variants with a variant allele fraction of <0.2%. (a) Among technical replicates drawn from Chan *et al.* 2024 (PMID 38485690), the coefficient of variation for clonal hematopoiesis (CH) mutations present at a variant allele fraction (VAF) <0.2% in one or more replicates is shown in relation to the total sequencing depth for that position. The red dashed line represents the sequencing depth of 17,000 which was defined as the new cut-off value for filtering. The blue line represents a linear regression fit to the data showing decreased variation with increased sequencing depth **(b)** The coefficient of variation among CH variants present at a VAF <0.2% is compared between DNA damage response CH variants detected in biologic replicates collected before and after chemotherapy and the technical replicates from Chan *et al.* 2024 (PMID 38485690) represented as boxplots with the bar indicating the median, interquartile range shown as the box edges and whiskers extending to 1.5x the interquartile range. Differences in the coefficient of variation was tested using linear regression without adjusting for any other covariates. *P <0.05, **P <0.01, ***P <0.001.

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