nature portfolio

Peer Review File

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

Corresponding Author: Dr Kelly Bolton

This manuscript has been previously reviewed at another journal. This document only contains information relating to versions considered at Nature Genetics.

Version 0:

Decision Letter:

14th Oct 2025

Dear Dr Bolton,

Your Article, "CDK4/6 inhibition mitigates chemotherapy-induced expansion of TP53-mutant clonal hematopoiesis" has now been seen by the same Reviewer #1 who reviewed the paper at Nature. You will see that they continue to raise issues with the work. We are interested in the possibility of publishing your study in Nature Genetics, but would like to consider your response to these concerns in the form of a revised manuscript before we make a final decision on publication. Our plan is to send the revised manuscript to a new reviewer and ask them to focus only on the way you have addressed the issues flagged in this round of review.

We therefore invite you to revise your manuscript taking into account all comments. Please highlight all changes in the manuscript text file. At this stage we will need you to upload a copy of the manuscript in MS Word .docx or similar editable format.

We are committed to providing a fair and constructive peer-review process. Do not hesitate to contact us if there are specific requests from the reviewers that you believe are technically impossible or unlikely to yield a meaningful outcome.

When revising your manuscript:

*1) Include a “Response to referees” document detailing, point-by-point, how you addressed each referee comment. If no action was taken to address a point, you must provide a compelling argument. This response will be sent back to the referees along with the revised manuscript.

*2) If you have not done so already please begin to revise your manuscript so that it conforms to our Article format instructions, available
here.
Refer also to any guidelines provided in this letter.

*3) Include a revised version of any required Reporting Summary: https://www.nature.com/documents/nr-reporting-summary.pdf
It will be available to referees (and, potentially, statisticians) to aid in their evaluation if the manuscript goes back for peer review.
A revised checklist is essential for re-review of the paper.

Please be aware of our guidelines on digital image standards.

EXTENDED DATA FIGURES

When re-submitting your manuscript, please ensure that any supplementary figures and tables that are crucial to the manuscript’s conclusions are converted into Extended Data figures and tables to increase visibility of these data. Extended

Data figures and tables are online-only (present in the online PDF and full-text HTML versions of the paper), peer-reviewed display items that provide essential background to the article but are not included in the main article due to space constraints. A maximum of ten Extended Data display items (figures and tables) is permitted.

Please use the link below to submit your revised manuscript and related files:

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Note: This URL links to your confidential home page and associated information about manuscripts you may have submitted, or that you are reviewing for us. If you wish to forward this email to co-authors, please delete the link to your homepage.

We hope to receive your revised manuscript within four to eight weeks. If you cannot send it within this time, please let us know.

Please do not hesitate to contact me if you have any questions or would like to discuss these revisions further.

Nature Genetics is committed to improving transparency in authorship. As part of our efforts in this direction, we are now requesting that all authors identified as 'corresponding author' on published papers create and link their Open Researcher and Contributor Identifier (ORCID) with their account on the Manuscript Tracking System (MTS), prior to acceptance. ORCID helps the scientific community achieve unambiguous attribution of all scholarly contributions. You can create and link your ORCID from the home page of the MTS by clicking on 'Modify my Springer Nature account'. For more information please visit please visit www.springernature.com/orcid.

We look forward to seeing the revised manuscript and thank you for the opportunity to review your work.

Sincerely,

Safia Danovi, PhD
Senior Editor, Nature Genetics
ORCiD: 0009-0007-7822-5479

Reviewers' Comments:

Reviewer #1 (Remarks to the Author):

The authors provide new reanalysis of PMID 38485690, arguing that the poor results shown for variants with VAF <0.2% in that paper are due to inclusion of lower depth sequencing samples. The only evidence in support of this is a recoloring of the data points Figure 4 of 38485690 by depth. Furthermore, they include variants with VAF>0.2% in this graph as well as in new Supplementary Table 3.

Please show the relative errors (where Relative Error = |(Measured Value - Expected Value) / Expected Value|) for variants with expected VAF less than 0.2% only. Please compare the relative errors of the technical replicates in Fig 4 of 38485690 to the range of fold changes seen in the longitudinal samples reported in the new manuscript. Please show a graph comparing relative error vs sequencing depth to show that sequencing depth is in fact driving poor reproducibility of these low VAF variants.

New Supplemental Figure 3 shows Pearson r=0.964 for all variants with VAF up to 2% VAF. Please include only values below 0.2% in this graph and for showing r.

I disagree about how to interpret the results when only values above 0.2% are used—the authors argue that this is a "sensitivity" analysis, but it is really an analysis to demonstrate whether the main result holds statistical significance when only the highest confidence variants are used. Unfortunately, the result for TP53 is null at this threshold. This does not necessarily mean that there is no effect, but the study lacks the power to show it.

Version 1:

Decision Letter:

Our ref: NG-A70328R

2nd Dec 2025

Dear Dr Bolton,

How are you? I hope you're well.

Thank you for submitting your revised manuscript "CDK4/6 inhibition mitigates chemotherapy-induced expansion of TP53-mutant clonal hematopoiesis" (NG-A70328R). As discussed with you, I decided to send the paper to a new reviewer (Reviewer #2) and restrict the scope of their review to the few remaining points that had been flagged by Reviewer #1.

I'm delighted to inform you that we'll be happy in principle to publish your paper in Nature Genetics, pending minor revisions to satisfy the referees' final requests and to comply with our editorial and formatting guidelines.

If the current version of your manuscript is in a PDF format, please email us a copy of the file in an editable format (Microsoft Word or LaTex)-- we can not proceed with PDFs at this stage.

We are now performing detailed checks on your paper and will send you a checklist detailing our editorial and formatting requirements soon. Please do not upload the final materials and make any revisions until you receive this additional information from us.

Thank you again for your interest in Nature Genetics Please do not hesitate to contact me if you have any questions.

Sincerely,

Safia Danovi, PhD
Senior Editor, Nature Genetics
ORCiD: 0009-0007-7822-5479

Reviewer #2 (Remarks to the Author):

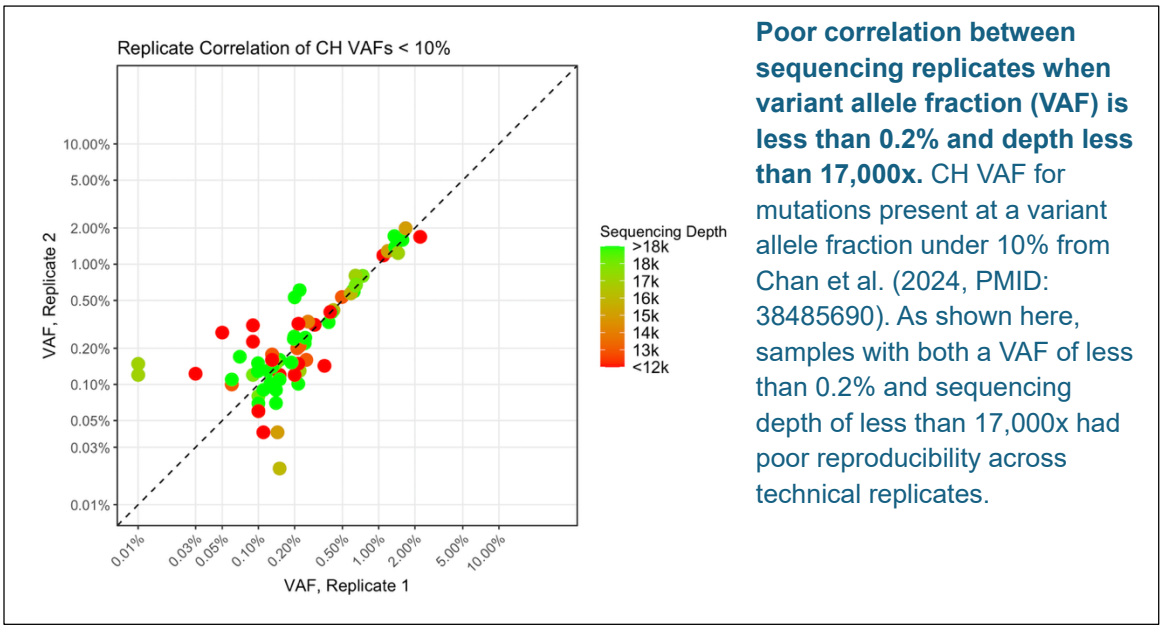
Therapy-related myeloid neoplasms (tMNs) are a recognised and frequently lethal consequence of cytotoxic chemotherapy for solid cancers. A significant proportion of such tMNs arise from pre-existing TP53-mutant CH clones that are afforded a relative fitness advantage over normal (TP53-WT) haematopoietic stem & progenitor cells (HSPCs) by cytotoxic chemotherapy. This results in TP53-mutant clonal expansion that increases the risk of downstream tMNs.
Chan et al investigate the impact of CDK4/6 inhibition on the clonal advantage/expansion of TP53- and other DDR gene-mutant CH observed during/after cytotoxic chemotherapies.
Specifically, they analyse data from 3 randomised placebo-controlled clinical trials in which the short-acting CDK4/6 inhibitor trilaciclib was given intravenously, together with cytotoxic chemotherapy for different cancers, to prevent myelosuppression (an FDA-licensed indication for this drug). Analyses of these data revealed that, whilst not abrogated, clonal expansion of DDR gene-mutant CH after cytotoxic chemotherapy was significantly reduced in those receiving trilaciclib (vs placebo). Findings were consistent amongst the different cytotoxic treatments given in these 3 trials, namely: i) carboplatin and etoposide with and without atezolizumab for ES-SCLS, ii) leucovorin/ fluorouracil/oxaliplatin/irinotecan (FOLFOXIRI) plus bevacizumab for metastatic colorectal cancer and iii) gemcitabine plus carboplatin for metastatic triple-negative breast cancer.
The authors go on to investigate experimentally the impact of CDK4/6 inhibition in a competitive transplantation setting involving HSPCs from a Tp53-R172H mouse model (mostly in a 1:9 ratio with CD45.1 WT HSPCs). They find that both intravenous trilaciclib administered 30 minutes prior to carboplatin and oral palbociclib at 100mg/kg daily for 5 days per week for 1 month, abrogated the relative expansion of TP53-mutant HSPCs in blood and bone marrow seen with carboplatin alone. Notably, the effect of trilaciclib on curtailing Tp53-mutant HSPC expansion persisted at 8 weeks after treatment cessation. Similar effects Tp53-mutant HSPC expansion were observed with a Cdk6 genetic knock mouse/allele.
Additionally, the authors explore the impact of CDK4/6 inhibition on the effects of carboplatin on haematopoiesis and find, amongst others, that: i) it promotes lymphopoiesis and suppresses the myeloid advantage seen with Tp53-mutant HSPCs in this context and ii) it increases apoptosis and cell cycle arrest of murine Tp53-mutant LT-HSCs.
Collectively, the manuscript makes a credible case that treatment with CDK4/6 inhibitors warrants investigation in the prevention of TP53-mutant tMN after cytotoxic chemotherapy for solid cancers. The concept that short-term CDK4/6 inhibition, contemporaneous with administration of cytotoxic chemotherapy, may be able to prevent TP53-CH clonal expansion and by extension tMN, makes for an attractive and clinically testable hypothesis.
Overall, this is a comprehensive manuscript that starts with clinical observations and moves on to validate these experimentally. From the rebuttal document, it is evident that the authors have made at least two rounds of revisions. I can see that one reviewer remained concerned about the robustness of mutation calling at low VAF, but I find the authors' response to this particular concern convincing.
In line with the above, I have no major criticism of the manuscript, but below are some minor comments aiming to further improve some aspects of the manuscript.

Minor comments
1. Line 106: the authors state that "Overall, 96% of all detectable CH mutations following therapy were present prior to therapy". They should reconcile (explain) this with their earlier observation that CH due to mutations in DDR pathway genes including TP53, PPM1D, and CHEK2 is increased in prevalence following chemotherapy
2. Line 156: the authors state that "Besides trilaciclib treatment, only older age at baseline (for TP53-CH and DDR CH) and smaller VAF at baseline (for DDR CH) was predictive of higher CH growth rate during therapy". Was the impact of age and smaller VAF observed in both groups (trilaciclib & placebo)? E.g. were age and smaller VAF also predictive of clonal growth in the placebo group?
3. Line 211: "…animals receiving both agents concomitantly experienced only a mild macrocytic anemia (Extended Data

Fig. 3d).” This is shown in Extended Data Fig 3b-c (not 3d). Also, the word “only” could be omitted as readers may infer that anemia (potentially worse than mild) was seen in other groups.
4. Line 215: “…hematopoietic cells to cytotoxic chemotherapy.” For clarity, the word “to” should be replaced with “during” or similar.
5. Lines 239-240: “…undergoing apoptosis at the conclusion of four weeks of mice receiving platinum chemotherapy”. The words “mice receiving” can be removed for clarity.
6. Line 257: “…the effect of genetically manipulating CDK6 in response to platinum chemotherapy”. Replace “in” with “on the” or similar
7. Line 260: “Cells expressing WT or mutant Cdk6”. Presumable these are “TP53-mutant cells expressing…”. Please clarify.
8. Line 333: remove “have”
9. Line 342: “… revealed positively enriched of…”. Replace “positively enriched” with “positive enrichment” or similar.
10. Line 365: “This includes those..”. Replace with “These include those…”

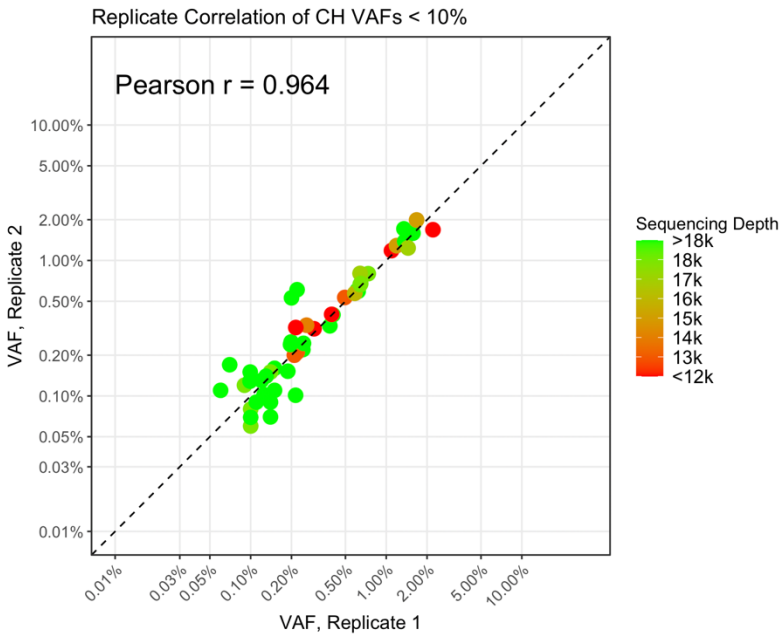
Response to Reviewer Comments: In this manuscript we provide multiple lines of robust evidence that administration of CDK4/6 inhibitors in conjunction with chemotherapy, mitigates the growth of *TP53* mutant clonal hematopoiesis (CH). The data derives from a highly unique dataset of genomic DNA sequencing of peripheral blood collected from patients with three distinct solid tumor types. These patients were enrolled in three separate randomized, placebo-controlled clinical trials of chemotherapy, with or without the short-acting intravenous CDK4/6 inhibitor trilaciclib administered prior to chemotherapy. In addition, we included a rigorous series of in vivo studies in mice with TP53-mutant clonal hematopoiesis to investigate the impact of CDK4/6 inhibitors on growth of TP53 mutant CH in the setting of platinum chemotherapy. The findings across each of these studies in both patients and mice are highly consistent: there is clear evidence that CDK4/6 inhibition prior to cytotoxic chemotherapy reduces expansion of *TP53* mutant clonal hematopoiesis. These findings are important and novel, as they represent the first demonstration of a pharmacologic strategy to prevent chemotherapy-related *TP53*-mutant CH expansion in patients. Moreover, CDK4/6 inhibitors are already a cornerstone of many cancer therapies, and this approach therefore has potential to be applied as a preventive strategy for patients with numerous common forms of cancer.

One Reviewer of this manuscript has raised a concern regarding misclassification of low VAF variants due to limits in precision at low VAFs. In our prior work in PMID 38485690 (Chan *et al.*, Bioinformatics 2024), we extensively characterized the precision, sensitivity, and specificity of the CH detection assay used in the present manuscript using tumor:normal dilution samples, orthogonal sequencing, and technical replicates. A point of confusion in the previous revision of the manuscript was the decreased precision of this assay for variants with a VAF under 0.2% shown in Figure 4 of PMID 38485690. However, when the data from Figure 4 of PMID 38485690 were evaluated based on total sequencing depth at each position where a variant was identified, it was very clear that a lower total sequencing depth (<17,000x) is associated with lower precision for low VAF variants (see figure below where we color-coded each variant based on sequencing depth at that position). Fortunately, the average sequencing depth in the manuscript under review is 27,665x which mitigates concern regarding precision for variants with a VAF under 0.2%.



Poor correlation between sequencing replicates when variant allele fraction (VAF) is less than 0.2% and depth less than 17,000x. CH VAF for mutations present at a variant allele fraction under 10% from Chan et al. (2024, PMID: 38485690). As shown here, samples with both a VAF of less than 0.2% and sequencing depth of less than 17,000x had poor reproducibility across technical replicates.

In the previous revision of the manuscript, we included a new figure (**Supplemental Figure 3**; shown below; now **Supplemental Methods Figure 1**) using data from Figure 4 in PMID 38485690 showing an excellent correlation in VAF between blinded technical replicates after removing variants with a VAF<0.2% at positions with a total sequencing depth of under 17,000x.



Supplemental Methods Figure 1. Accuracy of CH variant allele frequency for mutations present at a variant allele fraction under 10% using the ArCH Pipeline. Data taken from Chan *et al.*[26] after removing variants with a variant allele fraction (VAF) below 0.2% and a total read depth under 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.

Additionally, in the previous revision, to ensure consistently high precision for low VAF variants, we modified our CH filtering criteria for the clinical trial analysis. Specifically, we removed variants with a VAF<0.2% where the total sequencing depth at that position was under 17,000x. We retained other variants above 0.2% if the coverage was at least 5,000x.

Thus, in summary, in previous revisions, we have provided additional evidence of high precision even for low VAF variants (<0.2%) sequenced at a sufficiently high depth. We modified our CH filtering criteria to maximize excellent precision as informed by our technical replicate studies. In this current revision, the Reviewer seeks additional analyses which we perform below. These analyses continue to support the excellent precision of our assay.

We acknowledge that there can be measurement error when estimating VAFs for low-frequency variants, even with a high-depth assay. However, this error does not undermine our key findings. A lack of VAF precision introduces a form of random misclassification error. In molecular and genetic epidemiology studies, the well-established effect of random misclassification error is to bias results toward the null hypothesis. This means such error typically reduces the observed strength of association, making true differences harder to detect. The fact that we consistently observe a statistically significant effect (specifically, higher growth rates for DDR CH clones in the placebo arm compared to the trilaciclib arm across three

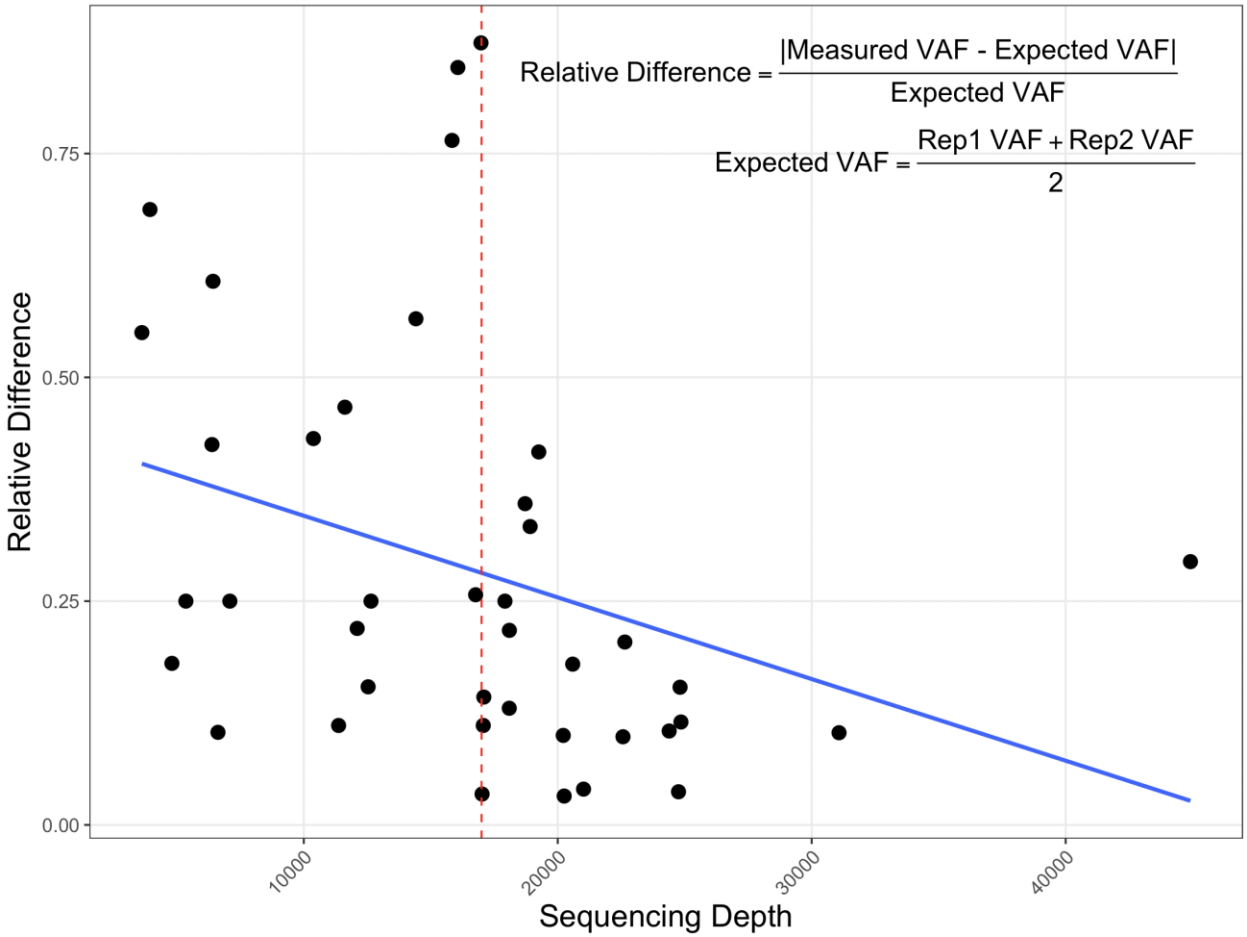
separate clinical trials) cannot be explained by random error. If our assay were perfectly precise (an unrealistic ideal), the difference we observe would likely be even more pronounced. Furthermore, the robustness of our conclusions is supported by external validation. We replicated the core finding from our clinical trials in an independent experimental animal model of *Tp53*-mutant CH, providing strong evidence that our results are due to a genuine biological effect rather than technical noise.

1) **Reviewer Comment:** "The authors provide new reanalysis of PMID 38485690, arguing that the poor results shown for variants with VAF <0.2% in that paper are due to inclusion of lower depth sequencing samples. The only evidence in support of this is a recoloring of the data points Figure 4 of 38485690 by depth. Furthermore, they include variants with VAF>0.2% in this graph as well as in new Supplementary Table 3. Please show the relative errors (where Relative Error = |(Measured Value - Expected Value) / Expected Value|) for variants with expected VAF less than 0.2% only. Please compare the relative errors of the technical replicates in Fig 4 of 38485690 to the range of fold changes seen in the longitudinal samples reported in the new manuscript. Please show a graph comparing relative error vs sequencing depth to show that sequencing depth is in fact driving poor reproducibility of these low VAF variants."

REPLY: We appreciate this suggestion and agree that expressing the difference between replicate samples relative to absolute value would be helpful. Relative error is used when comparing the results of an assay to an established "gold standard". Relative error is not typically used in evaluating technical replicates because no "expected value" from a "gold standard" would be available. For replicates samples, the coefficient of variation is more commonly used, defined as the standard deviation for the replicate values divided by their mean.

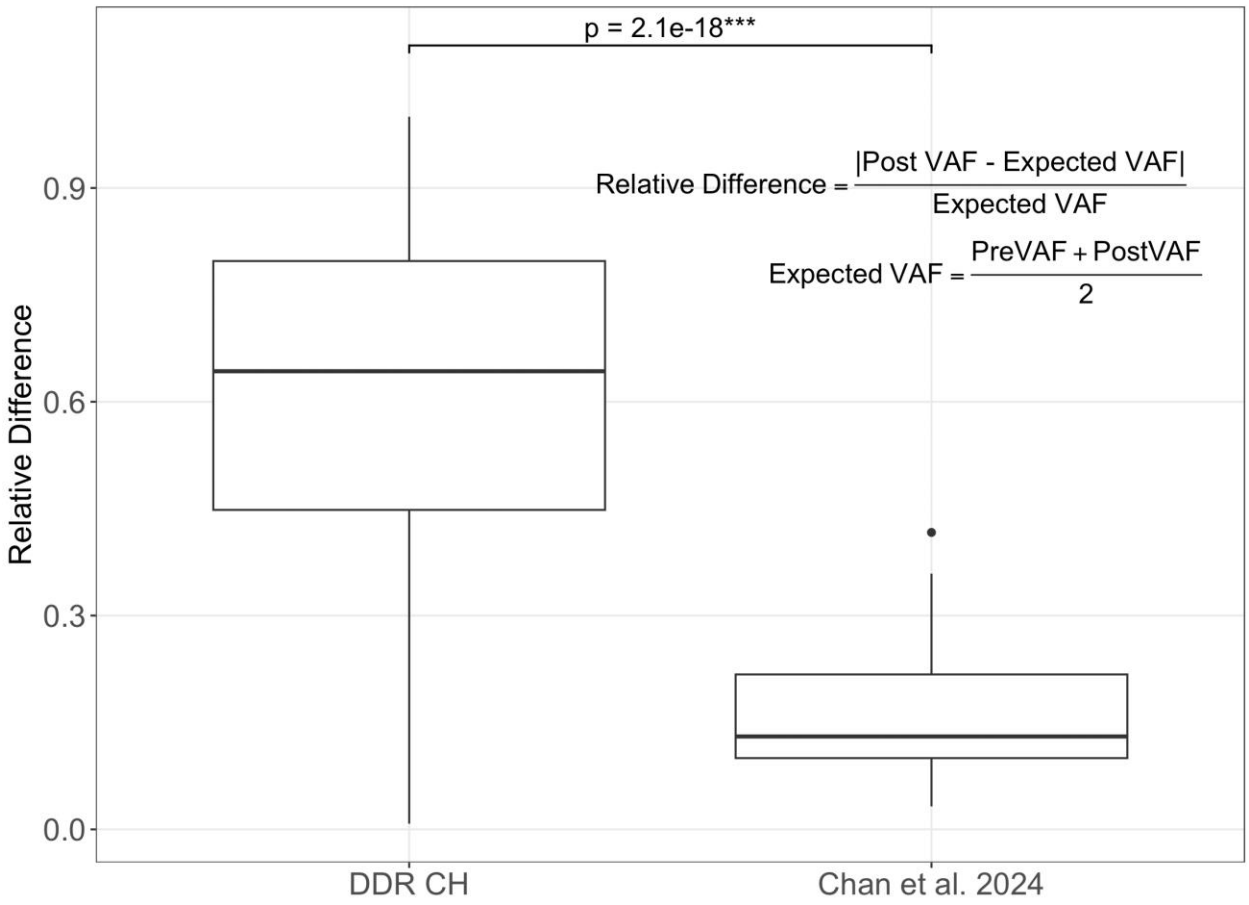
Nonetheless, as requested by the Reviewer, we present the "relative error" below, using the mean of technical replicate samples as the "expected value". For the "measured value", we select replicate 2 (this choice is arbitrary and virtually identical results are generated by using replicate 1). We label this as the "relative difference" between replicates instead of the "relative error" since there is no "gold standard" as a comparator group.

We plotted the relative difference between replicates for variants under 0.2% VAF as a function of the sequencing depth. As expected, the relative difference between replicates increases with decreasing total depth. We observed the most variability for variants where the total depth was under 17,000x as noted by the red dashed line, further supporting our revised filtering (from revision #3) to remove variants with a VAF under 0.2% and a total depth under 17,000x (see **Reviewer Figure 1** below).



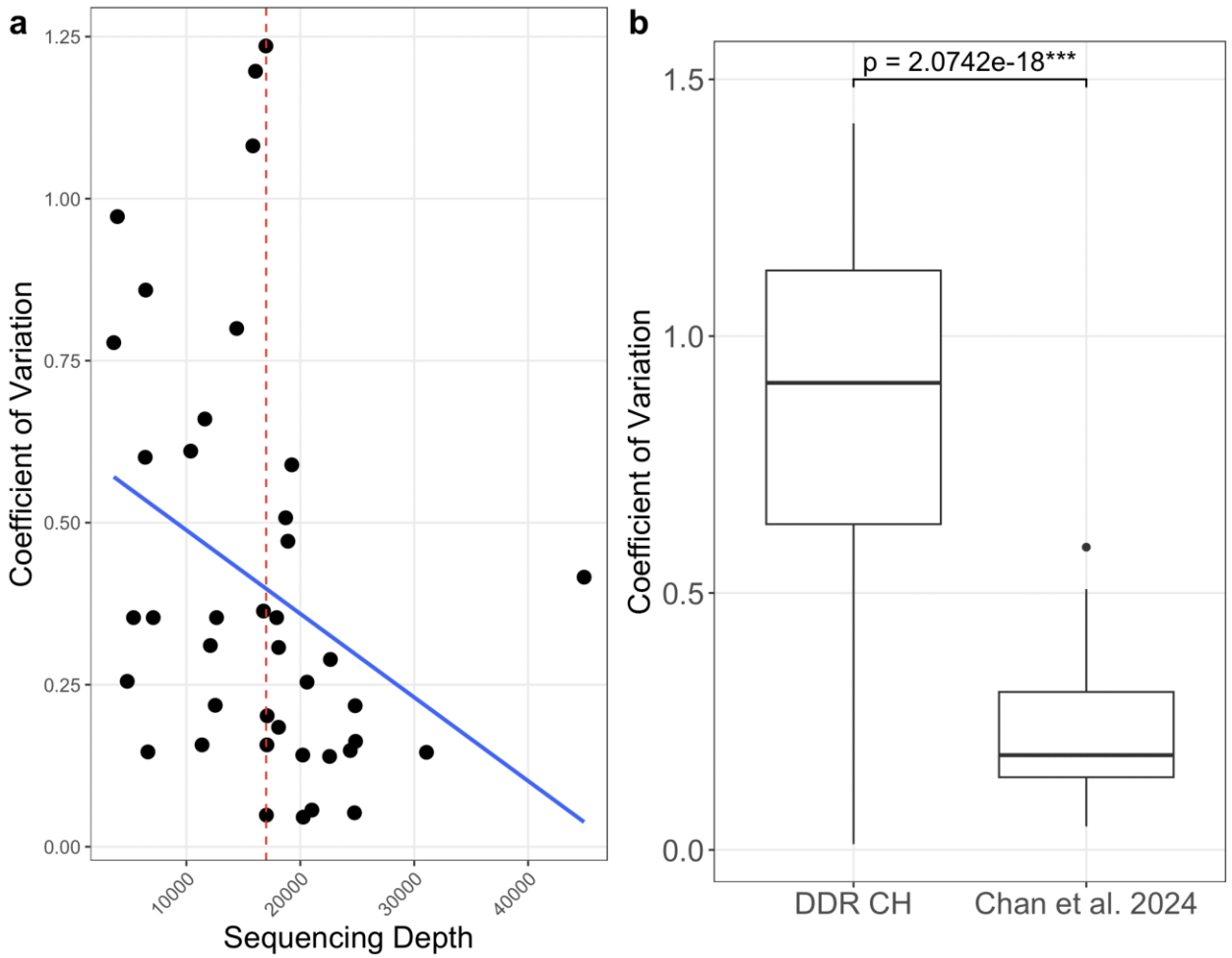
***Reviewer Figure 1**. The relative difference in the VAF estimates for variants <0.2% VAF between replicate samples by total sequencing depth.*

We next compared the relative difference in the VAF estimates between pre- and post-treatment DDR CH mutations in clinical trials of trilaciclib to the relative difference in VAF estimates for replicate samples. We limited this to mutations <0.2% VAF as requested by the reviewer. For both the replicate samples and pre-post samples, we exclude variants with a total depth under 17,000x (as was done in the manuscript). Compared to replicate samples, we observed substantially higher variability in the VAF between pre- and post-treatment samples (**Reviewer Figure 2**). This finding supports the conclusion that the observed changes in VAF we observe following treatment are substantially higher than observed by chance or measurement error alone.



Reviewer Figure 2. *The relative difference in the VAF estimates for DDR CH variants <0.2% VAF comparing technical replicates to biologic replicates drawn from pre-and post-treatment samples*

We observe similar results when using the coefficient of variation and include this as an additional supplemental method figure to justfy additional filtering criteria along with **Supplemental Methods Figure 1**.

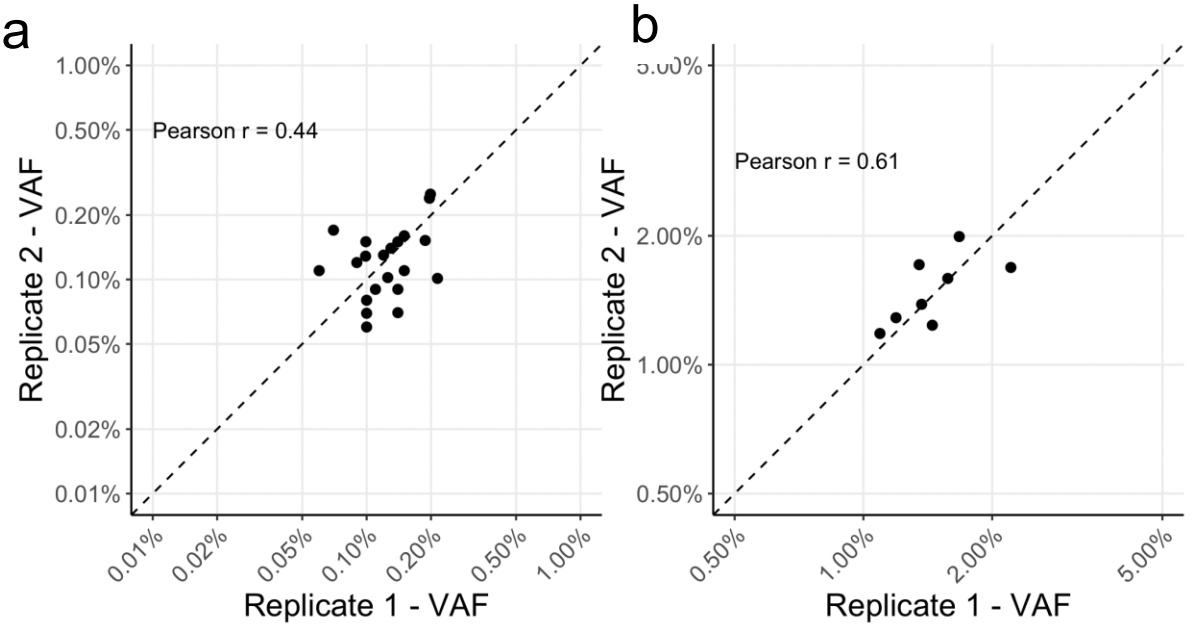


New Supplemental Methods Figure 2. Coefficient of variation among CH variants with a VAF of <0.2%. (a) Among technical replicates drawn from Chan et al. 2024, the coefficient of variation for CH mutations present at a VAF <0.2% in one or more replicates is shown in relation to the total sequencing depth for that position. (b) The coefficient of variation among CH variants present at a VAF <0.2% is compared between DDR CH variants detected in biologic replicates collected before and after chemotherapy and the technical replicates from Chan et al. 2024.

2) Reviewer Comment: "New Supplemental Figure 3 shows Pearson r=0.964 for all variants with VAF up to 2% VAF. Please include only values below 0.2% in this graph and for showing r."

REPLY: When correlation coefficients are calculated over a restricted subset of data compared to the full biological range, they are mathematically expected to appear smaller. Indeed, the correlation coefficient can artificially be pushed to zero if a range of values is selected that is so narrow that all variation is technical error instead of biological variation across a meaningful

range. To give an unrelated example, self-reported height was found to have an excellent correlation of *r* = 0.97 to measured values in a cohort study (Rimm *et al.*, Epidemiology 1990). If one were to repeat this analysis only for people who reported being between 5 foot 7 (170 cm) and 5 foot 8 (173 cm) tall, the *r* would be very small. In the case of VAFs, for variants between 0.1-0.2% VAF, we observed a correlation coefficient of 0.45 (Reviewer Figure 3a). Similarly, for variants of 1-2% VAF, the correlation coefficient would be 0.61 (Reviewer Figure 3b). These values are expected given the limited dynamic range and do not reflect an actual loss of biological correlation. Therefore we posit that these correlation coefficent metrics calculated over a narrow range are misleading.

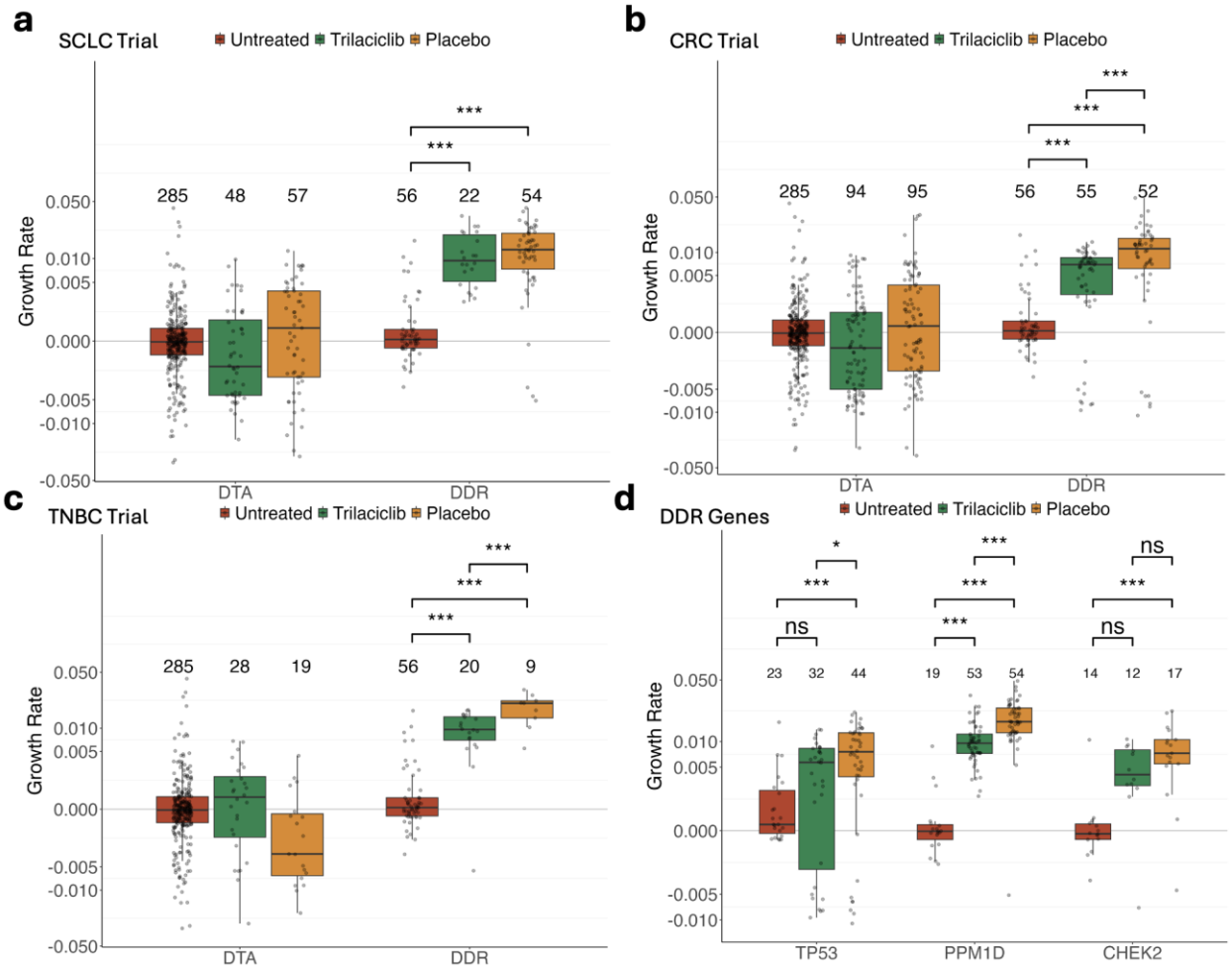


Reviewer Figure 3. Correlation between technical replicate samples for (a) variants between 0.1-0.2% VAF and (b) variants between 1-2% VAF.

3) Reviewer comment: "I disagree about how to interpret the results when only values above 0.2% are used—the authors argue that this is a "sensitivity" analysis, but it is really an analysis to demonstrate whether the main result holds statistical significance when only the highest confidence variants are used. Unfortunately, the result for TP53 is null at this threshold. This does not necessarily mean that there is no effect, but the study lacks the power to show it."

REPLY: The Reviewer appears to have overlooked the revised figure below (**Supplemental Figure 2**) which was provided in the most recent revision of the manuscript. There is a statistically significant association between Trilaciclib treatment and decreased *TP53* growth rate compared to placebo (p=0.02), even when restricted to mutations with a VAF above 0.2%. These data are shown again below.

The terminology of whether this analysis should be referred to as a "sensitivity analysis" or some other term does not alter the interpretation or conclusions of the data presented or the overall message of the manucript.



Supplemental Figure 2. Sensitivity analysis exploring the impact of trilaciclib on DTA and DDR CH limited to mutations present ≥0.2% VAF in either pre- or post-treatment samples. Growth rate of DTA CH and 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 SCLC trial, **(b)** the CRC trial, **(c)** the TNBC trial, and **(d)** pooled data across trials for individual DNA Damage response (DDR) genes. Differences in the growth rate of CH was tested using linear regression adjusted for age. *P <0.05, **P <0.01, ***P <0.001.

Reviewer Comments:

Therapy-related myeloid neoplasms (tMNs) are a recognised and frequently lethal consequence of cytotoxic chemotherapy for solid cancers. A significant proportion of such tMNs arise from pre-existing TP53-mutant CH clones that are afforded a relative fitness advantage over normal (TP53-WT) haematopoietic stem & progenitor cells (HSPCs) by cytotoxic chemotherapy. This results in TP53-mutant clonal expansion that increases the risk of downstream tMNs.

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Collectively, the manuscript makes a credible case that treatment with CDK4/6 inhibitors warrants investigation in the prevention of TP53-mutant tMN after cytotoxic chemotherapy for solid cancers. The concept that short-term CDK4/6 inhibition, contemporaneous with administration of cytotoxic chemotherapy, may be able to prevent TP53-CH clonal expansion and by extension tMN, makes for an attractive and clinically testable hypothesis.

Overall, this is a comprehensive manuscript that starts with clinical observations and moves on to validate these experimentally. From the rebuttal document, it is evident that the authors have made at least two rounds of revisions. I can see that one reviewer remained concerned about the robustness of mutation calling at low VAF, but I find the authors' response to this particular concern convincing.

REPLY: We thank the Reviewer for their supportive comments and interest in our manuscript. We greatly appreciate their careful review and thoughtful comments which we address below.

In line with the above, I have no major criticism of the manuscript, but below are some minor comments aiming to further improve some aspects of the manuscript.

Reviewer Comment: 'Line 106: the authors state that "Overall, 96% of all detectable CH mutations following therapy were present prior to therapy". They should reconcile (explain) this with their earlier observation that CH due to mutations in DDR pathway genes including TP53, PPM1D, and CHEK2 is increased in prevalence following chemotherapy'

REPLY: We agree that further explanation would be helpful to connect the two ideas. In figure 1e, we are comparing the frequency of CH in post-treatment samples to healthy controls to demonstrate that like previous work, we observe an increased frequency of DDR CH following therapy. However, most of these mutations are present prior to therapy but at very low levels (below the limit of detection of 0.1%). We have added the following sentence in red below to reconcile these observations.

> As expected, following chemotherapy, we observed an increased frequency (45% of ES-SCLC individuals) of CH in genes related to the DDR pathway including *TP53*, *PPM1D*, and *CHEK2* (Fig. 1e) compared to only 22% of untreated control individuals ($p=2.9e^{-2}$). Notably, this increase was not driven by the acquisition of new mutations as 96% of all detectable CH mutations following therapy were present prior to therapy but at levels below the limit of detection (0.1%) for our assay (Supplemental Table 3). This supports previous observations that the association between chemotherapy and DDR CH is largely driven by expansion of pre-existing clones[12].

Reviewer Comment: 'Line 156: the authors state that "Besides trilaciclib treatment, only older age at baseline (for TP53-CH and DDR CH) and smaller VAF at baseline (for DDR CH) was predictive of higher CH growth rate during therapy". Was the impact of age and smaller VAF observed in both groups (trilaciclib & placebo)? E.g. were aged and smaller VAF also predictive of clonal growth in the placebo group?

REPLY: This is an excellent suggestion. As requested by the reviewer, we tested for effect modification by including interaction terms between trilaciclib with baseline VAF and age into our multivariable linear regression model. Neither interaction terms were statistically significant. We also ran stratified analyses by treatment arm (placebo and trilacicllib separately) and observed similar results. Thus we believe there was no significant difference of the impact of baseline VAF or age on CH growth rate between placebo and trilaciclib arms.

Reviewer Table 1: Exploring the interaction between baseline VAF and Age between trilaciclib and placebo arms

Term	Grouping	Estimate	std.error	conf.low	conf.high	p.value	p.stars
DDR CH Growth Rate ~ Trilaciclib (Placebo Ref) + preVAF + Age + Covariates							
Placebo (Ref) vs Trilaciclib	**DDR Genes**	**-4.12E-03**	**9.72E-04**	**-6.03E-03**	**-2.22E-03**	**2.19E-05**	*******
preVAF	DDR Genes	-3.98E-02	1.80E-02	-7.50E-02	-4.50E-03	2.71E-02	*
Age	DDR Genes	1.14E-04	5.00E-05	1.62E-05	2.12E-04	2.23E-02	*
*DDR CH Growth Rate ~ Trilaciclib (Placebo Ref) + preVAF (Centered) + Age (Centered) + Trilaciclib*preVAF + Trilaciclib*Age + Covariates*							
Placebo (Ref) vs Trilaciclib	**DDR Genes**	**-3.97E-03**	**9.89E-04**	**-5.91E-03**	**-2.03E-03**	**5.97E-05**	***
preVAF	DDR Genes	-4.10E-02	1.98E-02	-7.99E-02	-2.14E-03	3.87E-02	*
Age	DDR Genes	1.61E-04	7.04E-05	2.35E-05	2.99E-04	2.18E-02	*
Trilaciclib x preVAF	DDR Genes	7.12E-03	4.72E-02	-8.53E-02	9.96E-02	8.80E-01	
Trilaciclib x Age	DDR Genes	-9.26E-05	9.66E-05	-2.82E-04	9.68E-05	3.38E-01	
TP53 CH Growth Rate ~ Trilaciclib (Placebo Ref) + preVAF + Age + Covariates							
Placebo (Ref) vs Trilaciclib	**TP53**	**-3.11E-03**	**1.34E-03**	**-5.73E-03**	**-4.85E-04**	**2.02E-02**	*****
preVAF	TP53	-3.99E-02	3.15E-02	-1.02E-01	2.17E-02	2.04E-01	
Age	TP53	2.42E-04	5.99E-05	1.25E-04	3.59E-04	5.30E-05	***
*TP53 CH Growth Rate ~ Trilaciclib (Placebo Ref) + preVAF (Centered) + Age (Centered) + Trilaciclib*preVAF + Trilaciclib*Age + Covariates*							
Placebo (Ref) vs Trilaciclib	**TP53**	**-3.12E-03**	**1.35E-03**	**-5.77E-03**	**-4.72E-04**	**2.09E-02**	*
preVAF	TP53	-3.71E-02	3.45E-02	-1.05E-01	3.05E-02	2.82E-01	
Age	TP53	2.33E-04	7.67E-05	8.24E-05	3.83E-04	2.41E-03	**
Trilaciclib x preVAF	TP53	-1.72E-02	8.65E-02	-1.87E-01	1.52E-01	8.42E-01	
Trilaciclib x Age	TP53	2.65E-05	1.25E-04	-2.19E-04	2.72E-04	8.33E-01	
PPM1D CH Growth Rate ~ Trilaciclib (Placebo Ref) + preVAF + Age + Covariates							
Placebo (Ref) vs Trilaciclib	**PPM1D**	**-6.21E-03**	**1.38E-03**	**-8.91E-03**	**-3.51E-03**	**6.66E-06**	*******
preVAF	PPM1D	-4.23E-02	4.91E-02	-1.39E-01	5.39E-02	3.89E-01	
Age	PPM1D	-5.17E-05	8.17E-05	-2.12E-04	1.08E-04	5.27E-01	
*PPM1D CH Growth Rate ~ Trilaciclib (Placebo Ref) + preVAF (Centered) + Age (Centered) + Trilaciclib*preVAF + Trilaciclib*Age + Covariates*							
Placebo (Ref) vs Trilaciclib	**PPM1D**	**4.08E-03**	**5.79E-03**	**-7.26E-03**	**1.54E-02**	**4.81E-01**	
preVAF	PPM1D	-2.00E+00	1.09E+00	-4.14E+00	1.46E-01	6.78E-02	
Age	PPM1D	1.54E-05	1.18E-04	-2.16E-04	2.47E-04	8.96E-01	
Trilaciclib x preVAF	PPM1D	1.96E+00	1.10E+00	-1.87E-01	4.11E+00	7.36E-02	
Trilaciclib x Age	PPM1D	-1.17E-04	1.53E-04	-4.18E-04	1.83E-04	4.44E-01	
CHEK2 CH Growth Rate ~ Trilaciclib (Placebo Ref) + preVAF + Age + Covariates							
Placebo (Ref) vs Trilaciclib	**CHEK2**	**-2.88E-03**	**1.95E-03**	**-6.69E-03**	**9.30E-04**	**1.38E-01**	
preVAF	CHEK2	-2.39E-02	1.96E-02	-6.24E-02	1.46E-02	2.24E-01	

Age	CHEK2	1.48E-05	9.92E-05	-1.80E-04	2.09E-04	8.81E-01
*CHEK2 CH Growth Rate ~ Trilaciclib (Placebo Ref) + preVAF (Centered) + Age (Centered) + Trilaciclib*preVAF + Trilaciclib*Age + Covariates*						
Placebo (Ref) vs Trilaciclib	**CHEK2**	**-2.76E-03**	**1.97E-03**	**-6.61E-03**	**1.10E-03**	**1.62E-01**
preVAF	CHEK2	-2.30E-02	2.00E-02	-6.22E-02	1.63E-02	2.51E-01
Age	CHEK2	1.59E-04	1.58E-04	-1.51E-04	4.68E-04	3.15E-01
Trilaciclib x preVAF	CHEK2	2.50E-02	1.32E-01	-2.33E-01	2.83E-01	8.49E-01
Trilaciclib x Age	CHEK2	-2.27E-04	1.93E-04	-6.05E-04	1.51E-04	2.39E-01

Reviewer Comment: 'Line 211: "… animals receiving both agents concomitantly experienced only a mild macrocytic anemia (Extended Data Fig. 3d)." This is shown in Extended Data Fig3b-c (not 3d). Also, the word "only" could be omitted as readers may infer that anemia (potentially worse than mild) was seen in other groups.

REPLY: We thank the Reviewer for this comment and have corrected this mistake in the manuscript.

4. Line 215: "…hematopoietic cells to cytotoxic chemotherapy." For clarity, the word "to" should be replaced with "during" or similar.

5. Lines 239-240: "…undergoing apoptosis at the conclusion of four weeks of mice receiving platinum chemotherapy". The words "mice receiving" can be removed for clarity.

6. Line 257: "…the effect of genetically manipulating CDK6 in response to platinum chemotherapy". Replace "in" with "on the" or similar

7. Line 260: "Cells expressing WT or mutant Cdk6". Presumable these are "TP53-mutant cells expressing…". Please clarify.

8. Line 333: remove "have"

9. Line 342: "… revealed positively enriched of…". Replace "positively enriched" with "positive enrichment" or similar.

10. Line 365: "This includes those..". Replace with "These include those…"

REPLY: We have made these suggested changes in the revised text