nature portfolio

Peer Review File

Multimodal antigenic escape to GPRC5D-targeted T-cell engagers in multiple myeloma

Corresponding Author: Professor Nizar Bahlis

This file contains all reviewer reports in order by version, followed by all author rebuttals in order by version.

Version 1:

Reviewer comments:

Reviewer #1

(Remarks to the Author)
Lee, Neri and Bahlis have become the World-leading group in translational research about determinants of resistance to immunotherapy in MM. This manuscript reflects it very well and I have only a few minor comments for potential improvement, and no major comments because the manuscript is superb.

1) Not all methods have been performed in all patients. This is of course a limitation and is not fully described in the main text. Please add it in the first paragraph of the Results section as well as in Figure 1A.

2) Please add at all times if the alterations being described are clonal or subclonal. This is not always clear and affects interpretation of the results. In addition to the overall frequency of GPRC5D alterations (13/21, 61.9%), please describe how many are clonal and how many are subclonal. A high percentage of subclonal resistance would warrant some discussion about these genetic alterations being a main, but not the only driver of resistance.

3) Would not give a percentage (12.5%) based on only 8 cases with available baseline samples.

4) I think the results in Figure 1B are very limited because of the number of patients and probably outside the scope of the study. Please consider removing it. Post-relapse treatment is not available in Table S1 to make proper interpretation but again, I think this is outside the scope.

5) The firs patient described (MM-03) is intriguing. Was the sum of the two GPRC5D mutants clonal? If yes, how is this associated with the residual GPRC5D expression detected by flow? Is it enough for cytotoxicity with other TCE or higher dose of Talq. Please discuss it for a more precise interpretation of the results.

6) Please describe in the main text what was the time elapsing between treatment and emergence of genomic alterations driving resistance. Not always easy to find this information.

7) I think the comment about CD38 expression in the post-relapse sample of patient MM-20 is out of scope cannot be interpreted in the absence of polyclonal and cytoplasmic staining of CD38. As is, it cannot be known if there was complete CD38 loss or this is an artefact due to treatment with Dara.

8) I insist in adding precise information of events that are clonal and those that are not. For example, in cases MM-54 and MM-62 there is no info about how many cells carry GPRC5D biallelic deletions. By contrast, in MM-18 it is mentioned that the biallelic GPRC5D deletion is present in 90% of cells. Are the other 10% contamination and this is clonal resistance? Or the other 10% of cells do carry MM-related genetic alterations but not the biallelic GPRC5D deletion?

9) For correct interpretation (at least of this Reviewer), in patient MM-10, antigen escape is being mediated by epigenetic silencing, the truncating frameshift mutation, or both events? Unclear to me, but also don't know if this can be fully dissected…

10) Maybe what I'm about to say does not make any sense, but given the ER entrapment of GPRC5D mutants, would it make sense to test proteasome inhibitors to rescue it? Just a wild idea of whether with ER stress these GPRC5D mutants

would no longer be entrapped. Because if it reaches the surface there could be binding with TCE despite these mutations.

11) Please clarify the interpretation of the p.Tyr257Ser GPRC5D mutant K562 cells. First there is minimal expression detected by flow cytometry (Figure 5I) but afterwards the authors say that the expression is below the limit of detection. It appears to be detectable and this of course explains the cytolytic activity of high-dose TCE.

12) The section about GPRC5D epigenetic silencing is in my opinion the one presenting more limitations. If I understood well, scATAC-seq was performed only in patients MM-10 and MM-20. This is insufficient to provide an estimate of how frequent this event may occur. Furthermore, I do not disagree with the rationale that t(11;14) may reprogram MM cells into more immature phenotypes and lower GPRC5D expression, but isn't the reduced expression enough for cytolytic activity with TCE? Is there any evidence from the ongoing studies that patients with t(11;14) do not respond, or show lower response rates and PFS? I'm afraid that there could be some incorrect interpretation from readers regarding t(11;14) as a high-risk feature in the context of anti-GPRC5D, unless of course this is well supported with data.

13) The authors end the manuscript considering finite-duration of TCE therapy to reduce the risk of antigenic drift. Because of this, I insist that the authors should describe in the main text how much time of treatment elapsed until genomic testing of the post-treatment sample. It is my impression that these events are observed after only a few cycles; in such case, it is hard to reconcile this with the short duration therapy of TCE.

Reviewer #2

(Remarks to the Author)
This manuscript provides an elegant, compelling and mechanistic analysis of antigen escape in a multicenter cohort of 21 myeloma patients treated with a GPRC5D-targeted T cell engager (TCE). The emergence of tumor subclones at relapse harboring biallelic deletions or monoallelic deletions + single nucleotide variants (SNVs) in GPRC5D that impair TCE binding was somehow expected and consistent with previously reported patterns of antigen escape, including those described by the same group in the context of BCMA-targeted TCE therapy. Notably, this study identifies mechanisms unique to GPRC5D, including the selection of SNVs affecting its transmembrane domain, leading to intracellular retention within the endoplasmic reticulum. Additionally, the authors report epigenetic silencing of GPRC5D, particularly enriched in patients with the t(11;14) translocation. These findings highlight novel and clinically relevant resistance pathways. Importantly, the authors demonstrate how TCEs targeting distinct GPRC5D epitopes may effectively eliminate mutant subclones, underscoring the therapeutic potential of epitope diversification. Overall, the data are robust and convincingly support the hypothesis that continuous TCE exposure, as currently practiced clinically, drives antigen escape by promoting clonal selection.

Few suggestions to further improve the significance of this work:

• Since the genetic classification of MM tumors can be inferred by WGS, it should be reported in supplementary table 1, considering that the authors make specific comments on t(11;14) patients and APOBEC mutational signature.

• Figure 1A reports that 8/21 patients relapsed without antigen loss. To drive this conclusion, the authors should demonstrate retained GPRC5D expression on myeloma cell surface. In particular, MM-71 and MM-73 relapsed after having achieved prolonged sCR, suggesting that antigen escape may be involved. In the absence of antigen escape, tumor extrinsic mechanisms likely drive relapse: it would be informative to measure T cell fitness in these patients in an in vitro killing assay as shown in figure 2F. Finally, the authors should report the disease burden at baseline of the "green" patients in comparison to the "red" ones (i.e. by measuring sBCMA), to account for lack of responses.

• The authors show high APOBEC mutational signature in patient MM-3. As stated, it is confusing: was it detected at relapse or present at baseline too, and therefore likely the result of the t(14;16)? Was this high ABOBEC signature identified in other cases?

• The ATAC sequencing data shown in figure 4E are suggestive of reduced chromatin accessibility around GPRC5D, but it would be more definitive (and simple) to demonstrate monoallelic GPRC5D expression by RNA sequencing.

Minor comments:

• Supplementary table 1, column L, "clinical" is misspelled.
• Figure 1A: I assume vertical dotted line indicates median PFS, but it should be explicitly indicated in figure legend.
• Figure 1A: in addition to the green/red labeling for the mutational status, it would be visually helpful to add symbols within the red dot to indicate the different mutational patterns
• In the figure legend for extended figure 1C please indicate the significance of the "1/1" labeling on the X axis.
• Killing assays reported in figure 2F use an E:T ratio of 10:1, which is not clinically relevant and may not be appropriate to test T cell fitness. A 1:1 or even 1:5 E:T ratio would be more meaningful.
• Figure 2D shows significantly reduced GPRC5D expression post relapse. Why? If I understood correctly, the only expressed allele carries a Asp239Asn that should not impair proper membrane localization (Figure 5E) or TCE binding (Figure 5F). Is the reduced expression simply due to loss of one allele? Please clarify.
• In Figure 3C, the annotation red and green for "normal" and "tumor" is a confusing. What does "normal" mean? It cannot refer to normal plasma cells, since they lack GPRC5D and largely TNFRSF17. Please clarify.
• In Figure 3D: residual but significant GPRC5D expression is noted in the "post" sample. It is coming from normal plasma

cells? In any case, the authors should revisit the statement in line 289 “undetectable membrane GPRC5D expression”.
• Extended figure 6A. Please label the X axis: what do the numbers refer to?
• Extended figure 6C, top: please label the Y axis. What does “TMB” indicate?
• Figure 5: if known, please indicate the binding sites for talquetamab and forimtamig.
• Extended figure 10: it is visually confusing to have red boxes labeling t(11:14) in 10A, and non t(11;14) in 10B. If possible, please invert the coloring in 10B.

Marta Chesi

Reviewer #3

(Remarks to the Author)
This is a well-written, comprehensive translational analysis exploring tumor-intrinsic mechanisms of resistance to GPRC5D-targeted, bispecific T cell engagers (TCE), an important new therapeutic option for myeloma. The authors integrate multiple complementary genomic and phenotypic approaches to identify 3 broad categories of antigenic loss or mutation that lead to resistance. While several of these have been previously described (by this group and others), the current study is the largest, and the first to perform functional in vitro studies demonstrating the consequences of these GPRC5D mutations, providing mechanistic insight into how they may limit surface protein expression or abrogate binding by currently-available TCE. Methodology and statistics are valid and conclusions are robust. Appropriate references to prior related work are included.
Comments/questions:
1) Patient MM-03 was noted to have a high APOBEC signature (SBS 2 and 13). How frequently was this signature seen in the patients studied, and was it more prevalent in the patients with antigen escape?
2) It seems that specific mutations didn’t correlate with presence or absence of GPRC5D mutations or loss (Extended Data Fig 6), but was there any correlation with total mutational burden?
3) In Fig. 2D/E, the Asp239Asn mutation leads to significantly decreased GPRC5D surface expression on MM-03’s myeloma cells, yet surface expression of GPRC5D harboring this mutation on K562 cells appears to be intact (Fig. 5E/F). Can authors speculate about reasons for this difference?
4) A table summarizing the observed genomic events in each patient should be included for the reader to understand the relative frequency of these various events and which mutations are most common. This is mentioned in the text (lines 203-204) but not included in the Supplementary Tables provided.

Version 2:

Reviewer comments:

Reviewer #1

(Remarks to the Author)
Thank you for thoroughly addressing the Reviewer’s comments. My only last comment emerges from the answer the authors provided to my first comment:

“Bulk WGS (100x) was performed on 20 patient cases (n=8 baseline and n=20 postrelapse) with matching normal when available (30x).”

Was matching normal unavailable in some cases? How many? Please described this information in the revised version of the manuscript as well as the procedures to overcome the limitation of matching normal unavailable. In addition, how these results can be confidently interpreted together with other data where matching normal germline DNA was available.

Reviewer #2

(Remarks to the Author)
Very elegant analysis. Recommend publication.

Reviewer #3

(Remarks to the Author)
The authors have responded adequately to the reviewer comments and the changes have significantly strengthened the manuscript.

Response to reviewers

We would like to thank all the reviewers for their valuable suggestions and comments to enhance our manuscript. We have carefully considered each comment and addressed them as below.

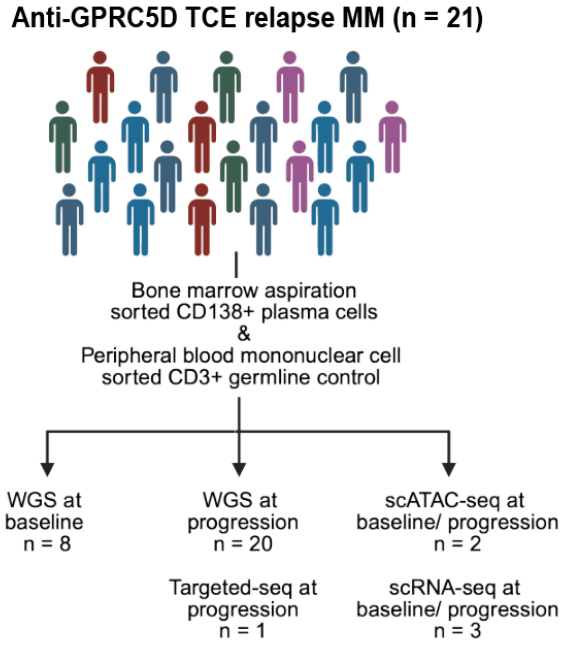
Reviewer #1 (Remarks to the Author):

1) Not all methods have been performed in all patients. This is of course a limitation and is not fully described in the main text. Please add it in the first paragraph of the Results section as well as in Figure 1A.

We thank the reviewer for this comment. As suggested, we have updated the first paragraph of Results (lines 191-199) to indicate types of samples and analyses performed for each patient and referred to **Supplementary Table 1** and the updated **Figures 1A,B** which now include sample collection status for each patient.

Bulk WGS (100x) was performed on 20 patient cases (n=8 baseline and n=20 post-relapse) with matching normal when available (30x). For cases, MM-03, MM-10, and MM-20 additional baseline and post-relapse scATAC and/or scRNA-seq were performed in addition to WGS. Case MM-61 underwent targeted sequencing due to paucity of primary DNA material from CD138+ cells.

Revised Main Figure 1A



2) Please add at all times if the alterations being described are clonal or subclonal. This is not always clear and affects interpretation of the results. In addition to the overall frequency of GPRC5D alterations (13/21, 61.9%), please describe how many are clonal and how many are subclonal. A high percentage of subclonal resistance would warrant some discussion about these genetic alterations being a main, but not the only driver of resistance.

Thank you for this important comment. To accurately infer clonality on all samples, we reanalyzed our data and computed the cancer cell fractions (CCF) using various tools to ensure concordance. To this end, we re-ran ASCAT[1] on all samples to compute purity, ploidy, B-allele frequencies, and manually calculated CCF as per the equation below.

$$\mathrm{CCF} = \mathrm{VAF} \times \frac{(\mathrm{purity} \times CN_{tumor}) + (1 - \mathrm{purity}) \times 2}{CN_{mut} \times \mathrm{purity}}.$$

In addition, we computed the CCF values using R tool Palimpsest[2] and confirmed concordance with the manually calculated CCF values. In the revised **Supplementary Table 4**, we have also added for each case, the sample purity, ploidy, GPRC5D mutation, observed VAF (not purity corrected), and reported the purity-corrected CCF from Palimpsest[2]. Throughout the manuscript text and figures, we have now indicated the CCF value for each GPRC5D variant. For each sample, we have also generated histograms representing the distribution of CCF for clonal and subclonal variants (**Supplementary Figure 7**)

Computed SNV/ indel clonality is a probabilistic estimate rather than a binary state. For clarity, we included the CCF estimates in **Supplementary Table 4** and **Supplementary Figure 7** with 95% confidence intervals (CCF.min, CCF.max) as derived from the binomial model that accounts for sequencing depth and variant read counts. We used a very conservative measure of clonality, with mutations classified as clonal only if the upper bound of the confidence interval (CCF.max) was ≥ 0.95. Mutations with CCF.max < 0.95 were classified as subclonal.

The copy number variant CCF (CCF_CNV) were generated using FACETS[3]. Of note the CCF_CNV for case MM-68 could not be accurately estimated by FACETS since the patient previously received an allogenic stem cell transplantation and hence the reference normal was influenced by the allogeneic germline chimerism.

Case MM-61 sample was only subjected to amplicon-based sequencing due to limitation in the available primary extracted DNA, and therefore the CCF could not be accurately estimated for this case.

We do concur with the reviewer that in cases where GPRC5 mutations were subclonal, other factors may have contributed to the acquired resistance such as T cell dysfunction. In addition, MM spatial heterogeneity and the nature of tumor sampling (mostly from bone marrow) may also contribute to the subclonal fractions estimates. To highlight the role of T cell dysfunction as a contributor of acquired resistance, we have

performed functional T cell evaluation (**Supplementary Figures 3 and 8**) on 4 samples (MM-03, MM-20, MM-71 and MM-73) where progression PBMCs were available. As shown in **Supplementary Figure 8**, T cell dysfunction was the primary driver of acquired resistance in samples MM-71 and MM-73. This data is now included in the manuscript in lines 344-352. The role of non-antigen dependent resistance mechanisms to TCEs is also added to the Discussion section lines 659-667.

3) Would not give a percentage (12.5%) based on only 8 cases with available baseline samples.

Thank you for this comment. We do agree that generating a percentage based on 1/8 patients having the monoallelic loss is not statistically robust. We have therefore removed the statement indicating the percentage.

4) I think the results in Figure 1B are very limited because of the number of patients and probably outside the scope of the study. Please consider removing it. Post-relapse treatment is not available in Table S1 to make proper interpretation but again, I think this is outside the scope.

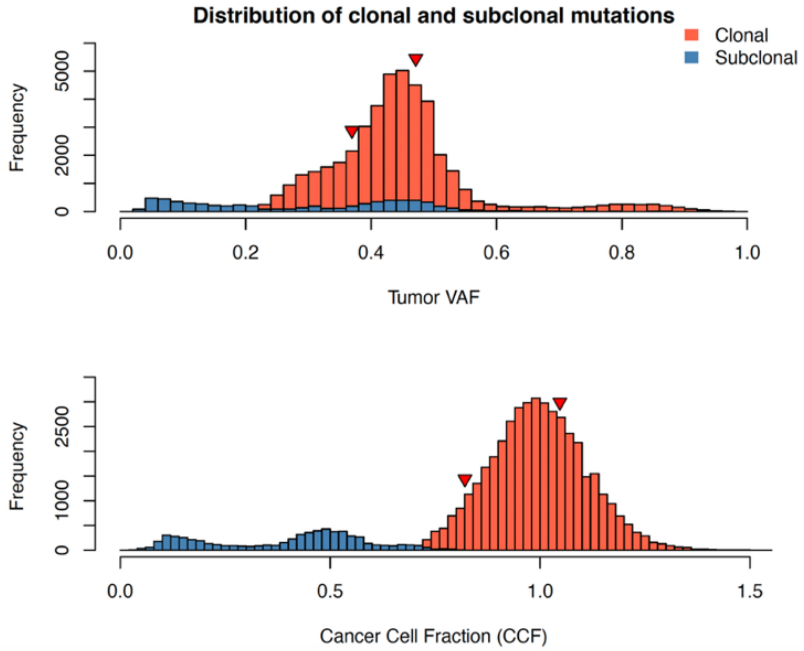
We thank the reviewer for this suggestion. This figure has now been removed.

5) The first patient described (MM-03) is intriguing. Was the sum of the two GPRC5D mutants clonal? If yes, how is this associated with the residual GPRC5D expression detected by flow? Is it enough for cytotoxicity with other TCE or higher dose of Talq. Please discuss it for a more precise interpretation of the results.

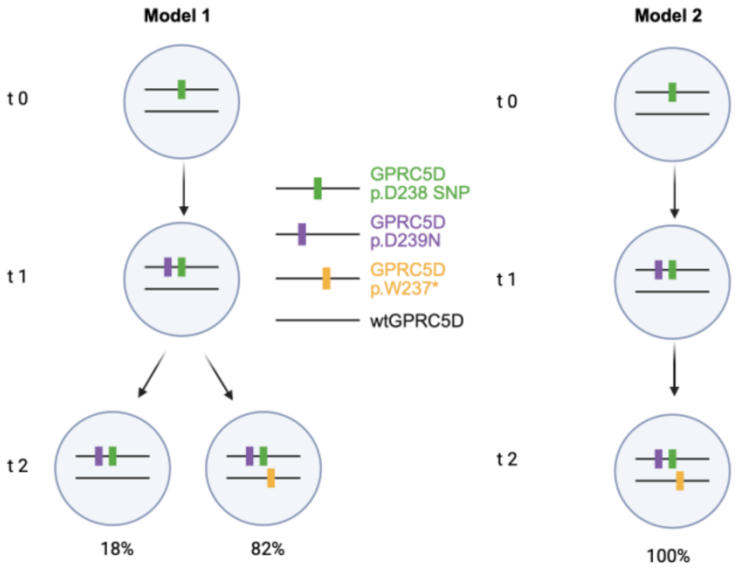
Thank you for this comment. Based on CCF analysis (Palimpsest VAF and CCF histogram **Figure A** below), GPRC5D p.Asp239Asn clone has a CCF of 1 (CCF.min 0.83; CCF.max 1.27), while the p.Trp237Ter clone has a CCF of 0.82 (CCF.min 0.62; CCF.max 1.04), as indicated by the red arrows in the **Figure A** below. These two mutations (p.Asp239Asn and p.Trp237Ter) are *in trans* (**Main Figure 2B**). As we noted above, CCF values are probabilistic estimates, and the default definition of clonal event in Palimpsest R tool is based on upper bound of the confidence interval (CCF.max) ≥ 0.95. Therefore, based on this definition, these two GPRC5D mutations are clonal (**Figure B** model 2 below). However, an alternate phylogenetic trajectory is also possible with more stringent definition of clonality (**Figure B** model 1).

Regarding the residual expression by flow, our *in vitro* cloning of p.Asp239Asn confirmed that this GPRC5D mutant does traffic to cell membrane and is expressed on the cell surface. In this case, GPRC5D protein expression is derived from a single allele (GPRC5D p.Asp239Asn) since p.Trp237Ter results in GPRC5D truncation. Furthermore, the *in vitro* cytotoxicity assays using K562 cells expressing p.Asp239Asn GPRC5D demonstrated that these cells required higher concentrations of talquetamab, or targeting with forimtamig, for elimination compared to wild-type GPRC5D-expressing K562 cells.

A.


Distribution of clonal and subclonal mutations
Clonal
Subclonal
Frequency
0
2000
5000
0.0
0.2
0.4
0.6
0.8
1.0
Tumor VAF
Frequency
0
1000
2500
0.0
0.5
1.0
1.5
Cancer Cell Fraction (CCF)

B.


Model 1
Model 2
t 0
t 1
t 2
GPRC5D p.D238 SNP
GPRC5D p.D239N
GPRC5D p.W237*
wtGPRC5D
18%
82%
100%

6) Please describe in the main text what was the time elapsing between treatment and emergence of genomic alterations driving resistance. Not always easy to find this information.

Thank you for this comment. All progression CD138+ samples were collected within 1-2 weeks of confirmed clinical disease progression by IMWG progression criteria. This statement is added to the first paragraph of the Results section in lines 193-195. Therefore, in the current cohort, the elapsed time between treatment and the emergence of mutant clones is equal to the PFS. Of note, we recently reported[4] that mutant clones may be detectable at timepoints prior to IMWG defined relapse in some bone marrow samples that were collected prior to clinical progression.

7) I think the comment about CD38 expression in the post-relapse sample of patient MM-20 is out of scope cannot be interpreted in the absence of polyclonal and cytoplasmic staining of CD38. As is, it cannot be known if there was complete CD38 loss or this is an artefact due to treatment with Dara.

We thank the reviewer for this comment. As suggested, we have removed the comment regarding CD38 expression in the text. To maintain consistency between the text and the figures, we have also removed CD38 staining flow dot plot results from the main figure.

8) I insist in adding precise information of events that are clonal and those that are not. For example, in cases MM-54 and MM-62 there is no info about how many cells carry GPRC5D biallelic deletions. By contrast, in MM-18 it is mentioned that the biallelic GPRC5D deletion is present in 90% of cells. Are the other 10% contamination and this is clonal resistance? Or the other 10% of cells do carry MM-related genetic alterations but not the biallelic GPRC5D deletion?

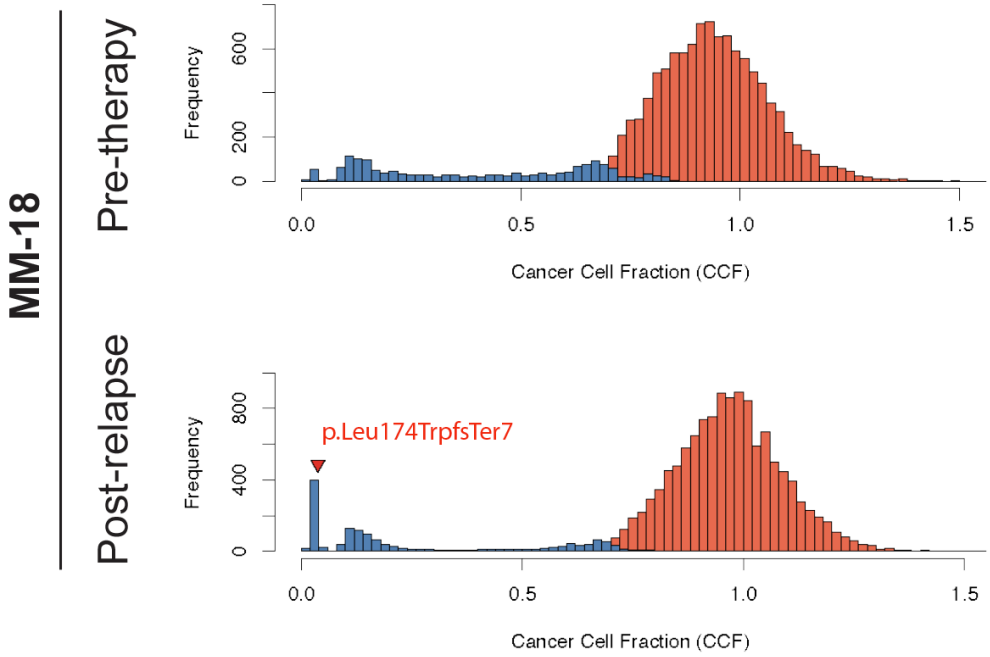
We thank the reviewer for raising this key point. As suggested, we have now included CCF values throughout the manuscript text and figures as well included a new **Supplementary Table 4**, with reference to sample purity, ploidy, GPRC5D mutation, observed VAF, purity-corrected CCF.

For case MM-54, CNVkit (added in **Extended Data Figure 5**) reported a focal copy number loss at the *GPRC5D* locus with a log2 ratio of -4. Tumor purity was 0.95 (ASCAT) and FACETS CCF_CNV was 0.92. Therefore, this is most consistent with a clonal biallelic GPRC5D loss rather than a subclonal event.

Similarly, ASCAT purity for sample MM-62 was 0.97 and FACETS CCF_CNV was 0.96 for the focal biallelic deletion of *GPRC5D* locus, representing a clonal antigen escape event (**Extended Data Figure 6**).

In case MM-18, the biallelic GPRC5D deletion had an estimated FACETS CCF_CNV of 0.95 (ASCAT purity of 0.96). This is consistent with the Palimpsest SNV CCF distribution depicting the major clone at approximately 90% (orange clone in Figure below) and a subclone harboring GPRC5D p.Leu174TrpfsTer180 with estimated CCF of

0.04. For added clarity, we have updated the CCF and CCF_CNV values in the manuscript text (line 310-311). Furthermore, the histograms of CCF distribution for the GPRC5D SNV/ indel for each sample are included in **Supplementary Figure 7**.



9) For correct interpretation (at least of this Reviewer), in patient MM-10, antigen escape is being mediated by epigenetic silencing, the truncating frameshift mutation, or both events? Unclear to me, but also don't know if this can be fully dissected…

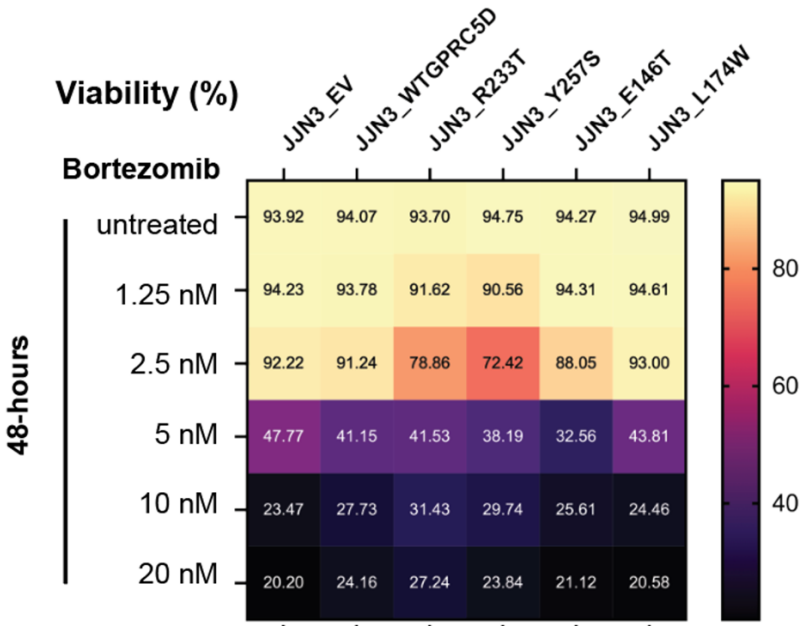
We thank the reviewer for this comment. The CCF of the GPRC5D frameshift mutation p.Thr88LeufsTer21 is 0.91 (ASCAT purity 0.59, ploidy 1.91). Reduced chromatin accessibility at the *GPRC5D* locus is observed by scATAC-seq as shown in **Main Figure 4E** and **Supplementary Figure 6.** The chromatin accessibility (scATAC-seq) at the *GPRC5D* locus and its enhancer (*EMP1*) are clearly reduced by over 50% in the post versus pre talquetamab treatment samples. In contrast, chromatin accessibility at *TNFRSF17* and *CCND1* loci were identical in pre and post samples. Furthermore, scRNA identified residual but reduced mRNA reads at the *GPRC5D* locus, hence the inferCNV analysis resulted in monoallelic *GPRC5D* loss (**Main Figure 4D**). In addition, single cell allele linkage analysis (scAllele computational tool[5] also revealed significant allele linkage skewing consistent with monoallelic expression (please see response to **Reviewer 2 query #4**). However, scAllele analysis should be interpreted with caution due to frequent dropout and the inherent allele bias with 3' scRNA sequencing technology. Lastly, flow cytometry analysis on a recent bone marrow sample collected from this patient at progression on immediate subsequent line of therapy post

talquetamab (post venetoclax) also confirmed the loss of GPRC5D protein expression (**Supplementary Figure 5**). Unfortunately, no residual cells were available for bulk RNA sequencing to confirm allelic bias from this sample.

Taken together, the only plausible explanation for the observed loss of GPRC5D protein expression in this case is that antigen escape occurred through 2 events: (i) a monoallelic clonal frameshift-mutant (CCF 0.91), and (ii) GPRC5D epigenetic silencing. The exact contribution of each of the epigenetic event on antigen loss cannot be accurately computed as stated by the reviewer.

10) Maybe what I'm about to say does not make any sense, but given the ER entrapment of GPRC5D mutants, would it make sense to test proteasome inhibitors to rescue it? Just a wild idea of whether with ER stress these GPRC5D mutants would no longer be entrapped. Because if it reaches the surface there could be binding with TCE despite these mutations.

We thank the reviewer for this comment. Given that the observed GPRC5D mutations resulted in the loss of the motifs involved in the protein trafficking from ER to cell membrane, we did not believe that inhibition of protein degradation would result in GPRC5D translocation to the cell membrane. However, as the reviewer suggested, we did examine whether the entrapped mutant GPRC5D in the ER, increased ER stress and hence sensitizes them to proteasome inhibitors. We therefore conducted bortezomib sensitivity assays in JJN3 cell line (with undetectable endogenous GPRC5D expression by flow cytometry) that we stably transduced with wild type or mutant GPRC5D. As shown below, these experiments did not result in conclusive evidence that the mutant GPRC5D expressing cells were any more sensitive to proteasome inhibitors than the wild type GPRC5D or empty vector expressing tumor cells.



For this bortezomib sensitivity assay, cells were resuspended at 500,000/ mL concentration in RPMI media and treated with bortezomib (0-20 nM) concentration for 48 hours. Cells were stained with propidium iodide and annexin V to assess cell death. The heatmap represents viable JJN3 cells after 48 hours of treatment with bortezomib.

11) Please clarify the interpretation of the p.Tyr257Ser GPRC5D mutant K562 cells. First there is minimal expression detected by flow cytometry (Figure 5l) but afterwards the authors say that the expression is below the limit of detection. It appears to be detectable and this of course explains the cytolytic activity of high-dose TCE.

We thank the reviewer for this comment. The case of the p.Tyr257Ser GPRC5D variant illustrates the importance of assay modality, limit of detection and analytical sensitivity/specificity. By standard flow cytometry staining with an anti-GPRC5D antibody, surface expression of the p.Tyr257Ser mutant was undetectable (**Main Figure 5F**). In TCE binding assay, talquetamab failed to bind the p.Tyr257Ser mutant (**Main Figure 5H**), while forimtamig showed minimal binding (**Main Figure 5I**) consistent with its higher avidity and different binding epitopes.

Consistent with their binding profiles, in functional cytotoxicity assays, we observed cytolytic activity of p.Tyr257Ser mutant with forimtamig but lesser than that of wild type GPRC5D. In contrast, modest killing (~30%) was noted with talquetamab only at high concentrations. No such effects were observed in the empty vector transduced negative control cells, suggesting that the p.Tyr257Ser mutant is expressed at low levels but remains below the detection threshold of direct antigen staining with anti-GPRC5D antibody by standard flow cytometry (**Main Figure 6D**).

Furthermore, we employed an orthogonal assay, membrane-focused confocal imaging with optimized staining protocols to selectively visualize surface-localized proteins. This approach confirmed the localization of p.Tyr257Ser GPRC5D on the plasma membrane (**Extended Data Figure 9**). Thus, although trafficking of the p.Tyr257Ser mutant to the membrane is inefficient, minimal residual surface expression is supported both indirectly by the cytotoxicity data and directly by sensitive confocal imaging. Collectively, these findings demonstrated that direct anti-GPRC5D staining by flow cytometry alone may be insufficient to reliably detect certain GPRC5D variants with reduced surface expression, and therefore we caution against using flow cytometry based assay for the prediction of response to anti-GRPC5D TCE.

12) The section about GPRC5D epigenetic silencing is in my opinion the one presenting more limitations. If I understood well, scATAC-seq was performed only in patients MM-10 and MM-20. This is insufficient to provide an estimate of how frequent this event may occur. Furthermore, I do not disagree with the rationale that t(11;14) may reprogram MM cells into more immature phenotypes and lower GPRC5D expression, but isn't the reduced expression enough for cytolytic activity with TCE? Is there any evidence from the ongoing studies that patients with t(11;14) do not respond, or show lower response rates and PFS? I'm afraid that there could be some incorrect interpretation from readers regarding

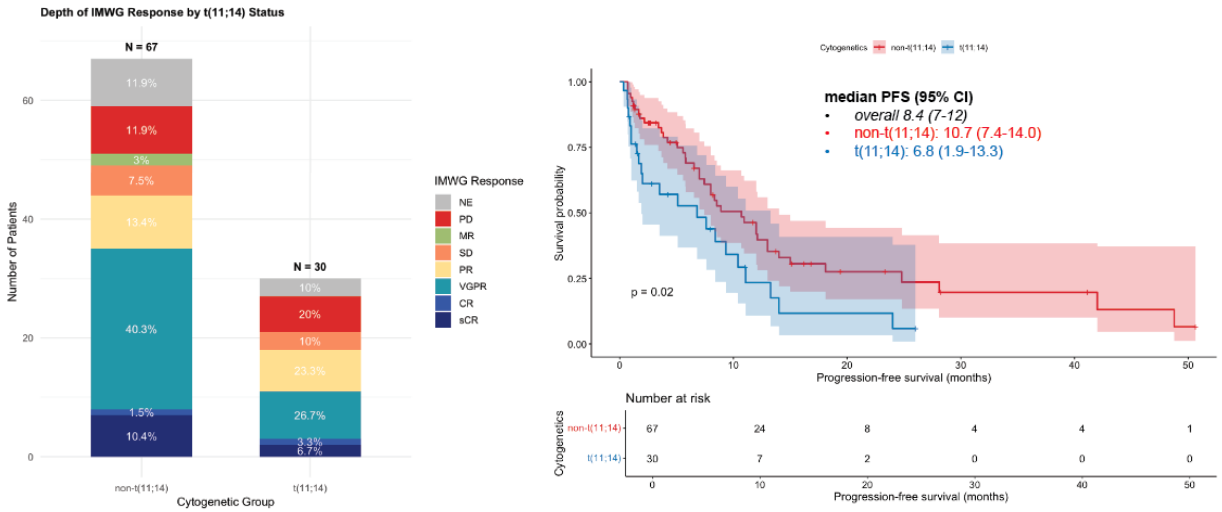
t(11;14) as a high-risk feature in the context of anti-GPRC5D, unless of course this is well supported with data.

We thank the reviewer for this comment. We agree that the frequency of epigenetic events cannot be reliably estimated from a single case (MM-10). Therefore, we analyzed our scATAC-seq data of 15 patients and observed significantly reduced GPRC5D chromatin accessibility in t(11;14) MM compared to non-t(11;14) (**Extended Data Fig10**).

As suggested by the reviewer, we retrospectively analyzed the overall response rate (ORR) and progression-free survival (PFS) in a cohort of 97 patients treated with talquetamab across three institutions (Würzburg, Heidelberg, and Calgary). The Wurzburg and Heidelberg cohort is a subset of the recently published German real-world data cohort[6]. Among patients with t(11;14) (n=30), ORR was 60% compared to 65.6% in patients with non-t(11;14) (n=67). Rates of ≥VGPR were 36.7% and 52.2% in t(11;14) and non-t(11;14) groups, respectively (**Figure left panel** below). The median PFS in the overall cohort was 8.9 months. In the t(11;14) subgroup (n = 30), the median PFS was 6.8 months (95% CI 1.9-13.3), whereas in the non-t(11;14) subgroup (n = 67), it was 10.7 months (95% CI 7.4-14.0) (p = 0.02) as shown in the **Figure right panel**.

Of note, we also sought data regarding t(11;14) patients from prospectively conducted trials with talquetamab (studies sponsored by Janssen). We were informed that while FISH for high-risk cytogenetics were collected for some of these studies, t(11;14) data was not available.

Cognizant of the limitation of relatively small cohort retrospective analysis, we have opted to provide this data to the reviewer, however, not to include it in the manuscript. As suggested by the reviewer, we also noted in the manuscript that t(11;14) patients still benefit from anti-GRPC5D targeting agents and that in these patients the resistance may be potentially reversed by chromatin modifying agents, a hypothesis that requires further testing in prospective clinical trials (line 650-654 and 657).



13) The authors end the manuscript considering finite-duration of TCE therapy to reduce the risk of antigenic drift. Because of this, I insist that the authors should describe in the main text how much time of treatment elapsed until genomic testing of the post-treatment sample. It is my impression that these events are observed after only a few cycles; in such case, it is hard to reconcile this with the short duration therapy of TCE.

We thank the reviewer for this important point. All post-relapse biopsies analyzed in this study were collected within 1-2 weeks of documented disease progression. We have now clarified this point in the Results section as noted above. Therefore, based on the current analysis the median time to mutation equals the median time to progression (PFS). However, since no bone marrow sampling was performed prior to progressive disease, we cannot accurately define the exact timing of these mutations. In a recent publication, we detected these mutants in some patients up to 4 months prior to clinical progression[4].

As we noted in the Discussion, we posit that these mutations occur stochastically over time, and therefore the longer the patient remains on therapy, the likelihood of selecting these mutant clones under therapeutic pressure is higher. We have revised the sentence in the Discussion to indicate that this is a hypothesis that requires clinical validation (lines 675-677)

Reviewer #2 (Remarks to the Author):

1. Since the genetic classification of MM tumors can be inferred by WGS, it should be reported in supplementary table 1, considering that the authors make specific comments on t(11;14) patients and APOBEC mutational signature.

We thank the reviewer for this comment. As suggested, **Supplementary Table 1** of the revised manuscript now includes Ig translocation status, determined using IgCaller[7].

To further indicate the genetic classification of each sample, we added a heatmap in **Supplementary Figure 9** showing Ig translocation, hyperdiploid status, and chr1q and delp17p/TP53 mutation status across all post-relapse samples. Ig translocation was assessed with the IgCaller R package[7] in combination with structural variant calls from SVABA, Manta, and LUMPY. Hyperdiploid status was defined as whole-chromosome gains in at least 3–5 odd-numbered chromosomes (3, 5, 7, 9, 11, 15, 19, 21), based on copy-number profiles from ASCAT and CNVkit. Chr1q gain/amplification and TP53 biallelic inactivation were assessed using ASCAT and CNVkit copy-number calls together with somatic SNV/indel calls from Mutect2, Strelka2, and Lancet.

Finally, APOBEC status was defined as the combined contribution of SBS2 and SBS13 mutational signatures using the mmsig R tool[8].

2 .Figure 1A reports that 8/21 patients relapsed without antigen loss. To drive this conclusion, the authors should demonstrate retained GPRC5D expression on myeloma cell surface. In particular, and MM-73 relapsed after having achieved prolonged sCR, suggesting that antigen escape may be involved. In the absence of antigen escape, tumor extrinsic mechanisms likely drive relapse: it would be informative to measure T cell fitness in these patients in an in vitro killing assay as shown in figure 2F. Finally, the authors should report the disease burden at baseline of the "green" patients in comparison to the "red" ones (i.e. by measuring sBCMA), to account for lack of responses.

We thank the reviewer for this comment and fully concur with the value of the additional data as requested. Unfortunately, no residual tumor samples are available for MM-71 and MM-73 for flow cytometry evaluation of GPRC5D expression. We also inquired with the trial sponsor (Janssen) and no residual tumor sample was available nor data was available for GPRC5D expression by flow cytometry.

We did, however, examine tumor extrinsic resistance mechanism involving T cell dysfunctions in PBMCs from cases MM-71 and MM-73 by *in vitro* cytotoxicity coculture assays. PBMCs collected at progression from talquetamab in these 2 cases were cocultured with OPM2 cells at varying E:T ratios (10:1, 3:1, 1:1, and 1:5) with or without talquetamab (1 or 10 nM) for 72 hours. Tumor cell cytotoxicity was assessed using propidium iodide and calcein AM staining by flow cytometry. We also examined the proportions of CD3, CD4 and CD8 by flow cytometry. As shown in **Supplementary Figures 3 and 8**, compared to healthy donor PBMCs, PBMCs for MM-71 and MM-73 demonstrated reduced cytolytic activities, with this effect being more pronounced at

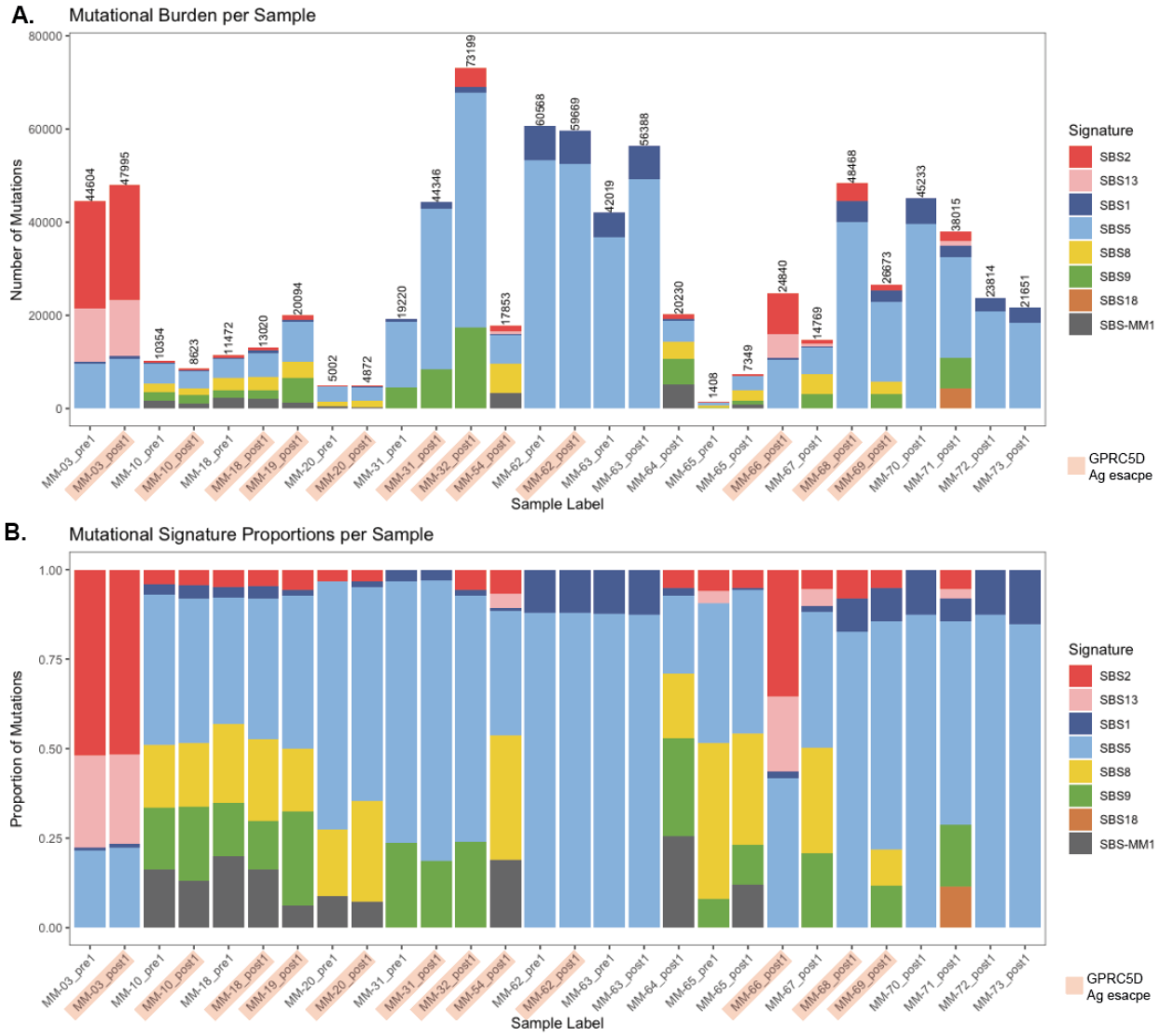
lower E:T ratios or TCE concentrations. Overall, this supported that in these two patients with relapse after durable response without GPRC5D antigen escape, reduced T cell cytolytic activity is likely the main contributing factor to their acquired resistance mechanism. A paragraph summarizing these findings were added to line 344-352.

We thank the reviewer for the comment regarding disease burden. While we recognize that tumor burden is a key determinant of TCE response, serum samples were not available from this patient cohort to measure sBCMA levels. While we do have bone marrow plasmacytosis data available in some of the patients included in the current study, we do not believe that bone marrow plasmacytosis accurately reflect disease burden due to the patchy nature of the bone marrow involvement as well as the spatial heterogeneity.

3. The authors show high APOBEC mutational signature in patient MM-3. As stated, it is confusing: was it detected at relapse or present at baseline too, and therefore likely the result of the t(14;16)? Was this high ABOBEC signature identified in other cases?

We thank the reviewer for this comment. We have clarified in the text (line 244-247)) that the high APOBEC mutational signature was present both pre-therapy and at relapse in MM-03 is associated with the MAF translocated t(14;16) MM in this case. In addition, we have further performed SBS signature analysis using mmsig[8] on all samples and included this updated data in **Supplementary Figure 1**, and shown below.

Revised Supplementary Figure 1

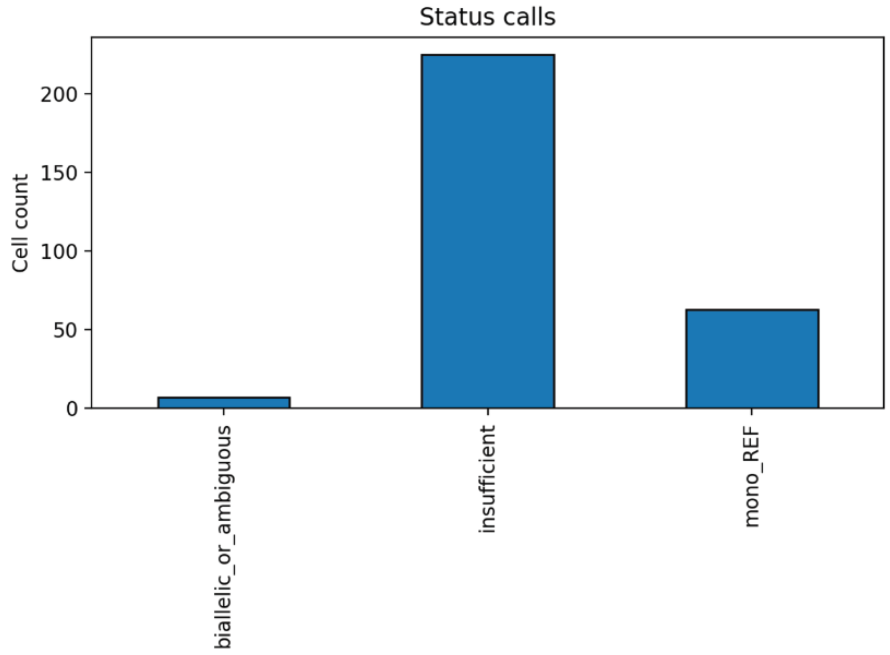


4. The ATAC sequencing data shown in figure 4E are suggestive of reduced chromatin accessibility around GPRC5D, but it would be more definitive (and simple) to demonstrate monoallelic GPRC5D expression by RNA sequencing.

We thank the reviewer for this valuable comment, and we fully concur with the suggestion regarding the use of RNA sequencing to demonstrate allelic skewing. Unfortunately, on this sample, we have no remaining material left to perform bulk RNA sequencing, and hence we are restricted to the scRNA-seq previously performed. Allelic linkage skewing analysis was conducted using scAllele[5], a computational tool that detects SNVs/ indels and their allelic linkage from scRNA data. This demonstrated significant monoallelic skewing with an imbalanced allele distribution (two-sided p value of 0) for the reads mapping to the GPRC5D locus (see **Figure** below). However, this analysis should be interpreted with caution due to inherent biases introduced by the

frequent dropout of scRNA sequencing, the limited number of reads, and a degree of allelic bias introduced by 3' or 5' scRNA-seq technologies.

The figure below represents the scAllele analysis depicting on the Y axis the number of cells (Cell count) with *GPRC5D* mRNA reads originating from both alleles (biallelic_or_ambiguous) or monoallelic (mono_REF). Reads with insufficient SNVs/ indels/ SNPs to define allelic linkage are classified as insufficient. In this analysis, monoallelic reference is defined as more than 90% of reads originating from a single allele.



metric	value
Total reference reads	1089
Total alternate reads	40
Total reference UMIs	817
Total alternate UMIs	27
n for test	844
Alternate fraction	0.03
p imbalance two sided	0

Minor comments:

5. Supplementary table 1, column L, "clinical" is misspelled

Thank you for this correction. We have updated **Supplementary Table 1.**

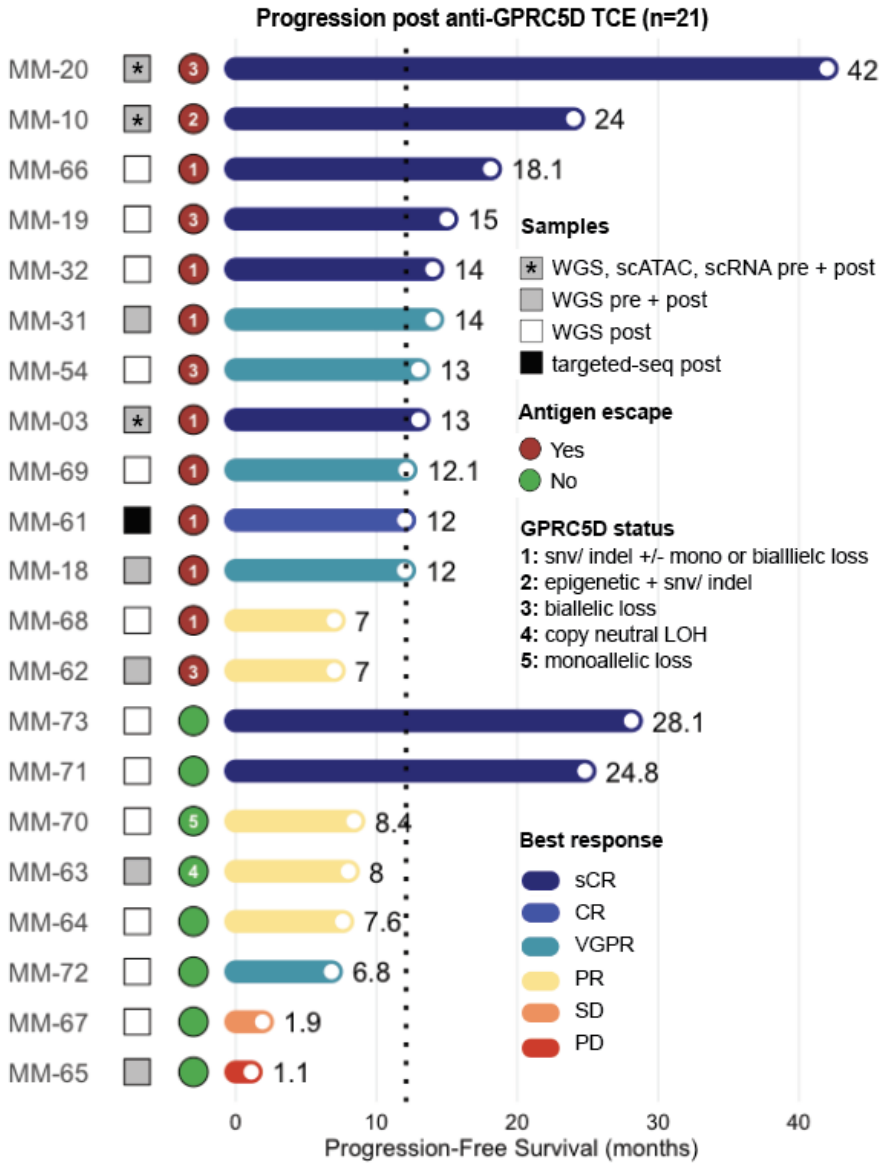
6. Figure 1A: I assume vertical dotted line indicates median PFS, but it should be explicitly indicated in figure legend.

Thank you for this comment. We have updated the figure legend to add description of the median PFS dotted line.

7. Figure 1A: in addition to the green/red labeling for the mutational status, it would be visually helpful to add symbols within the red dot to indicate the different mutational patterns.

Thank you for this comment. We have updated the figure (**Main Figure 1B** in the revised manuscript) and added annotations within the red/green labelling to indicate the type of genomic events that occurred in each patient. The genomic events on GPRC5D have been broadly categorized into the following categories including: 1. SNV/ indel with or without mono- or biallelic deletions, 2. Epigenetic silencing coupled with SNVs, 3. Biallelic deletion, 4. Copy neutral LOH, and 5. Monoallelic deletion.

Revised Main Figure 1B



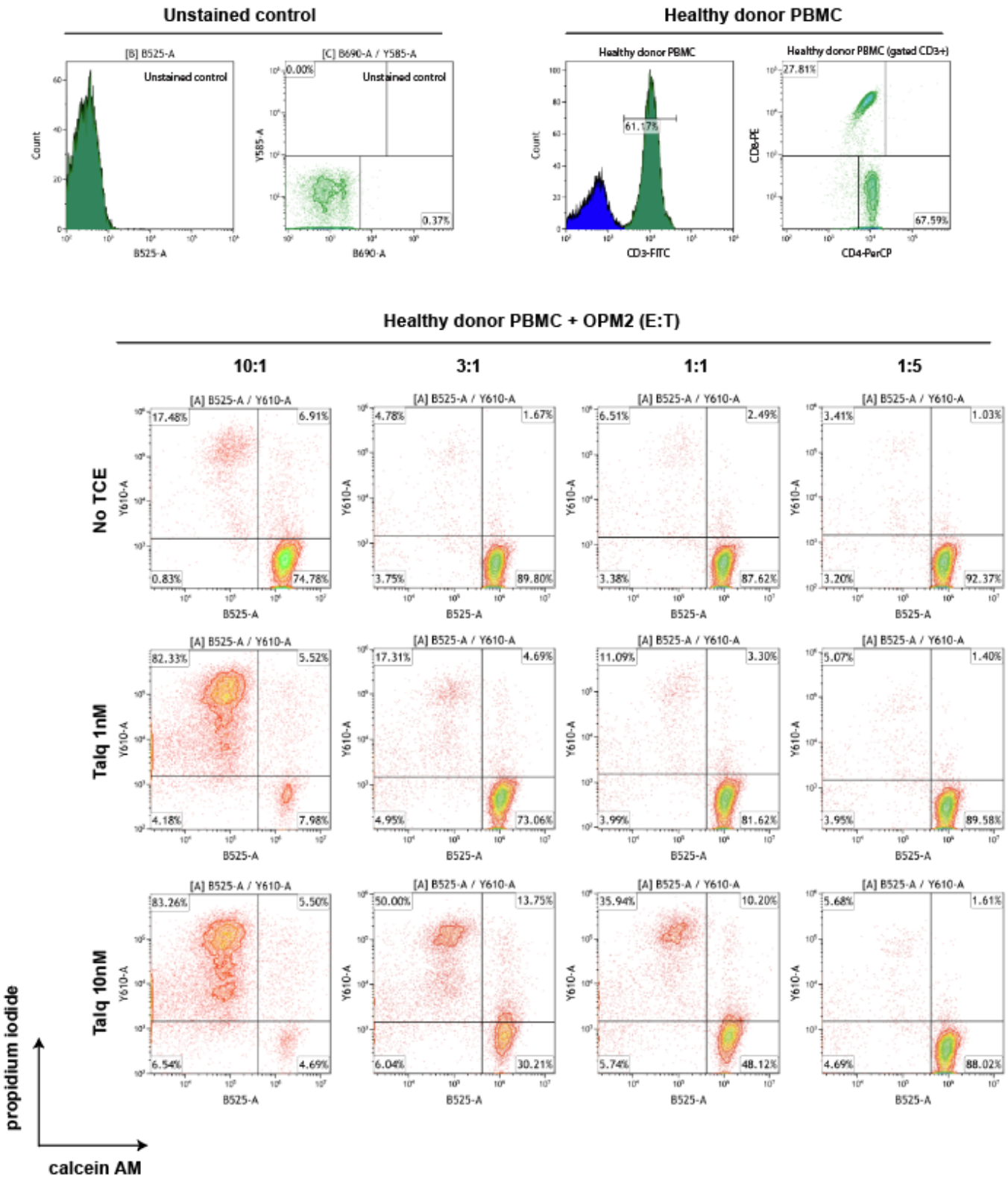
8. In the figure legend for extended figure 1C please indicate the significance of the "1/1" labeling on the X axis.

Thank you for this comment. The 1/1 labelling indicated that one out of one sample had the indicated SBS signature. To avoid confusion, we have removed this label in the X axis. In addition, we have now added the SBS signatures and the mutational burden for each sample in **Supplementary Figure 1.**

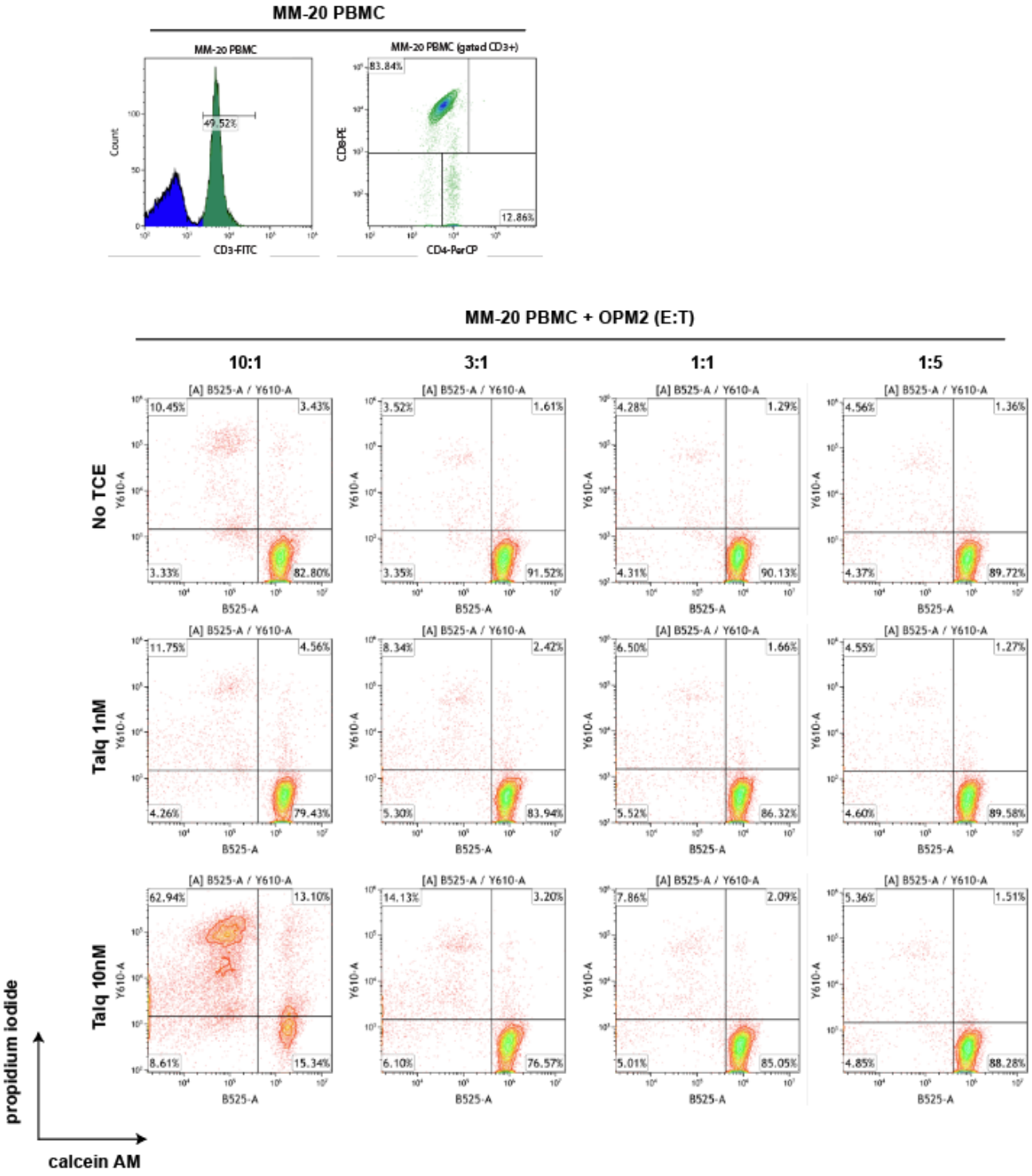
9. Killing assays reported in figure 2F use an E:T ratio of 10:1, which is not clinically relevant and may not be appropriate to test T cell fitness. A 1:1 or even 1:5 E:T ratio would be more meaningful.

Thank you for this comment. We agree that an E:T (PBMC to tumor) ratio of 10:1 favors T-cell killing and does not always reflect *in situ* bone marrow conditions with high disease burden. While our data strongly suggests that antigenic loss is the main driver of resistance to TCEs, this event does not preclude the possible co-occurrence of T cell dysfunction in particular in the context of chronic exposure to TCE. While PBMCs were not available from MM-03, we analyzed PBMC samples from MM-20, another case of antigen escape with biallelic *GPRC5D* deletion. Using OPM2 targets, we compared cytotoxicity of healthy donor PBMCs and MM-20 PBMCs across E:T ratios (10:1, 3:1, 1:1, 1:5) and talquetamab concentrations (1 nM, 10 nM) (**Figures A and B below**). Healthy donor PBMCs demonstrated more effective cytolytic activity at all E:T ratios, and even more pronounced at low E:T ratios compared to MM-20 PBMCs. Overall, E:T ratio of 10:1 may be appropriate for patients with adequate T cell counts and early biochemical relapse with low disease burden. However, we do concur with the reviewer that in higher disease burden settings, 1:1 E:T ratio is more representative, hence the importance of testing various E:T ratios and more importantly, accounting for the absolute T cell count in PBMCs in functional *in vitro* experiments. We have added the data from this additional analysis in **Main Figure 3E** and added the statement to the Discussion section (line 659-667).

A.


Unstained control
Healthy donor PBMC
[B] B525-A
Unstained control
Count
B525-A
[C] B690-A / Y585-A
0.00%
Unstained control
Y585-A
0.37%
B690-A
Healthy donor PBMC
Healthy donor PBMC
61.17%
Count
CD3-FITC
Healthy donor PBMC (gated CD3+)
27.81%
CD8-PE
67.59%
CD4-PerCP
Healthy donor PBMC + OPM2 (E:T)
10:1
3:1
1:1
1:5
No TCE
Talq 1nM
Talq 10nM
[A] B525-A / Y610-A
Y610-A
B525-A
17.48%
6.91%
0.83%
74.78%
4.78%
1.67%
3.75%
89.80%
6.51%
2.49%
3.38%
87.62%
3.41%
1.03%
3.20%
92.37%
82.33%
5.52%
4.18%
7.98%
17.31%
4.69%
4.95%
73.06%
11.09%
3.30%
3.99%
81.62%
5.07%
1.40%
3.95%
89.58%
83.26%
5.50%
6.54%
4.69%
50.00%
13.75%
6.04%
30.21%
35.94%
10.20%
5.74%
48.12%
5.68%
1.61%
4.69%
88.02%
propidium iodide
calcein AM

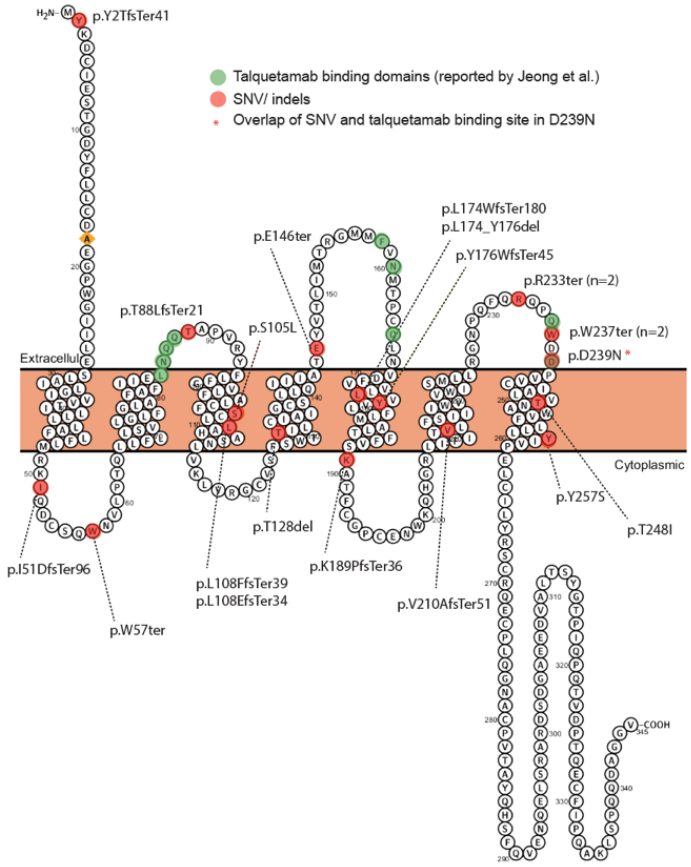
B.


MM-20 PBMC
MM-20 PBMC
MM-20 PBMC (gated CD3+)
49.52%
Count
CD3-FITC
83.84%
12.86%
CD8-PE
CD4-PerCP
MM-20 PBMC + OPM2 (E:T)
10:1
3:1
1:1
1:5
No TCE
Talq 1nM
Talq 10nM
[A] B525-A / Y610-A
B525-A
Y610-A
10.45%
3.43%
3.33%
82.80%
3.52%
1.61%
3.35%
91.52%
4.28%
1.29%
4.31%
90.13%
4.56%
1.36%
4.37%
89.72%
11.75%
4.56%
4.26%
79.43%
8.34%
2.42%
5.30%
83.94%
6.50%
1.66%
5.52%
86.32%
4.55%
1.27%
4.60%
89.58%
62.94%
13.10%
8.61%
15.34%
14.13%
3.20%
6.10%
76.57%
7.86%
2.09%
5.01%
85.05%
5.36%
1.51%
4.85%
88.28%
propidium iodide
calcein AM

10. Figure 2D shows significantly reduced GPRC5D expression post relapse. Why? If I understood correctly, the only expressed allele carries a Asp239Asn that should not impair proper membrane localization (Figure 5E) or TCE binding (Figure 5F). Is the reduced expression simply due to loss of one allele? Please clarify.

We thank the reviewer for raising this critical point. In case MM-03, genomic analysis demonstrates that the progression tumor has a clonal biallelic GPRC5D mutations *in trans*, p.Trp237Ter and p.Asp239Asn. The p.Trp237Ter nonsense mutation produces a truncated protein and the loss of the last 7th transmembrane pass and the cytoplasmic domain (**Extended Data Figure 8B**), and very likely undergoes nonsense-mediated mRNA decay, with loss of surface GPRC5D localization. The second missense mutation, p.Asp239Asn, affecting the remaining *GPRC5D* allele does not impair GPRC5D membrane trafficking, however, disrupts talquetamab binding (**Main Figure 6B**). We do concur with the reviewer that in this case, the reduced expression of GPRC5D by flow is most plausibly the result of monoallelic GPRC5D inactivation (p.Trp237Ter). Please also note our response to **query #5 from Reviewer 1** and **query #3 from Reviewer 3** regarding this topic.

Revised Extended Data Figure 8B



11. In Figure 3C, the annotation red and green for "normal" and "tumor" is a confusing. What does "normal" mean? It cannot refer to normal plasma cells, since they lack GPRC5D and largely TNFRSF17. Please clarify.

We thank the reviewer for this comment. We have clarified the annotation in **Main Figure 3C** and in the figure legend to indicate that the clusters represent CD138 positive versus CD138 negative cell populations.

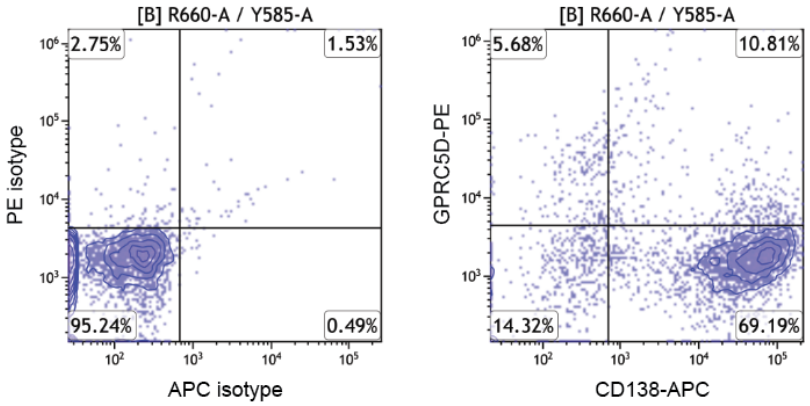
12. In Figure 3D: residual but significant GPRC5D expression is noted in the "post" sample. It is coming from normal plasma cells? In any case, the authors should revisit the statement in line 289 "undetectable membrane GPRC5D expression".

Thank you for raising this point. For this case MM-20, ASCAT and CNVkit both documented biallelic deletion of *GPRC5D*. Cancer cell fraction (CCF_CNV) using FACETS computed a CCF of 1 for this biallelic deletion (**Main Figure 3B**). Therefore we do not believe that there is residual clonal plasma cell fraction that is expressing GPRC5D.

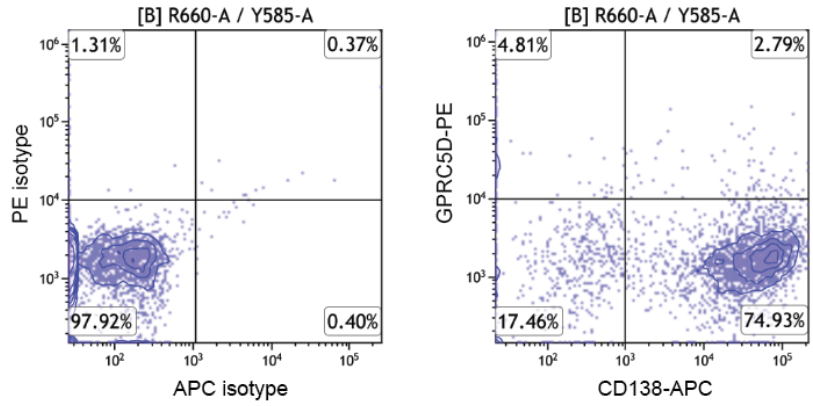
We also re-analyzed the flow data from this patient sample and noted evidence of PE signal spilling into the APC channel. We therefore applied minor compensation of 3.28 along the R660-A APC channel to correct this spill over. As shown in the Figure below, with this minor compensation and Y-axis gate correction, the proportion of CD138+ cells expressing GPRC5D is 2.79%. This is now updated in **Main Figure 3D**.

Revised Main Figure 3D

Original Main Figure 3D



Edited Main Figure 3D:
2 changes made
1) Y axis gated adjusted
2) compensation (3.28) applied to R660-A channel



13. Extended figure 6A. Please label the X axis: what do the numbers refer to?

Thank you for this comment. We have updated the figure to indicate that the X axis refers to mutational count.

14. Extended figure 6C, top: please label the Y axis. What does "TMB" indicate?

Thank you for this comment. We have updated the figure to indicate that TMB refers to tumor mutational burden.

15. Figure 5: if known, please indicate the binding sites for talquetamab and forimtamig.

We thank the reviewer for this comment. In the revised manuscript, we have added **Extended Figure 8B** with full length GPRC5D, and indicated the previously published[9] talquetamab binding epitopes in green as well as all the *GPRC5D* SNV/ indels (red) that we detected in our study.

16. Extended figure 10: it is visually confusing to have red boxes labeling t(11:14) in 10A, and non t(11;14) in 10B. If possible, please invert the coloring in 10B. – label with blue

Thank you for this comment. We have edited the colors of the boxes accordingly in the updated **Extended Figure 10**.

Reviewer #3 (Remarks to the Author):

Comments/questions:

1) Patient MM-03 was noted to have a high APOBEC signature (SBS 2 and 13). How frequently was this signature seen in the patients studied, and was it more prevalent in the patients with antigen escape?

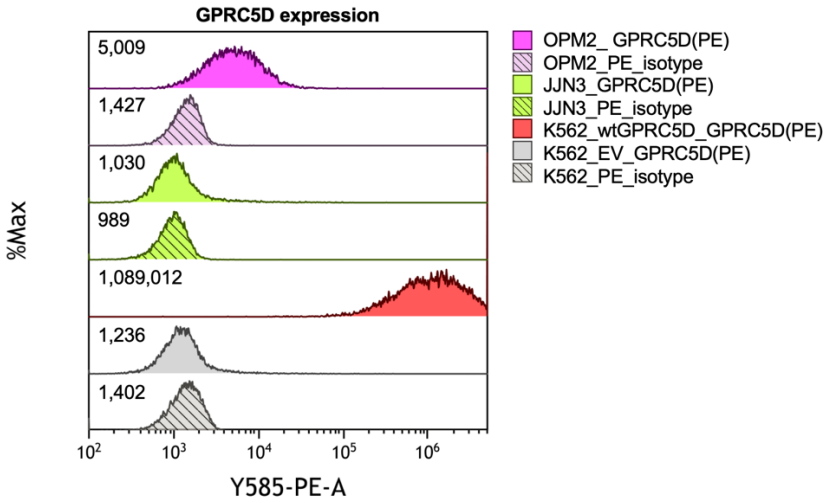
We thank the reviewer for raising this point. We performed SBS signature analysis using mmsig[8] in all patients and report this data in **Supplementary Figure 1**. In MM-03, MM-54, MM-66, MM-67 we detected hyper-APOBEC activity while in most remaining patients, there was low evidence of APOBEC activity (Please see attached figure to response to **query #8 from Reviewer 2** above). Therefore, APOBEC cannot explain all the mutational events we observed. A statement on APOBEC signature has been added to the manuscript in lines 368-371.

2) It seems that specific mutations didn't correlate with presence or absence of GPRC5D mutations or loss (Extended Data Fig 6), but was there any correlation with total mutational burden?

Thank you for the comment. We calculated total mutational burden of each sample using MutationalPatterns[10] (**Supplementary Figure 1**). Comparison of the pre- versus post-relapse total mutation counts was feasible in 8 cases wherein serial samples were available. This demonstrated that the total mutational burden was not significantly elevated in the post-relapse samples compared to pre-therapy. Please also refer to figure attached in our response to **Reviewer 2 query #3** as well as **Supplementary Figure 9**.

3) In Fig. 2D/E, the Asp239Asn mutation leads to significantly decreased GPRC5D surface expression on MM-03's myeloma cells, yet surface expression of GPRC5D harboring this mutation on K562 cells appears to be intact (Fig. 5E/F). Can authors speculate about reasons for this difference?

We thank the reviewer for this comment. The K562 cells were transduced with GPRC5D wild type or mutant lentiviral constructs expressed under the constitutive potent EF1a promoter. Hence, both wild type and mutant GPRC5D expression in transduced K562 cells is significantly higher than what is observed in primary patient samples or MM cell lines such as OPM2 (**Figure** below). This higher expression results from multi-site integration of the gene insert as well as the potent expression mediated by the EF1a promoter. In the case of MM-03, this patient harbored two mutations in GPRC5D (p.Trp237ter and p.Asp239Asn) affecting both alleles, resulting in GPRC5D lower protein expression. Please also note our response to **query #5 from Reviewer 1** and **query #10 from Reviewer 2** regarding this observation.



4) A table summarizing the observed genomic events in each patient should be included for the reader to understand the relative frequency of these various events and which mutations are most common. This is mentioned in the text (lines 203-204) but not included in the Supplementary Tables provided.

Thank you for this comment. We included an updated **Supplementary Table 4** that summarizes the GPRC5D genomic events observed in each patient. To further clarify this point, we have included in **Supplementary Figure 9** an annotated oncoplot displaying cancer cell fractions (CCF) of GPRC5D mutations across post-relapse samples, with sample-level features (antigen escape, mutational burden, APOBEC contribution, and cytogenetic alterations) and mutation frequency per row indicated on the right.

References:

1. Van Loo, P.*, et al.* Allele-specific copy number analysis of tumors. *Proceedings of the National Academy of Sciences* **107**, 16910–16915 (2010).
2. Shinde, J.*, et al.* Palimpsest: an R package for studying mutational and structural variant signatures along clonal evolution in cancer. *Bioinformatics* **34**, 3380–3381 (2018).
3. Shen, R. & Seshan, V.E. FACETS: allele-specific copy number and clonal heterogeneity analysis tool for high-throughput DNA sequencing. *Nucleic Acids Res* **44**, e131 (2016).
4. Papadimitriou, M.*, et al.* Timing genomic antigen loss in multiple myeloma treated with T-cell redirecting immunotherapies. *Blood Cancer Discov* (2025).
5. Quinones-Valdez, G., Fu, T., Chan, T.W. & Xiao, X. scAllele: A versatile tool for the detection and analysis of variants in scRNA-seq. *Science Advances* **8**, eabn6398 (2022).
6. Frenking, J.H.*, et al.* A German multicenter real-world analysis of talquetamab in 138 patients with relapsed/refractory multiple myeloma. *Hemasphere* **9**, e70114 (2025).
7. Nadeu, F.*, et al.* IgCaller for reconstructing immunoglobulin gene rearrangements and oncogenic translocations from whole-genome sequencing in lymphoid neoplasms. *Nature Communications* **11**, 3390 (2020).
8. Rustad, E.H.*, et al.* mmsig: a fitting approach to accurately identify somatic mutational signatures in hematological malignancies. *Communications Biology* **4**, 424 (2021).
9. Jeong, J., Park, J., Young Mo, G., Shin, J. & Cho, Y. Structural Basis for the Recognition of GPRC5D by Talquetamab, a Bispecific Antibody for Multiple Myeloma. *J Mol Biol* **436**, 168748 (2024).
10. Blokzijl, F., Janssen, R., van Boxtel, R. & Cuppen, E. MutationalPatterns: comprehensive genome-wide analysis of mutational processes. *Genome Medicine* **10**, 33 (2018).

Response to Reviewers

We thank all the reviewers for their constructive comments that enhanced the manuscript and added clarity.

Reviewer # 1:

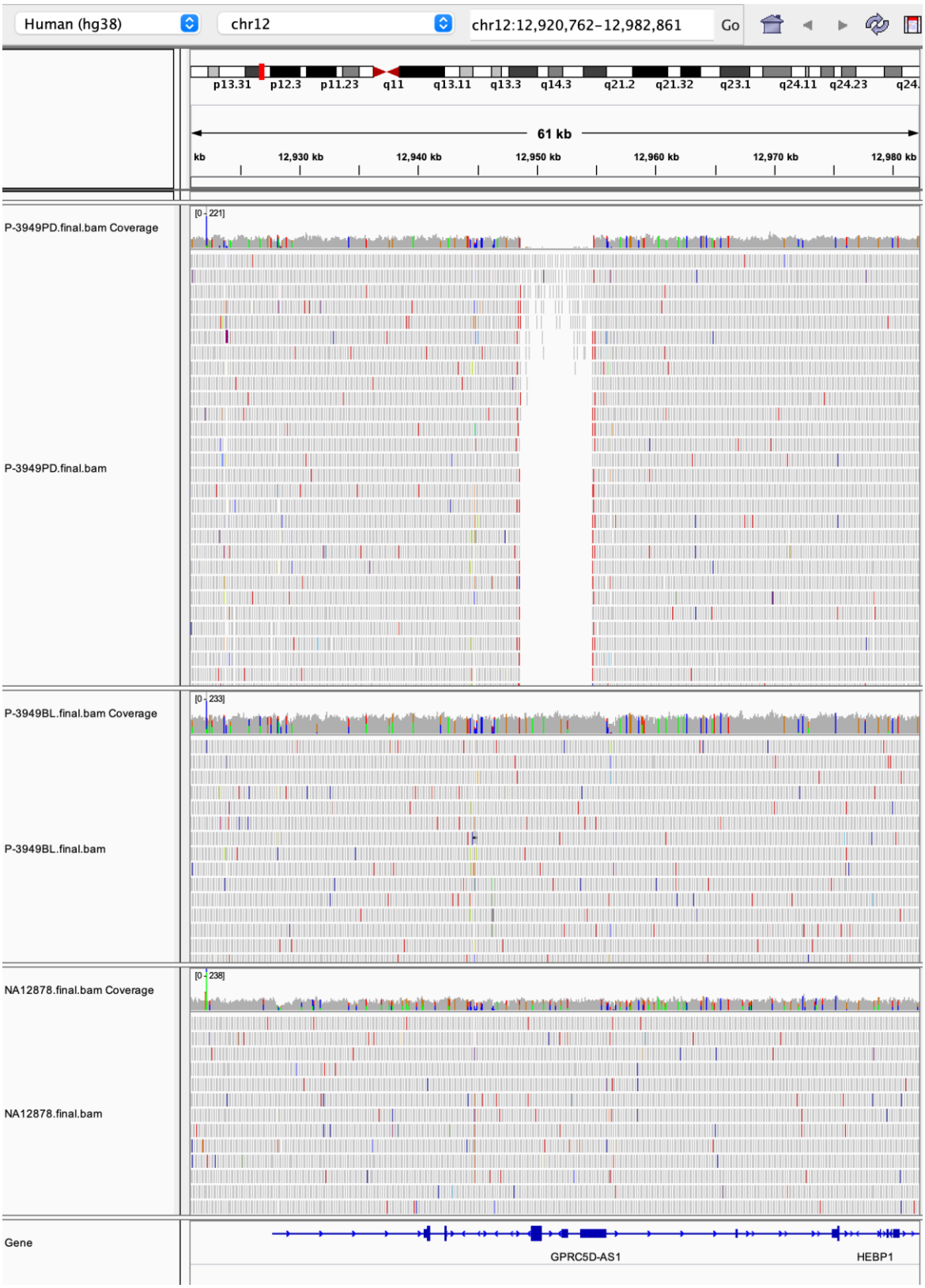
"Bulk WGS (100x) was performed on 20 patient cases (n=8 baseline and n=20 postrelapse) with matching normal when available (30x)."
Was matching normal unavailable in some cases? How many? Please described this information in the revised version of the manuscript as well as the procedures to overcome the limitation of matching normal unavailable. In addition, how these results can be confidently interpreted together with other data where matching normal germline DNA was available.

Response:

We thank the reviewer for this query.

Matched normal was available for all except 2 cases MM-62 and MM-63 as summarized in **Supplementary Table 1**. For these 2 cases (MM-62 and MM-63), genomic DNA from the contemporary normal NA12878 (from the Coriell Cell Repository GM12878 lymphoblastoid cell line, part of the 1000 Genomes Project) was used as germline control. NA12878, MM-62 and MM-63 were sequenced on the same Illumina platform and aligned to hg38. Germline WGS (NA12878) was processed with the same pipeline as the somatic DNA from MM-62 and MM-63 and used to (i) build a CNVkit WGS reference for copy-number normalization, and (ii) create a Mutect2 normal panel to filter recurrent technical artifacts. Somatic variant calls were further filtered using gnomAD (hg38) to mitigate germline contamination due to the mismatched normal. For added clarity, we have updated the Methods "**Whole genome sequencing and analysis"** section with this additional information.

In these two cases we do not call any SNVs at the GPRC5D locus. For patient MM-62, we reported a focal 6 Kb (chr12: 12,948,661-12,954,728) *GPRC5D* biallelic deletion at progression on talquetamab (P-3949PD) but not at baseline pre-therapy (P-3949BL) or in the germline control (NA12878) as shown by CNVkit in the **Extended Data Figure 6A-B,** as well as in the IGV image below (included NA12878 bam file as well).


Human (hg38)
chr12
chr12:12,920,762-12,982,861
Go
p13.31
p12.3
p11.23
q11
q13.11
q13.3
q14.3
q21.2
q21.32
q23.1
q24.11
q24.23
61 kb
kb
12,930 kb
12,940 kb
12,950 kb
12,960 kb
12,970 kb
12,980 kb
P-3949PD.final.bam Coverage
[0 - 221]
P-3949PD.final.bam
P-3949BL.final.bam Coverage
[0 - 233]
P-3949BL.final.bam
NA12878.final.bam Coverage
[0 - 238]
NA12878.final.bam
Gene
GPRC5D-AS1
HEBP1