Immune checkpoint inhibitors in mesothelioma

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Chapter 9

Tumor junction burden and antigen presentation as predictors of survival in mesothelioma treated with immune checkpoint inhibitors

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Abstract

Introduction

The favorable outcomes with immunotherapy for mesothelioma were somewhat unexpected since this tumor has a low tumor mutation burden which has been associated with benefit in other cancers. Since chromosomal rearrangements are common in mesothelioma and have neoantigenic potential, we sought to determine whether they are associated with survival in patients treated with immunotherapy.

Methods

Pleural biopsies of mesothelioma after at least one line of therapy were obtained from patients (n=44) prior to treatment with nivolumab alone (NCT29908324) or in combination with ipilimumab (NCT30660511). RNA and whole genome sequencing were performed to identify the junctions resulting from chromosomal rearrangements, and antigen processing and presentation gene set expression. Associations with overall survival were estimated using cox models. An overall survival cutoff of 1.5 years was used to distinguish patients with and without durable benefit for use in receiving operating characteristic (ROC) curves.

Results

While tumor junction burdens were not predictive of overall survival, we identified significant interactions between the junction burdens and multiple antigen processing and presentation gene sets. The "regulation of antigen processing and presentation of peptide antigen" gene set demonstrated an interaction with tumor junction burden and was predictive of overall survival. This interaction also predicted 1.5-year or greater survival with an area under the ROC of 0.83. This interaction was not predictive of survival in a separate cohort of patients with mesothelioma who did not receive immune checkpoint inhibitors.

Conclusions

Analysis of structural variants and antigen presentation gene set expression may facilitate patient selection for immune checkpoint inhibitors.

Introduction

Given the mixed results observed with immune checkpoint inhibitors for the treatment of mesothelioma, it is more important than ever to identify biomarkers that may predict outcomes and guide the use of these therapies. Unlike other tumor types with high tumor mutations burdens where clear survival benefits have been demonstrated with immune checkpoint inhibitors, mesothelioma has a very low mutation burden. Mesothelioma primarily arises as a result of the exposure to the carcinogen asbestos, although some cases develop after therapeutic radiation, or are inherited due to loss of function mutations in BRCA1 Associated Protein 1 (*BAP1*) (1). Recent studies reported very low tumor mutation burdens (TMB) using next-generation sequencing (NGS) to evaluate mesothelioma (2,3). This finding was unexpected because other tumors associated with carcinogenic exposures such as malignant melanoma, small cell and non-small cell lung cancer typically have a high TMB from ultraviolet radiation and tobacco exposure, respectively (4). High TMBs are thought to be a surrogate for an increase in neoantigens that can be recognized by the adaptive immune system and facilitate tumor elimination. Despite the reportedly low TMB in mesothelioma, the combination of the PD-1 inhibitor nivolumab and the CTLA-4 inhibitor ipilimumab was shown to be superior to treatment with cisplatin and pemetrexed chemotherapy in patients with unresectable mesothelioma, and is now approved by the United States Food and Drug Administration for frontline use (5).

Current clinically available NGS approaches do not fully characterize the genomic complexity of tumors. Cytogenetic studies have identified recurrent, structural chromosomal abnormalities in mesothelioma (6,7), yet these events are not commonly reported in more recent NGS studies (2,3). For this reason, in prior work, we used a sequencing approach that tiles the whole genome with large DNA fragments (2-5 kb compared to standard 200-500 bp) to improve the detection of structural variants such as insertions, deletions and translocations. Chromosomal rearrangements disrupt gene regions generating truncations or fusion transcripts reading into normally distal gene regions or noncoding DNA. We previously found multiple chromosomal rearrangements that resulted in discordant DNA junctions with the potential for novel fusions in mesothelioma (8). Many of these events fit a pattern of chromoanagenesis such as chromothripsis or chromoplexy (9). Since structural abnormalities like insertions, deletions, and chromosomal translocations have neoantigenic potential (8,10,11), we sought to determine their role in predicting outcomes in patients with mesothelioma treated with immune checkpoint inhibitors.

Materials and methods

Patients and specimens: Biopsies were obtained from patients just prior to treatment with nivolumab (NCT02497508) (12) or nivolumab with ipilimumab (NCT03048474) (13), after previous treatment with platinum-based chemotherapy. DNA and RNA were purified using the AllPrep DNA/RNA/miRNA Universal kit (Qiagen, #80224) following the instructions provided by the manufacturer. The buffer included β-mercaptoethanol for the specimens obtained from NCT02497508, and dithiothreitol for the ones obtained from NCT03048474. Otherwise, there were no differences in the handling of the specimens or nucleic acid purification. The clinical trials and translational studies were approved by the local institutional ethics committees. Characteristics of the patients included in our analysis were compared to those of patients who were excluded due to insufficient materials using the Fisher's exact test for categorical variables and the Mann Whitney U test for continuous variables. Survival between these groups was compared using the R packages "survival" and "survminer".

Determination of tumor junction burdens: Chromosomal rearrangements were reported by sequencing DNA prepared according to the mate-pair whole-genome library protocol (Nextera Library Prep Protocol). Sequencing results were mapped by BIMA, and the junctions of the chromosomal rearrangements were called by SVAtools. BIMA and SVAtools are Mayo Clinic in-house informatic pipelines (14,15). The junctions of the chromosomal rearrangements were annotated with 1) the position of the junction with a resolution of 200-500bp, 2) direction of the chromosomal rearrangement and 3) genes at the junction using NCBI RefSeq genes for GRCh38. The number of chromosomal rearrangements per sample was assessed by counting the number of unique genes hit by all junctions in the sample. All specimens had 60X or greater bridged coverage for the detection of junctions, except one which had 40X bridged coverage. Chromosomal rearrangements may refer to insertions, deletions, translocations, and inversions. Junctions are the locations of the breaks of these chromosomal rearrangements. There may be one junction (deletion, insertion, translocation), two junctions (inversion, balanced translocations) or multiple junctions (three-way, four-way etc. translocation) involved with each chromosomal rearrangement.

RNA-seq analyses: Mapping of the RNA-seq data and estimations of gene expression counts in each sample were performed by MAP-RSeq pipeline developed previously by the Mayo Bioinformatics Core (16). Raw "count" files were processed by the "edgeR" package to generate log 2 normalized gene expression values.

Antigen processing and presentation (APP): The Biological Processes Gene Ontology dataset in the Molecular Signature database was searched for gene-sets with names

that included "antigen" and "presentation." Of the 21 found hits, nine were eliminated for processes involving lipid, polysaccharide, exogenous antigens or processes representing dendritic cell or T-cell antigen processing and presentation. Single sample enrichment scores in the remaining 12 gene-sets were calculated by using the "ssGSEA" (single sample gene set enrichment analysis) algorithm in the "GSVA" package.

Survival and immune checkpoint inhibitor survival analyses: A statistical interaction is present when the association between two variables depends on a third variable. In our case, we hypothesized that the associations between tumor junction burden and survival (in terms of either hazard or odds ratio in cox or logistic regression models, respectively) depended on the APP capabilities of tumors. Therefore, we tested the statistical significance of APP and tumor junction burden interactions in predicting OS or $S_{1.5yr}$. Associations of interactions between gene-sets and log2 transformed junction burden (APP * log2[junction burden]) with overall survival (OS) were found by using the "coxph" (cox proportional hazard) program in the "survival" package. Associations of these interactions with response to immune checkpoint inhibitors in terms of survivals at 1.5-year ($S_{1.5yr}$) were calculated by logistic regression (LR) using the "glm" (generalized linear model) package. APP and junction burden interactions were considered significant when either or both of the following conditions were met: (i) log-rank p-values and the interaction terms in the OS models were significant ($p < 0.05$), or (ii) the interaction terms in LR analysis was significant and the LR model had an accuracy based on area under the curve (AUC) greater than 0.7. To create the Kaplan Meir plot representing an individual gene-set interaction with junction burden, samples were categorized as either "High" or "Low" by using the median multiplication product of gene-set scores and log2[junction burden] as the threshold. Reported p-values in the plot are associations of the interaction and the model (log rank test) with overall survival by "coxph" program.

Forest plots: Median enrichment scores in each of the APP gene-sets were used to group samples into high and low APP categories. In each category, hazard ratios representing associations between junction burdens and overall survival were calculated by "coxph" and plotted using the "forestplot" package.

Examination of existing models: Immunotherapy response models described elsewhere (17) were examined for predicting significant benefit (SB) and no significant benefit (NSB). Log2 transformed gene expression data were normalized in each row by subtracting average values across all samples according to the authors instructions. Normalized expression values were input to the python program "tidepy" to estimate individual tumor scores in 14 models. Logistic regression analyses were then used to estimate the accuracy of models with the CD8 model having been found as the best performer. Finally, "pROC" program was used to plot the ROC curves for TIDE, IFNG, PD-L1, and CD8 models.

Immune deconvolution: The immunedeconv package in R was used to assess the tumor microenvironment. immunedeconv contains six approaches (quantiseq, timer, cibersort_abs (and first generation cibersort), mcp_counter, xCell, and epic) to estimate the abundance scores of multiple cell types, including adaptive and innate immune cells, based on ssGSEA data. Statistical significance of differential cell type enrichment between cohorts of patients with high or low "REGULATION OF APP OF PEPTIDE ANTIGEN" gene set expression was compared the t test.

Results

Sixty-eight patients with pleural mesothelioma were treated with the PD-1 inhibitor nivolumab alone or in combination with the CTLA-4 inhibitor ipilimumab on the NivoMes (n=34) and INITIATE (n=34) clinical trials, respectively (12,14) (Supplementary Table 1). These patients had received at least one prior line of platinum-containing therapy. Biopsies were obtained on 65 of these patients just prior to the start of treatment with an immune checkpoint inhibitor(s), and 44 of these specimens had sufficient DNA and RNA content for analysis. There were no significant differences between the characteristics of the patients included in this analysis and those excluded based on sample insufficiency including sex, trial treatment, performance status, line of therapy, age, or overall survival. Despite the historic median survivals of less than six months with second or later line therapy in mesothelioma,(18) there was a separation in overall survival at 1.5 years ($S_{1.5yr}$) from start of treatment on trial which we selected to group patients into categories of significant benefit (SB, > $S_{1.5yr}$) and no significant benefit (NSB, ≤ $S_{1.5yr}$)(Figure 1A).

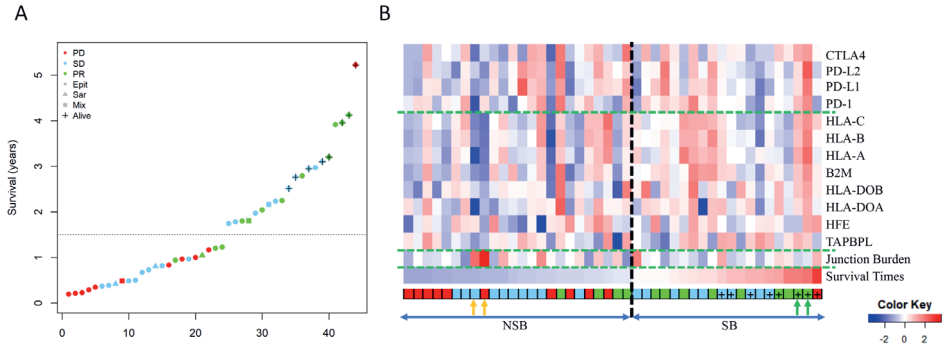

Figure 1: (A) Survival times of the study cohort. Red, blue, and green represent the best responses of progression of disease (PD), stable disease (SD), and partial response (PR), respectively. Circles, triangles, and squares represent epithelioid (Epit), sarcomatoid (Sar, including mesenchymal), and mixed (Mix) histology, respectively. "+" designates alive at the last follow-up. (B) Heatmap representing survival times, junction burden, antigen processing and presentation, and immune

checkpoint markers. The lower bar represents best responses with PD, SD, and PR as per Figure 1A. Orange arrows point to two cases with high junction burdens, short survival times, and low expression in genes involving antigen processing and presentation (APP). On the contrary, green arrows point to two cases with moderate junction burdens, long survival times, and robust APP expression.

There were no differences in overall survival between those who receive nivolumab with or without ipilimumab (Supplementary Figure 1). The biopsies obtained just prior to treatment were analyzed by mate-pair DNA sequencing and RNA-seq. There were many chromosomal rearrangements in each specimen (median 130 junctions, range 23-348), and a fraction of these involved unique genes (median 18, range 1-68). We selected the chromosomal rearrangements involving unique genes in each tumor for our analysis given their potential to be expressed and refer to them as the tumor junction burden from hereon.

Given our prior findings of the neoantigenic potential of chromosomal rearrangements, we sought to determine whether tumor junction burdens were associated with survival in patients with mesothelioma treated with immune checkpoint inhibitors. We did not find an association between tumor junction burden and overall survival (Cox model log rank $p > 0.5$)(Supplementary Figure 2A). Notably, two patients with the highest tumor junction burdens had very short survival times, whereas two other patients with moderate tumor junction burdens had a durable survival benefit (Figure 1B). The two patients with the highest tumor junction burdens and poor survival had low expression of genes involved in antigen processing and presentation (APP). On the other hand, patients with moderate tumor junction burdens and more durable survival had very robust expression of APP associated genes.

	P-IA-cox	*p-Log Rank*	*P-IA-lr*	*AUC*
REGULATION_OF_AP&P_OF_PEPTIDE_ANTIGEN	**0.0026**	**0.0031**	**0.0221**	**0.831**
AP&P_OF_ENDOGENOUS_PEPTIDE_ANTIGEN	**0.021**	**0.041**	**0.040**	**0.724**
AP&P_OF_PEPTIDE_ANTIGEN_VIA_MHC_CLASS_IB	**0.048**	**0.019**	**0.071**	**0.759**
AP&P_OF_ENDOGENOUS_PEPTIDE_ANTIGEN_VIA_MHC_CLASS_I_VIA_ER_PATHWAY	**0.049**	**0.010**	**0.072**	**0.811**
AP&P_OF_ENDOGENOUS_ANTIGEN	0.061	0.079	0.025	0.748
AP&P_VIA_MHC_CLASS_IB	0.154	0.045	0.023	0.800
NEGATIVE_REGULATION_OF_AP&P	0.034	0.145	0.072	0.702
REGULATION_OF_AP&P	0.065	0.253	0.079	0.697
AP&P_OF_PEPTIDE_ANTIGEN_VIA_MHC_CLASS_I	0.080	0.318	0.240	0.610
POSITIVE_REGULATION_OF_AP&P	0.197	0.382	0.100	0.660
AP&P	0.201	0.597	0.289	0.542
AP&P_OF_PEPTIDE_ANTIGEN	0.242	0.673	0.519	0.559

Table 1 legend. The statistical significance of interactions (P-IA-cox) and log-rank (p-Log Rank) in cox models, interactions (P-IA-lr) and area under the curve (AUC) in logistic regression models are listed. The gene sets with significant interactions are in bold. APP and junction burden interactions were considered significant when either or both of the following conditions were met: (i) log-rank p-values and the interaction terms in the OS models were significant ($p < 0.05$), or (ii) the interaction terms in LR analysis was significant and the LR model had an accuracy based on area under the curve (AUC) greater than 0.7.

Since the impact of tumor junction burdens appeared to be modulated by APP, we hypothesized that the neoantigenic potential of chromosomal rearrangements was dependent upon the capability of cancer cells to present neo-antigens to the immune system. To examine whether there was an interaction between APP gene sets and tumor junction burdens that impacted outcomes, we selected 12 APP gene sets from the Gene Ontology - Biological Processes dataset in the Molecular Signature Database and calculated their enrichment scores (Supplementary Table 2). We then used these scores to test for interactions between APP gene sets and junction burdens on survival and found significant interactions with six APP gene sets (Table 1). With these six APP gene sets, the hazard ratios representing associations between tumor junction burdens and overall survival favored patients with high APP scores (all hazard ratios <1) more so than patients with low APP scores (all hazard ratios >1)(Figure 2). There were no differences in survival between patients with high or low APP scores (Supplementary Figure 2B). In patients with low APP scores, those with a high tumor junction burden were at increased risk of death compared with patients with low tumor junction burdens (Supplementary Figure 2C). On the other hand, in patients with high APP scores, those with high tumor junction burdens were at reduced risk of death compared with patients with low tumor junction burdens (Supplementary Figure 2D).

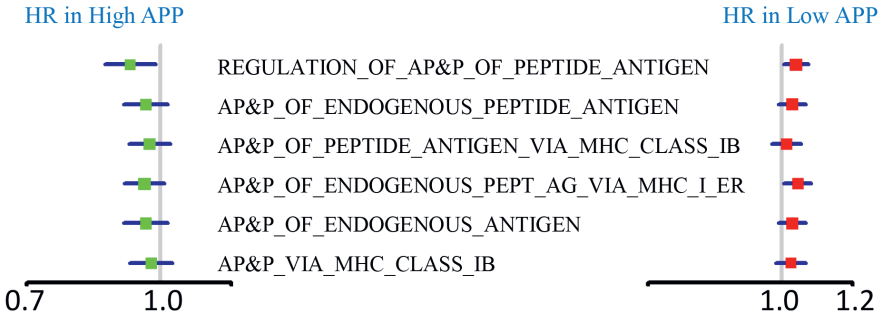

Figure 2: Forest plots displaying the hazard ratios for junction burdens and overall survival associations in samples with high and low APP gene set expression, respectively in gene sets identified as significant.

We further examined the interaction models that included the "REGULATION OF APP OF PEPTIDE ANTIGEN" gene set which included 6 genes (PYCARD, HFE, HLA-DOA, HLA-DOB, TREM2, and TAPBPL). Both the interaction parameter between this gene set and the tumor junction burden, and the survival model were highly significant (Table 1 and Figure 3A). Furthermore, this interaction was highly predictive of $S_{1.5yr}$ with an AUC of 0.831 (Figure 3B). For comparison, we tested several available gene models previously reported to associate with response to immune checkpoint inhibitors including TIDE, IFNG, PD-L1, CD8, and others.(17) In our cohort, none of these other models performed as well as the interaction of APP gene sets with tumor junction burdens in predicting $S_{1.5yr}$, but the most accurate of these gene models was the CD8 model with an AUC of 0.683 (Figure 3C). Based on this observation, and to account for the role of antitumor lymphocytes in survival with immune checkpoint inhibition, we included CD8A in our prediction model. This addition increased the accuracy of the model from 0.831 to 0.890 (Figure 3D).

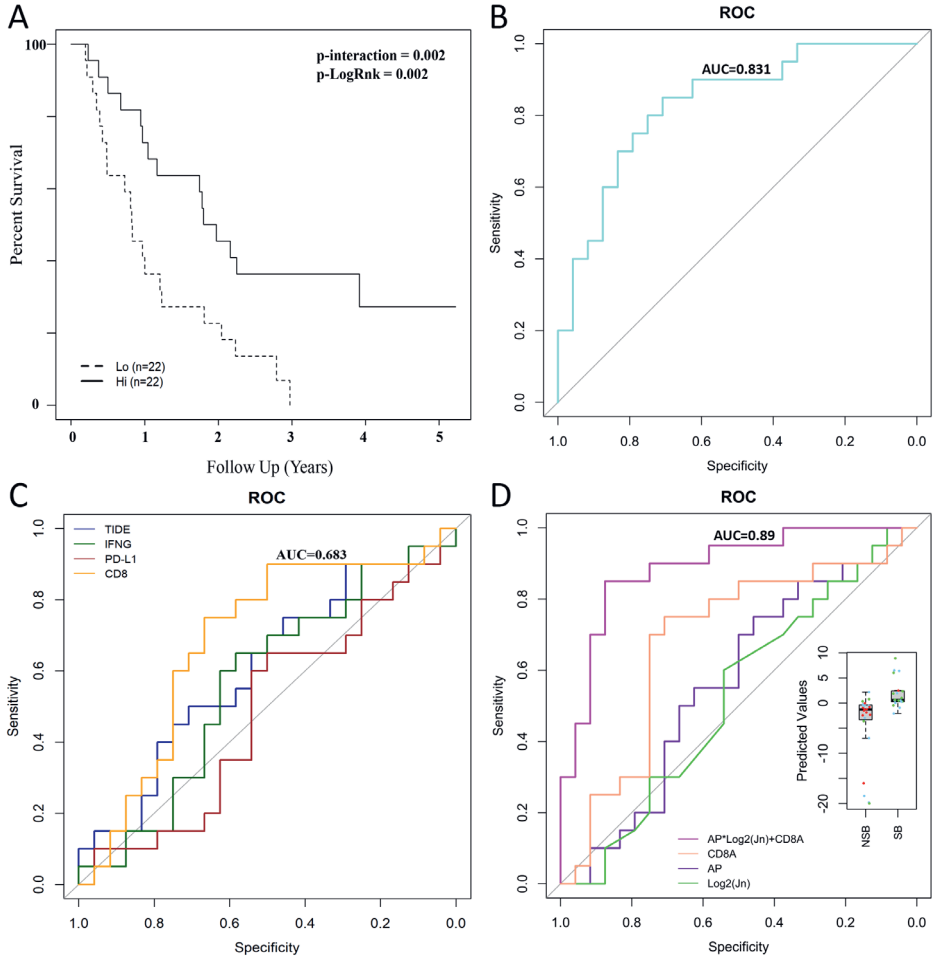

Figure 3: (A) The Kaplan Meier curve representing a survival model based on the interactions between "REGULATION OF APP OF PEPTIDE ANTIGEN" gene-set and junction burdens is shown. Both the interaction terms and the log-rank test were significant. (B) The ROC curve representing APP and log2[junction burden] interactions (cyan) in predicting NSB and SB is shown for the REGULATION_OF_AP&P_OF_PEPTIDE_ANTIGEN gene set. (C) ROC curves representing the accuracy of TIDE (blue), IFNG (green), PD-L1 (dark red), and CD8 (orange) models in predicting NSB and SB. (D) ROC curves representing APP (purple), log2[junction burden] (lime green), CD8A (light salmon), and the final model including APP / log2[junction burden] interactions and CD8A (magenta). The inlet is a boxplot and individual patient prediction values by the final model in NSB and SB categories. Colors represent radiologic responses as defined in Figure 1

We sought to determine if the interaction models were predictive of patient overall survival irrespective of treatment approach. To the best of our knowledge, the only available mesothelioma dataset that includes both chromosomal rearrangements from whole genome sequencing, and RNA-seq, is from our previous study of patients (n=24) who provided biopsy or surgical specimens prior to any cytotoxic systemic therapy (Mayo_2019 cohort) (8). The patients in the Mayo_2019 cohort did not receive immune checkpoint inhibitors as these therapies were not available during their lifetimes. There was a break in overall survival at 1.5 years from diagnosis in this cohort that was used as the threshold for categorizing patients as NSB and SB (Supplementary Figure 3). The Mayo_2019 cohort performed similar to other historic mesothelioma cohorts as a previously established mesothelioma survival signature gene set (19) had very high prognostic significance for both overall survival and $S_{1.5yr}$ (Supplementary Figure 4). We did not find an interaction between the tumor junction burdens and any of the 12 APP gene sets on overall survival to be statistically significant (Supplementary Table 3). In further analysis, we noted that the tumors in the Mayo_2019 cohort had fewer junctions than the current cohort (Supplementary Figure 5) which may have affected the predictive values of the interaction models.

Finally, we used RNAseq for computational immune deconvolution to compare the tumor microenvironment (TME) in mesotheliomas with low and high expression of the "REGULATION OF APP OF PEPTIDE ANTIGEN" gene set. The "immunedeconv" package used for our analyses provides results from 6 different computational approaches (see Methods). In all approaches, we observed a lower concentration of immune cells suggesting a "cold" TME in tumors with low compared to high APP gene set expression (Supplementary Figures 6-9). We found higher TME and immune scores (by xCell) and cytotoxicity score (by MCP-counter), and an enrichment of lymphocytes that are often associated with anti-tumor immunity such as B, T, and NK cells and M1 macrophages in tumors with high APP.

Discussion

Genomic structural variants are common in mesothelioma. In the current analysis the tumor junction burdens resulting from chromosomal rearrangements were associated with improved survival outcomes in patients treated with immune checkpoint inhibitors in the presence of antigen processing and presentation gene set expression. In contrast, tumor junction burdens in the absence of antigen processing and presentation gene set expression were associated with reduced survival despite treatment with immune checkpoint inhibitors. Our model was further improved by the inclusion of CD8A, a marker of cytotoxic lymphocytes. We interpreted these observations to be consistent

with our understanding of the mechanisms of adaptive anti-tumor immunity where antigen-specific T cell responses that are restored or generated by PD-1 and CTLA-4 inhibition require tumor cell presentation of neo-antigens. Since the interaction signature between the tumor junction burdens and APP gene sets did not favorably impact overall survival in a separate cohort of patients who did not receive immune checkpoint inhibitors, this signature is not likely to be predictive in settings outside of treatment with immunotherapy. Chromothripsis represents a complex pattern of multiple chromosomal rearrangements typically on a single chromosome. We previously identified that higher numbers of chromothripsis-like patterns detected from copy number segmentation data were a negative prognostic factor in mesothelioma (8), and others have suggested that chromothripsis is a negative prognostic marker across multiple tumor types (20). Despite the negative prognostic significance that has been attributed to increases in these complex patterns of chromosomal rearrangements in mesothelioma and other tumors, tumor junction burdens were associated with improved survival in the context of antigen processing and presentation gene set expression in this cohort of patients with mesothelioma treated with immune checkpoint inhibitors.

Given the marked differences between the TME in tumors with and without high APP gene set expression, we speculate that methods to manipulate the TME might be beneficial for these patients. Recently it was shown that low-dose radiotherapy in murine models promotes T cell infiltration, enabling response to combination immunotherapy (21). A clinical trial has recently activated to test this approach in mesothelioma (NCT04926948). Other work has suggested that oncolytic virotherapy may reprogram the TME to enable responses to immune checkpoint inhibitors (22). It is a major initiative across tumor types to identify means of converting tumors to be responsive to immune checkpoint inhibitors.

There have been inconsistent results with the use of immune checkpoint inhibitors for the treatment of mesothelioma. Based on the Checkmate 743 trial, the frontline use of ipilimumab and nivolumab clearly benefits patients with non-epithelioid mesothelioma, partially because chemotherapy is so ineffective for this group (5). The same degree of benefit was not observed in the epithelioid group, as chemotherapy is more effective for patients with that variant of disease. Since the survival analysis of all randomized patients was positive in the Checkmate 743 trial with a stratified HR of 0.74 (96·6% CI 0.60 0.91; p=0.0020), ipilimumab and nivolumab were approved by the United States FDA for frontline treatment of unresectable pleural mesothelioma regardless of histologic subtype. In second or later lines of treatment, single agent PD-1 inhibitors have been demonstrated to be superior to placebo in the CONFIRM (23) trial, but not superior to gemcitabine or vinorelbine in the PROMISE-meso trial (24); however, these studies both reported that there are responses with immune checkpoint inhibitors in patients with epithelioid disease. Surprisingly, the overall response rate with the PD-1 inhibitor

pembrolizumab was higher than that observed with chemotherapy (22% v. 6%) in the PROMISE-meso trial, although this difference did not translate into a survival benefit. PD-L1 expression was not able to discriminate benefit in the CONFIRM (23) or PROMISE trials (24), or in our cohort. Given the discrepancies with survival outcomes between these clinical trials, it is critical to develop better predictive biomarkers, especially for patients with epithelioid disease where benefit with immune checkpoint inhibitors is less certain.

There have been multiple efforts to identify predictors of benefit with immune checkpoint inhibitors (25). Mismatch repair deficiency is strongly associated with response to treatment across tumor types (26). TMB has also been proposed as a surrogate of neoantigens that can be recognized by the adaptive immune system for elimination. Recently, a PD-1 inhibitor has been approved for solid tumors with a TMB ≥10 mutations/Mb (27); however, these findings have been challenged by others who have failed to identify benefit across tumor types with this cutoff (28). There is significant heterogeneity in the approaches used to determine TMB, and use of population germline variant databases to filter calls can inflate scores and introduce racial bias (29,30). TMBs frequently do not assess or include structural variants or junction burdens. Also, TMB fails to incorporate the full complexity of an adaptive, anti-tumor immune response.

Immunograms may provide better predictors of response to immune checkpoint inhibitors as these would incorporate tumor foreignness (using comprehensive mutation burdens), the ability of tumors to present neoantigens with MHC proteins (antigen processing and presentation), lymphocytes and their ability to traffic to tumors, and the expression of immune checkpoints and other regulatory signals (31). Our findings represent one step towards adopting an immunogram to predict survival with immune checkpoint inhibitors in mesothelioma by incorporating antigen processing and presentation gene set expression in our analysis. These results also suggest that genomic approaches that identify and incorporate junction burdens can improve the determination of TMB, especially in tumors like mesothelioma that have relatively few single nucleotide mutations.

We tested the tumors of patients who had received prior platinum-based chemotherapy. Since the numbers of junctions were slightly higher in the current cohort than a separate cohort of patients who had not received prior platinum-based chemotherapy, it is possible that cytotoxic therapy introduced structural variants. Along these lines, our findings will need to be validated in a cohort of treatment-naïve patients. Also, given the DNA sample requirements to perform our analysis of structural variants, we did not have sufficient materials to perform traditional sequencing approaches to assess single nucleotide mutations. Given the reportedly low TMB in mesothelioma and our prior findings of large, complex rearrangements in this malignancy, we felt it was reasonable to focus our efforts on these structural variants. Finally, efforts are underway to develop chemoimmunotherapy

regimens for mesothelioma. We are not certain whether structural variants would retain their association with survival outcomes in the setting of combination cytotoxic and immunotherapy.

In conclusion, in the context of antigen processing and presentation gene set expression, tumor junction burdens were associated with improved survival in patients with mesothelioma treated with immune checkpoint inhibitors. In contrast, in the absence of antigen processing and presentation, tumor junction burdens were associated with poor survival. The inclusion of genomic approaches that can detect structural variants, and transcriptomics to assess antigen processing and presentation, may help refine the selection of patients to receive immune checkpoint inhibitors, especially for patients with mesothelioma.

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Supplementary data

Supplementary Table 1: Patient characteristics

Characteristics	Patients (n=44)
Sex, n (%)	
Male	37 (84%)
Female	7 (16%)
Histology, n (%)	
Epithelioid	38 (86%)
Biphasic	3 (7%)
Sarcomatoid	3 (7%)
Treatment, n (%)	
Nivolumab	24 (55%)
Nivolumab, Ipilimumab	20 (45%)
Line of therapy, n (%)	
2	38 (86%)
≥3	6 (14%)
Age, median (range)	66 (47-81)

Supplementary Table 2

Gene set Name	Included Genes
GO_REGULATION_OF_ANTIGEN_PROCESSING_AND_PRESENTATION_OF_PEPTIDE_ANTIGEN	PYCARD, HFE, HLA-DOA, HLA-DOB, TREM2, TAPBPL
GO_ANTIGEN_PROCESSING_AND_PRESENTATION_OF_ENDOGENOUS_PEPTIDE_ANTIGEN_VIA_MHC_CLASS_I_VIA_ER_PATHWAY	HLA-A, HLA-B, HLA-C, HLA-E, HLA-F, HLA-G, HLA-H, AZGP1
GO_ANTIGEN_PROCESSING_AND_PRESENTATION_OF_PEPTIDE_ANTIGEN_VIA_MHC_CLASS_IB	HLA-E, HLA-F, HLA-G, HLA-H, AZGP1, B2M, TAP2
GO_ANTIGEN_PROCESSING_AND_PRESENTATION_OF_ENDOGENOUS_PEPTIDE_ANTIGEN	ABCB9, HFE, HLA-A, HLA-B, HLA-C, HLA-E, HLA-F, HLA-G, HLA-H, IDE, ERAP1, AZGP1, B2M, ERAP2, TAP1, TAP2, TAPBP
GO_ANTIGEN_PROCESSING_AND_PRESENTATION_VIA_MHC_CLASS_IB	HLA-E, HLA-F, HLA-G, HLA-H, AZGP1, B2M, TAP2, AP3B1, AP3D1, CD1A, CD1B, CD1C, CD1D, CD1E
GO_ANTIGEN_PROCESSING_AND_PRESENTATION_OF_ENDOGENOUS_ANTIGEN	ABCB9, HFE, HLA-A, HLA-B, HLA-C, HLA-E, HLA-F, HLA-G, HLA-H, IDE, ERAP1, AZGP1, B2M, ERAP2, TAP1, TAP2, TAPBP, CD1A, CD1B, CD1C, CD1D, CD1E, ATG5, CD74

Supplementary Table 3: Antigen processing and presentation gene set analysis in Mayo_2019 cohort

	P-IA-cox	p-Log Rank	P-IA-lr
AP&P_OF_PEPTIDE_ANTIGEN_VIA_MHC_CLASS_IB	0.24	0.44	0.08
AP&P_OF_PEPTIDE_ANTIGEN_VIA_MHC_CLASS_I	0.18	0.37	0.20
AP&P_VIA_MHC_CLASS_IB	0.25	0.48	0.20
AP&P_OF_ENDOGENOUS_PEPTIDE_ANTIGEN	0.13	0.31	0.10
AP&P_OF_ENDOGENOUS_PEPTIDE_ANTIGEN_VIA_MHC_CLASS_I_VIA_ER_PATHWAY	0.24	0.39	0.06
REGULATION_OF_AP&P	0.37	0.52	0.38
NEGATIVE_REGULATION_OF_AP&P	0.46	0.54	0.36
POSITIVE_REGULATION_OF_AP&P	0.33	0.51	0.44
REGULATION_OF_AP&P_OF_PEPTIDE_ANTIGEN	0.88	0.41	0.24
AP&P	0.16	0.28	0.19
AP&P_OF_ENDOGENOUS_ANTIGEN	0.09	0.20	0.10
AP&P_OF_PEPTIDE_ANTIGEN	0.17	0.31	0.17

The statistical significance of interactions (P-IA-cox) and log-rank (p-Log Rank) in cox models, interactions (P-IA-lr) and area under the curve (AUC) in logistic regression models are listed for the Mayo_2019 cohort.

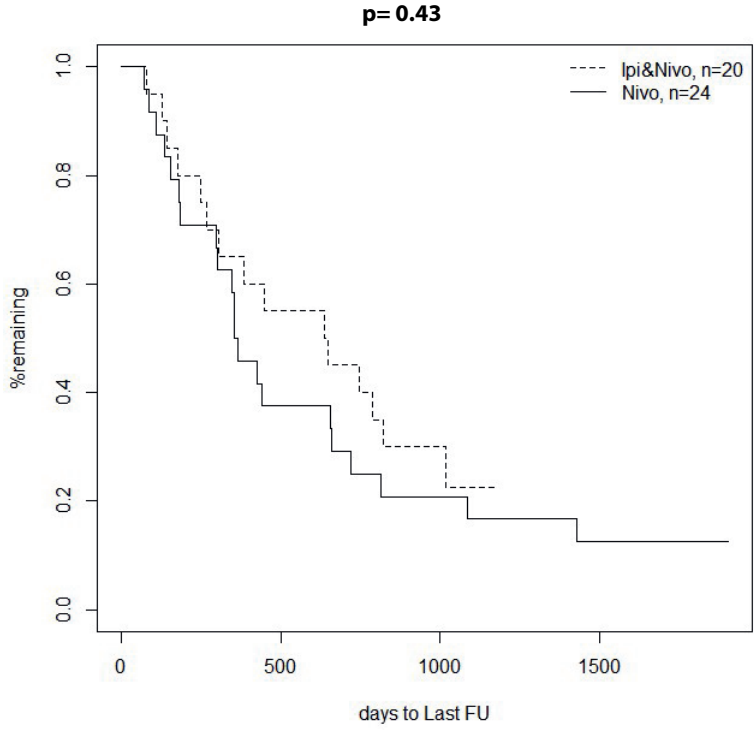

Figure S1: Kaplan-Meier plot of overall survival of patients with MPM treated with nivolumab with or without ipilimumab.

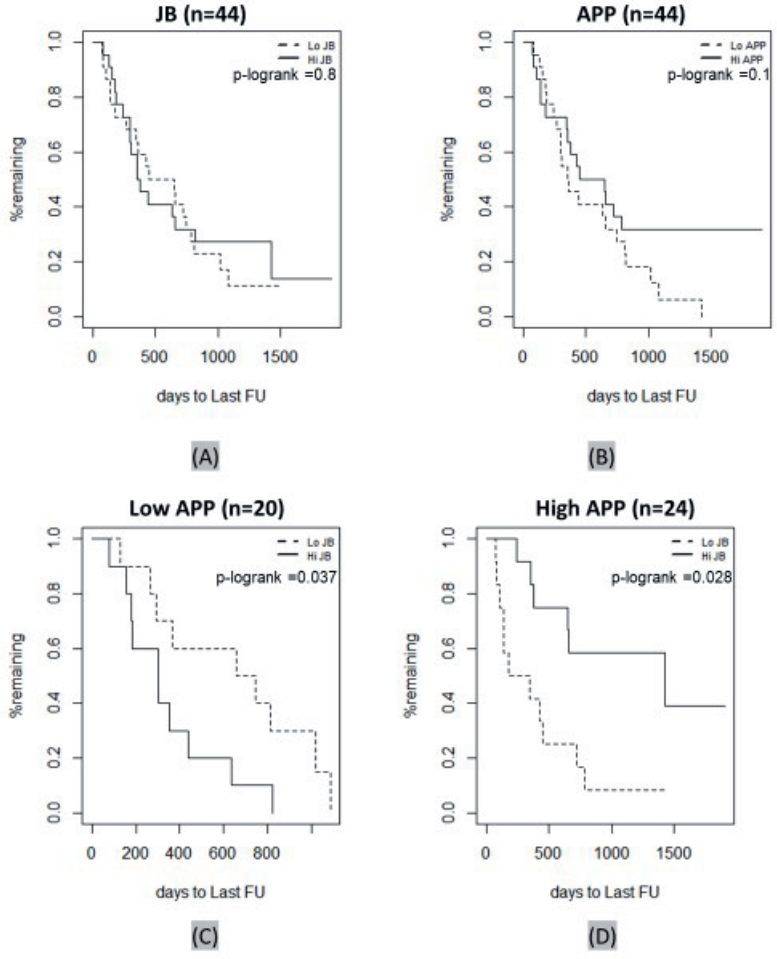

Figure S2: Kaplan-Meier plots of overall survival of patients with MPM are shown based on tumor junction burden (JB) categorizations (A) and "regulation of AP&P of peptide antigen" gene set expression categorizations (B). Similarly, the overall survival of patients with MPM with low (C) and high (D) "regulation of AP&P of peptide antigen" gene set expression based on tumor junction burdens is plotted.

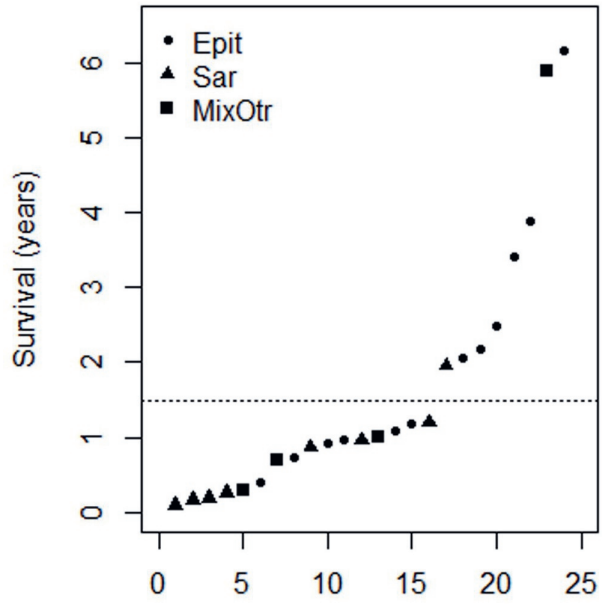

Figure S3: Overall survival times of patients with MPM from the Mayo_2019 cohort (8) are shown. Circles, triangles, and squares represent epithelioid (Epit), sarcomatoid (Sar, including desmoplastic), and mixed or other (MixOtr) subtypes.

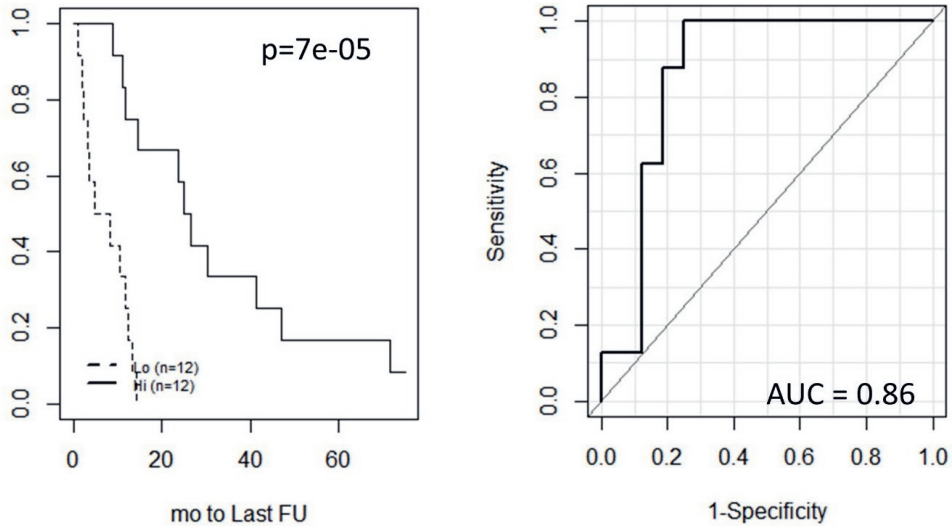

Figure S4: (A) Kaplan Meier plot representing associations between the gene signature "MESOTHELIOMA SURVIVAL OVERALL UP" from Lopez et al (19) and overall survival in the Mayo_2019 cohort are shown (8). (B) The ROC curve based on logistic regression predicting non-significant benefit (NSB) and significant benefit (SB) from the Mayo_2019 cohort is shown.

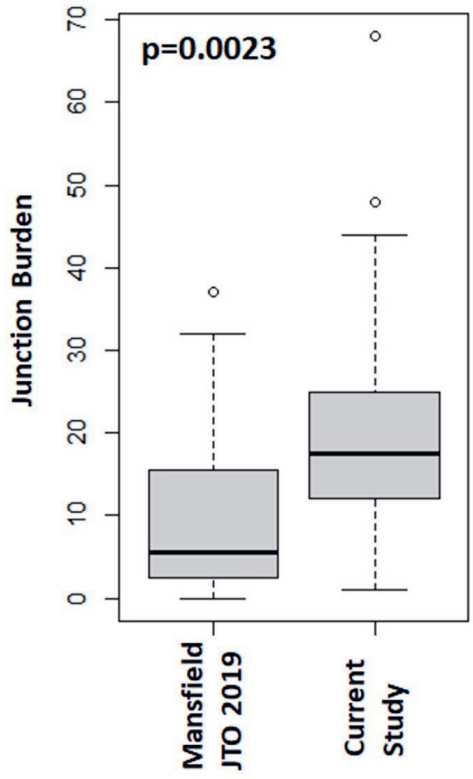

Figure S5: The number of junctions from the Mayo_2019 cohort (8) and the current study are presented.

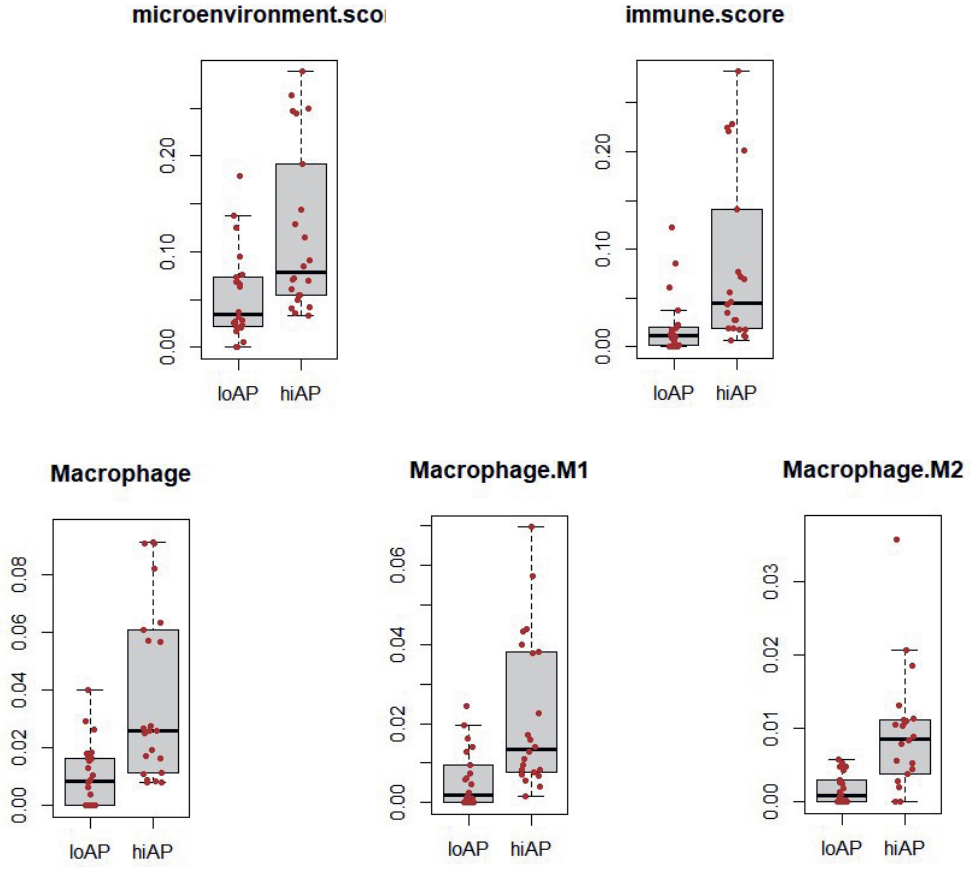

Figure S6: Immune profiling by xCell demonstrating higher TME scores, immune scores and macrophages and their subsets in tumors with high antigen processing and presentation gene set expression based on "REGULATION OF APP OF PEPTIDE ANTIGEN" gene set. The box plots represent the medians with the bars, the interquartile ranges with the boxes, and the ranges with the whiskers.

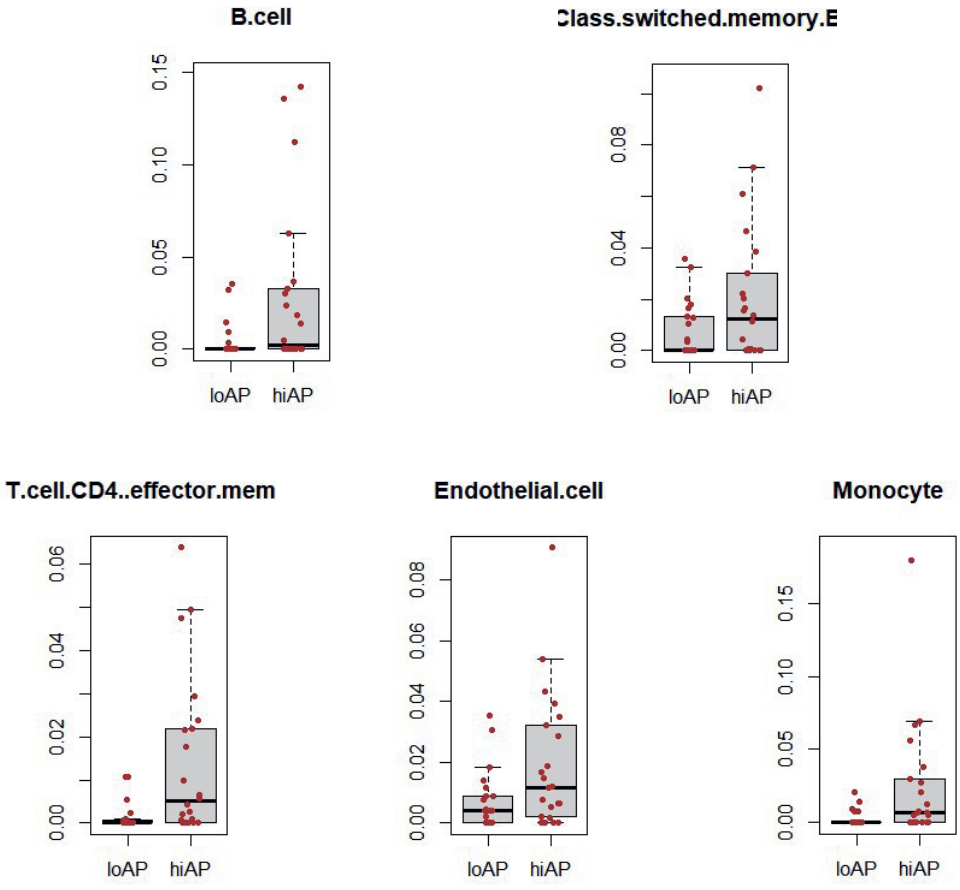

Figure S7: Immune profiling by xCell demonstrating higher B cells, T cells, endothelial cells and monocytes in tumors with high antigen processing and presentation gene set expression based on "REGULATION OF APP OF PEPTIDE ANTIGEN" gene set. The box plots represent the medians with the bars, the interquartile ranges with the boxes, and the ranges with the whiskers.

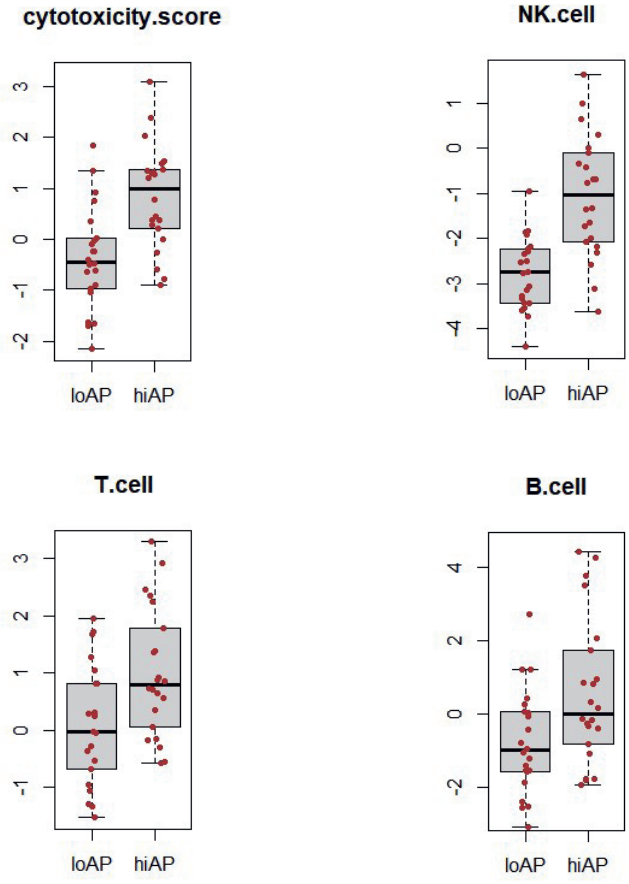

Figure S8: Immune profiling by MCP Counter demonstrating higher cytotoxicity scores, B cells, T cells and NK cells in tumors with high antigen processing and presentation gene set expression based on "REGULATION OF APP OF PEPTIDE ANTIGEN" gene set. The box plots represent the medians with the bars, the interquartile ranges with the boxes, and the ranges with the whiskers.

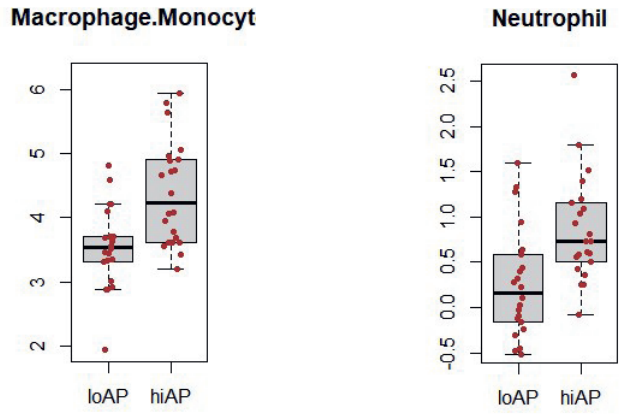

Figure S9: Immune profiling by MCP Counter demonstrating higher macrophages and monocytes, and neutrophils in tumors with high antigen processing and presentation gene set expression based on "REGULATION OF APP OF PEPTIDE ANTIGEN" gene set. The box plots represent the medians with the bars, the interquartile ranges with the boxes, and the ranges with the whiskers.