Effects of tumor mutation burden on the antigen presentation pathway

Enrique M. Garcia-Rivera[1], PhD, Jiho Park[1], Caroline Truntzer[2], François Ghiringhelli[3], Mitesh Borad [4,5], Aaron S. Mansfield[5,6], MD

[1]nference, Cambridge, Massachusetts, USA, [2]Genetic and Immunology Medical Institute, Dijon, France. [3]University of Burgundy Franche-Comté, France, [4]Division of Hematology and Oncology, Mayo Clinic, Scottsdale, AZ, USA, [5]Co-Directors of Precision Cancer Therapeutics within the Center of Individualized Medicine, Mayo Clinic, USA, [6]Division of Medical Oncology, Mayo Clinic, Rochester, MN, USA, mansfield.aaron@mayo.edu

Introduction

Tumor mutation burden (TMB) has emerged as an important biomarker to select patients with cancer to treat with immune checkpoint inhibitors (ICI)(PMID 32919526). With the application of next generation sequencing, TMB is frequently reported by commercial vendors and available for clinical interpretation. TMB has recently been accepted by the United States Food and Drug Administration as a biomarker to select patients to receive a PD-1 inhibitor based on the Keynote-158 clinical trial (32919526). Mismatch repair deficiency, which is associated with high TMB, has also been approved to select patients to receive immunotherapy (28596308). Somatic mutations that are translated into mutant proteins have the potential to be presented by tumor cell major histocompatibility complex (MHC) class I proteins to CD8 T cells. Several retrospective and post hoc analyses have correlated TMB with response or survival in multiple tumor types (28835386, 30643254, 25765070). So far, prospective clinical trials have not demonstrated an overall survival benefit based on the use of TMB.
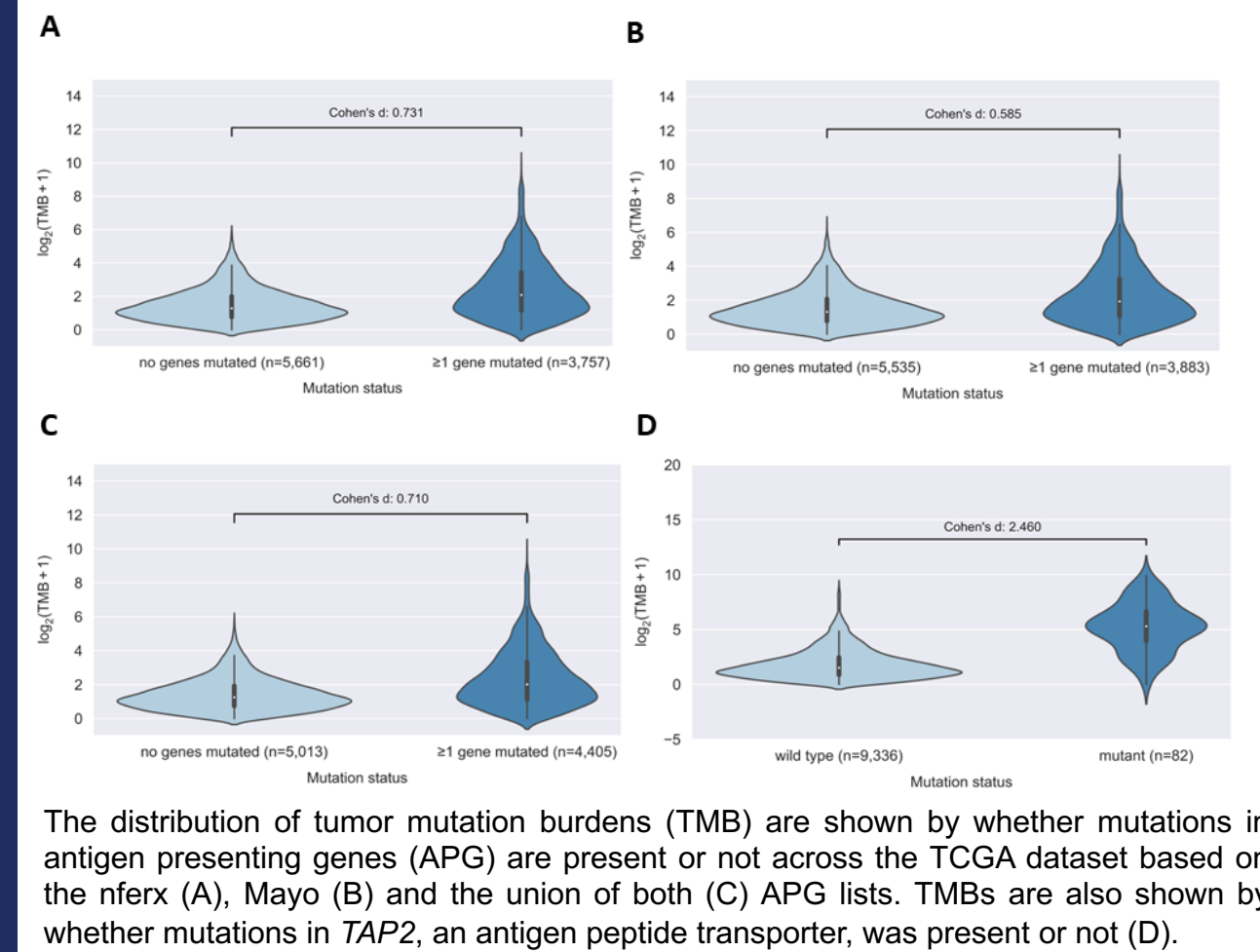
Acquired resistance to ICI has been reported to result from deleterious mutations in beta-2-microglobulin (*B2M*)(27433843), which is critical for antigen presentation. Given the role that loss of antigen presentation has in acquired resistance to ICI, we hypothesized that increasing TMB would more commonly involve antigen presenting genes (APG) and result in primary resistance to ICI. Accordingly, we sought to profile APG mutations across multiple tumor types and their association with TMB. We also sought to assess the outcomes of patients with mutant APG who were treated with ICI.

Results

To assess the associations of mutations in APGs with TMB, we first defined APGs. One APG set was defined using the nferx Signals application (nferX set = 44 genes), another set was defined by an author guided by a prior publication (Mayo set = 45 genes)(PMID 29900059) and the union of the two sets (union = 70 genes) was also used. When assessing 9,418 cancers from TCGA, mutations in one or more APGs had a large effect on the distribution of TMBs in the nferx set (Cohen's d=0.731), the Mayo set (Cohen's d=0.585) and the union of the two sets (Cohen's d=0.710)(**Figures 1A-C**). Across all tumors, the most common mutations in APGs involved genes encoding human leukocyte antigens. Mutations in *TAP2* and *HLA-DRB9* had the greatest effects on increased TMB (Cohen's d= 2.46 and 2.86, respectively; **Figure 1D**). When assessing individual mutations, there was a large increase in the distribution of TMB values of patients with the *TAP2* delC mutation (chr6:32838010) which was present in 0.15% of cases (Cohen's d=2.574). There were also large increases in the distributions of TMB values of patients with the *CANX* mutation (chr5:179722918) which was present in 0.19% of cases (Cohen's d=2.233), and the *B2M* delCT mutation (chr15:44711582) which was present in 0.31% of cases (Cohen's d=1.724).

There was significant variation in the effects of mutations in APGs on the distributions of TMBs across specific cancer types when looking at the union of both gene sets (**Figure 2A**), with the largest effect sizes seen in uterine corpus endometrial carcinomas and diffuse large B cell lymphomas. Negative effect sizes were seen in tenosynovial giant cell tumors and renal papillary cell carcinomas. In adenocarcinomas of the lung, *ITGAX*, a gene encoding an integrin, was one of the most frequently mutated APGs with a large effect on the distribution of TMBs (**Figure 2B**). *CIITA*, a transcriptional coactivator, had one of the largest effect sizes on the distribution of TMBs in adenocarcinomas of the colon (**Figure 2C**). LY75, an endocytic receptor, had one of the largest effect sizes on the distribution of TMBs in melanoma (**Figure 2D**). Progression-free survival was superior in patients with one or more mutations in APGs compared to those with wild type APGs (median PFS: 3.7 v 1.8 months; HR=0.54, p=0.04; **Figure 3**).

Figure 1

The distribution of tumor mutation burdens (TMB) are shown by whether mutations in antigen presenting genes (APG) are present or not across the TCGA dataset based on the nferx (A), Mayo (B) and the union of both (C) APG lists. TMBs are also shown by whether mutations in *TAP2*, an antigen peptide transporter, was present or not (D).

Figure 2

The effect sizes (Cohen's d) of the associations of TMB with mutations in APGs (from the union of both gene sets) is shown across the tumor types (A). When looking at the effect sizes of mutations in specific APGs with TMB, mutations in ITGAX (B), CIITA (C) and LY75 (D) had the greatest effect sizes in adenocarcinoma of the lung, colorectal cancer and melanoma respectively.

Figure 3

In a cohort of 62 patients who received the PD-1 inhibitor nivolumab as second or later lines of therapy, the overall survival (A) and progression-free survival (B) are shown between patients with and without mutations in APGs.
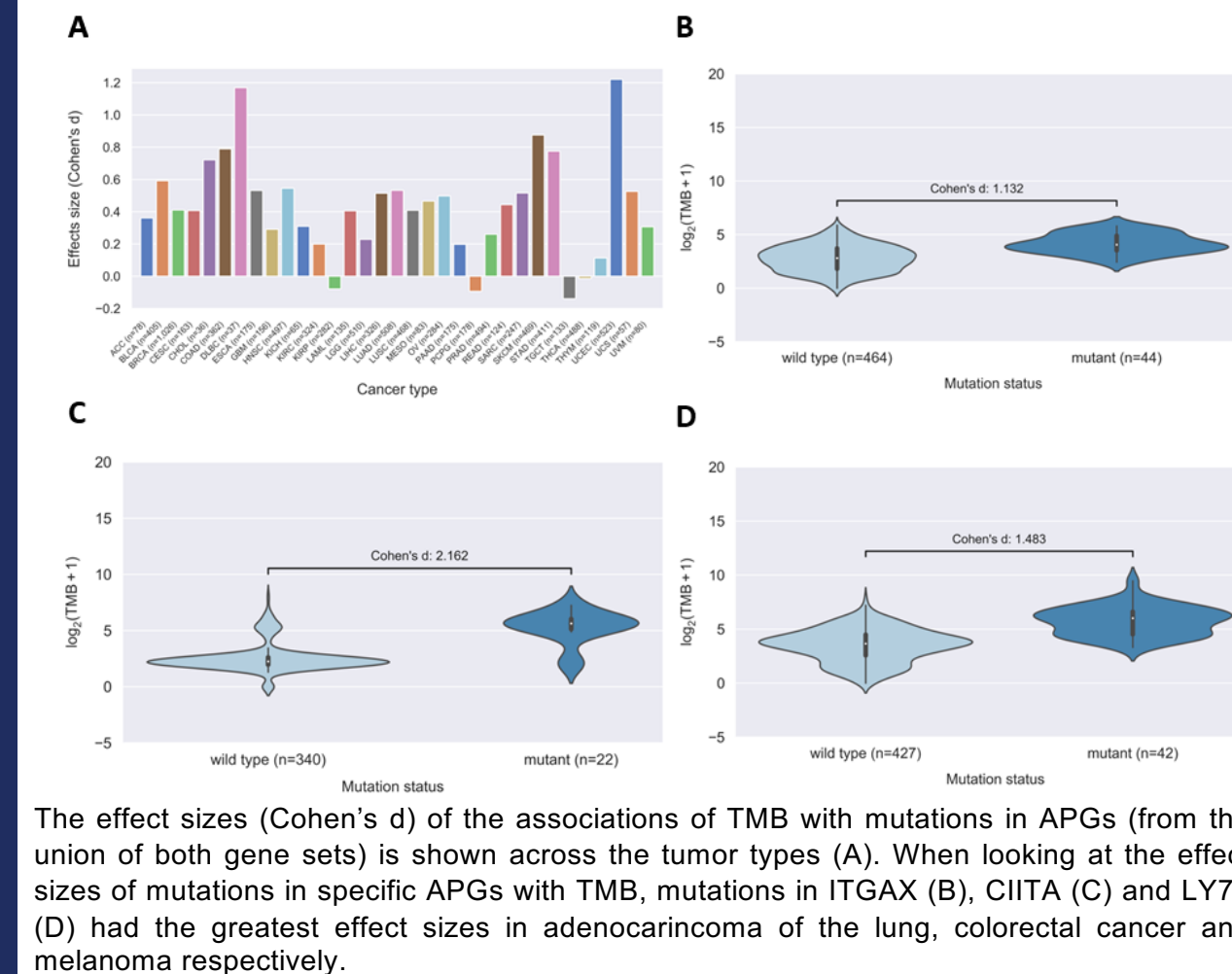
Discussion

Across all tumor types we analyzed, the distributions of TMB were significantly higher in specimens with mutations in one or more in APGs. When looking at specific tumor types, this effect was largest in uterine corpus endometrial carcinomas and diffuse large B cell lymphomas. Overall, mutations in genes that encode human leukocyte antigens were the most frequently involved APGs; however, mutations in *TAP2* and *HLA-DRB9* were associated with the largest increases in TMB. Contrary to our hypothesis, we did not see a survival benefit in patients with non-small cell lung cancer and mutant APGs treated with the PD-1 inhibitor nivolumab.

Similar to our findings that mutations in *TAP2* had one of the greatest effects on TMB across all tumors, others have reported that mutations in bare lymphocyte syndrome genes, which include *TAP2*, are associated with high TMB and neoantigen loads (31064401). Other genes associated with bare lymphocyte syndrome including *CIITA* and *TAPBP* also had large effects on TMB in our analysis. Just as germline loss of antigen presentation can cause severe immunodeficiency, somatic loss of antigen presentation may result in primary or acquired resistance to immunotherapy.

Beta-2-microglobulin is necessary for peptide-major histocompatibility complex class I formation at the cell surface. Mutations in *B2M* have been associated with acquired resistance to PD-1 inhibitors (27433843).
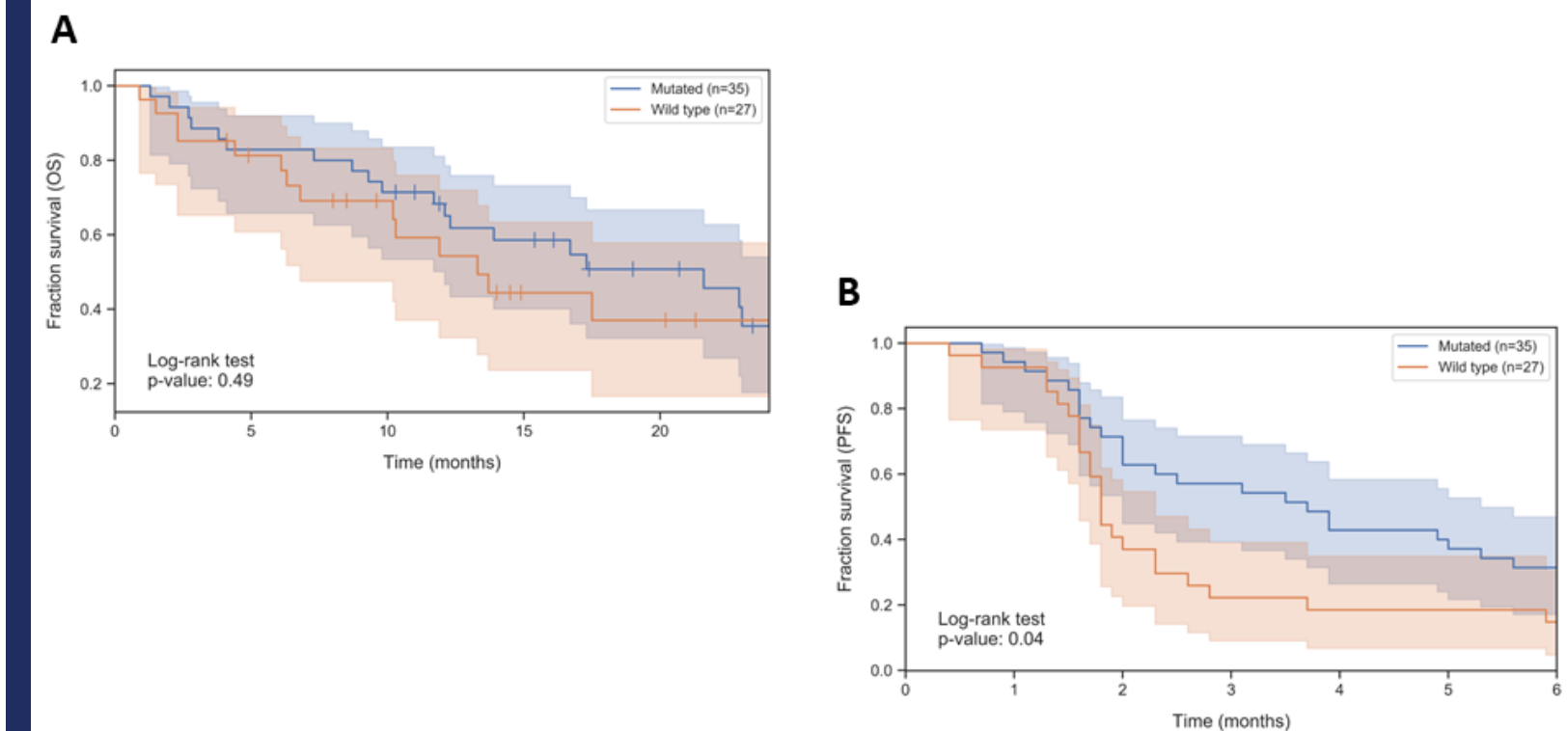
Discussion (continued)

Others have replicated these findings, demonstrating that mutations in *B2M* are associated with acquired resistance to anti-CTLA-4 and anti-PD-1 therapies in patients with melanoma, and additionally suggested that primary resistance to treatment can also result from mutations in *B2M* (29070816). When considering other forms of immunotherapy, others have also found that the *B2M* delCT mutation is preferentially selected in melanoma cells after T cell-based immunotherapy (15661905). We saw that mutations in *B2M* were associated with higher tumor mutation burdens.

Given the associations with mutations in APGs and acquired resistance to immunotherapies, we hypothesized that primary resistance could also be related to mutations in APGs. Although we saw that mutations in APGs were associated with large increases in TMB, our analysis of a cohort of patients with NSCLC identified a benefit in progression free survival when APGs were mutated, contrary to our initial hypothesis. Upon further inspection, most of the mutations in this cohort only involved one allele, suggesting that the adequate antigen presentation was possible in these cases with a preserved wild type allele. Furthermore, given the association we identified a benefit in progression free survival when APGs were mutated, contrary to our initial hypothesis. Upon further inspection, most of the mutations in this cohort only involved one allele, suggesting that the adequate antigen presentation was possible in these cases with a preserved wild type allele.

Discussion (continued)

Furthermore, given the association we identified with mutations in APGs and TMB, our analysis was aligned with the original report of this cohort demonstrating improved progression free survival in the patients with high TMB.

Retrospective and post-hoc analyses have suggested that tumor mutation burden is a predictor of response to or survival with immunotherapy (28835386, 30643254, 25765070). There are significant discrepancies in the scoring of TMB in some tumor types such as renal cell carcinoma where insertions and deletions have a greater effect on TMB than single nucleotide variants (28694034). Additionally, the combination of ipilimumab and nivolumab was recently approved for mesothelioma based on improvements in survival even though these tumors typically have the lowest mutation burdens of any carcinogen-related malignancy (26928227, 30322867). Recently, chromosomal rearrangements were reported to have neo-antigenic potential in mesothelioma (30316012) and head and neck cancers (31011208). These studies imply that insertions, deletions and rearrangements could improve upon TMB scores that only include single nucleotide variants. It has been suggested that HLA-correction (32320754, 32523802) and germline subtraction (32108894) further improve TMB scoring. Given the lack of a standardized approach to calculate TMB, there are calls to harmonize how this is done (PMID: 32217756, 30664300, 32522712).

Overall, we found that mutations in APGs are associated with increases in tumor mutation burdens. As others have suggested, bi-allelic loss of genes critical to antigen presentation such as *B2M* may result in primary and acquired resistance to immunotherapies. As efforts are ongoing to harmonize TMB scoring, attention to loss of APGs may further inform these efforts.