Original Research

Chemical complementarity between immune receptors and cancer mutants, independent of antigen presentation protein binding, is associated with increased survival rates

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ARTICLE INFO
Keywords: uterine cancer immunogenomics TCR-mutant amino acid complementarity scoring complementarity determining region-3

ABSTRACT
Uterine cancer has been associated with a T-cell immune response that leads to increased survival. Therefore, we used several bioinformatics approaches to explore specific interactions between T-cell receptor (TCR) and tumor mutant peptide sequences. Using endometrioid uterine cancer exome files from the The Cancer Genome Atlas database, we obtained tumor resident V-J recombinations for the T-Cell Receptor alpha gene (TRA). The charged-based, chemical complementarity for each patient’s LRP2 or TTN mutant amino acids (AAs) and the recovered, TRA complementarity determining region-3 (CDR3) sequences was calculated, allowing a division of patients into complementary and noncomplementary groups. Complementary groups with TTN mutants had increased disease-free survival and increased expression of complement genes. Furthermore, the survival distinction based on CDR3-mutant peptide complementarity was independent of programatically assessed HLA class II binding and was not observable based on the CDR3 AA chemical features alone. The above approach provides a potential, highly efficient method for identifying TCR targets in uterine cancer and may aid in the development of novel prognostic tools.

Introduction

Endometrial cancer is the most common form of uterine cancer and can be divided into subsets of serous and endometrioid cancer, with endometrioid comprising 75–80% of cases [1]. The five-year survival rate for endometrioid uterine cancer is 75 to 80% in contrast with serous uterine cancer, which has a slightly lower five-year survival rate [2,3]. A T-cell immune response as evidenced by the presence of T-cell, tumor infiltrating lymphocytes (TILs), has been shown to result in better prognosis for uterine cancer patients, i.e., without regard to the above endometrioid and serous subdivisions [4].

T-cells diversify and form clonotypes through recombination of V and J gene segments, leading to many unique TRA and TRB genes encoding polypeptides that constitute the alpha-beta, cell surface T-cell receptor (TCR). The recombinations of the TRA or TRB gene segments lead to complementarity determining region-3 (CDR3) amino acid (AA) sequences representing the gene segment recombination junctions and usually a significant TCR contact point for antigen binding. In addition, cancer mutant peptides have been shown to bind both the HLA antigen presenting molecules and patient TCRs [5, 6]. Recently, substantial correlative data have indicated that the CDR3 is an important part of the TCR for binding to cancer mutant peptides [7–10]. For example, survival distinctions representing numerous cancers have been observed with higher chemical complementarity between CDR3s and cancer mutant peptides, with greater complementarity associated with greater survival rates [7–10].

In this study, we built upon the above CDR3-cancer mutant peptide chemical complementarity approaches with the intention of establishing two advances. First, we sought to identify CDR3-mutant peptide combinations for uterine cancer that had the potential to be used for prognostic purposes and for design of immunotherapies whereby particular CDR3-mutant peptide interactions could be artificially engineered for patients. And second, for the first time, we sought to determine whether the survival distinctions associated with CDR3-mutant peptide comple-

Abbreviations: AA, amino acid; CS, complementarity score; KM, Kaplan-Meier; LRP2, low density lipoprotein-related protein 2; NCPR, net charge per residue; TCGA, The Cancer Genome Atlas; TCR, T-cell receptor; TIL, tumor infiltrating lymphocyte; TRA, T-cell receptor alpha; TTN, titin; UCEC, uterine corpus endometrial carcinoma; WXS, whole exome sequence.

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https://doi.org/10.1016/j.tranon.2021.101069
Received 7 September 2020; Received in revised form 19 January 2021; Accepted 8 March 2021
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mentarity were dependent or independent of HLA binding. As detailed below, the above goals were met, along with an unexpected, more refined understanding of the immunological distinctions between serous and endometrioid uterine cancers.

Methods

Recovery of the TRA recombination reads from uterine cancer exome (WXS) files

The TRA recombination reads were recovered from The Cancer Genome Atlas (TCGA) uterine corpus endometrial carcinoma (UCEC) WXS files via approved database of genotypes and phenotypes (dbGaP) project number 6300. The WXS file processing steps have been extensively described [11,12]. Briefly, the WXS files were downloaded to USF research computing facilities; the files were searched by sourcing 10-mers representing the TRA V- and J-gene segments, at the 3’ and 5’ ends of the segments, respectively. This search process was a low stringency pre-screen using overlapping 10-mers backing away from the ends of the V- and J-gene segments. The output of this primary search step was then subject to a high stringency verification leading to the TRA recombination reads and CDR3 AA sequences used in this study (supporting online material (SOM), Tables S1, S2). All code for the above processing, along with code used to do an initial, unsupervised search of CDR3-mutant AA pairs related to survival distinctions is freely available at https://github.com/bchobrut-USF/lgg_idh1. Another version of code used for recovery of immune receptor recombination reads from WXS or RNAseq files is available at https://hub.docker.com/repository/docker/bchobrut/vdj, including a readme file.

Validations of complementarity scores (CS)

The method of complementarity scoring is based on an approach documented in references [7–10]. Missense mutants for LRP2 and TTN were assigned a value based on change in charge from wild-type to mutant AA sequence. A value of negative one was given for a net negative electrostatic change, for example a change from Arg to Leu would yield a value of 0–1 or −1; zero for no electrostatic change; and one for a net positive electrostatic change, for example a change from Glu to Leu. For case IDs with more than one mutant AA for either TTN or LRP2, the average charge change was calculated and used for CS scoring. Next, maximum and minimum values for the net charge per residue (NCPR) were obtained for the TRA CDR3s for each case ID (SOM, Tables S3–S6). The maximum and minimum values were multiplied by the designated value for each mutant AA charge change as indicated above (tables S7, S8). The minimum value, i.e., most electrostatically attractive value, of the two products represented the CS for that case ID. That is, a negative CS represented a complementary TRA CDR3-mutant AA interaction whereas a positive or zero CS represented a noncomplementary TRA CDR3-mutant AA interaction (Tables S9, S10).

Assessment of survival distinctions using Kaplan–Meier (KM) analyses

TCGA-UCEC case ID survival data were obtained via cbioportal.org, and KM analysis was performed using GraphPad Prism software.

Gene expression analyses using RNAseq values

RNAseq-based RSEM values representing the level of expression for various genes were obtained through cbioportal.org. An original MATLAB reference program was used to compare RNAseq values for complementary and noncomplementary groups. The code for the program is available at github.com/MJD-BlanchGroup/MATLAB_for_Diaz_et_al_Feb2020. Significant distinctions in average RNAseq between complementary and noncomplementary groups were determined using a two-tailed t-test with significance level of 0.05 (Table S11).

Multivariate survival analyses

Multivariate analysis for clinical parameters including age, tumor grade, and POLE and TP53 mutation was conducted using Statistical Package for the Social Sciences (SPSS) statistical software (Table S12).

HLA class ii binding analyses

HLA-DRB1 alleles for each case ID were obtained via xHLA software. A peptide sequence was generated for each TTN mutant for each case ID. The sequence included the mutant AA and the 8 As on either side. The alleles and 17-AA peptide sequences were submitted to an IEDB webtool available at http://tools.iedb.org/mhcii/ which generated IC50 values representing binding affinity of the HLA-DR molecule to the mutant TTN peptide. The 10 smallest IC50 values representing the strongest binding were obtained for mutants of the top 10 most complementary and top 9 most noncomplementary case IDs based on the above indicated CS calculations (Table S13). The values were averaged for each group.

Results

Endometrioid versus serous comparisons for UCEC

TRA recombination read recovery differences. An extensive series of studies has validated a correlation between the number of TILs and the recovery of TRA recombination reads from tumor specimen WXS files [11,13–16]. Thus, to appreciate potential basic immunogenetics distinctions between the two prominent TCGA UCEC subsets, we first evaluated differences in the recovery of TRA recombination reads of the endometrioid versus serous UECW WXS files (Table 1; Tables S1, S2). The frequencies of the TRA recombination read recovery, represented by the number of case IDs with at least one TRA recombination read, were 63.41% and 47.82% for endometrioid and serous, respectively (Table 1, p-value < 0.05). Therefore, the frequency of the TRA-assessed immune response was greater for the endometrioid UCEC subset compared to serous subset, consistent with results obtained when the TRA recombination reads were sourced from RNAseq files [17] instead of WXS files (Table 2), although in the case of TRA recombination reads obtained the RNAseq files, the distinction in TRA read recoveries is based on a quantitative difference between the endometrioid and serous UCEC subsets rather than based on the recovery versus lack of recovery, as in the case of the TRA recombination reads obtained from the WXS files.

Endometrioid versus serous comparisons for survival rates: TRA recombination read recoveries versus all remaining samples

To evaluate how the UCEC subsets differed in survival rates, based on the recovery of TRA recombination reads, Kaplan Meier (KM) analyses were performed for case IDs with and without TRA recombination reads, with results indicating that only the endometrioid subset showed a difference in overall survival (OS) (Fig. 1A, p-value < 0.05), with case IDs representing TRA recombination read recoveries representing a higher OS rate. This result was consistent with a related approach to the recovery of the TRA recombination reads from RNAseq files [17](Fig. 1B). Thus, the remainder of this report is limited to the immunogenomics analyses of the endometrioid subset of UCEC. There was no significant difference for overall survival in the serous subset or for disease-free survival for either serous or endometrioid subsets, based on the recovery of TRA recombination reads from the genomics files.

Comparison of survival rates of case IDs with electrostatically complementary versus noncomplementary TRA CDR3-mutant AA pairs

Case IDs with TTN mutant AA, with TRA CDR3-mutant TTN AA complementarity, versus lack of complementarity (Methods), did not
show a significant difference for OS but did indicate a disease-free survival (DFS) distinction, with greater DFS associated with complementariness ($p = 0.054$) (Fig. 2A). This difference was consistent with a better DFS associated with TRA CD3-mutant TTN AA complementariness when the CD3Rs were sourced from RNAseq files [17] (representing the same set of case IDs as in Fig. 1A) instead of WXS files (Fig. 2B). To determine whether there were differences in gene expression between the complementary and noncomplementary groups, RNAseq-based, gene expression values for both groups were assessed in an unsupervised, discovery-based comparison. Complement genes C1QA, C1QB, and C1QC were all expressed at higher levels in tumor samples of the case IDs with the complementary TRA CD3-mutant TTN AAs (Fig. 2; Table 3). In contrast, there were high levels of expression of the ribosomal protein genes, RPLP1, RPL8, RPS6, RPS4X, and RPS18, in the noncomplementary case ID tumor samples (Fig. 3), as was the case for the elongation factor genes, EEF1A1, EEF1G, and EEF1A1P9 (Table 4). A survival distinction based on TRA CD3-mutant AA, electrostatic complementarity was also demonstrable, for the endometrioid subset of the UCEC case IDs having LRP2 mutations (Fig. 4), when the TRA recombination reads were sourced from either UCEC WXS or RNAseq files.

### Table 1

| Uterine cancer subtype | Total number of case IDs representing the indicated uterine cancer subtype | Case IDs with TRA recombination read recoveries | Frequency (Percentage of cases with TRA recombination read recoveries) |
|------------------------|-------------------------------------------------|-----------------------------------------------|-------------------------------------------------|
| Endometrioid           | 410                                             | 260                                           | 63.41%                                          |
| Serous                 | 115                                             | 55                                            | 47.82%                                          |

### Table 2

| Uterine cancer subtype | Total number of case IDs representing the indicated uterine cancer subtype | Case IDs with RNAseq based TRA recoveries | Frequency (Percentage of cases with RNAseq based TRA recoveries) | Average number of RNAseq based TRA recoveries per case ID |
|------------------------|-------------------------------------------------|-----------------------------------------------|-------------------------------------------------|--------------------------------------------------|
| Endometrioid           | 410                                             | 386                                           | 94.14                                          | 20.97                                           |
| Serous                 | 115                                             | 107                                           | 93.04                                          | 15.01                                           |

$p$-value $= 0.0274$ for average number of RNAseq based TRA recoveries per case ID.

### Table 3

| C1Q gene | Average RNAseq-based RSEM value for the TRA CDR3-TTN mutant AA complementarity group | Average RNAseq-based RSEM value for the TRA CDR3-TTN mutant AA non-complementarity group | Difference in RSEM values | $p$-value |
|----------|-----------------------------------------------------------------------------------|---------------------------------------------------------------------------------------|---------------------------|----------|
| C1QA     | 3124.54                                                                           | 1900.92                                                                               | 1223.62                   | 0.03     |
| C1QB     | 3580.55                                                                           | 2065.97                                                                               | 1514.58                   | 0.01     |
| C1QC     | 2679.65                                                                           | 1785.99                                                                               | 893.66                    | 0.03     |

### Table 4

| EEF1 gene | Average RNAseq-based RSEM value for the TRA CDR3-TTN mutant AA complementarity group | Average RNAseq-based RSEM value for the TRA CDR3-TTN mutant AA non-complementarity group | Complementary and noncomplementary gene expression difference | $p$-value |
|-----------|-----------------------------------------------------------------------------------|---------------------------------------------------------------------------------------|-------------------------------------------------------------|----------|
| EEF1A1    | 86,518.73                                                                         | 101,386.05                                                                            | 14,867.31                                                   | 0.03     |
| EEF1G     | 21,708.26                                                                         | 28,798.23                                                                             | 7089.97                                                     | 0.03     |
| EEF1A1P9  | 8376.35                                                                           | 10,000.12                                                                             | 1623.77                                                     | 0.03     |

Comparison of survival based on net charge per residue

To address the possibility that the above survival distinctions simply represented a consistent CDR3 NCPR difference that distinguished survival rates, rather than electrostatic complementarity of CDR3-mutant AAs, we compared case IDs representing the upper and lower 50th percentiles of the TRA CD3Rs, with respect to CDR3 NCPR values. Specifically, for the case IDs with TTN mutants there was no difference in OS (Fig. 5A) or DFS for those case IDs with a higher versus lower NCPR value (Fig. 5B). In addition, for case IDs with LRP2 mutants, there were no differences in either overall (Fig. 5C) or disease-free survival (Fig. 5D), for the high versus low NCPR values of the TRA CD3Rs. Thus, the above indicated survival distinctions, based on electrostatic complementarity of TRA CD3Rs and mutant AAs of TTN or LPR2 are not simply due to segregating case IDs into subdivisions of TRA CD3 NCPR values.

HILA-DR, binding affinities of peptides representing complementary and noncomplementary TRA CDR3-mutant AA pairs did not differ

Because the above gene expression analysis indicated a significantly higher level of complement protein expression in the complementary TRA CD3-mutant TTN AA group (Table 3), we reasoned that antigen presenting cells were common in that high surviving group and thus that HLA class II antigen presentation would be contributing to the apparent anti-tumor response resulting from the complementarity. Thus, we obtained the HLA-DRB1 alleles from the WXS files of the TCGA-UCEC set, using xHLA [18], as described in previous publications [19–22]. And, as indicated in Methods, we obtained the top 10 IC50s, based on variable and comprehensive positioning of the mutant peptide in the HLA-
Fig. 1. Kaplan-Meier overall survival (OS) analyses for TCGA-UCEC case IDs based on recovery of TRA recombination reads from the WXS files. (A) Comparison of the OS rates for case IDs with endometrioid uterine cancer representing recovery of TRA recombination reads (black; denoted by arrowhead) (n = 259) versus all remaining case IDs (grey) (n = 151; p-value = 0.049). (B) Comparison of the OS rates for case IDs with endometrioid uterine cancer representing the top 50% of the TRA recombination read recoveries (black; denoted by arrowhead) versus the case IDs representing the bottom 50% of TRA recombination read recoveries (total n = 386 case IDs), when the TRA recombination reads were sourced from RNAseq rather than WXS files [17] (p = 0.052). Note, in the case of the RNAseq-based, recombination read recoveries, the algorithm used different (lower) standards for identification of the TRA recombination reads, in comparison to the algorithm used for recovery of the TRA recombination reads from the WXS files, an algorithm detailed in ref. [12]. This lower standard was necessitated by the shorter RNAseq read lengths, which leads to a reduction in the certainty of the read verification as a TRA recombination read. Nevertheless, the same basic survival distinction obtains, with the distinction of OS representing the RNAseq-based TRA recombination reads based on quantity, whereas the distinction of OS based on the WXS-based TRA recombination reads is based on recovery of a single TRA recombination read or more, versus no TRA recombination read recoveries (Fig 1A). (C) Comparison of the OS rates for case IDs with serous uterine cancer representing recovery of TRA recombination reads from WXS files (black; denoted by arrowhead) (n = 60; p-value = 0.870).

Table 5
Average of allele-specific HLA-DRB1 IC50s for mutant TTN peptides representing complementary and noncomplementary case IDs as indicated in Fig. 2.

| Category                  | IC50 (See Methods) | p-value (not significant) |
|---------------------------|--------------------|---------------------------|
| Complementary TRA         | 3549 nM            | 0.564                     |
| CDR3-mutant TTN AAs       |                    |                           |
| Non-complementary TRA     | 5005 nM            |                           |
| CDR3-mutant TTN AAs       |                    |                           |

Fig. 2. KM disease-free survival (DFS) analysis for endometrioid uterine cancer case IDs representing TRA CDR3-mutant TTN combinations. (A) Comparison of the DFS rates for endometrioid uterine cancer case IDs representing electrostatically complementary (black; denoted by arrowhead) versus noncomplementary (grey) TRA CDR3 and TTN mutant combinations (p-value = 0.054). (B) Comparison of the DFS rates for endometrioid uterine cancer case IDs representing electrostatically complementary (black; denoted by arrowhead) versus noncomplementary (grey) TRA CDR3 and TTN mutant combinations, using TRA CDR3s sourced from RNAseq files [17], (p-value = 0.063) and the same case IDs used in Fig. 2A.

DRB1 binding groove, for the TTN peptides representing the 10 most complementary CSSs and the 9 least complementary CSSs. (The remaining noncomplementary case IDs all represented the CS product of zero and thus no particular, noncomplementary case ID among that group could be specifically chosen to be included in this comparison. See Methods for CS calculation process.) The average of these IC50s for each of the two distinct complementarity sets did not have a significant HLA-DRB1 binding difference (Table 5).
Figure 3. Box-and-whisker plots of RNAseq values representing ribosomal protein gene expression differences between complementary (black, left side) versus noncomplementary (grey, right side) TRA CDR3-mutant TTN groups, for endometrioid uterine cancer. The mean RNAseq-based, RSEM values of ribosomal protein genes RPLP1, RPL8, RPS6, RPS4X, and RPS18 for noncomplementary versus complementary groups are listed as follows. RPLP1: complementary (mean = 36,385.057); noncomplementary (mean = 48,509.600) (p = 0.017). RPL8: complementary (mean = 33,518.456); noncomplementary (mean = 44,038.424) (p = 0.018). RPS6: complementary (mean = 37,464.344); noncomplementary (mean = 47,280.283) (p = 0.023). RPS4X: complementary (mean = 35,089.163); noncomplementary (mean = 43,543.928) (p = 0.023). RPS18: complementary (mean = 26,308.811); noncomplementary (mean = 33,796.384) (p = 0.016).

Table 6
Multivariate analysis of clinical factors for case IDs with TTN mutations.

| Clinical Parameters | B   | SE   | Wald | df | Sig.   | Exp(B) |
|---------------------|-----|------|------|----|--------|--------|
| Age                 | -0.005 | 0.025 | 0.045 | 1 | 0.832 | 0.995  |
| Tumor Grade         | 0.409 | 0.406 | 1.016 | 1 | 0.311 | 1.506  |
| Complementarity     | -1.214 | 0.662 | 3.362 | 1 | 0.067 | 0.297  |

Table 7
Multivariate analysis of clinical factors including POLE and TP53 mutations for Case IDs with TTN mutations.

| Clinical Parameters | B   | SE   | Wald | df | Sig.   | Exp(B) |
|---------------------|-----|------|------|----|--------|--------|
| Age                 | -0.022 | 0.029 | 0.596 | 1 | 0.440 | 0.978  |
| Tumor Grade         | 0.471 | 0.432 | 1.185 | 1 | 0.276 | 1.601  |
| Complementarity     | -0.896 | 0.680 | 1.734 | 1 | 0.188 | 0.408  |
| POLE                | -1.484 | 0.811 | 3.348 | 1 | 0.067 | 0.227  |
| TP53                | 0.382 | 0.628 | 0.370 | 1 | 0.543 | 1.466  |

Multivariate analysis of the TRA CDR3-mutant TTN AA complementary group indicated that neither tumor grade nor age is a surrogate parameter.

TCGA-UCEC clinical data from cBioPortal were analyzed to determine whether TRA CDR3-mutant TTN AA complementarity was representative of either age or tumor grade, both important clinical parameters for UCEC. However, neither of these parameters substituted for the complementarity distinction in obtaining the indicated DFS distinction (Table 6; Fig. 2). Multivariate analysis was also done including the occurrence of POLE and TP53 mutation (Table 7) where the presence of POLE mutation also appears to be specifically associated with increased survival.

Discussion

This study assessed whether higher electrostatic complementarity between TRA CDR3 and mutant AAAs in LRP2 and TTN also represented a better survival rate for endometrioid uterine cancer, which was indeed the case. The survival distinction was consistent with the higher level of the expression of proteins related to protein translation in the lower surviving groups, as a higher level of protein translation would be expected in growing and thus more aggressive, more deadly tumors [23,24]. The results of the CS approach applied in this study are consistent with several previous studies representing other cancers [7–10], but as discussed below, the results from this study were explored in new ways. How-
ever, as all of these CS algorithm studies are correlative studies, underlying mechanisms explaining the basis of the association between TRA CD3-mutant AA complementarity and survival need to be determined. Obviously, one of the possible mechanisms facilitating longer survival would be a more successful immune response against the tumor, due to the impact of the binding of the TCR with cancer neoantigens, in turn reflected by the greater chemical attractiveness (complementarity) between the TCR CDR3s and the neoantigens. This possibility is consistent with higher levels of complement protein expression (Table 3), most likely representing high numbers of antigen presenting cells in the tumor microenvironment.

LRP2 encodes the LDL receptor related protein, also termed megalin. LRP2/megalin plays a role in cellular signaling for growth pathways by facilitating endocytosis of various ligands including hormones, lipoproteins, vitamins, and sterols [25]. The TTN protein is a large protein found in muscle and is responsible for passive elasticity. Its most well appreciated function is in proper structure and functioning of the heart sarcomere. TTN is one of the most highly mutated genes in many cancers, not surprising due to its very large size [26-29]. Thus, there is controversy as to whether TTN is cancer-development related, and further studies are indeed needed to elucidate the relationship between TTN and cancer [30].

The lack of survival differences between the average TRA CDR3, higher and lower NCPR, 50th percentile values, respectively, representing the case IDs for the complementary and noncomplementary groups, indicates that a CS does not simply segregate case IDs with a particular NCPR chemical feature independently of the corresponding mutant AA. This conclusion has not been obtained for any of the prior studies using CS values to assess survival rate distinctions. This assessment was facilitated in the case of endometrioid uterine cancer because of the presumed independence of the AA changes with respect to protein function. This would be in contrast to BRAF, where the vast majority of mutant AA alterations are the V600E change, owing to the glutamate conferring constitutive activation on BRAF, in turn due to the negative charge of the glutamate that mimics phosphorylation of BRAF. However, the presumption in the cases of LRP2 and TTN is that, at least to a much lesser extent than in the case of BRAF, there is no requirement for one specific charge change due to mutant AAs or a requirement for that change to occur at a specific AA position in the proteins. Thus, the distribution of the two possible charge changes, more negative or more positive, occurs sufficiently throughout the patient population to determine that a simple NCPR distinction among the corresponding CDR3s is not representative of the survival distinction. To put it another way, these data (Fig. 5), for the first time, establish the need for both mathematical partners, CDR3 and mutant AA, in the CS algorithm, to identify a survival distinction. And indeed, this was the case (Fig. 5) for all three CS-based survival distinctions of Figs. 2, 4.

Another potential confounding molecular factor has been HLA binding, in that tumor samples where there is a complementary relationship between CDR3s and mutant AAs may simply represent a condition
where the patient’s HLA type facilitates binding of neoantigens common to the tumor. This issue has not been addressed in any of the past CS-based survival distinction discoveries [7–10]. In this case, we focused on HLA class II binding, due to the apparent high level of antigen presenting cells in the endometrioid uterine cancer microenvironment. Results indicated that there were no statistically significant differences between HLA-DRB1 binding to the mutant peptides contributing to the CSs, using patient specific HLA-DRB1 alleles (Table 5). However, there did appear to be a trend in the direction of better HLA-DRB1 binding among the case IDs with complementary CDR3-mutant AA CSs. Also, noting the BRAF V600E discussion above, such a distinction may be more apparent when the relevant tumor mutant AA peptide repertoire is much less diverse. In any event, this issue will need further work for a more complete resolution. Having said that, there would also remain the question of whether TCR-neoantigen complementarity and good HLA binding together represent the most clear survival distinctions. This is a particularly apt question given recent work calling into question the relevance of very high neoantigen-HLA binding alone as a successful predictor of T-cell activation in the cancer setting [31].

Finally, revealed that clinical factors of age and tumor grade did not explain the survival distinctions seen between the complementary and noncomplementary groups, consistent with the idea, or the proposal that CSs could represent clinically useful, independent biomarkers for survival distinctions. However, POLE mutations, previously indicated as prevalent in endometrial cancer [32,33], may indeed represent a confounding factor (Table 7) in ways not yet apparent, a result which may represent a future area of research, possibly including the question of whether POLE mutants could be immune response targets.

Authorship statement detailing contribution of each author

MH: conceptualization, methodology, formal analysis, validation, writing original draft, review and editing. BC: conceptualization, methodology, software. MD: conceptualization, methodology, software. TIH: methodology, software, validation. SC: methodology; SZ: conceptualization; KJC: methodology, software. ECG: methodology, software; GB: conceptualization, validation, methodology, formal analysis, writing review and editing, supervision, project administration.

Declaration of Competing Interest

Authors have nothing to declare.

Acknowledgments

Authors thank the support of USF research computing; Ms. Corinne Waiters for managing dbGaP approval processes; and the tax payers of the State of Florida. MH, BC, and TH were all USF Morsani College of Medicine RISE fellowship recipients.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.tranon.2021.101069.
