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T-cell receptor V and J usage paired with specific HLA alleles associates with distinct cervical cancer survival rates

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ABSTRACT

Cervical cancer is more strongly associated with a specific virus, Human Papilloma Virus (HPV), in otherwise healthy individuals, than is any other cancer. Thus, there is an expectation that an adaptive immune signature of cervical cancer would be highly apparent. Here we used a genomics approach to investigate the relationship between T-cell receptor (TCR) V and J usage and survival for patients diagnosed with cervical cancer, relying exclusively on tissue and blood exome files. Specific TCR V or J segments, identified in recombination reads recovered from the exome files, were combined with the patient HLA alleles to identify V or J, HLA allele combination groups associated with distinct survival rates. For examples, the T-cell receptor-β (TRB) V6-5, HLA-A*02:01 combination was associated with a positive outcome, and the TRBV6-1, HLA-A*01:01 combination was associated with a negative outcome. Overall, these results point to V or J usage, HLA allele combinations as survival biomarkers, likely conveniently accessible with a noninvasive procedure, and the results may point the way towards immunological reagents useful in therapy designs.

1. Introduction

Much of the understanding of the adventof cancer revolves around the immune system or a failure of the immune system. In many cases, cancers with no known viral etiology have a strong immune component. Melanoma, for example, is highly immunogenic [1–4], and is considered to advance extensively due to an exhausted immune state, now being addressed with anti-immune checkpoint therapies [5]. In addition, melanoma is thought to progress due to loss of tumor cell immune functions that facilitate T-cell killing, such as loss of tumor cell HLA class I expression or loss of the tumor cell IFN-γ signaling pathway [6,7].Other cancers, as further examples being treated with immune checkpoint blockade therapies, include renal cell carincoma [8] and bladder cancer [9].

Other cancers with a viral etiology are first presumed to be initiated by a viral oncogene, followed by evasion of the immune system either by human DNA mutations or viral mediated down-regulated tumor cell immune functions, for example, down-regulation of HLA expression [10,11]. Cervical cancer was probably the first known, solid tissue cancer that requires a viral infection to precipitate a neoplasm. A concept of “necessary cause” has been proposed, implying that a woman without an HPV infection will not develop a cervical cancer. HPV infection leads to expression of the HPV E6 and E7 oncoproteins, which in turn mediate inactivation and degradation of the p53 and Rb tumor suppressor proteins [12], respectively. Despite being a preventable disease, via HPV-related vaccination, cervical cancer continues to be ranked the number two leading cause of death for women worldwide [13]. However, the list of HPV related cancers has expanded to include not only cancers in the parts of female body other than cervix but also cancers that affect males [13].

Recent developments have given more consideration to the impact of individual genetic variations in the capacity for an anti-tumor immune response. The progression of HPV infections to cancer depends on the individual’s immune response, which in turn relies on HLA alleles that vary extensively from person to person. The impact of HLA variations in disease are likely best understood in the context of autoimmunity where it has been appreciated for decades that certain HLA class I and class II alleles are strongly associated with particular autoimmune diseases, for examples, type 1 diabetes, multiple sclerosis, and Graves’ disease [14]. Similar but less extensive associations of HLA alleles with cancer development and viral infections have also be observed [15–17]. These types of HLA associations have been indicated to

Abbreviations: CESC, cervical squamous cell carcinoma and endocervical adenocarcinoma; DFS, disease free survival; HLA, human leukocyte antigens; OS, overall survival; TCGA, the cancer genome atlas; TCR, T-cell receptor; TRA, TRB, T-cell receptor alpha, beta; WXS, whole exome sequence file

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be due to the ability of certain HLA allelic variants to bind particular self antigens and present these antigens to T-cells. Thus, another aspect of the autoimmune response involves the relationship of T-cell receptor (TCR) usage (i.e., selection during recombination) of particular V and J gene segments to particular HLA allelic variants. TCR V and J usage, in combination with particular HLA alleles, is not only associated with the course of autoimmune diseases [18–22]; TCR V and J usage can be associated with either class I versus class II antigen presenting molecules [23,24]. Viral responses have also been associated with particular TCR V and J usage, HLA allele combinations [25], and more recently, cancer survival patterns have been associated with TRBV or TRBJ, HLA allele combinations [26–28]. In short, the molecular basis of MHC restriction is now well-established as the requirement for particular HLA alleles when certain TCR V or J gene segments are coding for an antigen binding, T-cell receptor.

As noted in the above paragraph, recent work indicates an association between TCR V and J usage, HLA combinations and cancer survival rates. Although only recently demonstrated, this is not surprising due to the immunological component of cancer progression or lack thereof. We considered the possibility that such TCR V and J usage, HLA allele combinations and associated survival rates would be readily demonstrable for cervical cancer keeping in mind the indisputable, and likely universal viral component to cervical cancer. In particular, again due to the viral and therefore likely systemic component of the HPV infection, as evidenced by serological detection of the virus [29,30], we considered the possibility that blood samples could reflect the TCR V and J usage, HLA allele combinations that in other cancers were established by relying on only tissue (i.e, cancer) resident T-cells [26–28]. Indeed, the results below do support a role for blood-available, TCR V and J usage information, useful for establishing a linkage between survival rates and antigen presentation components. This result extends similar information, also available from cervical cancer tumor specimens, but previously only available from tumor specimens.

2. Methods

Cervical cancer (CESC) exome (WXS) files indicated by barcodes were downloaded from the genomic data commons (GDC) website, https://portal.gdc.cancer.gov/repository via National Institutes of Health approved database of Genotypes and Phenotypes project number 6300. Recombination reads were retrieved for T-cell receptor-α gene (TRA) and T-cell receptor-β gene (TRB) from the WXS files using a previously described algorithm and script [31–33], available by email to the corresponding author. Briefly, the search algorithm occurred in two steps. In the first step, a low stringency of selection of reads was done by sourcing a series of 10-mers that match the TRA and TRB V- and J-gene segments close to the 3′ end and 5′ end, respectively, of the gene segment. Then, a high stringency match to known V and J gene segments was performed. The data from the initial output of the TRA and TRB search script were organized by separating productive reads from unproductive reads, i.e., reads with a stop code codon or out of frame joining sequence. The productive reads were further filtered using the following criteria: V match length > 19 bases, J match length > 19, V match percent > 89.9, and J match percent > 89.9. HLA alleles representing the WXS files were obtained using the xHLA software [34]. In particular, this software outputs both alleles for all three HLA class I and all three HLA class II genes. The software output has been verified by processing both tumor and blood WXS files for the same TCGA barcode (patient), with almost entirely identical results for both tissue samples. Survival data for all barcodes (i.e., patients) associated with the TRA and TRB data were obtained using cbioportal.org [35,36]. These data can be accessed by selecting the clinical tab for CESC (TCGA, provisional) and exporting the entire set of data as an Excel file for input for the Kaplan–Meier (KM) analyses, conducted using the cbioportal.org web tool and the GraphPad Prism software [26]. The definitions for disease free survival and overall survival are according to ref. [37].

3. Results

Primary tumor and blood WXS files for three hundred and seven CESC cases, representing a median survival time of 100.7 months, in aggregate, were downloaded from the GDC (Table S1, manifest) and searched for productive TRA and TRB recombination reads [31] (Methods) (Table S2, all recovered, TRB recombination reads). There were no statistically significant associations of read recovery with survival outcomes, however, recovery of the TRB recombination reads showed an overall survival (OS), KM analysis result that trended towards worse survival in comparison to all remaining samples, i.e., samples where no TRB recombination reads were recovered (data not shown).

3.1. TRB V or J gene segments identified in either tumor or blood exomes

We next examined the potential associations of HLA allele, TRB V or J usage combinations with survival outcomes. We first determined survival outcomes associated with TRB V or J, HLA allele combinations identified in either tumor or blood WXS files (Table 1). Of the eleven combinations statistically significantly associated with a survival outcome, based on a KM analysis, four TRB gene segment, HLA allele combinations revealed an association with a better survival rate, and the remaining seven represented reduced survival rates (Table 1; Table S3). In none of these eleven cases did the TRB gene segment or the HLA allele independently represent a statistically significant association with survival rates (Table S4).

The most significant correlation was observed for the TRBV6-1, HLA-A*01:01 combination, which showed a negative outcome for OS and disease-free survival (DFS) ($p = 0.018$, $p = 0.0016$ respectively). Another notable observation was the TRBJ2-1, HLA-A*01:01

Table 1
Association with TRBV and TRBJ gene segment, HLA allele combinations with cervical cancer survival rates when using TRB recombination reads recovered from both blood and tumor WXS files.

TCR gene segment	HLA allele	n total	n blood	Combined overall survival	Combined disease free survival	outcome
TRBV27	HLA-C*07:01	11	6	p = 0.040	p = 0.012	negative
TRBV6-5	HLA-A*02:01	19	11	p = 0.020	p = 0.048	*positive*
TRBV6-1	HLA-A*01:01	12	9	p = 0.018	p = 0.0016	*negative*
TRBV5-1	HLA-A*01:01	9	6	p = 0.24	p = 0.011	negative
TRBJ1-1	HLA-B*07:02	20	15	p = 0.039	p = 0.24	positive
TRBJ2-1	HLA-A*01:01	19	13	p = 0.20	p = 0.00017	negative
TRBV6-5	HLA-DPB1*01:01	9	8	p = 0.27	p = 0.039	negative
TRBJ2-7	HLA-DQB1*02:01	30	21	p = 0.22	p = 0.031	negative
TRBJ2-3	HLA-DRB1*07:01	15	9	p = 0.12	p = 0.047	positive
TRBJ2-1	HLA-DQB1*02:01	29	22	p = 0.20	p = 0.041	negative
TRBJ2-1	HLA-DRB1*07:01	25	14	p = 0.062	p = 0.029	*positive*

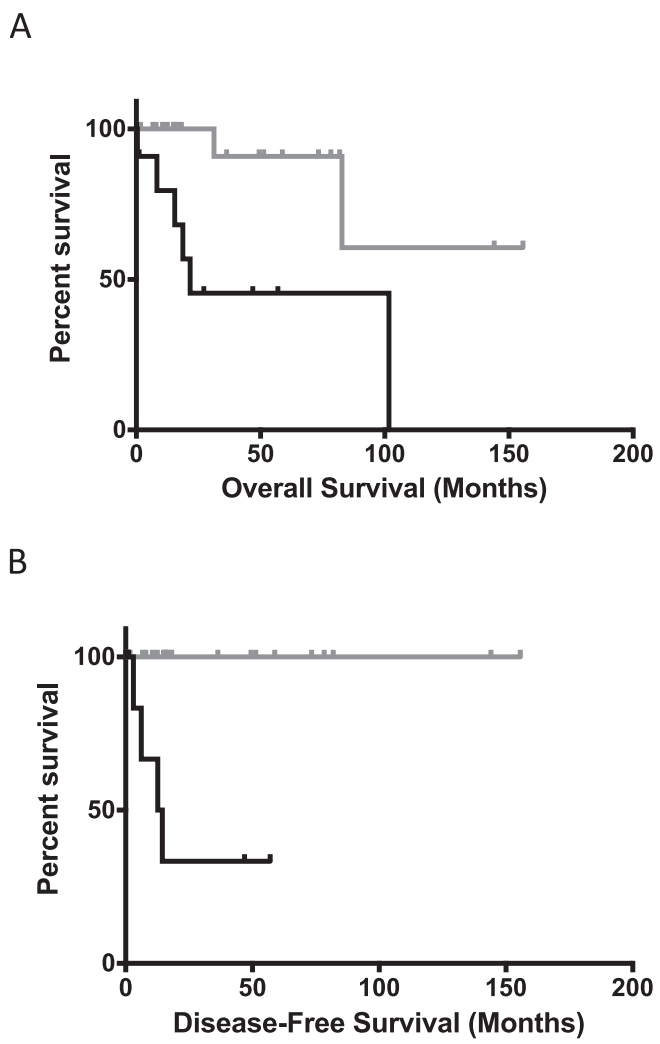

Fig. 1. (A) These data demonstrated statistically significant differences in OS respectively between the negative outcome of the TRBV6-1, HLA-A*01:01 combination (black) and the positive outcome of the TRBJ2-1, HLA-DRB1*07:01 combination (gray). In both cases, the barcodes of the KM analyses represent TCR recombination reads recovered from both tumor and blood WXS files. (B) Repeat of (A) for DFS. See Results text for p-values and median survival times.

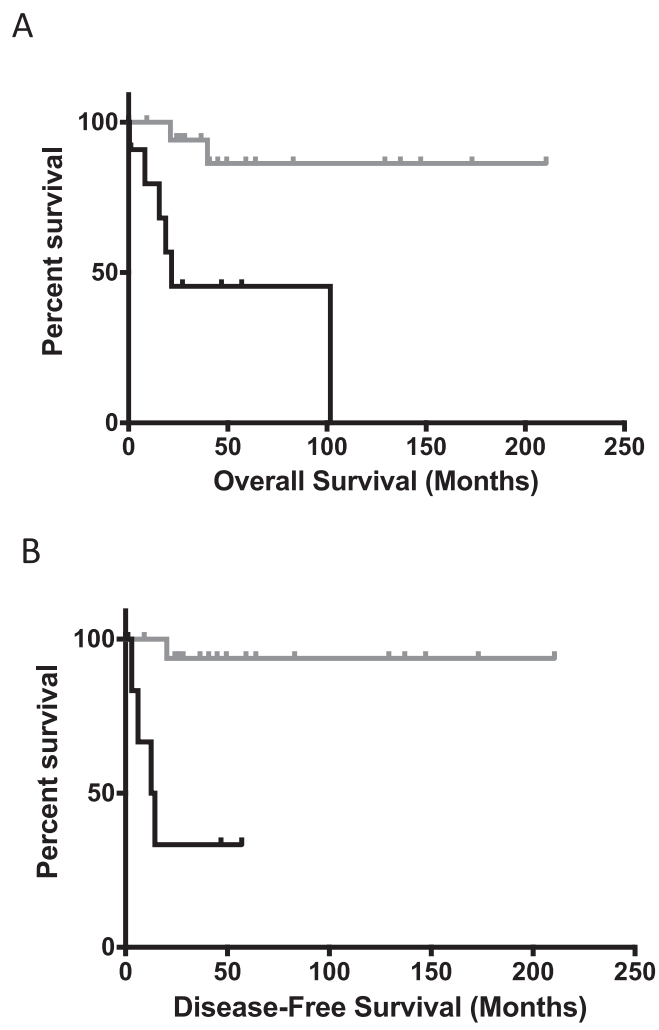

Fig. 2. (A) These data demonstrated statistically significant differences in OS respectively between the negative outcome of the TRBV6-1, HLA-A*01:01 combination (black) and the positive outcome of the TRBV6-5, HLA-A*02:01 combination (gray). In both cases, the barcodes of the KM analyses represent TCR recombination reads recovered from both tumor and blood WXS files. (B) Repeat of (A) for DFS. See Results text for p-values and median survival times.

combination, which was associated with a negative outcome for DFS ($p = 0.00017$).

To establish the extremes of contrasts, in survival rates, for TRB gene segment usage, HLA allele combinations, we first compared the survival rates for the barcodes representing the TRBV6-1, HLA-A*01:01 combination versus the barcodes representing the TRBJ2-1, HLA-DRB1*07:01 combination. The log-rank (Mantel-Cox) test for OS of TRBV6-1, HLA-A*01:01 combination (worse survival) versus the TRBJ2-1, HLA-DRB1*07:01 combination (better survival) demonstrated a p-value of 0.0055; a median survival time of 21.65 months for TRBV6-1, HLA-A*01:01; and an undefined value for survival time for the TRBJ2-1, HLA-DRB1*07:01 combination (consistent with the indicated better survival when this combination was compared to all remaining samples)(Fig. 1A). Additionally, a log-rank (Mantel-Cox) test was conducted for DFS for the TRBV6-1, HLA-A*01:01 combination versus the TRBJ2-1, HLA-DRB1*07:01 combination, revealing a p-value of 0.0001; a median DFS time of 13.55 months for TRBV6-1, HLA-A*01:01; and an undefined DFS time for the TRBJ2-1, HLA-DRB1*07:01 combination (Fig. 1B).

We similarly conducted a KM analysis of the TRBV6-1, HLA-A*01:01 combination versus the TRBV6-5, HLA-A*02:01 combination. The log-rank (Mantel-Cox) test for OS of TRBV6-1, HLA-A*01:01 (worse survival) vs TRBV6-5, HLA-A*02:01 (better survival) demonstrated a p-value of 0.0014; a median survival of 21.65 months for TRBV6-1; HLA-A*01:01; and an undefined median survival for the TRBV6-5, HLA-A*02:01 combination (Fig. 2A). A second log-rank (Mantel-Cox) test was conducted for DFS for the TRBV6-1, HLA-A*01:01 combination versus the TRBV6-5, HLA-A*02:01 combination, which demonstrated a p-value of 0.0005; a median survival of 13.55 months for the TRBV6-1, HLA-A*01:01 combination; and an undefined survival time for the TRBV6-5, HLA-A*02:01 combination (Table S5) (Fig. 2B).

3.2. TRB V or J gene segments identified in blood exomes only

To determine whether significant correlations of TRB V or J usage, HLA combinations with survival rates could be established using results from blood exomes alone, we conducted the KM analyses for all of the entries in Table 1 using the survival data represented by the barcodes representing TRB recombination read recoveries from only the blood WXS files (Table 2). Of the original eleven TRB gene segment, HLA allele combinations that represented a significant survival distinction when including data for barcodes representing both tumor and blood

Table 2
Association of TRBV or TRBJ gene segment, HLA allele combinations with cervical cancer survival rates when using TRB recombination reads recovered from blood WXS files. N/A = not applicable due to n < 9.

Gene segment	HLA allele	n blood	OS	DFS	Trend or statistically significant result
TRBV27	HLA-C*07:01	6	N/A	N/A	negative
TRBV6-5	HLA-A*02:01	11	p = 0.21	p = 0.28	positive
TRBV6-1	HLA-A*01:01	9	p = 0.0048	p = 0.000030	negative
TRBV5-1	HLA-A*01:01	6	N/A	N/A	negative
TRBJ1-1	HLA-B*07:02	15	p = 0.066	p = 0.16	*positive*
TRBJ2-1	HLA-A*01:01	13	p = 0.23	p = 0.00055	negative
TRBV6-5	HLA-DPB1*01:01	8	N/A	N/A	negative
TRBJ2-7	HLA-DQB1*02:01	21	p = 0.13	p = 0.0046	*negative*
TRBJ2-3	HLA-DRB1*07:01	9	p = 0.20	p = 0.14	positive
TRBJ2-1	HLA-DQB1*02:01	22	p = 0.22	p = 0.017	negative
TRBJ2-1	HLA-DRB1*07:01	14	p = 0.19	p = 0.12	positive

recombination read recoveries, four TRB gene segment, HLA allele combinations continued to represent statistically significant survival associations, in comparison to all remaining samples (Table 2).

A significant correlation was observed for the TRBJ2-7 gene segment, HLA-DQB1*02:01 combination, showing a negative trend for OS and a statistically significant association with reduced DFS (p = 0.13, p = 0.0046 respectively). Another notable correlation was for the gene segment TRBJ1-1, HLA-B*07:02 combination, a positive trend for OS (p = 0.066). A significant correlation was observed for the TRBV6-1 gene segment, HLA-A*01:01 combination, evincing a negative outcome for OS and DFS (p = 0.0048, p = 0.00003 respectively).

Next, we conducted KM analyses for direct comparisons of specific TRB gene segment, HLA allele combinations, using only the barcodes representing the recombination reads recovered from the blood WXS files. The log-rank (Mantel-Cox) test for OS for the TRBJ2-7, HLA-DQB1*02:01 combination (worse survival) versus the TRBJ1-1, HLA-B*07:02 combination (better survival) demonstrated a p-value of 0.0265; a median survival of 66.75 months for the TRBJ2-7, HLA-DQB1*02:01 combination; and an undefined median survival time for the TRBJ1-1, HLA-B*07:02 combination (Fig. 3A). A second log-rank (Mantel-Cox) test was conducted for DFS for the TRBJ2-7, HLA-DQB1*02:01 combination versus the TRBJ1-1, HLA-B*07:02 combination, which demonstrated a p-value of 0.0525; a median survival of 59.26 months for the TRBJ2-7, HLA-DQB1*02:01 combination; and an undefined survival time for the TRBJ1-1, HLA-B*07:02 combination (Table S5) (Fig. 3B).

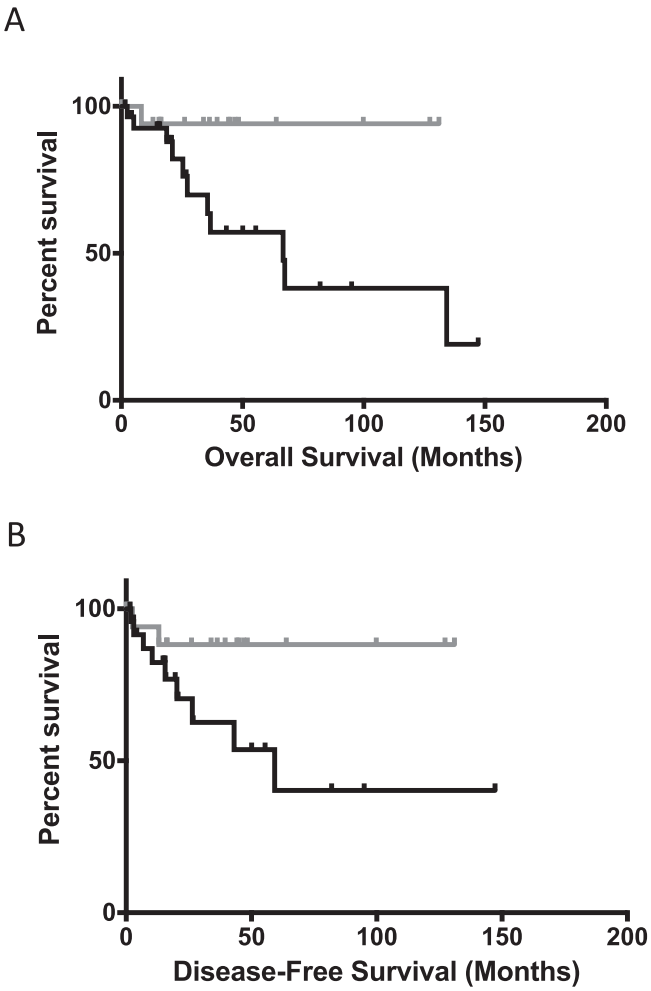

Fig. 3. (A) These data demonstrated statistically significant differences in OS respectively between the negative outcome of the TRBJ2-7, HLA-DQB1*02:01 (black) combination and the positive trend of the TRBJ1-1, HLA-B*07:02 combination (gray), which indicated a survival advantage for those with the TRBJ1-1, HLA-B*07:02 combination (gray). In both cases, the barcodes of the KM analyses represent TCR recombination reads recovered from blood WXS files only. (B) Repeat of (A) for DFS. See Results text for p-values and median survival times.

4. Discussion

Previous reports have indicated the relevance and usefulness of identification of immune receptor recombination reads from tumor specimen RNASeq and exome files [32,38–40]. The immune receptor recombination reads were presumed to be available in the RNASeq and exome files due to tumor infiltrating lymphocytes (TILs). This presumption has been extensively substantiated, particularly in the case of the exome files, with a large collection of correlative data consistent with the recovery of immune receptor recombination reads that represent TILs. For examples, TRB recombination read recovery correlates with a better outcome in the case of bladder cancer [41]; co-detection of TRA and TRB recombination reads in the same WXS file correlates with a high level of immune checkpoint proteins for melanoma and renal cell carcinoma [42,43]; more mutations correlates with higher recovery of TCR recombination reads, specifically in a lung cancer mouse model [44]; and in the case of pancreatic cancer, where work has shown that the presence of bacteria correlate with a worse outcome [45], recovery of immunoglobulin recombination reads also, independently, was shown to correlate with a worse outcome [46], consistent with a Th2 response expected with bacteria present. This list of correlations with recombination read recoveries has become quite extensive, recently, and may have been facilitated by algorithms that require a verifiable V and J on one sequencing read, thereby maintaining a high standard for the recombination event, including a virtually indisputable CDR3 amino acid sequence. This approach is in turn facilitated by longer read lengths for exomes as opposed to RNAseq data, where read lengths may limit recombination detections to read

contig building and statistical algorithms that establish the validity of the contig.

In addition to information indicated by basic recovery of the immune receptor recombination reads, more detailed information, namely the indications of V and J usage, as well as HLA types available from the exome files, has allowed an alignment of TRBV and TRBJ usage, HLA allele combinations with survival rates for a variety of cancers [26–28]. Another study has confirmed that certain HLA alleles are specifically associated with infection of a given HPV-type, consistent with an HLA-allele dependent development of cervical cancer [47]. Combining TRB recombination read recovery from the WXS files, with identification of the HLA alleles, represents a resolution of the information obtained with the TRB recombination reads alone. For example, data above indicated that in general, the recovery of TRB recombination reads is associated with a worse outcome (although only as a trend). However, in several cases, the combination of a particular HLA allele, and the recovery of the TRB recombination reads, is associated with better survival. While there is at this time no explanation for the apparent refocusing of the TRB impact on survival, when there is a specific HLA allele present, one hypothesis would be that the presence of a particular HLA allele favors successful antigen presentation to a cytotoxic T-cell while the lack of such an allele only facilitates T-cell mediated protection from tumor apoptosis or other T-cell mediated, pro-tumor developments.

In this report, we extended the cancer survival associated, TRBV and TRBJ, HLA allele combinations to cervical cancer by taking advantage of both the tumor and blood exome files. This approach was predicated on the assumption that in almost all cases, cervical cancer is due to HPV infection, which in turn would have a systemic “TRB footprint” [13]. In addition, many PCR-based studies of immune receptor repertoires point to apparently systemic, dominant clonotypes, i.e, dominant clonotypes in the periphery [17,48–50] but representative of specific immuno-reactivity. Thus, the preparation of the blood exome, particularly for a large number of subjects in a study, is likely to favor recovery and identifications of dominant clonotypes, in that the recovery of the immune receptor recombination reads is not systematically and comprehensively in play with an exome preparation.

Previous studies have indicated that specific HLA alleles can be associated with cervical cancer. For example, one such study discovered a reduced risk of cervical disease with HLA alleles DRB1*13:01, DRB1*13:02, and DQB1*06:03 while HLA allele B*07:02 was associated with an increased predisposition to cervical cancer [47].

Thus, the above data point towards three likely advances: (i) the identification of TRBV and TRBJ, HLA allele combinations that may be useful for prognosis and other patient-oriented goals, such as therapy design; (ii) the indication that the recovery of immune receptor recombination reads from blood exome files has the potential, at least in a large study, of providing immune response information related to clinical parameters; and (iii) the more precise indication of specific J1 or J2 subfamily members, e.g., J2-7, as being associated with survival rates, in combination with specific HLA alleles. In the previous analyses of TRBJ, HLA allele combinations associated with survival [26–28], only the J1 family, or the J2 family, could be statistically significantly associated with a survival distinction, in combination with a particular HLA allele.

As for the functional basis of the apparent association of TRBV or TRBJ gene segments with HLA alleles, i.e., apparent in that certain such combinations were associated with survival rates in this report, the presumption would be that certain HLA allelic variants only interact well with certain antigens; and that such HLA allelic variants only interact well with certain TRBV or TRBJ genome segments. Such a presumption, or tentative interpretation, would be consistent with results described in the Introduction regarding the HLA class and allelic variant, TRB gene segment combinations in viral and autoimmunity settings, where such combinations represent different outcomes [14–17]. Further ideas regarding mechanism may be in the offing with future studies of gene expression data associated with the tumor samples representing the barcodes in turn representing the distinct TRB gene segment, HLA allele combinations. (See Table S6.)

As noted above, just as in the case of the tumor exomes, it is likely that recovery of immune receptor recombination reads from the blood exomes represents, more often than not, recovery of immune receptors representing dominant usage, and the usefulness is particularly apparent when a very large sample size is available. However, a previous study examined the TCRs harvested from peripheral blood lymphocytes and cervical cancer infiltrating lymphocytes (TIL) and concluded that cancer resident lymphocytes were skewed towards reactivity with the cancers antigens [51]. Thus, such results may represent a contradiction to our results. It is possible a resolution of such a potential contradiction may be in the offing with applications of algorithms that can more broadly characterize and categorize TCRs and their antigen binding sites, especially the CDR3 region. With a broader collection of similar, yet not identical TCRs, certain TCR structural or chemical trends may be much more readily demonstrable for the blood derived TCRs. Examples of such TCR classification algorithms would include algorithms that apply phylogenetic dendrograms of bioinformatics based chemical assessments, for example, net charge per residue of the TCR CDR3.

Keeping in mind the less invasive aspect of obtaining blood exomes, identifying HLA alleles and TCR features with those exomes, holds the promise of more extensive studies, and possibly more patient-based approaches that could identify persons with distinct disease courses. More specifically, given that cancer is an inflammatory disease process that involves lymphocytes in circulation, a potential application of using blood exomes is that it could be used as a less invasive method of collecting very large numbers of patient samples for further cancer immuno-genomics analyses [52].

Conflict of interest

Authors have nothing to declare.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.humimm.2019.01.005.

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