Identification of targets for prostate cancer immunotherapy

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Background: We performed profiling of the immune microenvironment of castration-resistant (CRPC) and castration-sensitive (CSPC) prostate cancer (PC) in order to identify novel targets for immunotherapy.

Methods: PD-L1 and CD3/CD8 immunohistochemistry, PD-L1/2 fluorescent in situ hybridization, tumor mutation burden, microsatellite instability, and RNA-seq of 395 immune-related genes were performed in 19 CRPC and CSPC. Targeted genomic sequencing and fusion analysis were performed in 17 of these specimens.

Results: CD276, PVR, and NECTIN2 were highly expressed in PC. Comparison of CRPC versus CSPC and primary versus metastatic tissue revealed the differential expression of immunostimulatory, immunosuppressive, and epithelial-to-mesenchymal transition (EMT)-related genes. Unsupervised clustering of differentially expressed genes yielded two final clusters best segregated by CRPC and CSPC status.

Conclusion: CD276 and the alternative checkpoint inhibition PVR/NECTIN2/CD226/TIGIT pathway emerged as relevant to PC checkpoint inhibition target development.

KEYWORDS
aggressive prostate cancer, castration-resistant prostate cancer, castration-sensitive prostate cancer, CD226, CD276, immunotherapy, NECTIN2, PVR, TIGIT

1 INTRODUCTION

Immune checkpoint inhibition (ICI) in prostate cancer (PC) has yielded mixed results. Two large double blind randomized studies with metastatic castration-resistant prostate cancer (CRPC) cohorts, involving 799 and 400 patients, demonstrated improved progression-free survival with ipilimumab but no improvement in overall survival. The microsatellite status of the PCs in these two studies was not defined.1,2 Two of the patients who participated in these ipilimumab trials had long-term complete remission.3 Only one of these two patients had remnant material for additional testing and was found to have high infiltration of CD8+ and FOXP3 T-cells and intact expression of MLH1, MSH2, MSH6, and PMS2. An additional case of CRPC with complete PSA response to ipilimumab has also been reported.4 Two of 10 patients with CRPC have been reported as partial responders to pembrolizumab, one of whom was microsatellite unstable.5 In a phase 1 trial with nivolumab, 17 patients with CRPCs had no objective response.6 However, a CRPC patient with MSH2 and MSH6 loss in his neoplasm was reported to have had a robust response to nivolumab.7 Microsatellite instability is a promising predictor of immunotherapy response in PC with 1-2% of PC having microsatellite instability,8,9 which makes them eligible for pembrolizumab therapy.10 The most substantial checkpoint inhibition results have been observed with pembrolizumab. A pembrolizumab monotherapy study of 23 heavily pretreated PC with at least 1% positivity for PD-L1 immunohistochemistry in tumor or stroma cells demonstrated an
overall response rate of 13%. A subsequent pembrolizumab monotherapy study of 258 docetaxel-refractory metastatic CRPC demonstrated a disease control rate of 26%.

Our knowledge of the immune microenvironment of PC is limited. Human PC neoplastic cell clusters are surrounded by CD4+ T-cells that express the immunosuppressive regulatory T-cell markers CD25 and forkhead box P3 (FOXP3). C-C motif chemokine receptor 4 (CCR4) is expressed by a subset of PC-associated regulatory T-cells and may play a role in the recruitment process of myeloid-derived suppressor cells (MDSC). Lymphocytes surrounding PC, but not PC neoplastic cells, have been reported to express the immune exhaustion-related checkpoint receptor PDCD1 (better known as PD-1) and its ligand CD274 (also known as PD-L1; B7-H1). This evidence suggests that an adoptive T-regulatory cell-driven mechanism coupled with recruitment of MDSC is a part of an immunosuppressive microenvironment in PC.

In this study, we evaluated the immune microenvironment of 19 clinically aggressive PC and found high RNA expression of poliovirus receptor (PVR; alias CD155), nectin cell adhesion molecule 2 (NECTIN2; alias CD112, PVRL2), mechanistic target of rapamycin kinase (MTOR) pathway components, and the immune checkpoint molecule CD276 (also known as B7-H3) in addition to differential gene expression in CRPC versus castration-sensitive prostate cancer (CSPC) and primary versus metastatic PC. This study provides early limited evidence of additional immunosuppressive mechanisms present in aggressive PC.

2 MATERIALS AND METHODS

2.1 Samples

Formalin-fixed paraffin embedded material from 19 PC samples was tested with the Immune Report Card® and OmniSeq Comprehensive® (OmniSeq, Inc., Buffalo, NY) assays. All samples were either from patients with CRPC (progression following androgen-targeted therapy) or high-risk (Gleason 8 and above) high-volume CSPC. Although all of the patients had metastatic disease at the time of biopsy/surgery, 11 of the analyzed specimens are from the primary site (prostate). Patient and specimens characteristics are summarized in Table 1 and Supplementary Table S1. Eighteen specimens were tested under the non-human subjects research IRB-approved BDR #073166 at Roswell Park Comprehensive Cancer Center (Buffalo, NY). One specimen was tested after the patient was consented to participate in the PREDICT-UCSD (Profile-Related Evidence Determining Individualized Cancer Therapy, NCT02478931) study in accordance with UCSD IRB guidelines.

2.2 Genomic and fusion profiling

OmniSeq Comprehensive® uses FFPE tissue to test 144 genes for point mutations, insertions/deletions, copy number variants, and select fusions.

<table>
<thead>
<tr>
<th>TABLE 1</th>
<th>Patient and specimen characteristics</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of patients/specimens</td>
<td>19</td>
</tr>
<tr>
<td>Age range at diagnosis (years)</td>
<td>50-77</td>
</tr>
<tr>
<td>Sample collection date range</td>
<td>1/2008-12/2018</td>
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<tr>
<td>Report date range</td>
<td>8/2017-2/2018</td>
</tr>
<tr>
<td>CRPC</td>
<td>9</td>
</tr>
<tr>
<td></td>
<td></td>
</tr>
<tr>
<td>CSPC</td>
<td>10</td>
</tr>
<tr>
<td></td>
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</tr>
</tbody>
</table>

2.3 Immune microenvironment immune profiling

The Immune Report Card® uses FFPE tissue to evaluate multiple biomarkers including neoplasm-appropriate PD-L1 IHC, PD-L1/2 copy number by FISH, microsatellite instability (MSI) by PCR, tumor mutational burden (TMB) by DNA-Seq, CD3+ and CD8+ lymphocyte infiltration by IHC, and expression levels of multiple immunologically relevant genes, many of which are targets of immunotherapeutic agents currently in development, by RNA-Seq. The DNA-Seq and RNA-Seq components have been previously described.

2.4 Ranking, Wilcoxon rank sum test, heatmap, and determination of immunotherapeutic targets

A population of various solid tumors was used as a reference to subsequently produce normalized rankings of 397 gene transcripts. Details on this population have already been published. Expression levels of specific genes in each of the 19 cases of CRPC and CSPC were then compared to the reference population to derive gene- and case-specific rank results. A normalized rank of 75 was used as the cutoff to define an elevated rank. For any given gene, we calculated the percent of PC cases with an elevated rank in comparison to a separate clinical non-PC cohort of 450 cancer specimens. This non-PC population contains numerous cancer types including major cancer histological categories such as non-small cell lung carcinoma, urothelial carcinoma, endometrial carcinoma, ovarian carcinoma, breast carcinoma, colorectal carcinoma, pancreatic carcinoma, salivary gland carcinoma, thymic carcinoma, melanoma, sarcoma, and others. To determine the difference in expression between the PC and non-PC specimens, the mean rank expression of each gene was compared between PC samples (n = 19) and non-PC samples (n = 450) using the Wilcoxon rank-sum test with Benjamini-Hochberg corrected P values reported.

Unsupervised clustering was performed on all genes based on expression ranks. Pearson’s correlation was used as a measure of dissimilarity for hierarchical clustering of both the genes and the samples. Annotations of castration resistance, metastasis, rank of TILs (CD8 rank by RNA-seq) and CD8 infiltration pattern were included. The combined gene list of differentially expressed genes in CRPC versus CSPC and primary versus metastatic comparisons was used to construct a focused cluster.
We have separated the differential expression patterns in immunostimulatory, immunosuppressive, EMT-related, and other categories based on the expression direction and the function of the gene product as described in the available literature.

3 | RESULTS

3.1 | Genomic data and RNA sequencing comparison

Genomic data were available on 17/19 cases (Supplementary Table S2). One of the 17 cases (a castration-resistant case) had an androgen receptor (AR) gene copy number gain. Breast cancer 1 and 2 (BRCA1 and BRCA2) mutations or BRCA2 copy loss were seen in 5/17 cases, three of which were metastatic and/or castration resistant. Mutations or copy losses in PTEN were seen in 8/17 cases. Transmembrane serine protease 2-ETS transcription factor (TMPRSS2-ERG) fusions were present in 7/17 cases. There was no association (P > 0.05) between PTEN loss/mutation status with RNA-seq rank of any gene, CRPC versus CSPC status or primary versus metastatic status. No association (P > 0.05) was observed between the DNA mutational profile, CD3/8 IHC status, RNA-seq CD8, PD-L1 IHC, or TMB with the expression profile of any specific gene. In addition, there was no difference in the DNA mutational profile (P > 0.05) of CRPC versus CSPC or primary versus metastatic PC (data not shown).

3.2 | Immune infiltrate

IHC for CD8 revealed infiltration of nests of neoplastic cells by lymphocytes in 8/19 cases (5/9 CRPC and 3/10 CSPC), but CD8 RNA was not overexpressed in any cases with the exception of case 17. This case had a high RNA-seq CD8 rank, but the pattern of T-cell infiltration by IHC was non-infiltrating CD8-positive lymphocytes in the stroma between islands of neoplastic cells (Figure 1). The mean rank of CD8 RNA-seq expression levels was not significantly different between PC and non-PC cases (data not shown).

3.3 | PD-L1/PD-1 axis checkpoint blockade

One specimen (case 10) had weak PD-L1 expression by IHC with 5% of neoplastic cells staining and an equally low rank for PD-L1 (rank = 26) by RNA-seq. This was also the only specimen that was microsatellite unstable and had a very high TMB. A second case (case 8) had an elevated rank for PD-L1 (rank = 83) by RNA-seq and IHC staining was present only in immune cells (Figure 1). The mean ranks for PD-L1, PD-L2, and PD-1 by RNA-seq in all PC cases were 25, 27, and 18, respectively (Supplementary Table S4).

3.4 | Other checkpoint blockade

The most frequently overexpressed checkpoint blockade genes were PVR (mean rank = 84) and NECTIN2 (mean rank = 93). Both PVR and NECTIN2 had an elevated rank in 16/19 PC cases in addition to a relatively high mean-rank expression compared to non-PC cases (P = 0.00002 PVR; P = 0.00001 NECTIN2). TIGIT (mean rank = 25) and CD226 (mean rank = 28) were underexpressed compared to the non-PC cohort (P = 0.005 for both TIGIT and CD226). The checkpoint blockade ligand CD276 had an elevated rank in 11/19 cases and a significantly increased mean-rank expression (mean rank = 67) compared to the non-PC cohort (P = 0.008) (Table 2, Supplementary Table S4).

3.5 | MTOR pathway

Phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit alpha (PIK3CA), AKT serine/threonine 1 (AKT1), mechanistic target of rapamycin kinase (MTOR), and ribosomal protein S6 (RPS6) had an elevated rank in 10, 15, 11, and 16 of 19 PC cases, respectively, with mean rank values of 67, 82, 72, and 91, respectively. At least one of these four genes had an elevated rank in 18 of 19 PC cases and all genes had an elevated mean rank expression except for PIK3CA which had no difference from the non-PC cohort (PIK3CA P = 0.6; AKT P = 0.0000003; MTOR P = 0.00006; RPS6 P = 0.000001). Phosphatase and tensin homolog (PTEN) was highly ranked in 7/19 cases but its mean rank was not significantly different from the non-PC cohort (P = 0.9). PIK3CD had no cases with high rank and had a decreased mean rank compared to the non-PC cohort (P = 0.00006) (Table 2, Supplementary Table S4).

3.6 | CRPC/CSPC and primary/metastatic PC comparison

Hierarchical clustering based on the genes that were significantly differentially expressed between CRPC versus CSPC and primary versus metastatic PC showed two clusters of samples with each cluster overrepresented by either CSPC (cluster 1; 8/9 samples, 90%) or CRPC (cluster 2; 8/10, 80%) (Figure 2). Other associations such as primary versus metastatic status, CD8 rank by RNA-seq and CD8 infiltration by IHC did not segregate as well in the clusters (Figure 2, Supplementary Figure S1).

The heat map cluster constructed based on all the interrogated genes is shown in Supplementary Figure S1. Supplementary Table S3 contains IHC, TMB, MSI results, and the RNA ranks for all 19 cases. Only the genes that had significantly differential expression (P < 0.05) in the PC versus non-PC (Supplementary Table S4), CRPC versus CSPC (Supplementary Table S5), and primary versus metastatic PC (Supplementary Table S6) comparisons are shown in Supplementary Table S3.

4 | DISCUSSION

The current PC-related medical literature describes a few PD-1 axis checkpoint inhibitor therapy successes with some highly promising results with pembrolizumab monotherapy.1–5,7,11,12 The recent approval of pembrolizumab for MSI-H solid neoplasms is the only current indication for ICI therapy in PC. Despite these encouraging results, additional immune-related targets are needed.
The elevated mean rank expression of CD276 in PC compared to non-PC suggests an important function in aggressive PC and is a potential therapeutic target. Gleason score and castration status have been shown to correlate with the expression of specific genes. Some of these associations were not replicated in our case series.\textsuperscript{19–24}

The diminished expression of CD226 (also known as DNAM-1) and TIGIT and elevated expression of the ligands PVR and NECTIN2

\begin{figure}
\centering
\includegraphics[width=\textwidth]{figure1.jpg}
\caption{The spectrum of immunohistohemical findings in this PC series. Cases 6 and 1 have high and low infiltration by CD8+ cells and moderate rank (rank 66) and low rank (rank 38) of CD8 by RNA-seq, respectively. The neoplastic cells for both cases are negative for 22C3 and both have a low RNA-seq rank for PD-L1 (case 6: rank 27, case 1: rank 10). However, case 17 has no infiltration by CD8+ cells but does have a high CD8 rank by RNA-seq (rank 80). The presence of CD8+ cells in the stroma may explain this discrepancy. This case is negative for 22C3 with a corresponding low PD-L1 RNA-seq rank (rank 45). Conversely, case 10 has 5% neoplastic cells positive for 22C3 but a very low rank of PD-L1 by RNA-seq (rank 26). Infiltrating CD8+ cells are present but the CD8 rank is low (rank 44). Case 8 has a high PD-L1 rank (rank 83) by RNA-seq but no neoplastic cells stain with 22C3, possibly due to the presence of immune cells with 22C3 positivity. In addition, this case is infiltrated by CD8+ cells but has a low CD8 rank by RNA-seq (rank 34). [Color figure can be viewed at wileyonlinelibrary.com]}
\end{figure}
PVR and NECTIN2 are expressed in dendritic cells, T-cells, and neoplastic cells. CD226 is a costimulatory receptor expressed on T-cells, natural killer (NK) cells, B-cells, and monocytes. In preclinical models, CD226 is essential for CD8 T-cell costimulation in the presence of nonprofessional antigen-presenting cells and is required for NK cell activity. CD226 is downregulated by its CD112 ligand in AML and by its PVR ligand in ovarian carcinoma. CD226 is upregulated in PVR knock-out mice and anti-PVR antibodies cause T-cell CD226 upregulation in wild-type mice. TIGIT is a coinhibitory receptor expressed on T and NK cells and, similar to CD226, has NECTIN2 and PVR ligands. In a cell-line model, TIGIT outcompetes CD226 for the PVR ligand. It is unclear why the NK and T-cell-related CD226 and TIGIT transcripts are low considering that CD8+ lymphocytes and elevated CD8 transcripts were present in some of the cases (Supplementary Table S3). It is possible that only a small number of PVR and NECTIN2-expressing cells is required for CD226 and TIGIT suppression. We theorize that the coinhibitory TIGIT and its ligands may be important immunotherapy targets in PC in a manner similar to the interaction of PD-1 and PD-L1/2 in other tumor types. From a therapeutic perspective, using anti-TIGIT or anti-PVR antibodies to prevent the activation of TIGIT may enhance the proportional importance of the costimulatory receptor CD226, possibly leading to CD226-mediated, immunostimulatory anti-tumor signaling.

### TABLE 2  Wilcoxon rank sum test in PC versus non-PC cases

<table>
<thead>
<tr>
<th>Gene</th>
<th>Adj. P value</th>
<th>Mean PC</th>
<th>95CI-PC</th>
<th>Mean non-PC</th>
<th>95CI non-PC</th>
<th>Direction in PC</th>
<th>Effect in PC based on function and direction of expression</th>
</tr>
</thead>
<tbody>
<tr>
<td>PVR</td>
<td>2E-05</td>
<td>84</td>
<td>76–92</td>
<td>58</td>
<td>55–60</td>
<td>Overexpressed</td>
<td>Immunostimulatory/immunosuppressive</td>
</tr>
<tr>
<td>NECTIN2</td>
<td>1E-05</td>
<td>93</td>
<td>86–100</td>
<td>75</td>
<td>73–78</td>
<td>Overexpressed</td>
<td>Immunostimulatory/immunosuppressive</td>
</tr>
<tr>
<td>AKT1</td>
<td>3E-07</td>
<td>82</td>
<td>71–93</td>
<td>45</td>
<td>42–47</td>
<td>Overexpressed</td>
<td>Immunostimulatory</td>
</tr>
<tr>
<td>MTOR</td>
<td>0.0006</td>
<td>72</td>
<td>60–84</td>
<td>49</td>
<td>47–52</td>
<td>Overexpressed</td>
<td>Immunostimulatory</td>
</tr>
<tr>
<td>RPS6</td>
<td>1E-05</td>
<td>91</td>
<td>85–96</td>
<td>67</td>
<td>64–69</td>
<td>Overexpressed</td>
<td>Immunostimulatory</td>
</tr>
<tr>
<td>CD276</td>
<td>0.0075</td>
<td>67</td>
<td>52–81</td>
<td>47</td>
<td>45–50</td>
<td>Overexpressed</td>
<td>Immunostimulatory</td>
</tr>
<tr>
<td>PTPN11</td>
<td>0.0079</td>
<td>79</td>
<td>68–89</td>
<td>62</td>
<td>60–65</td>
<td>Overexpressed</td>
<td>Immunostimulatory</td>
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<tr>
<td>PTK7</td>
<td>0.0446</td>
<td>83</td>
<td>74–92</td>
<td>67</td>
<td>65–70</td>
<td>Overexpressed</td>
<td>Immune function unclear</td>
</tr>
<tr>
<td>MAPK1</td>
<td>0.0041</td>
<td>64</td>
<td>52–77</td>
<td>46</td>
<td>43–48</td>
<td>Overexpressed</td>
<td>Immunostimulatory</td>
</tr>
<tr>
<td>MIF</td>
<td>5E-05</td>
<td>66</td>
<td>55–76</td>
<td>39</td>
<td>37–42</td>
<td>Overexpressed</td>
<td>Immunostimulatory</td>
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<tr>
<td>RORC</td>
<td>4E-06</td>
<td>84</td>
<td>76–91</td>
<td>56</td>
<td>54–58</td>
<td>Overexpressed</td>
<td>Immune function unclear</td>
</tr>
<tr>
<td>TWIST</td>
<td>0.0185</td>
<td>68</td>
<td>57–80</td>
<td>52</td>
<td>49–55</td>
<td>Overexpressed</td>
<td>Immune function unclear</td>
</tr>
<tr>
<td>IKZF4</td>
<td>0.0204</td>
<td>70</td>
<td>54–86</td>
<td>56</td>
<td>53–58</td>
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<tr>
<td>MYC</td>
<td>6E-05</td>
<td>73</td>
<td>62–84</td>
<td>44</td>
<td>42–47</td>
<td>Overexpressed</td>
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<td>GUSB</td>
<td>0.0072</td>
<td>65</td>
<td>52–78</td>
<td>46</td>
<td>44–49</td>
<td>Overexpressed</td>
<td>NA (housekeeping gene)</td>
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<td>TBP</td>
<td>0.0180</td>
<td>64</td>
<td>49–79</td>
<td>48</td>
<td>45–51</td>
<td>Overexpressed</td>
<td>NA (housekeeping gene)</td>
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<td>B3GAT1</td>
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<td>79</td>
<td>67–92</td>
<td>37</td>
<td>34–40</td>
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<td>Immune function unclear</td>
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<tr>
<td>GADD45GIP1</td>
<td>0.0021</td>
<td>65</td>
<td>53–77</td>
<td>43</td>
<td>40–46</td>
<td>Overexpressed</td>
<td>Immune function unclear</td>
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<tr>
<td>CTAG1B</td>
<td>0.03377</td>
<td>21</td>
<td>6–36</td>
<td>10</td>
<td>8–12</td>
<td>Overexpressed</td>
<td>Immunogenic antigen</td>
</tr>
<tr>
<td>TARP</td>
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<td>93</td>
<td>83–104</td>
<td>45</td>
<td>42–48</td>
<td>Overexpressed</td>
<td>Immune function unclear</td>
</tr>
</tbody>
</table>

Only genes relatively overexpressed in PC are shown.

### FIGURE 2  Heatmap with genes that emerged as differentially expressed in castration-resistant prostate cancer versus castration-sensitive prostate cancer and primary versus metastatic prostate cancer. Unsupervised clustering produced two clusters. Cluster 1 has high representation of castration-sensitive prostate cancer and cluster 2 has high representation of castration-resistant prostate cancer. GEX, gene expression; PMR, primary/metastasis/recurrent; TILs, tumor infiltrating lymphocytes by CD8 rank. [Color figure can be viewed at wileyonlinelibrary.com]
The relative overexpression of AKT, MTOR, RPS6 and frequent mutation or loss of PTEN in our cases affirm the significance of the MTOR pathway in PC (Supplementary Table S4).\textsuperscript{42–45} RPS6 has been shown to be regulated by MTOR.\textsuperscript{46} In melanoma cell lines, PTEN loss is associated with immunosuppression and immune resistance.\textsuperscript{47} However, we did not see any associations between PTEN copy loss/ mutation with clinical characteristics or gene expression. Clinical trials utilizing MTOR inhibitors in PC have yielded disappointing results.\textsuperscript{48} Nevertheless, combinatorial therapy using AKT/MTOR pathway inhibitors in addition to checkpoint inhibition may be more efficacious.

A relatively diminished expression of numerous genes was also observed in PC compared to non-PC. IL21, which is associated with immune stimulation, was underexpressed.\textsuperscript{49} Genes which are associated with immune suppression and were underexpressed include CSF1R, CTLA4, PD-L1, and PD-1 (Supplementary Table S4).\textsuperscript{49}

CRPC versus CSPC and primary versus metastatic PC expression comparisons revealed a complex differential expression profile of potentially immunostimulatory, immunosuppressive, EMT-related, and other genes (Supplementary Tables S5 and S6).

The results of mutational profiling were overall consistent with previously published data.\textsuperscript{50} The only case which had microsatellite instability also had a high TMB, 5% PD-L1 by IHC, but low PD-L1 RNA-seq rank.

Overall, the PC immune microenvironment is complex and although it has immunosuppression characteristics, it may not fit in a simple “immunosuppressed” category relative to other cancers. Unsupervised clustering revealed two clusters best separated by CRPC and CSPC status and a slightly less impressive separation based on primary and metastatic sites (Figure 2). The fact that CRPC versus CSPC and primary versus metastatic status have such a close separation is likely because the majority of castration-resistant cases were metastases and the majority of castration-sensitive cases were from the primary site (prostate). The substantial segregation of these genes suggests an immunological difference between CRPC/CSPC that may be exploitable for the development of therapeutic agents.

However, the number of cases in each of the CRPC, CSPC, primary, and metastatic categories is too small to draw robust conclusions.

A major limitation of this study was the small sample size. This may be the reason we did not replicate the associations between clinical findings and some of the genes overexpressed in our dataset. Specifically, we did not detect any correlations between CD276, protein tyrosine kinase 7 (PTK7), RAR-related orphan receptor C (RORC), twist family bHLH transcription factor 1 (TWIST) and PSA, PC grade, or metastatic status (data not shown).\textsuperscript{19,20,51–53} Despite this limitation, we did identify potentially immune-related genes known to be overexpressed in PC, such as MYC proto-oncogene (MYC),\textsuperscript{54} cancer/testis antigen 1B (CTAG1B),\textsuperscript{55} and TCR gamma alternate reading frame protein (TARP) (Table 2, Supplementary Table S4).\textsuperscript{56–59} Second, our CSPC set included exclusively clinically aggressive cases. Therefore, the CSPC cases included in this study may not be equivalent to all CSPC.

Third, expression levels based on RNA-seq are not necessarily reflective of the functional importance of a final protein product. Fourth, our method of ranking is inherently limited by the specimens that were used to build the reference population, even though this reference population consisted of 19 different tumor types. Future studies should include an expanded cohort with confirmatory immunohistochemical studies.

In conclusion, we have described a segment of the clinically aggressive/castration-resistant PC transcriptome, including the relative differences between PC/non-PC, CRPC/CSPC, and primary/metastatic PC with a focus on potential immune targets. The PVR/NECTIN2/CD226/TIGIT axis is a potentially biologically and therapeutically important target in PC.

**DISCLOSURES**

APS, SP, FLL, PDP, MN, JC, SG, and CM are all employees of OmniSeq, Inc. (Buffalo, NY) and hold restricted stock in OmniSeq, Inc.; YY, GC, JC, SG, and CM are employees of Roswell Park Comprehensive Cancer Center (Buffalo, NY). Roswell Park Comprehensive Cancer Center is the majority shareholder of OmniSeq, Inc. GC is on an Advisory Board for Immuno-Oncology for AstraZeneca. RK receives research funding from Incyte, Genentech, Merck, Serono, Pfizer, Sequenom, Foundation Medicine, and Guardant, as well as consultant fees from X Biotech, Loxo, NeoMed, and Actuate Therapeutics, speaker fees from Roche, and has an ownership interest in Curematch, Inc. YY and SK have no conflicts of interest to disclose.

**DATA ACCESS, RESPONSIBILITY, AND ANALYSIS**

APS and CM had full access to all the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis.

**AUTHORS’ CONTRIBUTIONS**

APS, YY, SP, FLL, PDP, and CM contributed to the design and conduct of the study collection, management, analysis, and interpretation of
the data. All authors contributed to the preparation, review, and approval of the final manuscript and the decision to submit the manuscript for publication.

CONSENT FOR PUBLICATION

OmniSeq’s analysis utilized deidentified data that were considered non-human subjects research under IRB-approved protocol (BDR #073166) at Roswell Park Comprehensive Cancer Center (Buffalo, NY). One specimen was tested after the patient consented to the PREDICT-UCSD (Profile Related Evidence Determining Individualized Cancer Therapy, NCT02478931) study in accordance with UCSD IRB guidelines.

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REFERENCES


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Additional Supporting Information may be found online in the supporting information tab for this article.