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A meta-analysis of epitopes in prostate-specific antigens identifies opportunities and knowledge gaps

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Abstract

Background ––The Cancer Epitope Database and Analysis Resource (CEDAR) is a newly developed repository of cancer epitope data from peer-reviewed publications, which includes epitope-specific T cell, antibody, and MHC ligand assays. Here we focus on prostate cancer as our first cancer category to demonstrate the capabilities of CEDAR, and to shed light on the advances of epitope-related prostate cancer research.

Results ––The meta-analysis focused on a subset of data describing epitopes from 8 prostate-specific (PS) antigens. A total of 460 epitopes were associated with these proteins, 187 T cell, 109 B cell, and 271 MHC ligand epitopes. The number of epitopes was not correlated with the length of the protein; however, we found a significant positive correlation between the number of references per specific PS antigen and the number of reported epitopes. Forty-four different class I and 27 class II restrictions were found, with the most epitopes described for HLA-A*02:01 and HLA-DRB1*01:01. Cytokine assays were mostly limited to IFNg assays and a very limited number of tetramer assays were performed. Monoclonal and polyclonal B cell responses were balanced, with the highest number of epitopes studied in ELISA/Western blot assays. Additionally, epitopes were generically described as associated with prostate cancer, with little granularity specifying diseases state. We found that *in vivo* and tumor recognition assays were sparse, and the number of epitopes with annotated B/T cell receptor information were limited. Potential immunodominant regions were identified by the use of the ImmunomeBrowser tool.

Conclusion ––CEDAR provides a comprehensive repository of epitopes related to prostate-specific antigens. This inventory of epitope data with its wealth of searchable T cell, B cell and

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MHC ligand information provides a useful tool for the scientific community. At the same time, we identify significant knowledge gaps that could be addressed by experimental analysis.

Keywords

epitope; CEDAR; database; prostate; T cell; antibodies; cancer; MHC ligand

INTRODUCTION

Despite advances in prostate cancer therapies, prostate cancer (PCa), the most common solid tumor in men, is still the second leading cause of cancer death among American men, only surpassed by lung cancer. According to the NIH, the 5-year survival rate has increased to 96.8% from 2012–2018, but nevertheless, an estimated 268,490 new cases and 34,500 deaths are still expected in 2022[1].

Immunotherapeutic strategies for prostate cancer, a cold tumor with low mutational burden, are being developed and are presenting promising treatment options. Some forms of immunotherapy are already used routinely[2–5], especially for castration-resistant prostate cancer, such as sipuleucel-T and immune checkpoint inhibitors (ICI)[6–8]. Immunotherapies and diagnostic strategies based on defined T cell and B cell epitopes could provide significant improvements. Tumor-associated proteins that are mainly overexpressed in the normal and malignant prostate and that are potentially recognized as targets for tumor specific T cells have been identified[9]. Epitopes from these prostate-specific antigens, which can be recognized by tumor-specific T cells or antibodies, are potential targets for immunotherapy and diagnostics development.

CEDAR (cedar.iedb.org) is a recently developed database that catalogues all cancer epitope-related data extracted from peer reviewed scientific literature[10,11]. The database was initiated in 2021, leveraging experience of nearly 20 years by its sibling database, the Immune Epitope Database and Analysis Resource (IEDB)[12–15]. It captures specific experimental conditions, including host, immunization protocols, disease state, type of assay, and the assay antigen. CEDAR includes epitope-related data for T cell, antibody, and MHC assays, which can aid researchers in developing effective therapeutic approaches. If available, immune receptors (T cell and B cell receptors) and Protein Data Bank (PDB)[16] structures are also captured. The PDB database contains 3D structural data of large biological molecules, usually obtained by X-ray crystallography, NMR spectroscopy, or, increasingly, cryo-electron microscopy, along with the biomolecule's species source, its biological function, and other details. Initial CEDAR curation focused on two prototype categories, neoepitopes and prostate cancer. These two categories cover orthogonally distinct data sources; one derived from well-defined and non-mutated antigens associated with "cold" tumors, and one associated with highly mutated tumors targeted by neoepitopes.

Here, we present a meta-analysis of prostate-associated antigens to illustrate the capabilities, and to identify strengths and weaknesses of the newly created cancer epitope bioinformatics resource, CEDAR. We generated a list of epitopes described in prostate cancer-related references and analyzed eight PS antigens in detail, which account for close to 50% of all epitopes identified.

METHODS

Data inclusion criteria

The analysis included available data for T cell, B cell, and MHC ligand epitopes associated with prostate neoplasms in human and murine hosts. The data are derived from peer-reviewed literature from PubMed. Epitope definitions (length and mass restrictions) and inclusion criteria can be found in the Curation Manual[17] that was developed for the IEDB, and updated to reflect cancer curation rules for CEDAR. For the purpose of this report, epitopes represent the unique molecular structures (minimal sequences, linear and discontinuous regions) experimentally shown to react with a B cell or T cell receptor. Epitopes from 8 prostate-specific antigens were analyzed in detail.

CEDAR queries and analysis

All queries were performed using the CEDAR search interface (cedar.iedb.org)[11]. Search criteria are provided in the figure legends. Search results were exported in Excel format for detailed analysis. Prostate disease queries were performed using the disease finder on the home page. Antigen queries included all data independent of the prostate disease state of the host. Figures and Tables were produced in Excel.

ImmunomeBrowser analysis

The ImmunomeBrowser allows the user to map the results of a CEDAR query onto a reference protein or genome. The response frequency of each epitope is plotted by residue along the entire length of the reference protein. This allows for visualization of regions that are more immunodominant or more frequently studied for a specific response type (CD4, CD8, T cell, B cell), thereby providing a way to analyze and compare the cumulative data. The reference antigens used to compare response patterns were: PSA [P07288], PSMA [Q04609], PAP [P15309], PSCA [O43653]. Full-length proteins were used to accommodate all defined epitopes onto the refence antigen.

Statistical analysis

The statistical analysis of the data was completed in a multi-step process, first by (i) exporting the available information from the ImmunomeBrowser, as mentioned above, for each of the four primary prostate antigens (PSA, PSMA, PAP, PSCA). A CEDAR search was completed for each antigen for the available T cell class I, T cell class II and B cell data in CEDAR, and the resulting data was exported into an Excel format. The amino acid positions and response frequencies (ranging from 0 and 1) were obtained. (ii) Excel was used to compare the response frequencies for each antigen (T cell class I v T cell class II, T cell class I v B cell, T cell class II v B cell). Importantly, amino acid positions where no response frequency was recorded for either pair were removed (i.e., removed blanks) to enable further statistical calculations, and scatter plots of each relationship were created. (iii) The Analysis ToolPak in Excel was used to complete regression analyses for each relationship, and probability (P) values were obtained. (iv) The following website was utilized to calculate the Spearman correlation coefficient reported in the results (https://www.socscistatistics.com/tests/spearman/default2.aspx).

RESULTS

Inventory of published references describing epitopes derived from prostate antigens

Papers describing prostate cancer-associated epitopes were identified applying a curation pipeline developed in the context of the IEDB "sibling" database [12–15]. Briefly, a broad bi-weekly search of the PubMed database[18] and the Protein Data Bank (PDB)[16] identifies papers potentially containing epitope-related information. An automatic text classifier [19,20], periodically retrained on the basis of the outcome of actual paper curations, parses those papers related to infectious disease, autoimmunity, allergy, transplantation or cancer. This high-level classification has categorized 11,675 papers as cancer related papers potentially containing curatable cancer epitope information.

After this initial categorization, each paper is further classified in broad categories, such as the type of infectious agent (e.g., mycobacteria, influenza) or type of autoimmune disease (e.g., multiple sclerosis, diabetes). In the case of cancer papers the further initial classification was based on a general type/source of the cancer antigens, and prostate-associated antigen is one such example. The papers classified as prostate cancer include papers related to prostate cancer epitopes recognized in the context of prostate cancer as a disease state. In addition, the category also captures papers that describe mouse assays, or MHC binding or elution assays, or data obtained from healthy human subjects. The comprehensiveness of the search for prostate cancer-specific epitope papers was further verified by running ad-hoc keyword searches in the over 11,000 papers identified as potentially curatable by the process above.

The scope of this analysis is papers with prostate specificity, and we have not yet curated studies in which the antigens were not prostate-specific, or the study was not associated with solely prostate cancer (e.g., papers that examined MAGE epitopes in mixed breast, colon and prostate cancer patient populations, or papers related to Tn, a B cell antigen commonly expressed in many different tumors including prostate). Curation of these additional studies is ongoing and we plan to report on those as curation of papers related to each antigen is completed. Overall, our analysis is related to a total of 160 identified and curated prostate-specific papers, defined as described above.

Prostate-specific vs prostate-associated epitopes

The 160 papers included epitopes derived from 96 different proteins (Supplemental Table 1). The majority of the papers (99/160, 62%) contained epitopes derived from eight common antigens, corresponding to antigens regularly described in the literature [21–31] as prostate-specific (PS) antigens. As shown in Table 1, these eight PS antigens account for about half (49%) of the total epitopes. The same analysis revealed a large fraction of epitopes are derived from several other tumor antigens (such as for example allikrein-4; or epidermal growth factor receptor (Supplemental Table 1), which were associated with a variety of different tumor types and are by definition not prostate-specific. In conclusion, the analysis revealed that PS antigens account for about half of prostate-associated epitopes. In the following sections, we present a meta-analysis of the PS epitope data contained in these papers.

Inventory of cancer epitopes derived from prostate-specific antigens

We next inventoried the specific epitopes reported in the literature that are derived from the PS antigens. The common names of each antigen are listed in Table 2, which also lists common abbreviations and synonyms. The CEDAR interface allows users to query for the records associated with these antigens by entering either the protein name, abbreviations or synonyms with a simple type recognition function. This function can be accessed by entering the protein name into the antigen field in the 'Epitope Source' box on the CEDAR home page (cedar.iedb.org). The interface contains six main search panels, which allow for the selection of specific criteria. Queries can be performed by protein or epitope of interest, and can be narrowed down to certain subsets of data. Reference proteome accession numbers, overall protein lengths and total curated references related to each antigen are also listed in Table 2.

Querying CEDAR for PS antigens returned a total of 460 epitopes, including T cell, B cell, and MHC binding and elution data, filtering the data to include positive results. PAP, PSA and PSMA were the most represented in terms of epitope numbers. By focusing only on T and B cell records with positive reactivity, a total of 309 epitopes were curated as of December 14, 2022. PAP and PSA were associated with a total of 112 and 62 B cell and T cell epitopes, respectively, and PSMA and PROS with 57 and 42 epitopes, respectively. PSCA and STEAP were associated with 18 and 10 epitopes, respectively, and TRP-P8 and TARP are associated with less than 10 epitopes each.

No correlation was found between protein size and number of epitopes ($r_s = 0.2857$, $p = 0.4927$), while a significant correlation existed between the number of papers published for each antigen and the number of identified epitopes ($r_s = 0.8809$, $p = 0.0039$). These results suggest that the higher number of epitopes are not influenced by size of the protein, but might be influenced by how intensely the specific proteins have been studied.

In terms of host species distribution, most T and B cell epitopes (192 epitopes total) were studied in human systems, but numerous studies were also conducted in murine systems (87 epitopes total). The ratio of epitopes studied in human vs mouse systems varied as a function of antigen considered. A balanced human/mouse representation was observed for PAP and PSA. For all other proteins, most epitopes were defined in human systems.

Functionality of cancer epitopes derived from prostate-specific antigens

In terms of type and functionality of epitopes associated with the various antigens, 109 (35%) were B-cell epitopes, 116 (37%) were class I and 62 (20%) were class II T cell epitopes (Table 2). In relative terms, B vs T cell epitope representation was balanced for PSMA, more B cell epitopes than T cell epitopes were recorded for PROS, and more T cell than B cell epitopes were recorded for all other antigens.

Within T cell epitopes, the ratio of class I and class II epitopes was fairly balanced only in the case of PAP and TARP, and in all other cases the class I epitopes were most numerous, possibly reflective of the relative focus on induction of CD8 responses in the context of cancer immunotherapy (Table 2). In parallel, we examined how many epitopes were associated with either MHC binding or MHC ligand elution data. We found that

epitopes from all prostate cancer-associated proteins were studied in MHC binding assays, and identified in MHC ligand elution assays (Table 2), with the total number of described epitopes varying by more than ten-fold (range 1 for TARP to 63 for STEAP).

MHC restriction of T cell and MHC binding epitopes

In the case of T cell and MHC ligand assays (Table 2), defined MHC restrictions have been reported for 368 of 382 (96%) of the epitopes. A total of 164 T cell epitopes (92% of T cell epitopes) and of 201 MHC binding epitopes (74% of MHC binding epitopes) were associated with one or more defined MHC restrictions. Table 3 shows the number of different MHC class I and class II alleles restrictions identified in T cell, and MHC ligand assays. Because of their prominence as a tool for T cell characterization, the number of MHC alleles for which specific tetramer reagents have been reported is also listed, as a subset of the T cell counts.

Overall, data exist for 71 different MHCs. Consistent with the data presented above, the number of MHC restricting molecules was highest for PAP and PSA. The specific alleles for which restrictions have been described are detailed in Figures 1a, b, which shows how many unique epitopes have been defined for each MHC allele. In terms of T cell assays, most identified PS epitopes were class I restricted with a total of 116 individual epitopes for class I and 62 epitopes for class II (Table 2). Unexpectedly, a very limited number for tetramer assays were described with only 12 class I epitopes and 1 class II epitope with specific serotypic or allelic restrictions (Figures 1a, b). Even though a total of 34 different class I restrictions were recorded for MHC assays, most epitopes were restricted by HLA-A2, HLA-A*02:01 or HLA-A*24:02. The epitopes recorded in T cell assays followed the same pattern with most of the epitopes described for the same three restrictions. In contrast, 19 different class II alleles were identified in MHC assays with the highest number of epitopes described for HLA-DR. For class II allelic restricted T cell assays, HLA-DRB1*01:01 had the highest number of epitopes with 19 epitopes. The MHC molecules for which restricted epitopes are more frequently described are HLA-A*:02:01 or HLA-DRB1*01:01 (Figures 1a, b). This result likely reflects investigation-bias, since these are HLA class I and class II alleles common in Caucasians and highlight the need for a more balanced coverage of HLA polymorphism.

Type of assays associated with T and B cell epitopes

To address the functionality of the epitope-associated responses, we also investigated which type of cytokine release assays were associated with the T cell epitopes from the main four PS antigens. As shown in Table 4, IFNg has been studied most extensively, and is the only cytokine tested for epitopes from PSCA. For PAP, while data is available for several other cytokines, none of these cytokine assay types are well represented. Since a diverse pattern of cytokine production is associated with cancer-specific T cell responses, both in terms of diverse anti-tumor effector responses and potential for negative regulatory and suppressive responses [32–34], this highlights a significant knowledge gap.

B cell assays related to both polyclonal and monoclonal responses are captured. For PSMA, PAP, and PSCA polyclonal responses are relatively more frequently reported in

the literature, with the exception of PSA, where the reverse is true (Table 5). The main assay types utilized were direct binding assays (ELISA and Western blot), followed by antigen inhibition assays. X-ray crystal structures were available only in 5 instances, all related to PSA epitopes.

Thus, in several cases monoclonal responses have been mapped to the epitope level. However, much still needs to be done for further characterizations, such as determining the specific BCR sequences (discussed below), and/or the 3D structure of antibody-epitope complexes. Definition of epitopes recognized by monoclonal responses is also of interest as it represents a prelude for development of CAR-T cell applications.

In vivo assays associated PS epitopes

We specifically queried for epitope assays associated with *in vivo* activity; as a result, these epitope assays records are a subset of those described in Tables 2, 3, and 5. Unexpectedly, PS antigens are not intensely studied in *in vivo* contexts (Table 6). Only 13 T cell assays were curated, including assays that measure decreased disease, tumor burden, or survival.

Only two proteins had epitopes that were described in a tumor model measuring decreased disease. For PSMA, 4 assays were described and for PSA 1 assay was captured (Table 6). Likewise, only four proteins had epitopes that were described in a tumor model measuring tumor burden. For PAP, 3 assays were described, while for PSMA and for PSCA only one assay was captured (Table 6). In addition, two assays were captured for STEAP. Finally, one PAP epitope assay was described related to survival. The lack of in vivo data might be a result of the limited number of mouse prostate tumor models. In addition to several xenograft models for prostate cancer, the TRAMP model was the only genetically engineered tumor model used in the curated publications.

Assays associated with recognition of cancer cell lines

Assays demonstrating that a particular response can recognize and kill cancer cells and/or primary tumor cells are of particular significance in cancer immunology. Using CEDAR to analyze the number of T cell epitopes that are described in the killing of tumor cells returned 62 assays for the top 4 PS antigens (Table 7). In addition, 4 IFNg and 1 proliferation T cell assay in which the antigen was a tumor cell, were also curated. For the remaining 4 PS proteins, a total of 23 IFNg assays with the antigen being a tumor cell were recorded. In addition, a low number of cytotoxicity, TNFa, IP-10, GM-CSF, and MIG assays were recorded. We also identified several B cell assays that tested a tumor cell as the antigen, however, these B cell assays were only associated with PSMA (3 assays measuring decreased disease, and one each of antibody mediated cytotoxicity, immunostaining, and immunohistochemistry).

It should be noted that in CEDAR, querying for specific assay antigens is not yet available. However, it is possible to export results for cytotoxicity assays that target tumor cells, by downloading the data for all cytotoxicity assays into an excel spreadsheet, and identifying the assays which used tumor cells as antigen.

In conclusion, these results show that the PS proteins are only studied in a very limited number of assays that involve tumor cells as antigens. This highlights a significant knowledge gap for the prostate cancer community.

Sequences of PS T cell and B cell receptors, and structural data

If available, the CEDAR database curates the specific immune receptors, T cell receptors (TCRs) and B cell receptors (BRCs), associated with the curated PS-derived epitopes[35]. Only one TCR and three BCR sequences are currently available in the curated data (Table 8). All of the described immune receptors are for PSA. This represents a significant knowledge gap, also in light of the fact that thousands of TCR and BCR sequences are available for other more in depth characterized antigens.

As mentioned above, like for the sibling database IEDB, CEDAR also captures PDB structures when available and links out to the PDB database[36]. Unexpectedly, very few structures were available at the time of analysis. Only five PDB structures were recorded, four for B cell epitopes from PSA, and one for a B cell epitope from STEAP (Table S2). No T cell epitope structures were reported at the time of this analysis.

Type of prostate pathology associated with the epitopes from prostate-specific antigens

For each of the studies from which the epitope data is curated, CEDAR will record the particular type of cancer described in the study, following disease ontology (DO)[37–39], which includes the following prostate diseases: prostate cancer, experimental prostate cancer, prostate adenocarcinoma, castration-resistant prostate carcinoma, benign prostate phyllodes tumor, lymphoepithelioma-like acinar prostate adenocarcinoma, prostatitis, and prostate carcinoma. Records associated with each type of pathology are searchable using the ‘Immune Exposure’ box located on the left-hand side of the result page. In addition, disease stage descriptions are also captured by the curation process, although they are currently not directly searchable in CEDAR.

Considering either number of epitopes or number of assays (Table 9), most of the epitope-related studies were associated with was a generic “prostate cancer” designation and experimental prostate cancer. The second most frequent pathology description found in the studies was prostate adenocarcinoma. A smaller but still appreciable number of records was associated with castration-resistant prostate carcinoma. Much fewer records were associated with other pathology designations.

In conclusion, these data suggest that the vast majority of records are designated as being related to generic prostate cancer, highlighting a potential need to define epitope repertoires in other pathology designations.

Epitope distribution as revealed by ImmunomeBrowser analysis

In the next series of investigations, we probed the distribution of the epitopes along the sequence of the various PS antigens, using the ImmunomeBrowser tool[40] which can be accessed by clicking on the ImmunomeBrowser icon (bar graph) in the ‘Antigens’ tab on the results page. The tool computes the number of assays and epitopes associated with each

amino acid position along the antigen sequence and visualizes these counts in a graphic format. The user can select the specific type of epitope (all epitope, B only, class II, human only, etc.). Here, we considered the PSA, PAP and PSMA antigens, which were associated with the largest numbers of epitopes, thus enabling a meaningful analysis of epitope density.

In the case of PSA (Figure 2a), the highest class I reactivity is centered around amino acid (AA) positions 152–197, with lesser frequency found from AA 65–74 and 240–258. For class II, four main positive regions were found (AA 68–102, 115–144, 169–210, 221–258) (Figure 2b). Finally, in the case of antibody epitopes response patterns were distributed along the entire antigen (Figure 2c).

A similar analysis of the epitope distribution of PAP revealed class I reactivity was observed over several regions along the protein with the highest response seen in the region between AA 198–222 (Figure 2d). Class II epitopes were widespread, with most immunogenic areas located between AA 111–213, 228–251, and 351–367 (Figure 2e). Antibody reactivity was also mapped to several regions, with the main reactive stretch mapping to residues 213–222, which overlaps with the 198–222 area of highest class I reactivity (Figure 2f).

Finally, for PSMA we found short stretches of class I reactivity, with two dominant regions located at the C terminus (AA 3–10 and 26–35) and N terminus (AA 624–633 and 701–718) (Figure 2g). Class II reactivity is not extensively studied, and six short regions with low response frequency were identified, (Figure 2h). The antibody reactivity was associated with three prominent regions (AA 235–288, 310–332, and 624–632, with the latter overlapping with a high frequency class I region (Figure 2i).

The significance of correlations between different types of reactivity was addressed by performing a Spearman correlation analysis, plotting the response frequencies associated with each residue position with different epitope types (class I, class II, B cell) for all 4 main prostate specific antigens (Figure 3). While no significant correlations were observed for PMSA, a significant correlation was observed for all reactivity types for PSA. In addition, significant correlation was seen for class I and class II for PAP. A weak negative correlation between T cell class I and antibody epitopes were also observed. In conclusion, the ImmunomeBrowser analysis identifies several discrete immunodominant regions, of potential interest for further studies.

DISCUSSION

We performed this metanalysis to catalog epitope data related to prostate cancer-specific proteins, and raise awareness of the newly developed Cancer Epitope Database and Analysis Resource (CEDAR). As of December 14, 2022, we curated 160 (97%) of identified prostate cancer epitope-associated publications that were identified by our classifier. We present an in-depth analysis of T cell, B cell, and MHC binging data for identified epitopes from top 8 literature identified PS antigens (PAP, PSA, PSMA. PROS, PSCA, STEAP, Trp-p8, and TARP). Herein we report that all 8 proteins are well represented in CEDAR, with a total of 460 positive epitopes. The number of individual epitopes per protein varied, and we showed that the variation was not dependent on protein length. However, we found a

positive correlation between the number of published papers for each protein and the number of identified epitopes. One hundred and fifteen PAP epitopes were described in 51 papers, the highest number of all 8 prostate specific antigens. In contrast, only 6 epitopes were identified in 5 references for TARP.

A relatively low number of epitope-specific in vivo assays, including decreased disease, tumor burden, and survival were published for the analyzed PS proteins. In addition, assays which measure the killing of prostate tumor cells were also sparse. That lack of data could be explained by the lack of suitable genetic prostate cancer mouse models, with most of the in vivo studies performed in xenograft models. Only one genetic mouse model was used in the study of prostate specific antigens, the transgenic adenocarcinoma of the mouse prostate model (TRAMP)[41], in which mice spontaneously develop prostate tumors, thereby closely reflecting human prostate cancer.

Another reason for the scarcity of *in vivo* experiments with defined epitopes is that for one of the most relevant human PS antigens, PSA, no mouse counterpart has been identified, and that the mouse prostate differs from the human prostate significantly[42]. While the human prostate is one singular gland with several zones, the mouse prostate consists of anterior, dorsal, ventral, and lateral lobes. This lack of *in vivo* data for epitopes of PS antigens reflects a potential gap in prostate cancer research.

Of the 8 main PS antigens, PSA is a secreted protein[43], PAP can be detected intracellularly and secreted[44], five are transmembrane (PSMA, Pros, PSCA, STEAP, Trp-p8)[27–29,45,46], and one is a cytoplasmic protein (TARP)[31]. PSA and PAP, the only two secreted PS proteins, account for the highest number of epitopes, potentially reflective of the relative ease of experimental work with soluble secreted proteins.

A special feature of CEDAR is that epitope-specific TCR and BCR sequences are captured and are easily accessed. Unfortunately, the data is very limited with only one of the 8 PS antigens, PSA, associated with epitopes with described TCR (1 TCRs) and BCR (3 BCRs). This lack of receptor data associated with PS antigens clearly shows that much needs to be done to identify prostate cancer-associated immune receptors.

We used the disease finder search capability in CEDAR to evaluate the number of epitopes related to prostate-specific diseases; for example, prostate cancer, experimental prostate cancer, prostate adenocarcinoma, castration-resistant prostate carcinoma, benign prostate phyllodes tumor, lymphoepithelioma-like acinar prostate adenocarcinoma, prostatitis, and prostate carcinoma. As expected, the highest number of T cell and B cell epitopes and assays were described for prostate cancer, a parent node of the prostate cancer categories. This is followed by experimental prostate cancer, castration-resistant prostate carcinoma and prostate adenocarcinoma for T cell and B cell epitopes respectively. The total number of MHC ligand epitopes was highest for experimental prostate cancer, however, most of these epitopes were not subject to further evaluation in T cell or B cell assays.

We took advantage of the ImmunomeBrowser, a tool that aggregates and visualizes immunological data for a protein of interest[40]. The data, represented as calculated response frequencies, are displayed as tables and figures. The response frequencies are plotted against

a reference proteome, thereby calculating and visualizing the data along the entire epitope source protein. Of the three PS proteins we analyzed, we found several areas of interest in PSA. Performing Spearman analysis, we found correlations of class I, class II and antibody responses frequencies for PSA. In addition, a correlation was seen for class I and class II response frequencies for PAP. A weak negative correlation between T cell class I and antibody epitopes were seen for PSCA. The ImmunomeBrowser offers a valuable tool for identifying immunodominant regions of interest for potential antibody or T cell vaccine design.

Overall, we presented a meta-analysis of epitopes of PS antigens by using the newly established CEDAR database. We showed that CEDAR contains epitope-specific data for all 8 literature-identified PS antigens. Even though there are several gaps, including a lack of *in vivo* data, the curated data provides the scientific community with a wealth of searchable information and identified immunodominant regions for the most prominent antigens, which have potential implications for vaccine strategies. We also realize that several limitations exist in this report. Namely, both the design of CEDAR and curation efforts are ongoing and thereby the analysis results are likely to evolve over time. In that respect, we will appreciate any specific feedback on any paper containing PS data that was overlooked, and general feedback on the database structure and functionality, as these feedback sources are of great value to improve the CEDAR resource content and usability. You can submit your feedback via email to cedar@lji.org.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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ABBREVIATIONS

PS	prostate-specific
PAP	Prostatic acid phosphatase
PSA	Prostate-specific antigen
PSMA	Prostate-specific membrane antigen
PROS	Prostein (Solute carrier family 45 member 3)
PSCA	Prostate stem cell antigen
STEAP	Metalloreductase STEAP1
Trp-p8	Transient receptor potential cation channel subfamily M member 8
TARP	TCR gamma alternate reading frame protein

MHCLE	MHC ligand elution
BCR	B-cell receptor
TCR	T-cell receptor
SPR	surface plasmon resonance
IHC	immunohistochemistry

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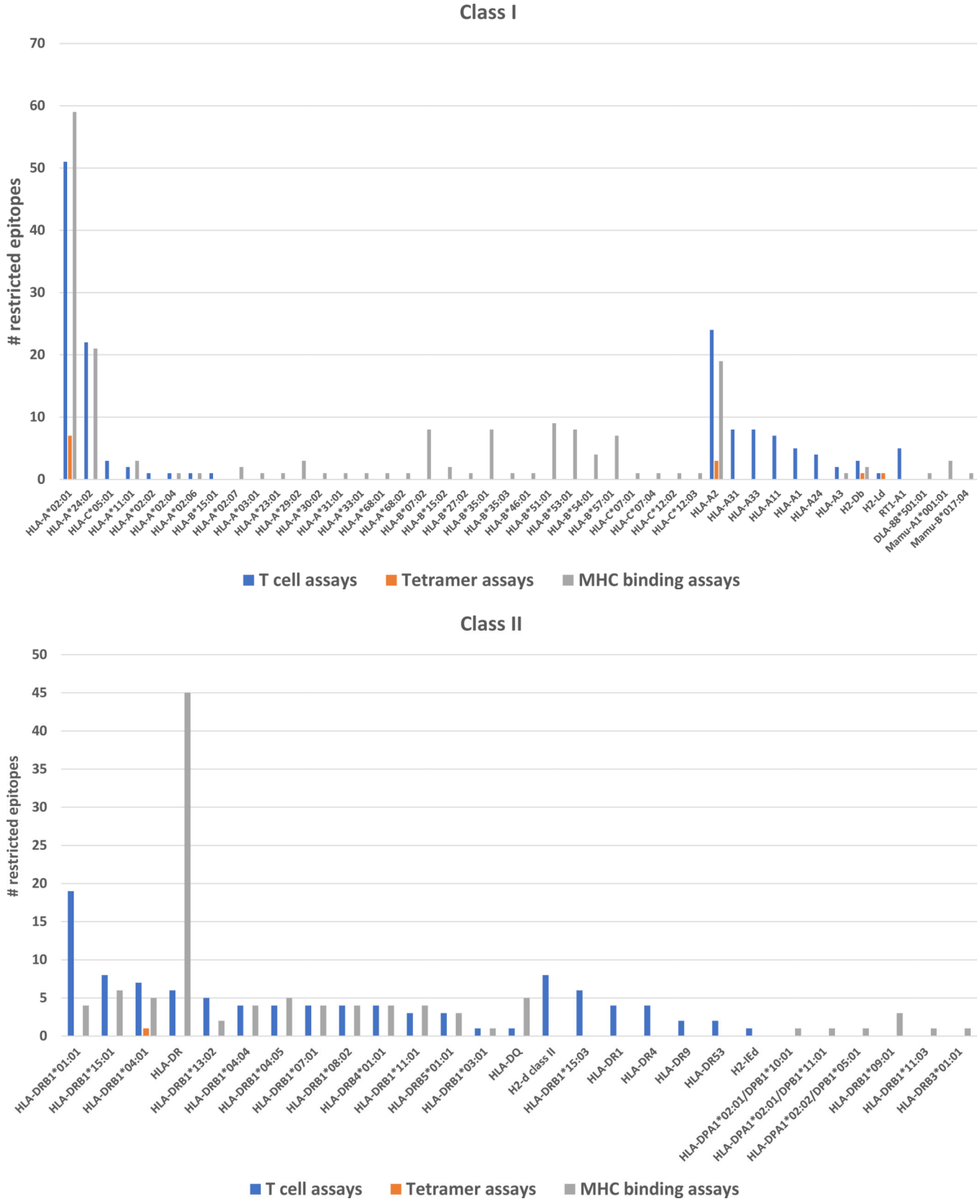
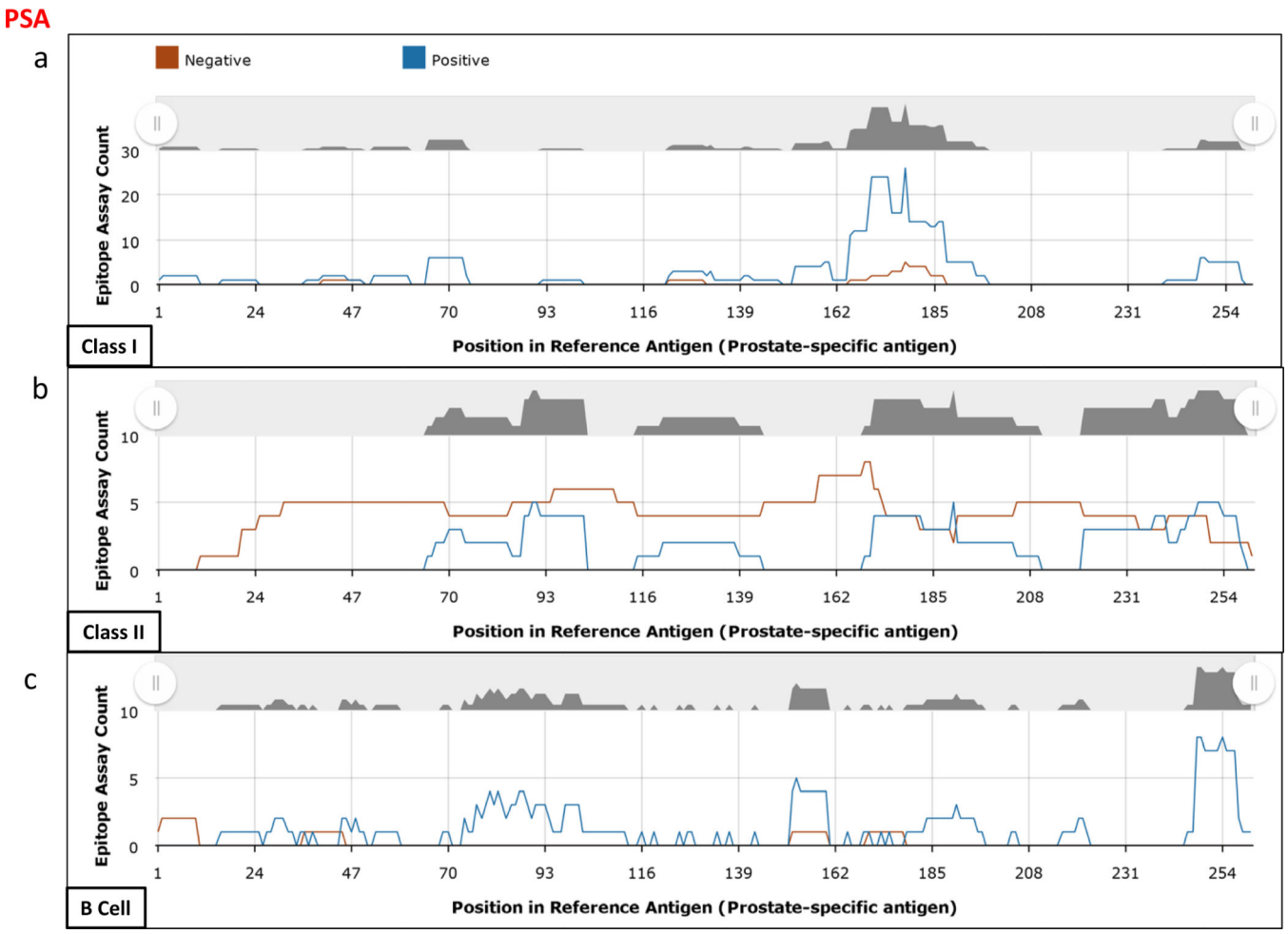
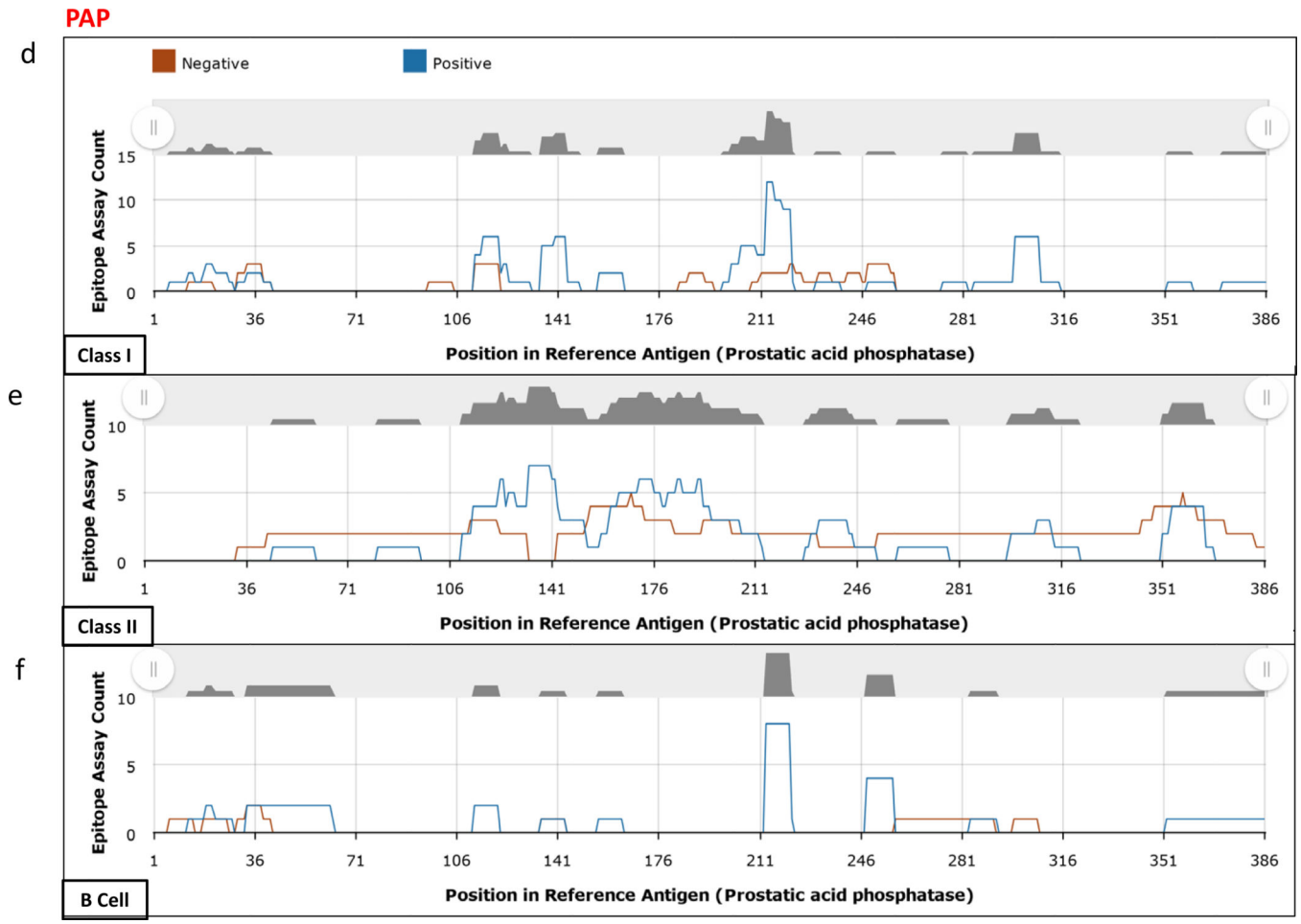


Figure 1. Defined class I and class II restrictions of epitopes from prostate-specific antigens

Allelic and serotypic restricted epitopes were compiled for all (A) 44 class I and (B) 27 class II molecules. Included are data for human, mouse, rat, rhesus macaque and canine class I and human and mouse class II restrictions. Number of epitopes per restriction is depicted.





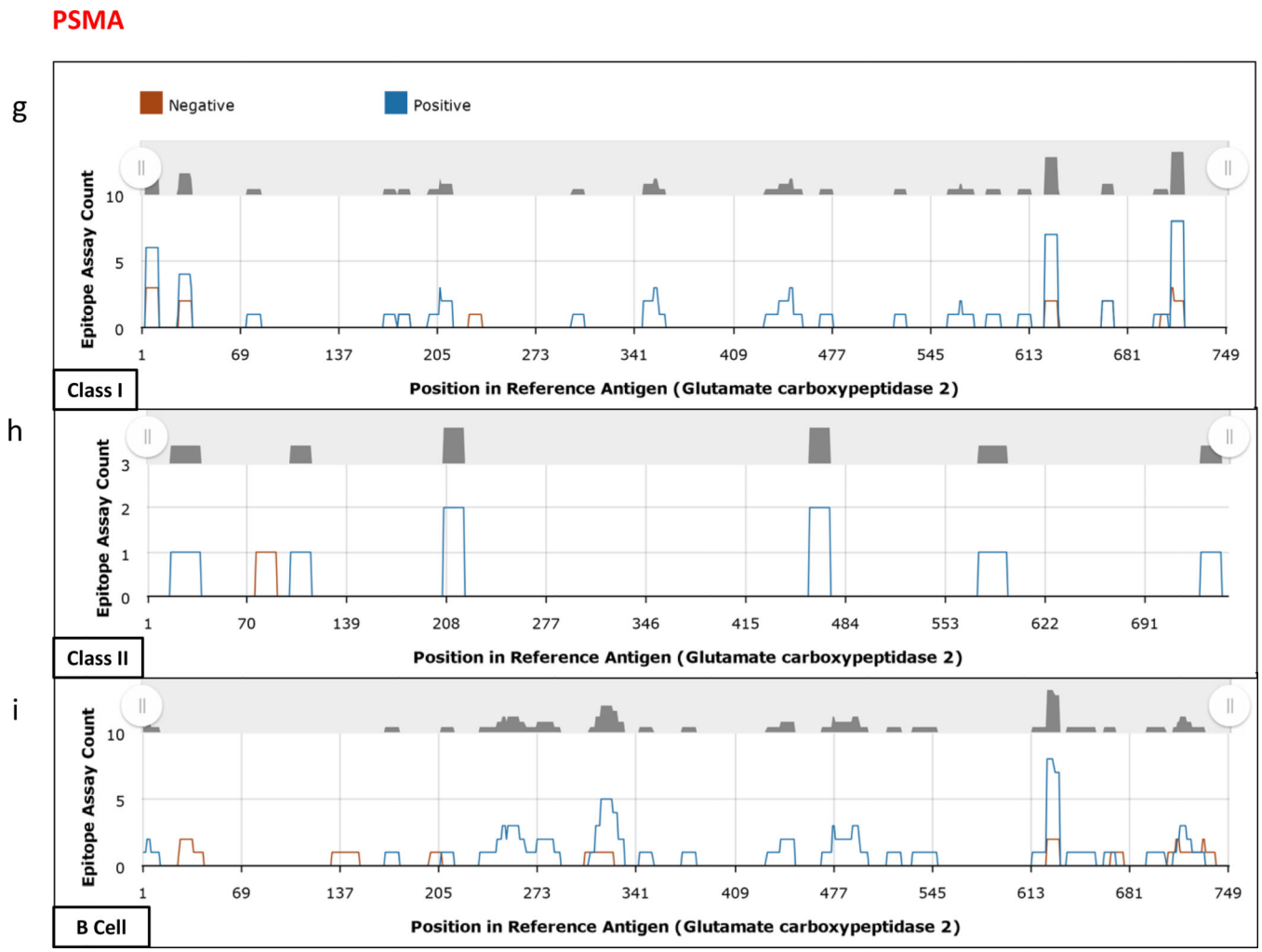


Figure 2. Immunodominance patterns

The line plot shows the number of positive (blue) and negative (red) assays or number of responder and not-responder subjects along the positions in reference protein. Depicted are ImmunomeBrowser results for class I (top), class II (middle) and antibody assays (bottom). Immunodominance patterns are shown for PSA (a-c), PAP (d-f), and PSMA (g-i).

A

Protein	Spearman Coefficient (P-value)		
	Class I vs Class II	Class I vs Ab	Class II vs Ab
PSA	**0.382 (p=1.04E-06)***	**0.245 (p=0.0094)***	**0.216 (p=0.0075)***
PSMA	-0.045 (p=0.7269)	-0.086 (p=0.2859)	-0.009 (p=0.9497)
PAP	**0.211 (p=0.0029)***	0.019 (p=0.2118)	-0.131 (p=0.1005)
PSCA	N/A	-0.378 (p=0.00083)	N/A

B

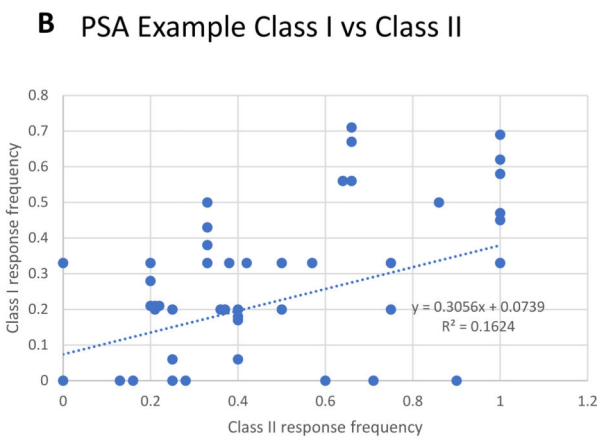


Figure 3. Correlation between response frequency of different reactivity types

A) Spearman coefficient (*p-value*) was calculated between the response frequency of class I, class II and antibody epitopes of the 4 PS antigens; PSA, PSMA, PAP, and PSCA. B) Example plot for significant correlation between class I and class II epitopes from PSA. *Correlations were calculated using https://www.socscistatistics.com/tests/spearman/default2.aspx

Table 1.

Epitopes identified in papers categorized as prostate cancer related.

Number of individual epitopes and references identified for PS and non-PS proteins.

Type of antigen	Number of epitopes	Percent total epitopes
Prostate-specific antigens	275	49
Non-prostate-specific antigens	287	51
All antigens	562	100

Table 2.
Inventory of epitopes from prostate-specific antigens

Epitope data for 8 prostate-specific antigens found in CEDAR. The number of epitopes is the sum of epitopes identified in the human, and if available, mouse and rat proteins. The number of refences displays the number of curated publications that include epitopes from the individual prostate-specific antigens. Response type (antibody and/or T cell, MHC binding, MHC ligand elution) are further parsed to show the total number of individual epitopes per assay type. Total epitopes are the sum of all individual epitopes that were identified. Host human/mouse refers to the individual epitopes tested in either human or mouse systems. The data include positive reactivities only.

Protein	Abbreviation	Species	Proteome Accession	Protein Lengths	References	Epitope Number	Epitopes[2]							
						Grand Total	T & B cell	Host Human	Host Mouse	B Cell	T Cell	T Cell Class I	T Cell Class II	MHC Bindi
Prostatic acid phosphatase	PAP; PAcP	human, mouse, rat	P15309, Q8CE08, A0A0G2K4B4	386 381 381	51	115	112	35	32	11	61	28	33	29
Prostate-specific antigen	PSA, KLK3	human	P07288	261	64	75	62	35	34	19	47	30	19	23
Prostate-specific membrane antigen	PSMA	human, mouse	Q04609 O35409	750 752	55	86	57	49	13	33	32	25	6	26
Prostein (Solute carrier family 45 member 3)	PROS [3]	human, mouse, rat	Q96JT2 Q8K0H7 D3ZPP5	553 553 564	23	71	42	42	0	38	4	4	0	6
Prostate stem cell antigen	PSCA	human	O43653	114	9	21	18	18	1	7	17	17	0	12
Metalloreductase STEAP1	STEAP	human, mouse	Q9UHE8 Q9CWR7	339 339	26	73	10	6	6	1	9	7	2	2
Transient receptor potential cation channel subfamily M member 8	Trp-p8	human, mouse	Q7Z2W7 Q8R4D5	1104 1104	16	13	3	2	1	0	3	2	0	5
TCR gamma alternate reading frame protein	TARP	human	A2JGV3.1	58	5	6	5	5	0	0	5	3	2	3
Total						460	309	192	87	109	178	116	62	106

Table 3.

Inventory of T cell assays with class I and class II restrictions of epitopes from prostate-specific antigens

CEDAR was queried for T cell (including tetramer), tetramer, and MHC ligand (MHC binding and MHCLE) assays for epitopes with defined restrictions from PS antigens. “Other” refers to the combined numbers for PSCA, STEAP, Trp-p8, and TARP.

	Antigen Name	Parent Protein	Class I Alleles	Class II Alleles
T cell assays with restriction [1]	PSA	P07288	11	16
	PSMA	Q04609	9	3
	PAP	P15309	12	13
	PSCA	O43653	4	0
	Other[3]		3	6
Tetramer	PSA	P07288	4	1
	PSMA	Q04609	1	0
	PAP	P15309	2	0
	PSCA	O43653	0	0
	Other		0	0
MHC ligand [2]	PSA	P07288	15	14
	PSMA	Q04609	13	1
	PAP	P15309	13	14
	PSCA	O43653	1	0
	Other		13	6

[1] Includes tetramer assays

[2] Includes MHC binding assays and MHC ligand elution (MHCLE) assays

[3] Includes numbers for PSCA, STEAP, Trp-p8, and TARP

Table 4.
Distribution of cytokine assay types of T cell epitopes by antigen

Number of cytokine response assays of epimiddlees from prostate-specific antigens are listed. “Other” refers to the combined numbers for PSCA, STEAP, Trp-p8, and TARP. *Note: The total number of assays can be greater than the number of epimiddlees as there are multiple assays for each cytokine (e.g., ELISA, ELISPOT, ICS, etc.).*

Protein	IFNg	TNFa	IL-10	IL-12	IL-13	IL-2	IL-4	IL-5	IP-10	MIG	GM-CSF	GrB
PSA	151	5	1	3	2	4	5	5				1
PSMA	62					2						
PAP	179	1				1						2
PSCA	30											
Other [1]	23	5							1	1	5	

[1] Includes numbers for PSCA, STEAP, Trp-p8, and TARP

Table 5.
Distribution of assay types of B cell epitopes by antigen

Number of antibody response assays of epimiddlees from prostate-specific antigens are listed. “Other” refers to the combined numbers for PSCA, STEAP, Trp-p8, and TARP. *Note: The total number of assays can be greater than the number of epitopes.*

Protein (epitopes)	Monoclonal Response	Polyclonal Response	ELISA/ Western	Antigen Inhibition	Mass Spec/ SPR[2]	Immuno Staining	IHC[3]	Micro Array	X-ray Crystallography	Electron microscopy	Biological Activity[4]	Other binding[5]	I
PSA *(19)*	44	18	32	4	11				5		4	6	
PSMA *(33)*	27	39	36	3	6	1	1	12			1	3	
PAP *(11)*	4	28	17	2		1		2				10	
PSCA*(7)*	0	8	8										
Other[1] *(39)*	2	65	6					59		1		1	

[1] Includes numbers for PSCA, STEAP, Trp-p8, and TARP

[2] SPR: surface plasom resonance

[3] IHC: immunohistochemistry

[4] Antibody-dependent cellular cytotoxicity, antibody activity inhibition

[5] Binding assay, cross blocking, phage display, chromatography

Table 6.

In vivo T cell assays

Four prostate specific antigens were queried for three different types of in vivo T cell responses; decreased disease, tumor burden, survival. Number of assays are shown. “Other” refers to the combined numbers for PSCA, STEAP, Trp-p8, and TARP.

Protein	Decreased Disease	Tumor Burden	Survival
PSA	1	1	
PSMA	4	1	
PAP		3	1
Other [1]		2	

[1] Includes numbers for PSCA, STEAP, Trp-p8, and TARP

Table 7.

Assays with tumor cells as antigen

Four PS antigens were queried for all assay with antigen type “tumor cell”. A) Numbers and types of T cell assays are shown. B) Numbers and types of B cell assays are shown. *The only protein with positive B cell assays was PSMA.*

A	Protein	Total T cell Assays	Tumor Burden (in vivo)	Cytotoxicity	Proliferation	IFNg	TNFa	IP-10	GM-CSF	MIG
	PSA	22		21	1					
	PSMA	8		6		2				
	PAP	26	2	24						
	PSCA	13		11		2				
	Other [1]	45	2	8		23	5	1	5	1

B	Protein	Total B Cell Assays	Decreased Disease (in vivo)	Cytotoxicity	Immunostaining	Immuno-histochemistry
	PSMA	6	3	1	1	1

[1] Includes numbers for PSCA, STEAP, Trp-p8, and TARP

Table 8.
Receptor data captured in CEDAR for prostate-specific antigens

Epimiddlees from prostate-specific antigens with defined BCR and TCR sequences are listed. PSA is the only prostate specific antigen with curated receptor sequences. The TCR is of human origin, the BRCs from mouse.

TCR sequences

Epimiddlee	Antigen	Antigen	Species	Response Type	MHC Allele	Type	Chain 1 CDR3	Chain 2 CDR3
KLQCVDLHV	PSA	P07288	Homo sapiens	T cell	HLA-A* 02:01	αβ	CAVREEDYKLSF	ASSFRGPNLYTEAF

BCR sequences

Epimiddlee	Antigen	Antigen	Species	Response Type	Type	Chain 1 CDR3	Chain 2 CDR3
H98, P99, L100, Y101, D116, H119, T143, K191, K194, F195, H248, Y249, R250, K251, W252, K254	PSA	P07288	Mus musculus	B cell	HL	ARADYGFNSGEAMDY	QQSNEDPYT
I25, W29, E30, C31, E32, K33, S35, Q36, W38, H82, D134, K137, Y153, K169, K170, C173, D175, G204, G205, N220, G221, V222	PSA	P07288	Mus musculus	B cell	HL	ARDGYRYYFDY	MQHLEYPVT
R45, G46, A48, N69, K70, L74, R77, L80, P83, G87, Q88, V89, Q91, V92, S93, H94, R125, S127, E128, F165	PSA	P07288	Mus musculus	B cell	HL	ARSGRLYFDV	QQTHEDPYT

Table 9.

Total number of epimiddlees and assays by prostate disease

CEDAR was queried for different prostate diseases. The number of T cell, B cell, and MHC binding assays are shown *(left)*. The table contains the number of epimiddlees per assays type recoded for each prostate disease *(right)*.

Disease State	Assay Type				Number of Epimiddlees			
	T Cell	B Cell	MHC	Total	T Cell	B Cell	MHC	Total
Prostate cancer	666	432	0	1098	196	223	0	343
Experimental prostate cancer	3	4	127	134	1	1	122	124
Prostate adenocarcinoma	78	45	0	123	31	45	0	62
Castration-resistant prostate carcinoma	126	126	0	252	46	36	0	53
Benign prostate phyllodes tumor	0	6	0	6	0	6	0	6
Lymphoepithelioma-like acinar prostate adenocarcinoma	0	6	0	6	0	6	0	6
Prostatitis	54	0	0	54	4	0	0	4
Prostate carcinoma	4	0	0	4	1	0	0	1