Repurposing of Known Drugs as Potential Therapeutics for Cancer Immunotherapy for Patients with Solid Tumors: Modeling Small Molecule Interactions with PD-1 Binding Sites

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Abstract: Small molecule inhibition of T cell immune checkpoints for cancer immunotherapy has seen a recent increase of attention after the discovery of CTLA-4 and programmed death protein, PD-1. As of now, no small molecules have been approved for treatment although many compounds are going through clinical trials. This paper uses public web tools to discover potential small molecules to inhibit the PD-1/PD-L1 signaling pathway. Computational arrays and programs are used to find the most suitable target for binding, and ZINC databases, as well as PocketQuery, are used to find potential small molecules. Finally, SwissDocking and SwissADME are used to discover the molecule with the highest probability of success. This research enables future discussion of found ZINC compounds as well as potential modification to existing compounds, creating small molecule inhibitors.

Keywords: Immunotherapy, PD1/PD-L1, small molecule Inhibition, computational arrays.

1. Introduction

Based on data collected from 2015 to 2017, roughly 39.5% of people will contract cancer sometime during their life span [1]. With 559,601 mortalities in just 2019, cancer is the second-highest cause of death in the United States, closely following heart disease at 659,041 mortalities [2]. Cancer is a potentially fatal disease that hinders the body's normal cell division and programmed death mechanism [3]. In broad terms, cancer cells ignore orders from the body to die and they reproduce at an abnormal rate without instructions from nearby cells [3]. This unprecedented increase of cells in a specific location of the body leads to the creation of tumors, inflated masses of tissues that can be benign (non-cancerous) or malignant (cancerous) [3]-[5]. Additionally, through a process classified as metastasis, cancer cells can travel through the blood and/or lymph system to other locations in the body [4]-[6]. Moving away from the primary cancer (beginning location of the cancer cells), these erratic cancer cells can form metastatic tumors (tumors that form away from the primary cancer) [4]. These tumors can lead to dangerous complications in many locations of the body, resulting in inhibited organ function, blocked blood flow, and potentially death [4], [6], [7]. Given the prominence of cancer throughout history, many treatment methods have evolved into the modern day of medicine [8], [9].

Throughout the 19th and 20th centuries, surgery and radiation were the faces of cancer treatment [9], [10]. Surgery treatment simply aimed to eliminate the entire portion of the body which contained apparent cancer

cells, usually by means of removing tumors [11]. This treatment method saw success in specific cancers such as breast cancer but struggled to eliminate all the damaged cells from a metastasized cancer [11]. Radiation therapy, on the other hand, utilizes ionizing radiation, which forms electrically charged particles, depositing energy into the cells covered [12]. The energy will damage deoxyribonucleic acid (DNA) in the cancer cells, inhibiting their ability to proliferate and causing further damage in the body. It was not until 1960, when survival rates fluctuated around 33% due to unpredicted micrometastases, that chemotherapy started getting attention in the medical community [10], [12]. Although creating a balance between rigorous surgical procedures and radiation treatment, chemotherapy still did prevent the issue of healthy body cells being eliminated along with cancer cells (Fig. 1) [10], [12].

Moving into the modern day of cancer treatment, immunotherapy has been of keen interest since the mid-1900's when a regression of solid tumors in experimental mice was observed after inducing chemicals into the tumors [13]. In a broad sense, Immunotherapy is a cancer treatment method that interactively modifies an immune system to better target cancer cells in the body (Fig. 1) [14]. By stimulating the immune cells in a cancer patient, their own body can readily identify passing cancer cells as well as kill solid and/or metastasized tumors (Fig. 1) [14].

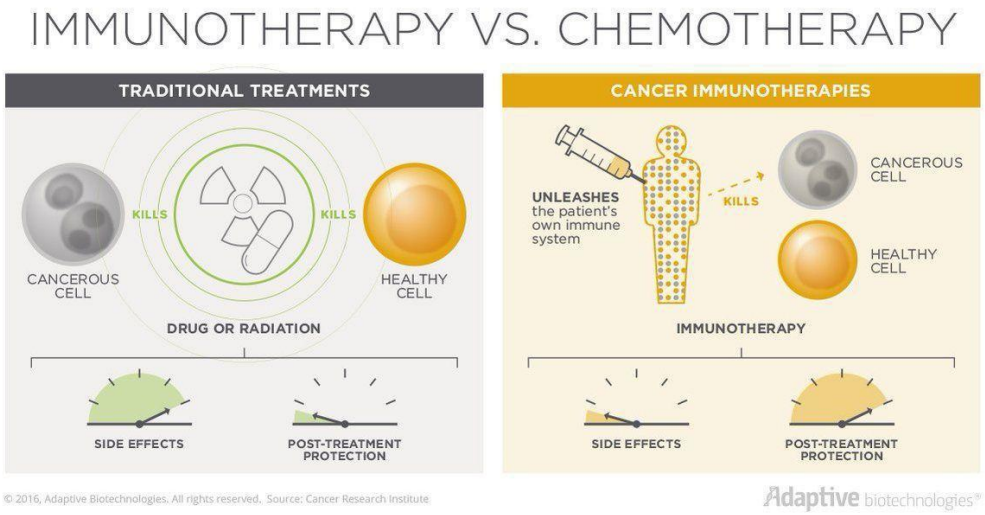


Fig. 1. Diagram displaying the differences between cancer treatment methods chemotherapy and immunotherapy.

Although being a possibility of cancer treatment for over a century prior to the aforementioned experiment, no developments were sought out and immunology wasn't heavily studied [13], [15], [16]. However, by the '70s and '80s, countless clinical trials arose experimenting with antibodies that would bind to cancerous tumors in patients' serum [13]. Success in immunotherapy first began by investigation of Cytokines for treatment in breast cancer, renal cell cancer, lymphoma, glioblastoma, and melanoma [13]. Cytokines are soluble proteins secreted by some immune system cells and they make up the fourth major class of intercellular signaling molecules [16], [17]. These proteins carry the important role of building and/or reconstructing tissues throughout the entire body [17]. By the time immunology research began on these proteins, the only identified cytokines were interferon-alpha (1957), and interleukin 2 (1976), or IL-2 for short [14]. IL-2 was then cloned in 1983 to be put through many clinical trials [14]. The immunotherapy found great success in shrinking tumors by way of enhancing lymphocytes T production in the immune system [13], [14]. Thus, IL-2 was approved as an immunotherapeutic treatment in 1991 for metastatic kidney cancer and in 1998 for metastatic melanoma [13], [14]. Around the same time, studies regarding recombinant

interferon-alpha proved the cytokine a useful immunoregulatory, bringing metastasized tumor response rates of 10% to 20% in conducted trials [16], [18]. Thus, in 1996, IFN-a was approved as an immunotherapeutic treatment for stage IIB/III melanoma [16], [18]. Moving into more modern-day research, the 1980's also brought attention to cytotoxic T-lymphocyte antigen 4 (CTLA-4), a protein receptor on the surface of T cells [15], [19], [20]. CTLA-4 is an activation molecule for T cells, inhibiting T cell immune responses by binding to molecules of the B7-family [19]-[21]. Acknowledging this new information, immunologists sought out anti-bodies to inhibit CTLA-4 T cells that could detect and kill cancer cells without being blocked [15]. Antibodies were developed for this case, specifically ipilimumab and tremelimumab, and success has been shown in clinical stages [20]-[22]. Another well-researched immune checkpoint/T cell inhibitor that has gained interest recently is the programmed death cell PD-1 [15], [20]-[22]. However, to better understand CTLA-4 and PD-1 and their potential for cancer treatment, an understanding of the role of immune checkpoints is necessary.

Immune checkpoints (examples include CTLA-4 and PD-1) are immune cell surface receptors that control and/or inhibit the activation of cellular immune responses [18], [23]-[25]. These immune checkpoints are key for T cell success as cellular immune responses protect the development of primary or metastatic tumors [18], [23]-[25]. For immune checkpoints to activate T cell responses against cancer, CD28 binding with CD80 or CD86 expressed by APCs/Dendritic cells (Fig. 2) [23]. Additionally, a recognition of a peptide antigen through MHC must be shown for T cells to gain cytotoxic status and secrete effector molecules such as IFN-y or IL-2 (Fig 2). Cytotoxicity refers to T cells actively releasing granzymes and perforin, both of which disrupt tumor cells' plasma membranes leading to their death [23]. IFN-y also helps in reducing tumor cell population by increased expression of MHC class I and interrupted tumor cell multiplication [23].

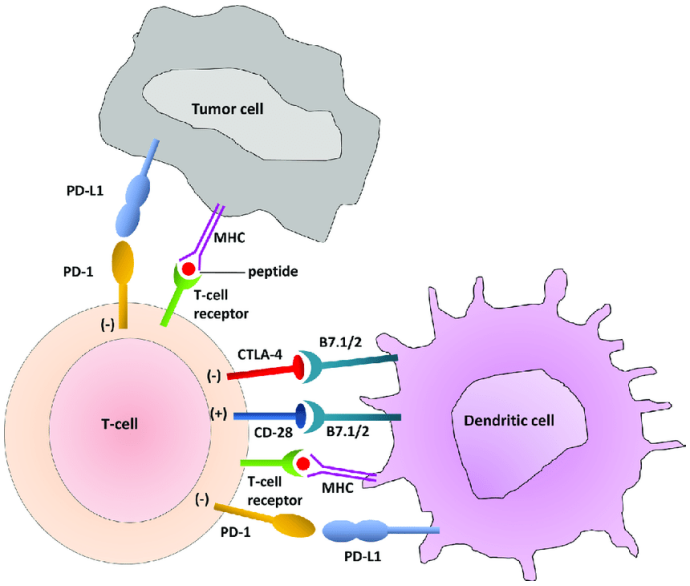


Fig. 2. Diagram displaying exhausted T-cell after PD-1/PD-L1 interaction from tumor cell.

Given the significance of T-cell cytotoxicity in tumor regression, considerable research has been done analyzing the few immune checkpoints we have discovered as of now [23]-[25]. One of these checkpoints, being PD-1, has been proved as a key inhibitor of T cell activation through PD-1 ligand expression on tumor cells [23]-[25]. To better understand PD-1's interference on T cell activation, we need to fully understand the protein itself.

PD-1, also known as programmed death-1, is a protein member of the CD28/B7 family which helps activate and inhibit T cell immune responses [26], [27]. The cell receptor can be commonly found on T regulatory cells

(T reg or Tregs) and $CD4^+$ and $CD8^+$ T cells [28]. The extracellular domain of the protein is made up of two sheets connected by a disulfide bridge [28]. These sheets contain a total of seven β strands and have an lg-like fold, like other proteins in the immune system [28]. One function of the PD-1 protein lies in its regulation of Tregs [29], [30]. Tregs are a subpopulation of $CD4^+$ T cells which inhibit T cell immunity in case of severe activation of nearby T cells [30]. Studies have shown the PD-L1 ligand increases Foxp3 expression and strengthens the immune homeostasis and self-tolerance role of Treg cells [30]. Additionally, through the use of Akt, mTOR, ERK2, and PTEN, PD-L1 can convert normal $CD4^+$ cells into Treg cells [30]. In addition to PD-1's role in immunological tolerance, the protein plays a part in many circumstances such as infection, autoimmune diseases, and peripheral tolerance [30]. However, as a widely studied immune checkpoint, the PD-1/PD-L1 checkpoint has been shown to regulate the activation of T cells against proliferating tumor cells [26]-[31]. When T cells are activated in autoimmune responses, they express PD-1 on their surface and induce PD-L1 responses from nearby tissues [26], [29], [31]. The binding between PD-1 and its ligands PD-L1 and PD-L2 prevents the T cell from exercising its cytotoxicity on cells containing such ligands. Thus, in a healthy autoimmune system, the PD-1/PD-L1 pathway controls the damage done by activated T cells and keeps immune tolerance to self-antigens [26], [29], [31]. However, in many cancers, tumor cells have consistently carried PD-L1 ligands for interaction with the immune system's T cells [26], [29], [31]. Interaction between a tumor's PD-L1 ligand and the T-cell's PD-1 cell receptor inhibits the immune response directed by T-cells, leaving tumor cells safe from cytotoxicity and death [26], [29], [31]. Tumor cells displaying both a PD-1 ligand to interact with T-cell PD-1 and an MHC peptide to interact with T-cell receptors are successful in inhibiting T-cell function [26]-[31]. Given the severity of this inhibition, extensive research has been done on blocking the PD-1/PD-L1 signaling pathway between tumor and T cells [26]-[31].

Recently, scientists have been studying the use of monoclonal antibodies to interrupt the PD-1/PD-L1 signaling pathway between the two cells [32]-[38]. Monoclonal antibodies are man-made antibodies that bind to the same antigen as antibodies already in your immune system [33]. First arising in 1975, the earliest method for creating monoclonal antibodies was through the cloning of a single B-lymphocyte known as hybridoma technology [33]-[34]. These B-lymphocytes are fused with immortal myeloma cells lacking any immunoglobin-producing cells and the HGPRT gene. The hybridomas, or the fusion between primary lymphocytes and myeloma cells, create a mixture of polyclonal antibodies [33]. After identifying the necessary antibodies for medical use, the highlighted polyclonal antibodies are cloned and tested for use [33]. Over time, methods have been improved for generating the necessary antibodies for medical use, and ideas such as phage display through the conversion of human mRNA to cDNA have risen to popularity [32]-[34]. These antibodies bind to antigens located on different targeted cells, inhibiting, or boosting their roles in the immune system [32]-[34]. This process has found success in countering many diseases such as cancer, chronic inflammatory diseases, cardiovascular diseases, infectious diseases, and transplantation [33]. By 2014, 30 monoclonal antibodies were approved as therapeutics in the medical world and that number has risen in the modern-day [33]. Following the discovery of immune checkpoints such as CTLA-4 and PD-1, countless antibodies were put on trial to inhibit protein function, and success was quickly shown [32], [35]. Now, due to already approved cancer-treating antibodies such as ipilimumab that inhibit CTLA-4 function, attention is quickly turning to discover monoclonal antibodies to inhibit the function of the programmed death protein PD-1 [32]. However, the production and use of antibodies do not come out without its drawbacks [36]-[38]. Through clinical testing of antibody use against multiple cancers, there have been countless common symptoms: fatigue, rash, nausea, diarrhea, pruritus, and many more [37], [38]. Additionally, antibodies come with a hefty price tag for their production and use [36]-[38]. While the materials needed to create antibodies are not expensive, the process can take up to two weeks, requiring an absurd amount of money to run [38]. In just 2014, antibody sales were expected to increase to 166 billion dollars, making for 30% of the

prescription market [38].

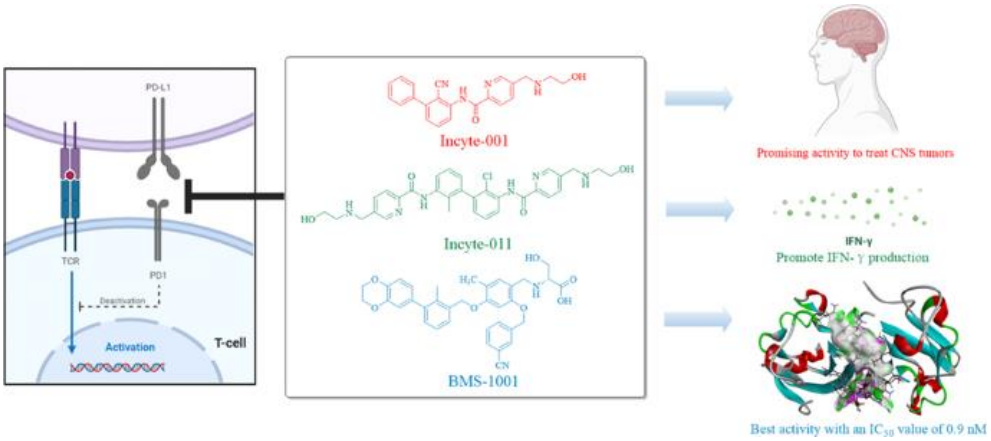


Fig. 3. Diagram displaying current research on the use of small molecules to stop the binding of the PD-1/PD-L1 signaling pathway.

Regarding the unavoidable challenges face using antibodies for cancer treatment, immunologists have turned their attention to other methods to block protein to protein interaction [39]-[43]. One of these methods, which has shown potential for successful cancer therapeutics in clinical trials, is the use of small molecules to inhibit the binding between T cells and tumor cells (Fig. 3) [39]-[43]. Small molecules in cancer therapeutics come with many distinct advantages such as oral bioavailability, greater penetration of tumor micro-environments, and easily controllable dosing to avoid pharmacodynamic challenges [39], [40]. However, the greatest benefit of the use of small molecules in the world of cancer therapeutics, lies in its cost-efficient production and distribution [39], [40]. Unlike antibodies, which take up a majority of medical revenues, small molecules are cheap to produce due to the nature of the goods themselves [39], [40]. This is crucial to the medical world as stable and accessible cancer treatment can open unimaginable possibilities in the future [39], [40]. So far, many pathways have been clinically tested and success with small molecule inhibition has been evident [41]-[43]. However, immunologists have struggled to advance small molecule research on the PD-1/PD-L1 signaling pathway because of challenges brought by the hydrophobic interface [41]. Given the benefits of introducing small molecules to inhibit PD-1/PD-L1 function, many compounds have been tested and success has been shown in a few small molecules such as BMS-1001 and BMS-1166 [42]. Immunologists discovered these small molecules by first judging the toxicity of all BMS compounds by exposing them each to modified T cells for around 48 hours [42]. After BMS-1001 and BMS-1166 were found to be the least toxic of the molecule family, their effects on PD-1/PD-L1 were tested through exposure to activated T cells with the presence of human sPD-L1 [42]. The results of the experiment showed a strong potential for BMS-1001 and BMS-1166 to likely inhibit the PD-1/PD-L1 signaling pathway. Additionally, small molecules such as BMS-57 and BMS-71 were tested towards PD-1/PD-L1 inhibition through NMR-titration, differential scanning fluorimetry, and cell-based Blockade Bioassay [42]. Another promising small molecule for the inhibition of the immune checkpoint is AUNP-12 [43]. Binding assays have shown AUNP-12 to successfully inhibit PD-1 and PD-L2 interaction for a stable 24 hours, and animal experiments have started to show promise for PD-1/PD-L1 inhibition [43]. Furthermore, a team of scientists in 2020 created a compound (named 2k in their research summaries) through the amination of isolated aldehydes with sodium cyanoborohydride [44]. The compound was shown the most promising out of the many different compounds tested, and the researchers argue it could successfully inhibit the PD-1/PD-L1 signaling pathway. Despite the research efforts arising in the past few years, no small molecules have been approved for cancer

immunotherapy to this day (some are going through clinical trials) [41]-[42], [45]. The purpose of my experiment is to identify potential small molecules to bind to PD-1/PD-L1 binding sites to inhibit tumor cell binding. My experiment will first identify the most probable target (PD-1 or PD-L1) for small molecule inhibition, and then identify compounds using a variety of technological tools. The overarching goal of my experiment is to find small molecules to interfere with PD-1 and PD-L1 binding for cancer therapeutics.

2. Methodology and Experiment Results

To identify small molecules which can inhibit PD-1/PD-L1 binding, the prime protein to push further research on had to be determined. Thus, the first experiment conducted was using three different methods: geometric, energy-based, and machine-learning-based, to discover which protein had the most binding sites for small molecule binding. The first tool used for this research experiment was an online tool named Protein*Plus.* After submitting the protein codes of PD-1 and PD-L1 to the site's search box, the results were calculated and shown below (Fig. 4, Fig. 5). This website located these sites geometrically by scanning the protein through a grid and applying a difference of Gaussian (DoG) filter [46]. This will highlight portions of the protein where spherical objects can potentially fit [46]. Then, through combinations of these sub pockets, binding sites are predicted and shown above [46]. In the protein PD-1, the algorithm found four different binding sites along the surface. However, in the protein PD-L1, the algorithm found 11 binding sites in total, showing a higher probability for small molecule binding.

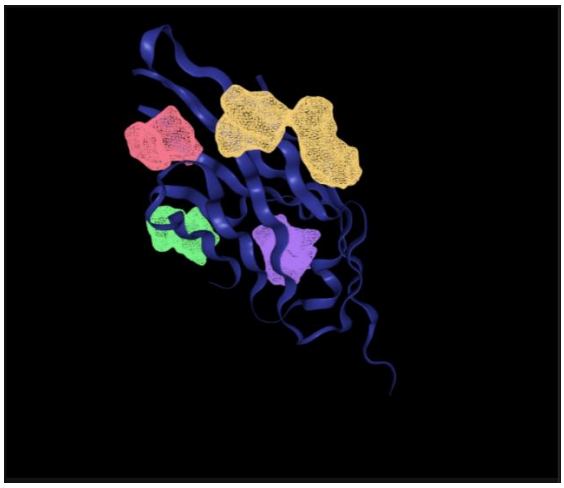


Fig. 4. Blue string model representing detected ligand binding sites (colored sections) in the protein PD-1 when run by the website Protein*Plus.*

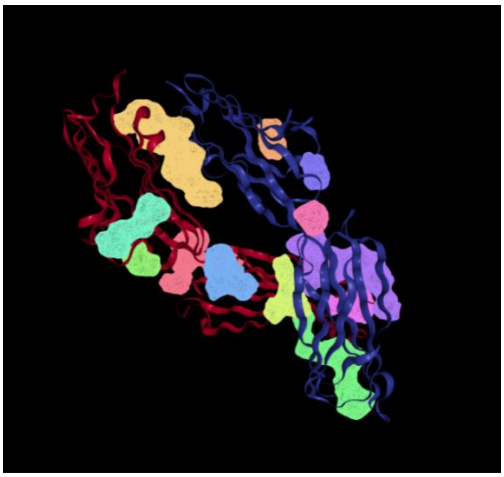


Fig. 5. Blue String model representing detected ligand binding sites (colored sections) in the protein PD-L1 when run by the website Protein*Plus*.

The results of the first tool, Protein*Plus,* showed a significantly greater binding site count in PD-L1 over PD-1, highlighting the protein as the better target for further experiments. However, in case of inaccuracy due to the geometric method, two other tools were used to highlight the ligand-binding sites on both PD-1 and PD-L1. The second tool accessed was a website named FtSite. The information displayed in the models below was found through FTSite's energy mapping algorithm (Fig. 6, Fig. 7) [47]. To identify potential binding sites and interactive amino acids, 16 different molecular probes find positions on a densely gridded protein through empirical free energy functions [47]. Then, the individual probes are clustered together and ranked by average free energy [47]. These probe clusters can then overlap, and cluster groups are ranked by the number of interactions between the protein and the cluster's probes [47]. This method found a total of three different binding sites on the protein PD-1, and they are displayed belong along with the contacted amino

acids. The method also found three binding sites on the surface of protein PD-L1, the equivalent of that on PD-1.

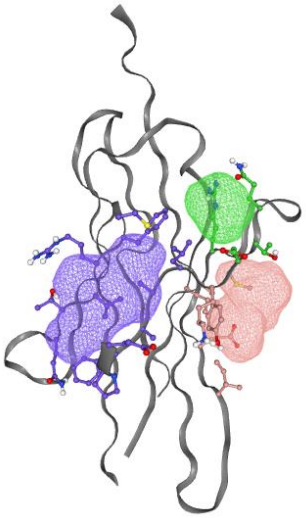


Fig. 6. String model representing detected ligand binding sites (colored sections) in the protein PD-1 when run by the website FTSite.

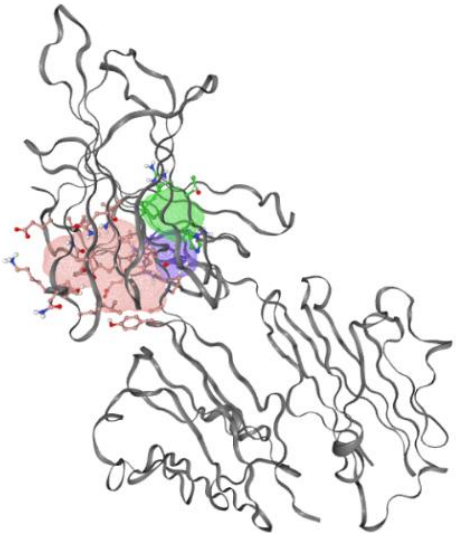


Fig. 7. String model representing detected ligand binding sites (colored sections) in the protein PD-L1 when run by the website FTSite.

Despite the geometric method showing a significant number more ligand binding sites on PD-L1 over PD-1, the energy-based method conducted by the FTSite showed an equal number of binding sites between the two proteins. The final tool used to discern the best protein for small molecule inhibition was a website named PrankWeb. This website generated predictions through a well-trained machine learning algorithm that finds the most relevant ligands in each protein [48]. After receiving an inputted protein, an algorithm ranks the importance of ligands based on several conditions taught to the machine-learning algorithm [48]. Narrowing down the results of the protein, the PrankWeb algorithm found one binding site (blue section) in the PD-1 protein (Fig. 8). However, the PrankWeb algorithm found two potential ligand-binding sites (blue and red section) in the PD-L1 protein (Fig. 9).

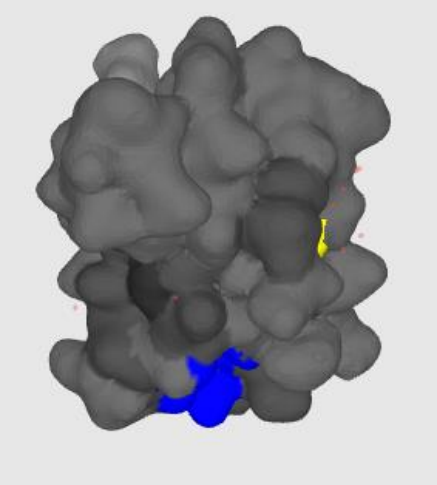


Fig. 8. LiteMol and Protael generated visualization of PD-1 protein and its ligand binding sites found by the PrankWeb algorithm.

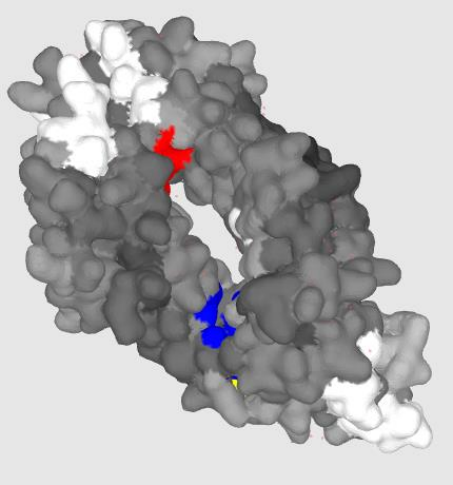


Fig. 9. LiteMol and Protael generated visualization of PD-L1 protein and its ligand binding sites found by the PrankWeb algorithm.

The results of the three tools show that the protein PD-L1 has a greater chance of small molecule inhibition, as the experiment showed a general trend of the protein containing more predicted ligand binding sites than that of PD-1. Thus, the next step of the experiment is to evaluate the binding interaction of PD-L1 ligands and find suitable small molecules to inhibit its function.

After identifying the programmed death cell ligand PD-L1 as more probable for small molecule inhibition, the next step was to discover potential small molecules for further testing. To do this, a web tool named PocketQuery (http://pocketquery.csb.pitt.edu) was used to collect PD-1/PD-L1 interaction clusters with a high "drug" score: an estimate of small molecule interfering in the connection [49]. A score of one signifies a 100% probability of small inhibition and a score of 0 signifies a 0% chance. PocketQuery was developed to explore PPI (protein-protein interaction) and generate cluster residues that can be exported to pharmacophore mapping [49]. The PD-1/PD-L1 protein complex was submitted to the website through its protein code, 3BIK. The website, which contains a list and analysis of every cluster residue for PDB coded proteins, then generated all the clusters updated for the PD-1/PD-L1 complex. The list was sorted by draggability score and the five clusters with the highest score were selected for pharmacophore mapping (Table 1).

Table 1. Table of Selected PD-1/PD-L1 Interaction Clusters Generated by the PocketQuery Web Tool

Cluster	# Of Amino Acids	Drug Score
1	2	0.788639
2	1	0.787095
3	3	0.74594
4	2	0.737558
5	1	0.73537

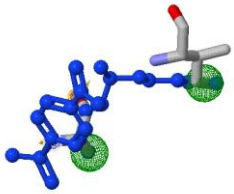
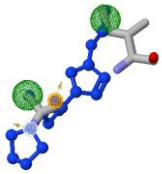
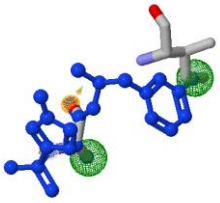
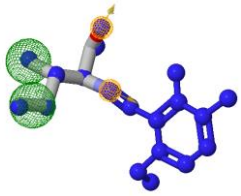
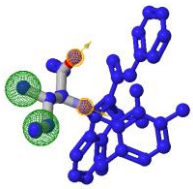
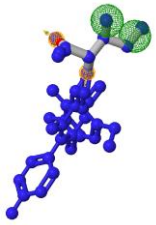
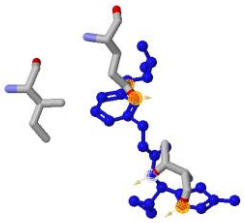
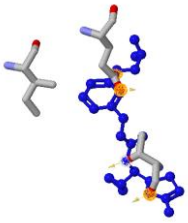
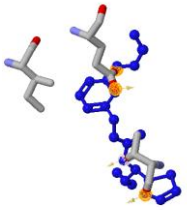
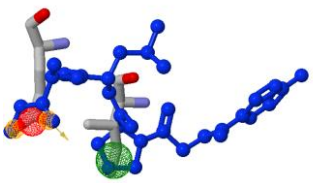
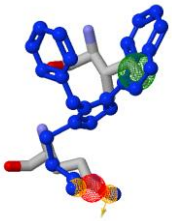
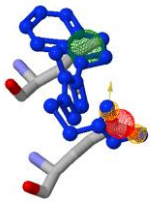
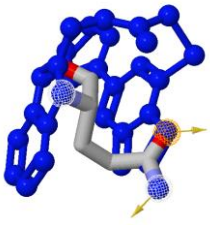
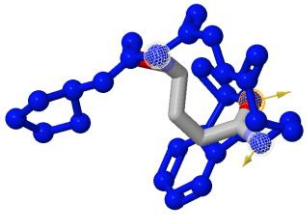
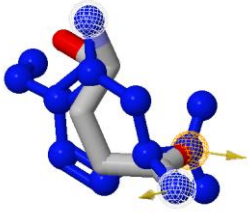
After selecting the top five cluster residues, each cluster was exported to ZINCPharmer, a web tool designed to search for potential small molecules to interfere with protein-protein interaction [50]. Once exported to the web tool, a pharmacophore map is shown, displaying the binding ligand to be blocked and the cluster residues collected from the PocketQuery tool. A tab on the website allows for the selection of specific pharmacophore features which can be selected and/or deselected in the case of a lack of found compounds. After clicking the submit query button on the site, the tool begins to generate a list of found ZINC compounds for the corresponding ligand and pharmacophore classes [50]. ZINCPharmer discovers potential compounds by searching through the constantly updated ZINC database and matching them to interaction pharmacophores on the ligand structure [50]. After all the compounds are discovered and verified, a list will be formed on the web tool, and tabs are available to sort the compounds. Like the PocketQuery drug score, the ZINCPharmer measures the potential of each compound for inhibition with a score (0 represents the highest probability and 1 represents the lowest probability). The top three compounds for each PocketQuery cluster were selected and a graph of the results is shown below (Table 2).

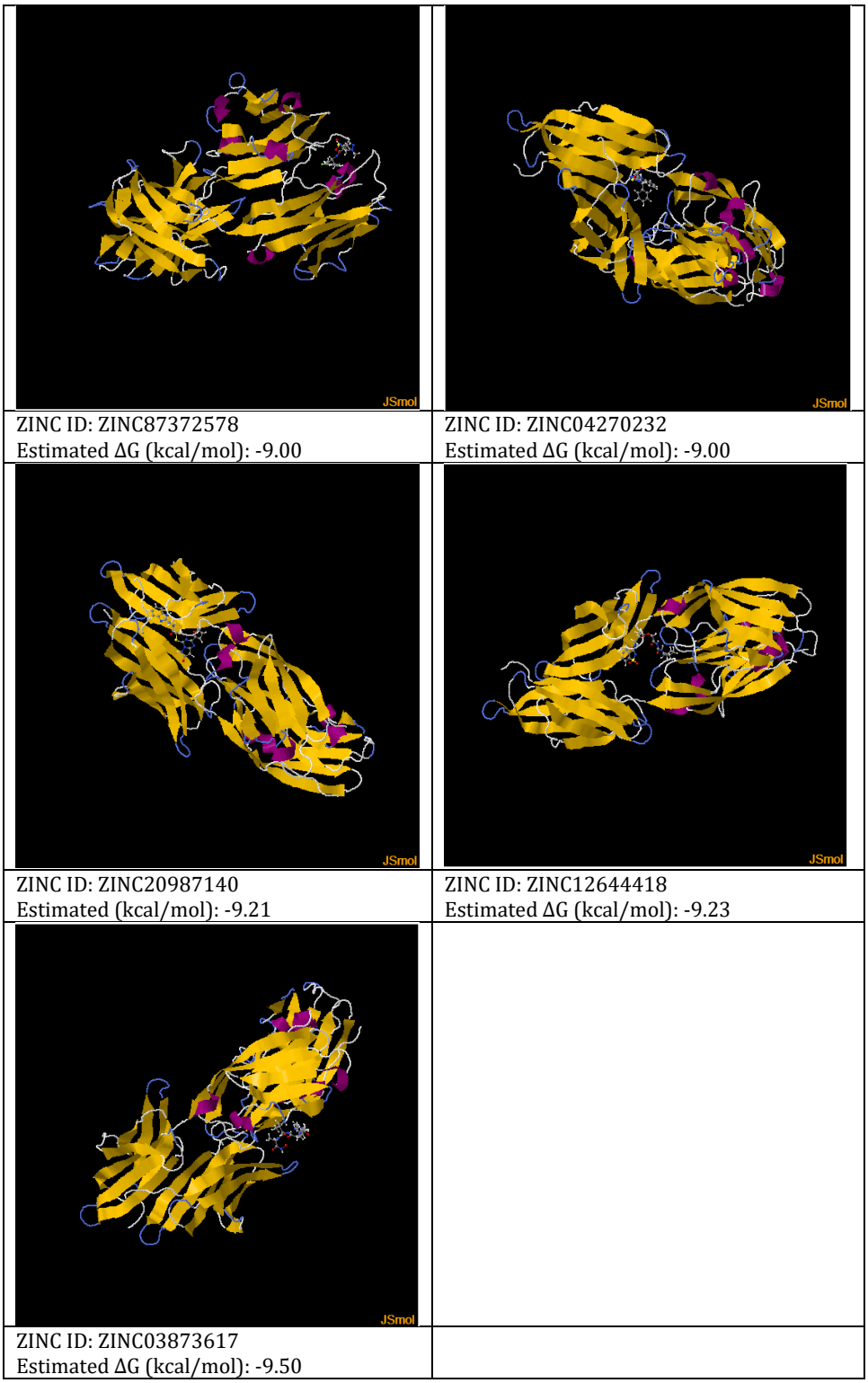
The figure above displays the top three ZINC compounds organized by score for each cluster residue generated by the PocketQuery tool. The gray sticks represent the protein-ligand, and the blue sticks represent the ZINC compounds to bind with the ligands. The results of this experiment are 15 of the lowest scoring ZINC compounds for potential small molecule inhibition of specific PD-1/PD-L1 protein complexes.

After discovering 15 potential ZINC compounds for small molecule inhibition, they were further tested to narrow down the 5 most probable interactions. To do this, a web tool named SwissDock was used to analyze the potential of each compound successfully interacting with PD-L1. SwissDock is a website created to dock

inputted small molecules onto inputted proteins [51]. For many purposes such as selecting amino acids to interrupt protein-protein interaction, obtaining molecular probes, and investigating molecule to protein interaction in detail, SwissDock has been crucial in a variety of protein research experiments [51]. Using the website's simple interface, the PD-L1 protein file was uploaded along with each of the 15 ZINC compounds chosen in the pocket query experiment. After submitting all the jobs to the web server, the results were collected, and the top five ZINC compounds were selected using the website's analysis (Fig. 10).

Table 2. Table Displaying All Selected ZINC Compounds Generated by the ZINCPharmer Web Tool for Each PocketQuery Cluster Residue

Cluster	Compound	Compound	Compound
1	 ZINC80297148 (0.063)	 ZINC91605650 (0.101)	 ZINC87372578 (0.109)
2	 ZINC33915444 (0.057)	 ZINC68561613 (0.066)	 ZINC20358184 (0.067)
3	 ZINC94057711 (0.257)	 ZINC94057978 (0.257)	 ZINC94057871 (0.258)
4	 ZINC03873617 (0.251)	 ZINC04270232 (0.255)	 ZINC04270227 (0.256)
5	 ZINC20987140 (0.28)	 ZINC12644418 (0.32)	 ZINC83411188 (0.35)



ZINC ID: ZINC87372578 Estimated ΔG (kcal/mol): -9.00	ZINC ID: ZINC04270232 Estimated ΔG (kcal/mol): -9.00
ZINC ID: ZINC20987140 Estimated (kcal/mol): -9.21	ZINC ID: ZINC12644418 Estimated ΔG (kcal/mol): -9.23
ZINC ID: ZINC03873617 Estimated ΔG (kcal/mol): -9.50	

Fig. 10. Combined image displaying the top five ZINC compounds interacting with the PD-L1 protein along with the molecule ID and the estimated energy at the location of interaction.

The five chosen ZINC compounds were selected by the estimated energy of the interaction between the compound (ball and stick group) and the PD-L1 protein-ligand (yellow string). The estimated energy displayed by the website represents the amount of interaction between the submitted molecule and the submitted protein [51]. Thus, this number represents the likelihood of the molecule successfully interacting and inhibiting with the protein (the more negative the number, the greater the interaction) [51]. SwissDock

generates these energy estimates through a process of steps similar to that of EADockDSS, another docking program available for use [51]. First, the website generates around 5000 to 15000 predicted binding modes [51]. Then using a CHARMM force field and 32 computing nodes, the binding modes are calculated for their CHARMM energies [51]. The most favorable of these binding modes are selected and displayed in a table for the user to analyze. After sorting each of the 15 submitted ZINC compounds, the algorithm found their energies and the five compounds chosen were as such: ZINC87372578 (ΔG:-9.00), ZINC04270232 (ΔG:-9.00), ZINC20987140 (ΔG:-9.21), ZINC12644418 (ΔG:-9.23), ZINC03873617 (ΔG:-9.50). With these final five compounds, the last step is to experiment with which molecule best interacts and inhibits the PD-1/PD-L1 protein-protein interaction.

To find the best molecule for inhibiting PD-1/PD-L1 function, the five ZINC compounds from the SwissDock experiment were analyzed for their compatibility using a set of rules named Lipinski's rule. Lipinski's rule is a commonly used method to determine the solubility and permeability of a compound designed by a team of researchers led by Christopher A. Lipinski [52]. In short, Lipinski's team discovered five parameters to determine if a drug would have success in the medical world, deemed the "rule of 5": less than 5 H-bond donors, a molecular weight less than 500, a Log P under a score of 5, and less than 10 H-bond acceptors [52]. This conditional set was used to test the top 5 ZINC compounds found from the SwissDock experiment (ZINC87372578, ZINC04270232, ZINC20987140, ZINC12644418, ZINC03873617) through a web tool named SwissADME. SwissADME utilizes countless *in silico* (computer-based/computational scanning) methods to determine the ADME parameters (Absorption, Distribution, Metabolism, and Excretion) of a compound and the calculations for the five given ZINC compounds are shown below (Table 3) [53].

Table 3. Table Displaying the SwissADME Calculated Values of Each SwissDock ZINC Compound for Lipinski's Drug Success Rule of Five

ZINC ID	# Of Hydrogen Bond Donors	Calculated LogP	Molecular Mass	# Of Hydrogen Bond Acceptors	# Of Violations
ZINC87372578	2	2.47	368.43 g/mol	6	0
ZINC04270232	3	2.08	379.44 g/mol	4	0
ZINC20987140	3	1.97	421.43 g/mol	6	0
ZINC12644418	3	2.39	436.48 g/mol	7	0
ZINC03873617	6	2.33	490.55 g/mol	6	1

The results of the SwissADME web tool show that four of the five submitted ZINC compounds (ZINC87372578, ZINC04270232, ZINC20987140, ZINC12644418) had a total of 0 violations for Lipinski's rule of five. However, ZINC compound ZINC03873617 had six hydrogen bond donors, violating Lipinski's rule of having less than five hydrogen bond donors. Thus, ZINC03873617, although having the highest energy score from the SwissDock experiment, will not be as efficient as the other compounds for drug solubility and permeability. Next, to decide on the most probable molecule out of the four ZINC compounds for PD-1/PD-L1 inhibition, the compound with the highest energy was selected as the best potential small molecule drug. In this case, ZINC12644418, with an estimated ΔG (kcal/mol) of -9.23 and zero violations of Lipinski's rule of five is the best potential small molecule inhibitor of the PD-1/PD-L1 signaling pathway.

3. Conclusion

In conclusion, this experiment has introduced a potential small molecule, ZINC compound ZINC12644418, for inhibition of the PD-1/PD-L1 signaling pathway. Through a set of computer-based web tools, ZINC12644418 was found as the best candidate for binding to PD-L1 and solubility and permeability as a drug. Additional clinical testing should be conducted on ZINC12644418 as this experiment followed the usage of strictly free, online web tools. Given the chance of inaccuracy of computer technology as well as unknown errors in the used tools, methods can be introduced to verify the potential of ZINC12644418 as a small

molecule inhibitor. Additionally, this experiment found other small molecules which can successfully inhibit the PD-1/PD-L1 complex with minimal adjustments. For example, ZINC03873617 was found to have the highest estimated ΔG (kcal/mol) of -9.50, showing a higher interaction with the PD-L1 ligand. However, the molecule was found to have violated one of Lipinski's rules by having more than five hydrogen bond donors. If future experiments could remove a couple of hydrogen bond donors from the molecule, a very probably small molecule for inhibition would be discovered. Overall, this work will enable future research in a couple of discovered small molecules.

Conflict of Interest

The author declares no conflict of interest.

Author Contributions

AH designed and carried out the experiments. AH recorded the data and analyzed the results. AH wrote the manuscript draft. AH supervised the study; all authors had approved of the final version.

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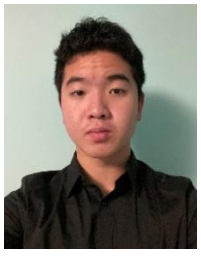

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