Computational approach for the discovery of small molecule inhibitors of the PD-1/PD-L1 immune checkpoint in dendritic cell-based vaccines for improved cancer immunotherapies

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INTRODUCTION

Immunotherapy plays a significant role in the treatment of particularly severe tumours, demonstrating remarkable benefits over traditional chemotherapy. Immunotherapy using dendritic cell (DC)-based vaccination is a promising approach due to the capability of DCs to activate cytotoxic T-cells, thereby boosting antitumour responses. Nevertheless, clinical responses have been disappointing, with very limited classic objective tumour responses (1–3).

The DCMatters project intends to develop an improved and innovative DC vaccine manufactured in the presence of small molecule inhibitors of immune checkpoints, thus enhancing the potential for T-cell activation and anti-tumour activity. For this purpose, a tailored *in silico* approach has been explored to build machine learning (ML) QSAR classification models to predict the inhibition of the PD-1/PD-L1 axis (**Figure 1**). These models, built using experimentally-validated compound datasets, have been applied to a library of off-patent approved drugs and virtual hits have been prioritized using computer-aided drug design (CADD) software (**Figure 2**).

Drug repurposing, or finding new uses for existing approved drugs, can substantially reduce drug discovery time and costs.

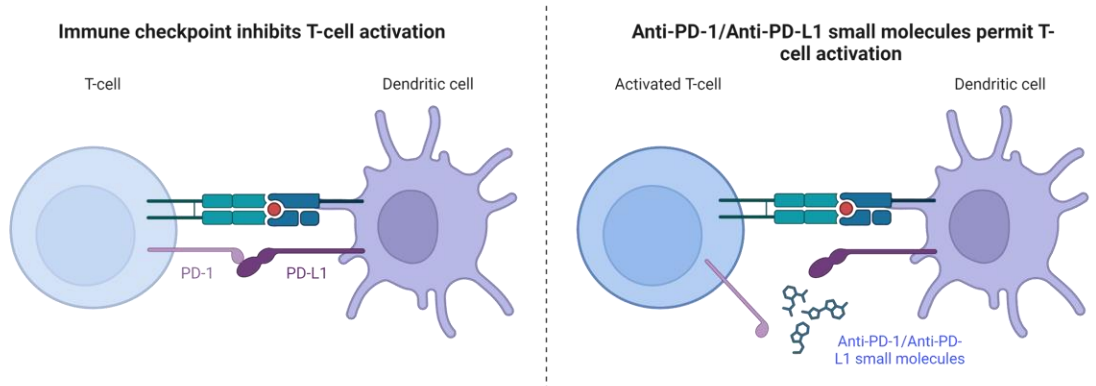

Figure 1. Inhibition of the PD-1/PD-L1 axis by small molecules, permitting T-cell activation.

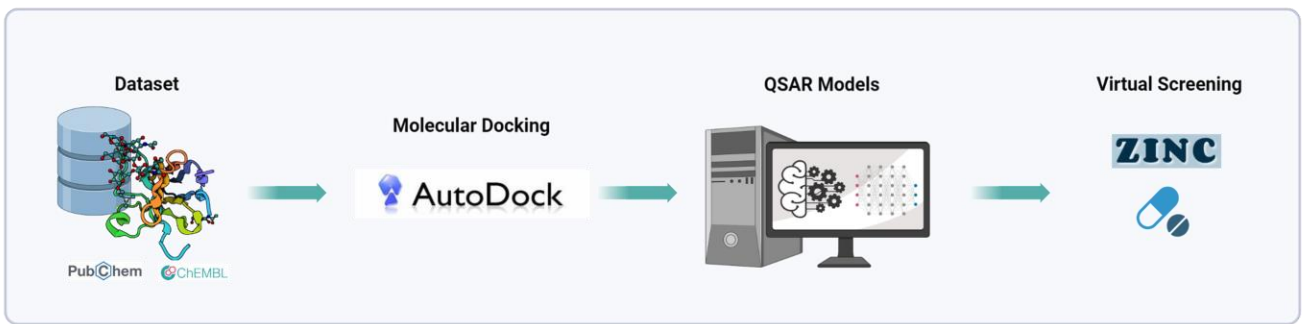

Figure 2. Workflow of the virtual screening strategy to find promising inhibitors of the PD-1/PD-L1 pathway.

METHODS

- The dataset used to construct the models was built with compounds previously tested on the PD-1/PD-L1 pathway, present on curated databases such as ChEMBL (https://www.ebi.ac.uk/chembl/) and PubChem (https://pubchem.ncbi.nlm.nih.gov/). Each dataset compound was labelled as "positive" or "negative", according to its ability to interact/inhibit PD-L1. The dataset includes 29197 compounds randomly divided in a train set and in a test set.
- Molecular docking against PD-L1 protein (PDB ID 5N2F, https://www.rcsb.org/structure/5N2F) was performed on the entire data set. 3D descriptors were calculated using GUIDEMOL (4), an innovative program created in the scope of this project that calculates 3D molecular descriptors based in grid representations of 3D molecular structures using the electrostatic potential or voxels.
- 1D&2D molecular descriptors and fingerprints (FPs) were also calculated, namely Morgan FPs, MACCS FPs and other RDKit descriptors.
- The predictive performance of the FPs, 1D&2D and 3D molecular descriptors calculated using the Random Forest (RF) algorithm (for the training set with OOB estimation) is represented in **Figure 3.** The performance of the best set of descriptors using RF algorithm was evaluated and was achieved the best results with a **Q= 0.999 and MCC= 0.972**.

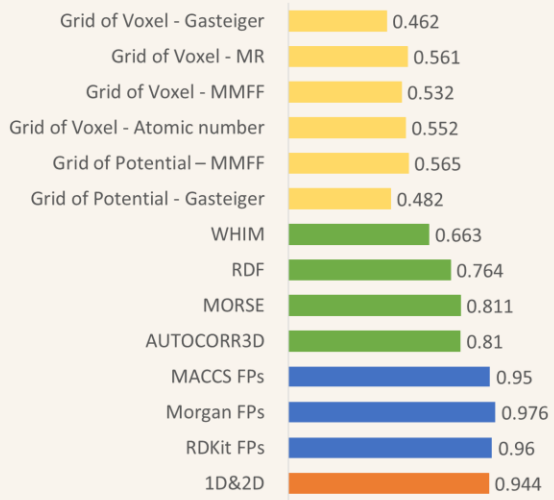

Figure 3. Influence of descriptors in RF model performance. Values indicate the MCC for each model.

- A comparison of three ML techniques using RF, Support Vector Machine (SVM) and Artificial Neural Network (ANN) for building the PD-L1 models with the 50 most important descriptors was studied (**Table 1**).

Table 1. Exploration of different ML algorithms using the 50 most important descriptors (Morgan FPs, 3D RDKit and Molar Refractivity grid voxel descriptors).

		SE[1]	SP[2]	Q[3]	MCC[4]
RF	Tr[5]	0.975186	0.999570	0.999223	0.972381
	Te[6]	1.000000	0.998843	0.998861	0.965533
ANN	Tr[5]	1.000000	0.998603	0.998623	0.954196
	Te[6]	1.000000	0.997685	0.997722	0.934331
SVM	Tr[5]	0.945409	0.998388	0.997634	0.918344
	Te[6]	1.000000	1.000000	1.000000	1.000000

[1] Sensitivity, the ratio of true positive to the sum of true positive and false positive. [2] Specificity, the ratio of true negative to the sum of true negative and false negative. [3] Overall predictive accuracy, the ratio of the sum of true positive and true negative to the sum of true positive, true negative, false positive and false negative. [4] Matthews correlation coefficient. [5] Training set. [6] Test set.

- The virtual library comprises 1576 off-patent approved drugs (FDA, EMA and other agencies), which are also commercially available compounds, extracted from the ZINC database (https://zinc.docking.org/).

RESULTS

- Two virtual hits were predicted as active for all three models (**Table 2**). In addition, the positive control (the inhibitor present originally in the crystallographic structure used for molecular docking, PDB ID 5N2F) had a similar docking score to the virtual hits, which reinforces this prediction.

- One of the virtual hits has a very similar binding pose to the positive control, sharing a great number of residues, namely Tyr56, Asp122, and Lys124, residues that play important roles in ligand binding to PD-L1 (**Figure 4**).

Table 2. Activity predictions of the two resulting virtual screening hits and the positive control in accordance with QSAR modelling and molecular docking.

	RF		SVM		ANN		
ID	**Active**	**Inactive**	**Active**	**Inactive**	**Active**	**Inactive**	**Docking Score (kcal/mol)**
157	0.72000	0.28000	0.85983423	0.14016577	0.988287	0.01171	-11
247	0.64000	0.36000	0.86889995	0.13110005	0.9914836	0.00852	-11.8
PC	1.00000	0.00000	1.00000000	0.00000000	1.0000000	0.00000	-11.7

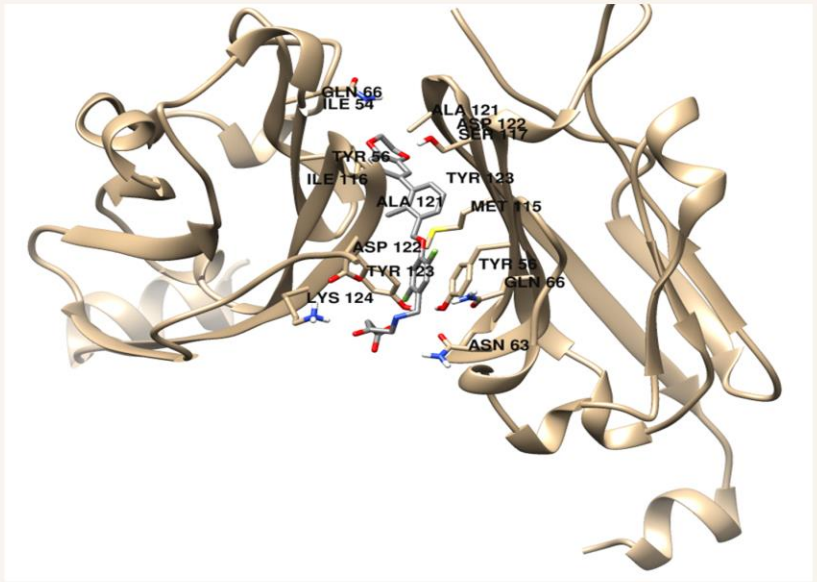

Figure 4. Predicted binding of positive control in the binding site of PD-L1. Structure data were obtained from PDB structure 5N2F.

CONCLUSIONS

Two virtual hits were obtained as potential inhibitors of PD-1/PD-L1, which can be of great value for the development of a new generation of DC based vaccines.

Future work:

1. Test *in vitro*:

- the effect of lead small molecules in inhibiting the interaction of PD-1/PD-L1 axis;
- the efficacy of lead small molecules in improving DC maturation and increasing T-cell activation and proliferation in DC-T cell co-cultures
- the efficacy of lead small molecules in improving T cell anti-tumour activity.

2. Clinical trials.

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