Targeting TIGIT: Can small molecules do the job?

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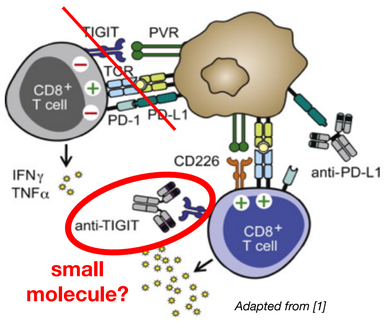
CD8+ T cells are a group of cytotoxic T-cells that kill cancerous or damaged cells. They identify their targets through the interactions between the CD8 protein on their plasma membrane and the target cell's MHCI protein.

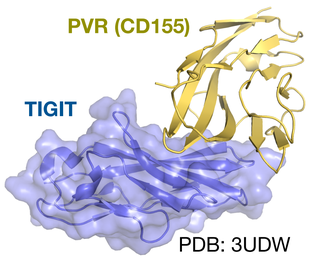
Some cancer cells can inhibit this immune response by expression of the proteins PVR (CD155) and PD-L1 on their surface. The T cell binds to PVR with the TIGIT protein and to PD-L1 with the PD-1 protein. These interactions down-regulate the immune response and allow the cancer cells to continue to grow.

In this project we apply computational modeling towards the identification of the first small molecule inhibitor of the TIGIT/PVR interaction.

Although the TIGIT/PVR interaction can be disrupted by an antibody [1], a small molecule is more desirable from a therapeutic standpoint. Compared to an antibody therapy, small molecules (drugs) are typically less expensive, simpler to administer, and do not risk triggering an immune response.



Adapted from [1]

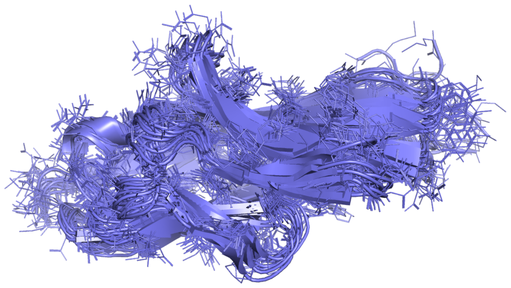


The TIGIT/PVR complex has been resolved through X-ray crystallography [2]. This static structure has a classical large and flat protein-protein interface with no obvious pockets that a small molecule can bind to.

In order to rationally target the TIGIT/PVR interaction with a small molecule, we computationally explored the dynamics of the TIGIT protein to identify a putative druggable allosteric pocket. Fragment docking and pharmacophore search were then used to virtually screen commercially available molecules and identify a 1μM inhibitor.

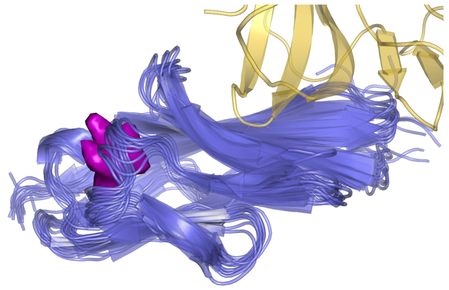
1. Molecular Dynamics Simulations

Multiple 100ns simulations of the TIGIT monomer in explicit water were performed using AMBER in order to characterize the dynamics of the protein.



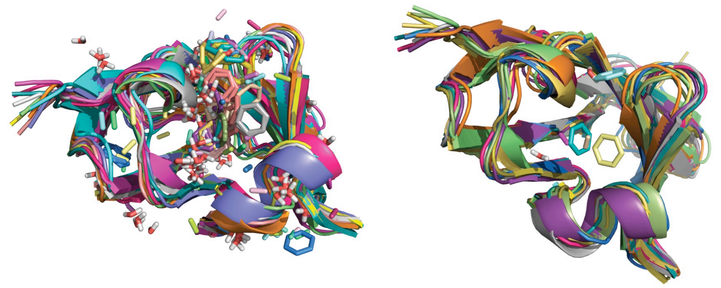
2. Pocket Analysis

Applying MDPocket [3] to the simulations identified a single significant pocket that was removed from the PVR interface site.



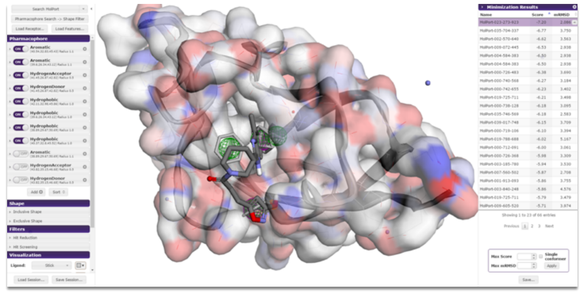
3. Fragment Docking and Selection

Small molecular fragments (benzene, ethyne, and water) were docked with smina [4] into the putative pocket in multiple simulation snapshots. The fragments with high frequency and predicted affinity were selected as representing potential interactions.



4. Pharmacophore Virtual Screening

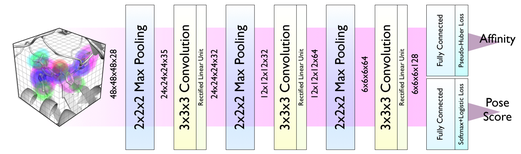
The selected fragments were used to define multiple 3D pharmacophores in Pharmit [5]. The MolPort library of >7 million commercially available compounds was screened for matches.



5. Scoring and Ranking

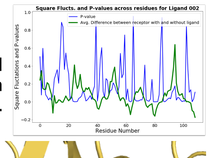
Matching compounds were energy minimized, scored and ranked with respect to an ensemble of receptor snapshots using both the empirical AutoDock Vina [6] scoring function and our convolutional neural network (CNN) scoring function [7]. Top ranked compounds for each scoring routine were selected.

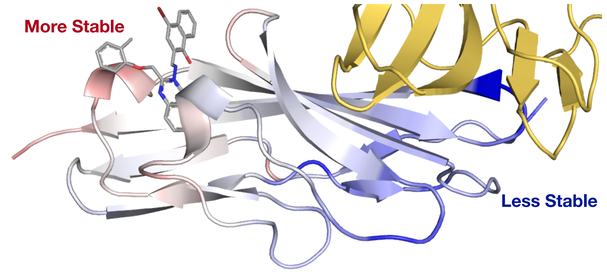
Name	CNN Affinity	CNN Score	Vina
Compound 1	7.70	0.9948	85.95
Compound 2	5.58	0.0180	-8.13
Compound 3	6.74	0.0625	-9.82
Compound 4	6.88	0.9535	-3.81
Compound 5	6.33	0.2098	-8.60
Compound 6	5.69	0.0437	-8.99
Compound 7	4.37	0.022	-9.35
Compound 8	4.81	0.072	-6.82
Compound 9	5.22	0.032	-6.26
Compound 10	6.67	0.361	6.11



6. Allostery Analysis

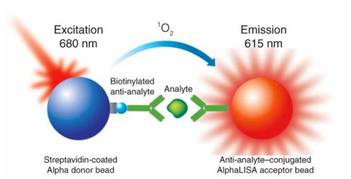
Ligand-bound monomers were simulated and the change in dynamics characterized with root mean squared fluctuations (RMSF). Bound ligands destabilized the PVR interface.

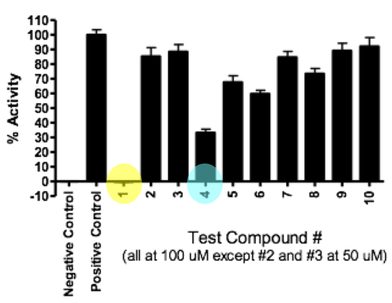


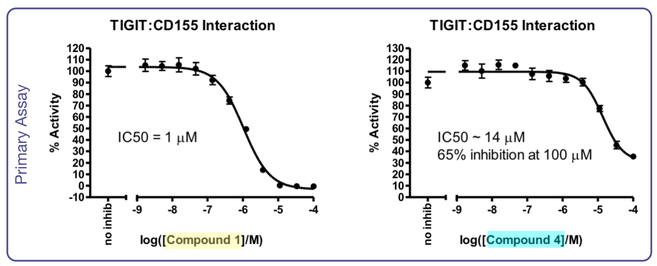


7. Biochemical Protein Interaction Assay

An AlphaLISA assay was performed by BPS Bioscience to measure the ability of the top ranked compounds to inhibit the TIGIT/PVR interaction. Two compounds exhibited inhibition at 100μM. Compound 1, the compound top-ranked by CNN scoring, achieved a 1μM IC50 in the primary assay while having no meaningful effect in the assay control (PD-1:PD-L1).

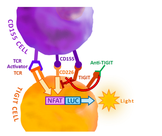


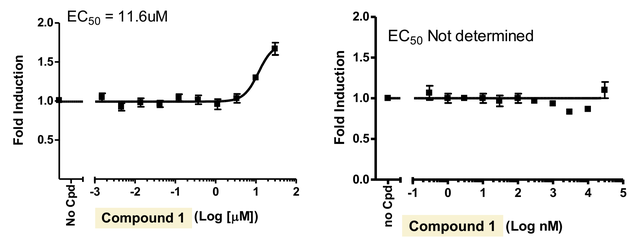




8. Cellular Assay

Results of cell-based testing were inconclusive. The first trial exhibited an EC50 of 11μM, but subsequent trials failed to show any activity.





What's Next?

We are attempting to procure the resources needed to construct our own in-house suite of assays to properly validate, characterize, and optimize the current lead compound. It is important to validate the molecular target of the compound with direct binding assays and better understand its effect on cells. We welcome any collaborations in this pursuit.

References

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