Atezolizumab and Granzyme B as Immunotoxin Against PD-L1 Antigen; An Insilico Study

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Abstract

Background

Programmed death-ligand 1 (PD-L1), also called B7 homolog 1 (B7-H1), is a human protein playing an important biomarker role in highly proliferation cells (like cancer cells) which is encoded by the CD274 gene. The interaction between PD-1 and PD-L1 inhibits T cell growth and cytokine secretion. Therefore, PD-L1 negatively regulates immune responses and permits tumor cells to evade immune surveillance. Thus, PD-L1 seems to be a suitable target for designing the immune-therapeutics.

Results

The predicted structure of this protein indicated that the chains were linked by (Gly4Ser)₃ linker. In that line, using different simulation software, the structure of Granzyme B (GrB), a serine protease exists in cytotoxic lymphocytes granules as an apoptosis mediator, was attached to its specific antibody structure (Atezolizumab) via an adaptor sequence. Evaluation of accuracy, energy minimization and characterization of biological properties of the final processed structure were done. Our computational outcomes showed that the method of employed structure prediction has been successfully managed to design the immunotoxin structure.

Conclusion

It is necessary to mention that the precise and accurate design of the immune-therapeutic agents against cancer cells, can be confirmed by employed in-silico approach. So, using this method, we have designed a capable immunotoxin targeting the PD-L1 in an accurate orientation and cause the cancer cell destruction by its toxin domain.

Introduction

Programmed death-1 (PD-1) is a protein expressed on the T and B lymphocytes, monocytes, natural killer T cells as well as dendritic cells. PD-1 has two ligands: PD-L1 (B7-H1, CD274) which is constitutively expressed on both hematopoietic and non-hematopoietic cells and PD-L2 (B7-DC, CD273) which is expressed on dendritic cells, macrophages, mast cells and certain B cell population. The functional interaction between PD-1 and PD-L1 inhibits T cell growth and cytokine secretion. Therefore, PD-1 negatively regulates immune responses and permits tumor cells to evade immune surveillance (1, 2). In the recent years, the PD-1/PD-L1 axis has been considered as a cancer immunotherapy target (3). An activated T cell can express PD-1 on its surface upon antigen recognition and also can produce interferons inducing PD-L1 expression in multiple tissues. Binding between the PD-1 and its ligand (PD-L1) limits T-cell activity and prevents excessive immune stimulation. This immunological mechanism maintains the immune tolerance to self-antigens by negative regulation of the immune response (4).

The human protein of PD-L1, also known as B7 homolog 1 (B7-H1), is a 40 kDa type 1 transmembrane protein playing a vital role in suppressing the adaptive arm of immune system during some events such as pregnancy, tissue allografts, autoimmune disease as well as other disease like hepatitis (5).

The crystal structure of the human PD-1/PD-L1 complex has already been solved by Zak et al. Significant differences have been documented by this study regarding the binding properties of murine and human PD-1/PD-L1. Moreover, the structures of the PD-1/PD-L1 complexes from different species and their individual components have been thoroughly compared(6). Analyses on this information have led to characterization of hot-spot pockets of human PD-1/PD-L1 complex which is crucial to block PD-1/PD-L1 interaction. The suggested pharmacophore model for this protein-protein interaction could be employed as a solid starting point for the design of chemical probes and potential small-molecule therapeutics targeting the PD-1/PD-L1 protein (6).

PD-L1 overexpression in the tumor microenvironment applies this strategy to handicap immune response in various tumor cells such as lymphoma, melanoma, lung, breast cancer, glioblastoma, ovarian, kidney tumors and bladder cancers (7). The PD-1/PD-L1 interaction might induces apoptosis in tumor-specific T cells to inhibit T-lymphocyte proliferation and subsequently, cytokines release and cytotoxicity. The strategies of protein-protein blockage for manipulation of PD-1/PD-L1 interaction might lead to reversion of exhausted T-cell phenotype to activate antitumor response. These properties provide the rationale to design targeted therapies against PD-1/PD-L1 interaction in the context of cancer immunotherapy. It is expected that reversal of exhausted T-cell phenotype might provide a therapeutic advantage to treatment of chronic viral infections (7).

Currently, Atezolizumab, also known as MPDL3280A, is a fully humanized IgG1 (N298A) monoclonal antibody targeting the PD-L1/PD-1 interaction. Studies has been showed that the Atezolizumab disrupts the interaction of the PD-L1/PD-1 inducing reactivation of anti-tumor immune (8).

Engineering antibodies such as improving the binding affinity is one of the most promising areas of antibody in the treatment of human diseases continues, which can enhance the therapeutic efficiency of the antibody (9, 10).

Immunotoxins combine the specificity of antibody therapeutics with the cell-killing power of some of nature's most toxic proteins. They are chimeric molecules that consist of a protein toxin fused to a targeting moiety. The targeting domain is the most commonly antigen-binding fragment of a monoclonal antibody. The antibody brings the toxin to a cancer cell, then the toxin enters and kills the cell.

Immunotoxins as highly promising therapeutic agents are composed of two parts: a toxic part derived from different kinds of toxins such as fungal, bacterial and human apoptotic agents and a coupled antibody part with high affinity to a specific receptor on the surface of malignant cells. Immunotoxins, using their toxic parts, tend to knock-down the target cells after being bound (11).

As an effective molecule, Granzyme B (GrB) lead to activate apoptosis pathway in tumor or virus-infected cells. Because of the enzyme cytotoxicity, GrB should be amended to candidate as immunotoxin (12).

In this study, we designed an immunotoxin composed of the GrB molecule and Atezolizumab (anti-PD-L1 antibody molecule). In this line, we applied an integrated in silico approach and different computational tools/software. Contemporary, bioinformatics tools/software facilitate to understand the molecular mechanisms of different macromolecules helping the rational design of novel diseases therapeutics. The bioinformatics prediction/modeling might lead to less failure of the experimental methods and less costly approaches to find novel results. Our finding showed that the designed immunotoxin has an effective interaction with the PD-1 causing the GrB part applies its toxic effects on the target cells.

2. Methods

2.1. Sequence availability

To find the Atezolizumab antibody 3D structure, the Protein data bank (PDB) was investigated. No record found for Atezolizumab antibody 3D structure on Protein data bank. further, registered patents Were searched for [registered sequences](#) of the antibody. PD-L1 and GrB sequences were achieved from UniProtKB.

2.2.Atezolizumab 3D structure prediction

Rosetta Antibody Protocol , PIGS)A web server for the automatic modeling of immunoglobulin variable domains based on the canonical structure method) and SAbPred (An antibody modelling tool and prediction software from the Oxford Protein Informatics Group (OPIG)) employed To predict the 3D structure of the Atezolizumab sequence.

RamPage Ramachandran Plot Analysis available was used to determine the quality of the built models and selection the best structure. A Ramachandran plot is a way to visualize energetically allowed regions for backbone [dihedral angles](#) ψ against ϕ of [amino acid](#) residues in [protein structure](#).

2.3. Linker sequence Addition

As regards, the light and heavy chains of antibody are considered separately without any linking amino acids in 3D structure prediction, for recombinant production of the antibody, it seemed necessary to add a linker to join two chains. The (Gly4Ser)_n is the most prevalent linker employed in joining two chains of antibody.

Three repeats of the sequence of linker, (Gly4Ser)₃, were added to join the heavy and light chains. modeler 9.21 software was applied to predict the antibody 3D structure with linker. The predicted antibody 3D structure in previous step was used as the template structure in modeler 9.21 software for homology modelling. To estimate the homology between the final antibody after linker addition and the

predicted antibody structure without linker, Discovery Studio software and Dali Server were used to compute the RMSD and Z-Score between them respectively.

2.4. Immunotoxin design and modelling

GrB structure as the toxic domain of the immunotoxin obtained from protein data bank (PDB). The acquired structure was in its dimeric form and with ligand interactions, accordingly MOE software was employed to eliminate its ligands and one of the monomers. In this regard, an adaptor sequence was attached before the interpolation of the antibody and the GrB to increase the GrB molecule toxicity. In the following, to predict the immunotoxin final structure combined of the GrB and antibody structures modeler homology modeling software was employed. Homology modeling predict based on template structure. In this regard modeler aligns the immunotoxin sequence with the pre-existing structures. Modeller software was applied to attain the immunotoxin structure based on Antibody (containing linker) and preparing GrB as templates.

2.5. Loop and structure refinement

Structure refinement was applied on the immunotoxin structure to achieve a more native and more stable molecule. We used the loop model class in MODELLER to refine the conformation of the loops. The loop optimization method relies on a scoring function and optimization schedule adapted for loop modeling.

ModRefiner at is an algorithm for atomic-level, high-resolution protein structure refinement, which can start from either C-alpha trace, main-chain model or full-atomic model. Both side-chain and backbone atoms are completely flexible during structure refinement simulations, where conformational search is guided by a composite of physics- and knowledge-based force field.

One aim of structure refinement is to draw the initial starting models closer to their native state, in terms of hydrogen bonds, backbone topology and side-chain positioning. It also generates significant improvement in physical quality of local structures. The standalone program also supports ab initio full-atomic relaxation, where the refined model is not restrained by the initial model or the reference model.

2.6. Model validation

Estimating the quality of protein structure models is a vital step in protein structure prediction. QMEAN Qualitative Model Energy Analysis employed for the quality estimation of immunotoxin structure model. RamPage (Ramachandran Plot Analysis) available was used to validate the quality of the immunotoxin 3D model before and after the refinement.

One of the ways that can corroborate the 3D structure accuracy is [secondary structure determination of the sequence .in order to predict the immunotoxin secondary structure following software and services were used: Jpred, PSIPRED and SOPMA](#)

In the next step, the secondary structure of the modeled molecules was compared with the predicted secondary structure acquired from MOE software to indicate if they are in concordance with each other.

2.7. Immunotoxin characterization

Further characterization of the immunotoxin was carried out using following software and services: ProtParam at is a tool which allows the computation of various physical and chemical parameters. The computed parameters include the molecular weight, theoretical pI, amino acid composition, atomic composition, extinction coefficient, estimated half-life, instability index, aliphatic index and grand average of hydropathicity (GRAVY).

Periscop is a sequence-based predictor that estimates the expression level and yield of soluble protein in the periplasm of E. coli.

ccSol predicts protein solubility using physico-chemical properties.

Further research were done to investigate the recombinant protein antigenicity and toxicity.

VaxiJen is a tool for prediction of protective antigens and subunit vaccines. ToxinPred at is an in silico method, which is developed to predict and design toxic/non-toxic peptides. AlgPred at was used to determine the possible allergic feature of the recombinant molecule. To specify and characterize the existing epitopes within the immunotoxin sequence, various software was used. Propred and Propred-1 were utilized to specify T-cell epitopes 1 and 2, respectively.

In order to identify the B cell epitopes, Ellipro , Bepipred and SVMtrip were used.

2.8. Immunotoxin binding efficiency

The interaction possibility between the immunotoxin and its receptor can be investigated using its 3D structure for molecular docking study. First, PD-1 3D structure was obtained from PDB database and edited using MOE software. The obtained structure was in Complex with Atezolizumab-Fab form. Hence, MOE software was applied to remove extra domains and ligands. The possible interaction between PD-1 and immunotoxin was predicted by Zdock server ClusPro server can be used to explore the designed immunotoxin functionality. To calculate the Gibbs free energy of reaction between these two proteins PRODIGY server was applied.

3. Results

3.1. Sequence retrieval

sequence source for Atezolizumab antibody was drug bank with Accession Number DB11595. The sequences under the UniProtKB ID of Q9NZQ7 and P10144 was obtained for the PD-L1 and GrB protein (Supplementary Figure 1).

3.2. Atezolizumab 3D structure prediction

All of the employed software were employed to model the antibody chains (Figure 1). The modeled antibodies quality was determined by the Rampage quality assessment software. The results indicated the Rosetta Antibody Protocol provided the best prediction (Figure 1a). Table 1 shows the results of the Ramachandran plot evaluations of the predicted structures.

Table 1. Ramachandran plot evaluations for the predicted structures.

	Favoured region	Allowed region	Outlier region
<i>Rosetta</i>	98.6%	1.4%	0.0%
<i>PIGSPro</i>	94.1%	5.0%	0.9%
<i>SAbPred</i>	97.7%	1.8%	0.5%

3.3. Linker sequence addition

Modeler 9.21 was effective in modeling the antibody structure with the linker sequences. 5 different structures predicted by Modeler 9.21. Discovery Studio software and Dali Server were applied to determine the homology between the structures after the linker addition and original Atezolizumab. The best structure with the lowest RMSD and highest Z-Score selected for further analyzes. Table 2 shows the results for the RMSD and Z-Score investigations of the predicted structures.

Figure 2 demonstrates the best selected structure in which two chains of the antibody are linked as a single chain. The RMSD was calculated to be 1.4 Å. The results showed linker sequence addition does not make any significant change in the original antibody structure. Figure 3 shows the superimposed structures of the antibody structure before/after linker addition.

Table 2. RMSD and Z-Score evaluations for the predicted structures.

	Z-Score	RMSD
1	36.8	1.4
2	36.8	1.5
3	36.8	1.4
4	36.7	1.4
5	36.7	1.5

3.4. Immunotoxin assembly

The structure under the PDB record of 1FQ3 was found for the GrB structure. To obtain a monomeric GrB, all non-protein atoms and the extra GrB structures were discarded by MOE software. All sequences of the antibody, GrB, adaptor and linker sequences were applied to synthesize the final immunotoxin sequence (Supplementary Figure 2). Figure 4 shows GrB structure before/after monomerization. Homology modelling successfully determined whole of the immunotoxin structure.

3.5. Loop and structure refinement and Model validation

Ramachandran plot indicates that some amino acids in the immunotoxin predicted structure are located at outlier region. Hence, refinement of the model could improve its quality. One aim of ModRefiner is to draw the initial starting models closer to their native state, in terms of hydrogen bonds, backbone topology and side-chain positioning. It also generates significant improvement in physical quality of local structures. Ramachandran plot of the refined model was improved. In initial model the percent residues in the favored region was 69.2%while 76.6% in the loop refined and 86.3% in final structure refined model. 12.5% residues in outlier region in the initial model decrease to 9.6% in the loop refined and 3.4% in final structure refined model (Table 3). The QMEAN (Qualitative Model Energy Analysis) score value of immunotoxin structure before and after refinement calculated -22.72 and -7.73 respectively (the higher score, the more reliable is the structure). Figure 5 shows the Ramachandran plot and Qmean structural validation of the immunotoxin before and after refinement coloring based on Local Quality Score. Figure 6 shows the final structure of the immunotoxin coloring based on different domains.

Table 3. Ramachandran plot result of initial loop refined and final structure refined model.

	Favored region	Allowed region	Outlier region	QMEAN Score
Initial model	69.2%	18.3%	12.5%	-22.72
Loop refinement by modeller	76.6%	13.8%	9.6%	-10.42
loop and structure refinement by modeller and modrefiner	86.3%	10.3%	3.4%	-7.73

Figure 6. The final structure of the immunotoxin in CPK(left) and solid ribbon model(right). The antibody light chain structure is in blue, antibody heavy chain structure is in yellow, the linker structure is in red, the adaptor structure is in violet and the GrB structure is green.

Based on the results, the secondary structure of the modeled structure and the predicted secondary structure of the immunotoxin molecule were compared. The predicted secondary structure is in accordance with the secondary structure of the predicted 3D model which indicates the reliability of the predicted 3D model and employed approach. The accuracy of the secondary structure prediction software is often better than the 3D protein model predictions. Thus, the consistency between the secondary structure prediction and the secondary structure associated with the predicted 3D model could be a good indication of high quality 3D structure prediction.

3.7. Immunotoxin characterization

Based on the ProtParam results, the molecular weight of the immunotoxin was estimated to be 55199.40D, the pI was predicted to be 9.51 and 10 hours was predicted to be the protein half-life in *E. coli*. The protein expression content of the recombinant gene in *E. coli* was expected to be 9.2622 mg/L by Periscope server. Although, the recombinant protein was estimated to be soluble. ToxinPred predict no toxic peptide along the immunotoxin sequence. The allergenicity prediction results indicated the designed immunotoxin contains no significant allergen potency (Score= -1.0586434, Threshold= -0.4). The results for Overall Prediction for the Protective Antigen demonstrated that the designed immunotoxin is a Probable ANTIGEN (Score= -0.6881, Threshold= -0.4).

According to the prediction provided by Propred and Propred-1, the immunotoxin contains 75 and 94 T cell epitopes of type 1 and type 2, respectively. In addition, according to the Ellipro prediction results the immunotoxin contains 19 linear and 4 structural B cell epitopes. Bepipred and SVMtrip identified 24 and 8 linear epitopes in the immunotoxin sequence respectively.

3.8. Immunotoxin binding efficiency

The PDB record code 5Wt9 was obtained as the Complex structure of PD-1 and Nivolumab-Fab molecule. The 5Wt9-G structure contains the first 167 amino acids of the PD-1 molecule. This region is The Extracellular Domain of Human Pd-1 that interacts in binding with antibody. All non-protein atoms and the extra 5Wt9-G structures were removed to have a monomeric 5Wt9-G using MOE software. 5Wt9-G contains only PD-1 structure. Figure 7 shows 5Wt9-G structure in comparison with 5Wt9 PDB record.

The Zdock, Cluspro and Hex8 results demonstrated the possibility of the immunotoxin interaction to the PD-1. The results demonstrated the PD-1 loops contribute in reaction with the immunotoxin. The directional binding of the immunotoxin shows that this molecule is able to bind to PD-1 via integrin binding region. Such interaction enables the immunotoxin to hamper leukocytes from binding to PD-1 expressing cells in addition to directing the immunotoxin to its desired target. This phenomenon enhances its cytotoxic activity. Figure 8 indicates the interaction of the immunotoxin to PD-1. to calculate the Gibbs free energy of reaction between these two proteins was used from HAD duck server.

The Gibbs free energy of the docked immunotoxin with the PD-1 receptor is expected to be -8.7 which points up a stable interaction.

4. Discussion

Analyzing the functional properties of a designed macromolecule is one of the essential requirements of biological modeling studies. Since the structural and functional analyses are highly costly and time consuming, exploiting molecular modeling methods and protein 3D structures could circumvent numerous challenges ahead empirical experimentation. Even models with low accuracy could be useful to make assumptions about the fictional model of an unknown antigen (13, 14). Using molecular

modeling methods would help the researchers to decrease the number of necessary experimental assessments and to avoid different ethical issues (15–18). Given these properties of molecular modeling methods, we have launched an *in silico* study to model and assess an immunotoxin for its functionality against PD-L1 molecule. Various analyses were carried out to assess the designed immunotoxin which indicated promising functional properties for the final molecule.

The designed immunotoxin is composed of the Atezolizumab antibody sequence as the targeting moiety and the GrB as its toxic moiety. The Atezolizumab monoclonal antibody as a fully humanized IgG1 would bring about proper targeting of the PD-L1/PD-1 interaction on the cancer cells. This would prompt the successful delivery of the immunotoxin to the cancer cell location. Moreover, being fully humanized would diminish the possible adverse effects and undesired immune activation against the immunotoxin. These humanized antibodies would compensate for reduced efficacy of the mouse-derived antibody sequences owing to their higher immunogenicity (19). An approved sequence of the Atezolizumab was used to predict its 3D structure with different modeling approaches. Using more than one approach to model the 3D structures would lead to higher accuracy of the final model and the chance to find the best approach to get a native like structure for the protein. It has already been shown that changing the length of the linker sequence to join the variable domains of the light and heavy chains of an antibody (to form single-chain antibody fragment (scFv)) would have a drastic effect on the orientation of their assembly and the valence of the formed complex. Different applications of scFv for therapeutic and imaging purposes are directly affected by the valency, flexibility and size of them. Inclusion of linkers with at least 12 amino acids in length predominantly leads to formation of monomers with varying amounts of dimer and higher molecular mass oligomers in equilibrium (20). An ideal linker sequence should effectively reduce the interference amongst domains and have the lowest effect on the folding properties of the joined domains. Our results indicated that linking two variable domains of the Atezolizumab light and heavy chains using a 12 amino acids glycine and serine rich sequence did not change the overall structure of the linked domains. The obtained RMSD values confirmed the lowest effect of added linker on the joined domains. Using scFv as the targeting moiety instead of whole antibody molecule (which were employed in early generations of immunotoxins) reduces the final size of the immunotoxin. Smaller antigens are endowed with better penetration into the tissues and slower renal plasma clearance (21, 22).

The GrB moiety was used to confer the toxicity effect of the immunotoxin. GrB is a serine protease which induces apoptosis via activation of several pathways within the cytoplasm and nucleus of target cells. Proteolytic activation of caspase 3 is one of the major pathways of GrB mediated apoptosis activation (23, 24). Various substrates of apoptotic induction, like the inhibitor of caspase activated deoxyribonuclease and Lamin B, could be produced following the direct proteolysis of caspase 3 via GrB (25). GrB mediated cleavage of Bcl-2 and Bid protein could also initiate the caspase 3 pathway associated with caspase-8 activation (24, 26). Bid cleavage ultimately leads to permeabilization of the mitochondrial outer membrane and the release of pro-apoptotic factors like Smac/DIABLO, Omi/HtrA2, cytochrome c and apoptosis inducing factor (25). The formation of apoptosome complex is induced by release of Cytochrome c which in turn leads to downstream activation of Caspase 3. Permeabilization of

the mitochondrial outer membrane could be further promoted by GrB mediated cleavage of anti-apoptotic Mcl-1 and following release of Bim protein as a member of pro-apoptotic Bcl-2 family (27). The substrates of the GrB activated effector caspases including NuMa, ICAD, Tubulin, PARP-1 and Lamin B are also among the substrates of GrB (23, 25, 28). Alternatively, apoptosis activation could occur via another immune cell/GrB-mediated route. Perforin multimerization is needed in this route to build pores in the target membrane through which the GrB trafficking into the cell would be possible (25). Given these facts, targeting the GrB against cancer cells could eventually lead to activation of apoptosis in these cells and their elimination.

The 3D modeling of the final immunotoxin indicated that each component of the immunotoxin does not experience any drastic change. The calculated RMSD values confirmed the retained structural properties of each component of the immunotoxin. The predicted high chance of the immunotoxin to have soluble expression in the periplasm of *E. coli* is accounted as a desirable property. This property is important due to existence of 3 disulfide bonds of the GrB molecule; the periplasmic space is an oxidizing compartment in genetically un-modified *E. coli* cells which is equipped with various chaperones and foldases (29). Given these properties this compartment could bring about the suitable environment to form these bonds. Forming these bonds is imperative to restore the original biological functions of the designed immunotoxin. It has also been shown that the designed immunotoxin could be expressed as a soluble protein. It should be noted that soluble proteins are associated with much more simple downstream purification processes and they are more likely to be biologically functional with proper folding. Therefore, the designed immunotoxin could have proper therapeutic function upon expression and easier purification processes. The interaction between the immunotoxin and the integrin binding region of the PD-1 molecule indicates that the designed antigen could properly target the cancer sites and hamper the interaction between leukocytes and PD-1 expressing cells. Low binding energy between the immunotoxin and PD-1 expressing cells was calculated by docking analyses. It implies that the interaction between these two molecules would be strong enough to exert the immunotoxin functions. High antigenicity and lack of toxicity and allergenicity are also the desirable properties that confirmed the suitable design of the final immunotoxin.

In conclusion, our results indicate that the designed immunotoxin bears desirable structural, physicochemical and immunological properties to exert its functions at their target site. The interaction between the immunotoxin and the integrin binding region of the PD-1 molecule indicates its proper orientation, while the low binding energy confirms the stability of this interaction. These properties confirm the suitability of this immunotoxin to be employed in fight against cancer. However, performing in vitro analyses would be required for further analyses of the immunotoxin.

Abbreviations

PD-1: Programmed death-1, GrB: Granzyme B, PDB: Protein data bank, OPIG: Oxford Protein Informatics Group. RMSD: Root-mean-square deviation.

Declarations

Availability of data and materials:

Data presented in this manuscript is available upon request.

Ethics approval and consent to participate:

This article does not contain any studies with human participants or animals performed by any of the authors

Consent for publication:

All authors have read and approved the final version of the manuscript.

Conflict of interests

All authors declare no potential conflicts of interest.

Competing interests:

The authors declare that they have no competing interests.

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Authors' contributions:

All authors participated in writing. Writing-review and language correction.

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Figures

Figure 1

The predicted structure of Atezolizumab by (a) Rosetta (b) PIGS and (c) SAbpred.

Figure 2

Atezolizumab with linker. Light chain sequence is followed by the heavy chain sequence and the linker is shown in yellow.

Figure 3

Atezolizumab with linker. Light chain sequence is followed by the heavy chain sequence and the linker is shown in yellow.

Figure 4

Granzyme B 3D structure. (a) is the 1FQ3 and (b) is the GrB without extra non-protein and protein molecules.

Figure 5

QMEAN and Rampage structural validation of the immunotoxin. (a: Initial model, b: Loop refinement by modeller and c: loop and structure refinement by modeller and modrefiner)

Figure 6

The final structure of the immunotoxin in CPK(left) and solid ribbon model(right). The antibody light chain structure is in blue, antibody heavy chain structure is in yellow, the linker structure is in red, the adaptor structure is in violet and the GrB structure is green.

Figure 7

(a) 5Wt9: The Complex structure of PD-1 and Atezolizumab-Fab and (b) is the 5Wt9-G without extra non-protein and protein molecules.

Figure 8

Immunotoxin bound to PD-1 (6 position with the best dock score). The immunotoxin is shown in violet and PD-1 in yellow.