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# Small Molecule Inhibitor Design for Anaplastic Lymphoma Kinase Inhibition

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# Small Molecule Inhibitor Design for Anaplastic Lymphoma Kinase Inhibition

THEODORE D. HANSEL, DAVID J. GRABOVSKY

The Anaplastic Lymphoma Kinase (ALK) gene has been linked to tumorigenesis in a number of human cancers, including anaplastic large cell lymphoma (ALCL) and neuroblastoma. While ALK mutations in ALCL and many other cancers occur as a result of gene fusions with wild type kinase domains, those in neuroblastoma stem from single nucleotide polymorphisms (SNPs) in the kinase domain. These lead to autophosphorylation and constitutive signaling by ALK for cell growth and division, ultimately causing cancer. Crizotinib, an ATP-competitive ALK inhibitor, has proven to be an effective inhibitor of both ALK<sup>WT</sup> and ALK<sup>Mutant</sup> kinase domains, and is in the middle of clinical trials for neuroblastoma treatment. This review used the PyMOL and AutoDock Vina computational biology programs to predict the binding affinities of Crizotinib, Ceritinib (LDK378), and PF-922 to three different ALK kinase mutations in order to determine the most effective inhibitor. The EGFR inhibitors gefitinib and erlotinib were also analyzed in complex with ALK as negative controls to verify the specificity of the ALK inhibitors. The crystalline complexes were then qualitatively analyzed to uncover the mechanics behind the docking results. Based on the results generated by Vina, PF-922, representative of the second generation of ALK inhibitors, is predicted to be the most effective out of the tested compounds. These results may be used to predict the inhibitor that will require the lowest dosage to achieve the greatest inhibitory effect, hopefully leading to fewer side effects from treatment.

## Introduction

The Anaplastic Lymphoma Kinase (ALK) receptor tyrosine kinase has been implicated as a major oncogene in a variety of different cancers, ranging from adult non-small cell lung cancer (NSCLC) and anaplastic large-cell lymphoma (ALCL) to neuroblastoma in children<sup>1</sup>. The majority of these mutations are fusion proteins, such as NPM-ALK in ALCL<sup>2</sup> or EML4-ALK in NSCLC<sup>3</sup>. In these fusion proteins, the ALK kinase domain generally remains intact and wild type. However, ALK mutations in neuroblastoma are unique as they are frequently point mutations within the kinase domain. The mutations may be somatic or germline, with those causing tumorigenesis leading to constitutively active kinase domains<sup>4</sup>. Crizotinib is a dual ALK/MET inhibitor produced by Pfizer, and was one of the first ALK inhibitors introduced. Although first used with non-mutated (WT) ALK kinase domains in NSCLC, Crizotinib has also demonstrated efficacy preclinically in ALK<sup>Mut</sup> cell lines, and clinically in Phase I clinical trial patients<sup>5</sup>.

For the purposes of this review, the PyMOL and AutoDock Vina computational programs were used to compare the binding affinity of Crizotinib with that of ATP, the natural ligand, as well as other ALK inhibitors. These other inhibitors include Ceritinib (LDK378) produced by

Novartis, and a second-generation ALK inhibitor designed by Pfizer called PF-06463922 (PF-922). All of these inhibitors are ATP-competitive and function by binding preferentially to the ALK kinase domain and preventing ATP from entering the binding pocket, inhibiting the kinase activity. Theoretically, a higher binding affinity correlates with greater efficacy and reduced dosages to achieve the same inhibitory effect. *In vitro*, this effect would be shown by measuring ALK inhibition through western blotting or ELISA assays. This review analyzes the binding affinities associated with each ligand, in addition to one designed for this study, for three different ALK point mutations. The first, C1097S, is used as a stand-in for the WT kinase domain found in ALK fusion proteins. This mutation stabilizes the model of the ALK kinase domain, and is found in all other models of the protein<sup>6</sup>. The R1275Q mutation represents the most common neuroblastoma mutation, while the F1174L mutation is the most resistant mutation commonly seen in the clinical setting<sup>7</sup>.

## Methods

AutoDock Vina is a molecular docking program that takes in the molecular structures of a receptor and a ligand, runs computations simulating the ligand's docking into a specified binding pocket on the receptor, and predicts the ligand's binding affinity to its receptor<sup>8</sup>. Using

structures for the C1097S, R1275Q, and F1174L ALK mutations<sup>6</sup>, as well as for each inhibitor<sup>9-11</sup>, a docking analysis in Vina for each ligand-receptor pairing was run to determine binding affinities for each pairing. Vina also produces files containing the ligand's docked pose responsible for the reported affinity; these files were opened in the PyMOL molecular viewer and analyzed qualitatively in terms of their conformations.

Before running Vina, special .pdbqt files describing each molecule involved in docking were prepared. PDB files (descriptions of the spatial arrangement and types of atoms in a molecule) containing each compound's structure were downloaded from [pdb.org](http://pdb.org) and isolated in PyMOL. ALK<sup>WT</sup> was downloaded in complex with a piperidine-carboxamide inhibitor<sup>6</sup>, which was removed; the other two ALK mutations were downloaded in apo<sup>6</sup> (ligand-free) form. All three drugs (Crizotinib, Ceritinib, and PF-922) were originally downloaded in complex with ALK<sup>9-11</sup>, which was subsequently removed from each ligand.

Each ligand file was then opened in AutoDockTools (ADT), a GUI extension of Vina, and subsequently saved in .pdbqt format, which preserves the atom locations specified in PDB format, but also gives each atom partial charges (q) and an AutoDock-specific atom type (t). The same was done with each receptor; however, crystallographic water molecules were removed and polar hydrogens were added (as preferred by Vina) before saving each file in .pdbqt format. The final step in molecule preparation involved defining a search space on the receptors where Vina would search for a binding pocket. Each protein was visually inspected in ADT until the binding site was found; the search space was then specified as a box around the binding site, with (x, y, z) coordinates for its center and x, y, and z-lengths for its sides.

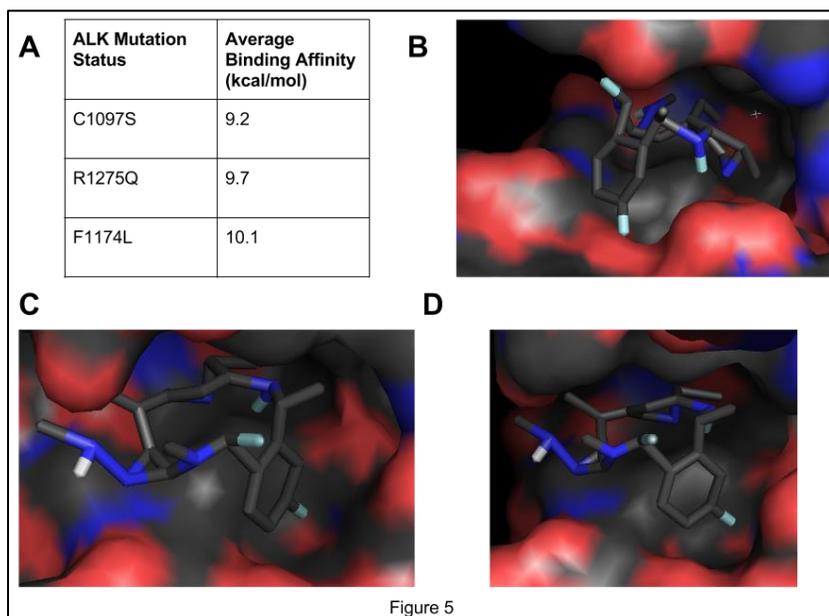
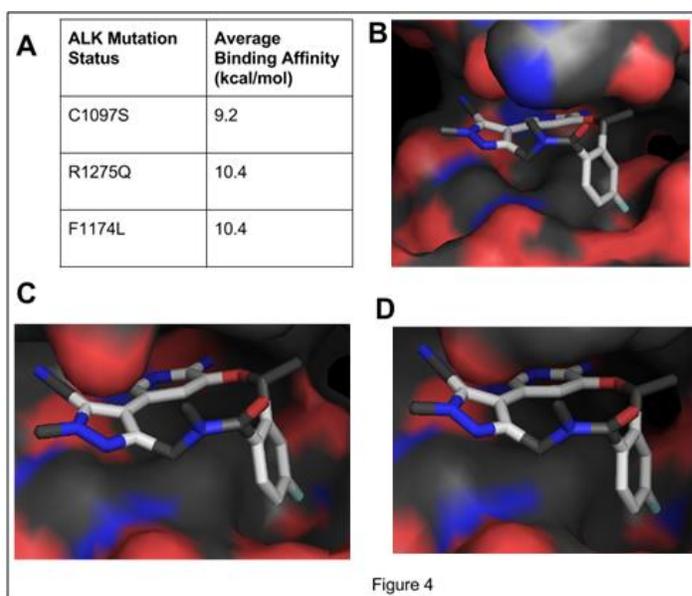
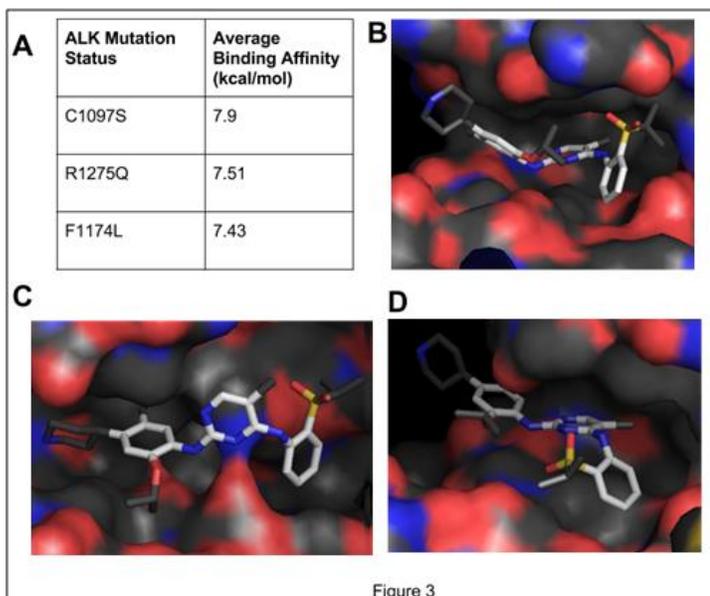
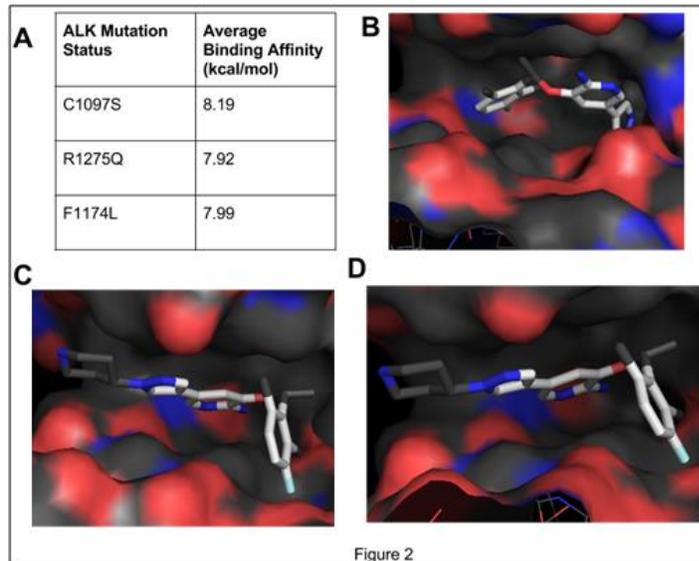
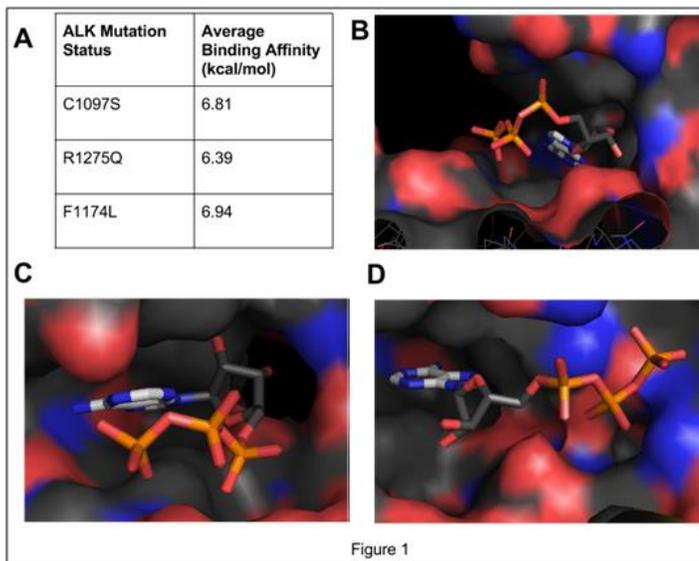
Once the .pdbqt files for all receptors and proteins were saved, a configuration (config) file was written and Vina was run. The config file gave Vina the filenames of the ligand and receptor to be docked, as well as the coordinates and size of the search space box found graphically in ADT. (See Appendix A for the format and parameters of the config file.) Vina was run from a Windows command prompt: after changing the computer's directory to the folder where vina.exe was located, the command `"\\Vina\vina.exe" --config conf.txt --log log.txt` read the config file and created a log of its output as the analysis ran.

Vina's output takes the form of .pdbqt files containing each ligand's 9 most likely docking poses, as well as the log of the 9 affinities corresponding to those poses. The .pdbqt files were saved for visualization and comparative analysis, and only the highest affinity for each docking run, representing the ligand's most likely pose, was recorded. Because Vina uses a random seed for each docking run, its results are nondeterministic; the program must therefore run several times for each ligand-receptor pairing to produce reliable data. Affinities from 10 docking runs were recorded in a Microsoft Excel spreadsheet and then averaged to produce each pairing's mean affinity.

## Results: Computational Analysis

Figures 1 – 5 below summarize the results of our study, listing each ligand's binding affinities to all three proteins (A), followed by images of the ligands docked with each of the three versions of ALK: C1097S (B), R1275Q (C), and F1174L (D). Figure 1 shows data on ATP, Figure 2 on Crizotinib, Figure 3 on Ceritinib, Figure 4 on PF-922, and Figure 5 on TDH-01, a drug designed by one of the authors based on the structure of PF-922. Comparison of the ligands bound to ALK<sup>WT</sup> shows that PF-922 (Figure 4 (B)) and TDH-01 (Figure 5 (B)) both had the highest affinity, followed by Crizotinib (Figure 2 (B)), Ceritinib (Figure 3 (B)), and finally ATP (Figure 1 (B)). Binding affinities associated with both mutated proteins exhibited the same order, with PF-922 pulling slightly ahead of TDH-01. In every simulation, ATP was the weakest binding partner. Another table summarizing our results, which compares ligands instead of proteins, can be found in Appendix B.

Comparison of the receptors yielded less obvious patterns. ATP bound to ALK<sup>WT</sup> (Figure 1 (B)) with a lower affinity than to ALK<sup>F1174L</sup> (Figure 1 (D)), but with higher affinity than to ALK<sup>R1275Q</sup> (Figure 1 (C)). Crizotinib did not follow this trend, binding weakest to ALK<sup>R1275Q</sup> (Figure 2 (C)), slightly stronger to ALK<sup>F1174L</sup> (Figure 2 (D)), and strongest to ALK<sup>WT</sup> (Figure 2 (B)), for which it was designed. Ceritinib behaved differently, binding to ALK<sup>R1275Q</sup> (Figure 3 (C)) with higher affinity than to ALK<sup>F1174L</sup> (Figure 3 (D)); yet still the bond was not as strong as to ALK<sup>WT</sup> (Figure 3 (B)). PF-922, the overall best ALK inhibitor, interestingly bound with higher affinity to ALK<sup>Mut</sup> proteins (Figure 4 (C-D)) than to ALK<sup>WT</sup> (Figure 4 (B)). TDH-01 also fared better with mutated proteins (Figure 5 (C-D)) than with ATP<sup>WT</sup> (Figure 5 (A)), actually binding best to the notoriously resistant ALK<sup>F1174L</sup> (Figure 5 (D)). These trends in PF-922 and TDH-01 show their functional similarity, as well as their specificity for mutated ALK variants.



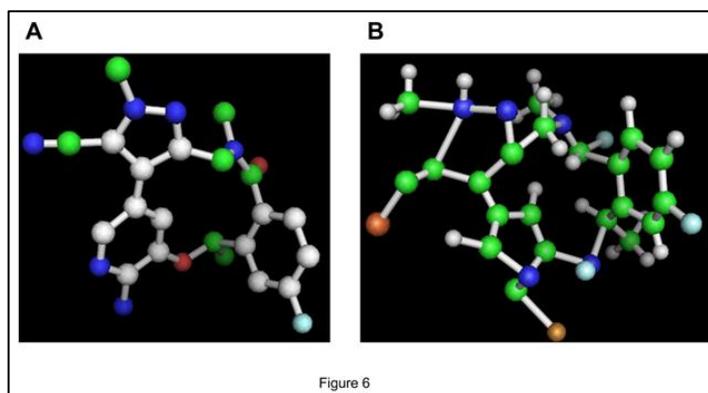
## Qualitative Comparison

The binding affinity between a protein and its ligand are a direct result of how tightly the ligand fits into its binding pocket, as well as how well the ligand matches “hot spots” in the pocket. Hot spots are regions in the protein of high electrical charge that can create strong intermolecular forces to hold the ligand inside the binding site. As previously noted, all of the inhibitors demonstrated a greater binding affinity than ATP for all ALK mutations, which is necessary for the inhibitor to function. By analyzing the crystal structure of the inhibitors in complex with the ALK kinase domain, it becomes clear that the more effective compounds, such as PF-922, TDH-01, and Crizotinib, bind more preferably than Ceritinib or ATP.

Figure 1 (B) shows the crystalline structure of ATP in the WT kinase domain. The fit of the molecule inside the pocket is relatively loose, and there are only a few hotspots linked to electronegative regions in the molecule. These explain the relatively low binding affinity. There are even some electronegative regions of the molecule near negative hotspots, which would suggest repulsion. These same principles are also evident in ALK<sup>R1275Q</sup> in figure 1 (C) and ALK<sup>F1174L</sup> in figure 1 (D). Crizotinib, shown in complex with ALK<sup>WT</sup> in figure 2 (B), has a similar linear structure to that of ATP, but features a highly electronegative fluorine attached to an aromatic ring, giving it the ability to bind with a hot spot in the pocket ATP was unable to reach, likely giving Crizotinib its preferential affinity. Crizotinib also binds strongly to this hotspot in both ALK<sup>R1275Q</sup> (figure 2 (C)) and ALK<sup>F1174L</sup> (figure 2 (D)). Ceritinib also binds more preferentially than ATP to all three ALK mutations (Figure 3). Since Ceritinib does not appear to directly bind with many hot spots, its increased affinity is likely due to a tighter fit than ATP's, although it also features a linear structure similar to ATP and Crizotinib but lacks the highly interactive fluorine.

PF-922, representing the second generation ALK inhibitors, displays the highest binding affinity out of all of the reviewed inhibitors (figure 4 (A)). Like Crizotinib, it features the aromatic ring-linked fluorine to strongly bind to hot spots, but departs from the linear design in favor of a ringed shape. Resulting in an altogether wider molecule in the main horizontal plane, PF-922 fits more snugly into the binding pocket than the other inhibitors, allowing it gain a stronger affinity to the walls of the pocket while interacting with particular hotspots (figure 4 (B-D)).

TDH-01 was designed by one of the authors based on the PF-922 geometry, and attempted to gain a higher binding affinity than PF-922 by creating more regions of high electronegativity in the molecule. While TDH-01 interacted with many of the same hot spots as PF-922 and had a nearly identical docking pose, the additional electronegativity also increased its repulsion of the binding pocket's negatively charged regions. While also present in ALK<sup>WT</sup> and ALK<sup>F1174L</sup> (figure 5 (D)), this is especially evident with the proximity of the anterior nitrogen atoms and the negative region of ALK<sup>R1275Q</sup> (figure 5 (C)). Figure 6 below is a side-by-side comparison of the structures of PF-922 (A) and TDH-01 (B). The increased polarity within TDH-01, as evidenced by the abundance of fluorine, nitrogen, and oxygen atoms, is most likely liable for its slight decrease in binding affinity.



## Discussion and Conclusion

Based on our results, PF-922 is predicted to be the most effective ALK inhibitor, especially for the resistant F1174L mutation. Our data matches that of other published studies<sup>9</sup>. Additionally, the protein models accurately reflect the observed *in vitro* resistance of the F174L ALK mutation due to its increased ATP binding affinity<sup>12</sup>. This review has demonstrated the beneficial effect of not only conformational compatibility, but also hotspot targeting in rational drug design. The inhibitors that demonstrated the highest binding affinities were those with the greatest amount of conformity to the pocket and those which targeted the most hotspots.

The most obvious path for future research is preclinical and clinical testing of these compounds. Crizotinib has already proven its efficacy in the clinical setting, and the other drugs are also ambling in this direction. TDH-01, at present only a theoretical model, would have to be produced in the laboratory prior to any bench testing. While direct clinical or preclinical comparisons of these compounds are unlikely, this review

serves as a good basis for the comparison of ALK inhibitor efficacy from computational and qualitative perspectives. Computational results gathered during preclinical testing of new compounds allows researchers to better focus on testing drugs with higher chances of success, allowing for more efficient use of limited research funding. The current

process for drug design (high throughput screening) is not only laborious, but also incredibly expensive. By integrating data gathered through computational methods, pharmaceutical engineers may more intelligently create drugs, streamlining the development and production of more effective novel treatments.

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There was no funding for this project. PyMOL and AutoDock Vina were obtained free under educational use licenses, while the free WebMO demo server was used to create TDH-01.

## Appendix A: The Configuration File

```
receptor = filename.pdbqt
ligand = filename.pdbqt

out = out.pdbqt

center_x = 20.0
center_y = 15.0
center_z = variable:
    C1097S: 63
    R1275Q: 10
    F1174L: 11

size_x = 20
size_y = 20
size_z = 20

exhaustiveness = 8
```

## Appendix B: Average Inhibitor Binding Affinities

### Inhibitors

(kcal/mol)	ATP	Ceritinib	Crizotinib	PF-922	TDH-01
C1097S	6.81	7.9	8.19	9.20	9.20
R1275Q	6.39	7.51	7.92	10.40	9.70
F1174L	6.94	7.43	7.99	10.40	10.10

### Negative Controls

(kcal/mol)	Gefitinib	Erlotinib
C1097S	7.61	6.55
R1275Q	7.22	6.66
F1174L	7.50	6.65

Note: All binding affinities listed above and in Figures 1 – 5 are given in terms of the energy released as binding occurs.