ORIGINAL ARTICLE

Behavior of the anti-apoptotic BCL2 protein in non-small cell lung cancer cell models in response to treatments with ALK inhibitors and its potential dual BCL2-ALK inhibition

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ABSTRACT

Objective: To describe the behavior of the BCL2 protein in non-small cell lung cancer (NSCLC) cell models in response to treatments with ALK (anaplastic lymphoma kinase) protein tyrosine kinase inhibitors (crizotinib and alectinib) and their potential dual BCL2-ALK inhibition.
Materials and methods: Three NSCLC cell models were used: Ba/f3 EML4-ALK^{WT}, Ba/f3 EML4-ALK^{L1196M} and Ba/f3 EML4-ALK^{G1202R}, generated by site-directed mutagenesis. These were treated with crizotinib and alectinib in a dose-responsive manner, and an apoptosis assay was also conducted to confirm pharmacological susceptibility. Subsequently, BCL2 protein expression was measured under three treatment conditions (no treatment, 100 nM crizotinib and 50 nM alectinib). Finally, a search for BCL2 and ALK ligands was performed for molecular docking simulation and interaction energy calculation, measured in kcal/mol using the YASARA™ program.
Results: The WT model evidenced sensitivity to crizotinib and alectinib, with apoptosis percentages of 23 % and 74 %, respectively; G1202R showed resistance to both drugs (apoptosis: 5 %), and L1196M resistance to crizotinib (apoptosis: 12 %) and sensitivity to alectinib (apoptosis: 25 %). BCL2 expression revealed overexpression in the WT and G1202R models, while L1196M showed expression close to baseline. Finally, bioinformatics findings identified ABT-199 (which is part of small molecule libraries) as the best candidate to inhibit BCL2, while its interaction with ALK^{L1196M} revealed interaction energies higher than those obtained in the interaction with crizotinib and alectinib.
Conclusions: The cell models exhibited the pharmacological susceptibility described in the literature, BCL2 expression during treatments remained overexpressed in WT and G1202R, while L1196M showed no variation. Finally, bioinformatics findings suggest ABT-199 as a potential dual action inhibitor due to its higher interaction energy with ALK.

Keywords: Small Molecule Libraries; Carcinoma, Non-Small-Cell Lung; Inhibitors, Tyrosine Kinase; Proto-Oncogene Proteins c-bcl-2; Anaplastic Lymphoma Kinase; Molecular Docking Simulation (Source: MeSH NLM).

INTRODUCTION

Lung cancer is the second most common cancer type diagnosed worldwide, specifically non-small cell lung cancer (NSCLC) in the United States, accounting for 81 % [1,2]. Regarding the various risk factors for NSCLC, oncogenes, such as KRAS (29 %), EGFR (19 %) and ALK (3 %), are among the main genetic markers responsible for causing this neoplasm [3,4]. The anaplastic lymphoma kinase (ALK) gene encodes for an ALK transmembrane protein with a tyrosine kinase domain that, when fused with the EML4 gene [5,6], leads to constitutive phosphorylation of the ALK protein and triggers a cascade of tumor signaling pathways within the cell [7]. Constitutive phosphorylation in NSCLC cells is being circumvented with the ALK tyrosine protein kinase inhibitor "crizotinib", and directs the tumor environment toward cell death. Nevertheless, point mutations in specific amino acids, such as L1196M, G1202R, C1156Y and others, have resulted in drug resistance. Therefore, the need has arisen to search for novel molecules such as ceritinib, alectinib, brigatinib and lorlatinib, which are already approved by the US Food and Drug Administration (FDA) as treatment algorithms to overcome resistance and lead tumor cells to apoptosis [8-10]. However, as described above, point mutations within the ALK protein seem to be endless; therefore, each point mutation will always necessitate the search for additional selective ALK inhibitor molecules.

On the other hand, the BCL2 protein is directly responsible for activating apoptosis, and has currently been described as an oncological therapeutic target due to its high expression in many tumors [11,12]. One of the molecules targeting BCL2 is ABT-199, approved by the FDA for clinical use in chronic

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lymphocytic leukemia [13]. Moreover, encouraging results have been observed in some solid tumors such as sarcoma, as well as in combination therapy [14,15].

According to this rationale, this study identified BCL2 as another therapeutic target to lead tumor cells toward apoptosis and prevent the ALK protein from acquiring point mutations. In this context, we propose to study the behavior of the anti-apoptotic protein BCL2 in NSCLC cell models under treatment with ALK inhibitors (crizotinib and alectinib) and to evaluate, through bioinformatics studies, the interaction between the BCL2 inhibitor and the ALK protein, with the expectation of observing dual inhibition.

MATERIALS AND METHODS

Study design and population

The design of this study was descriptive both in silico and in vitro. Our study population consisted of three NSCLC cell models: Ba/F3 EML4-ALK^{WT}, Ba/F3 EML4-ALKL^{1196M} and Ba/F3 EML4-ALK^{G1202R}, which were generated by in vitro site-directed mutagenesis [16]. The cell models were maintained in RPMI® culture medium supplemented with 10 % inactivated fetal bovine serum and 1% penicillin/streptomycin. To ensure cell growth, they were incubated under conditions and temperature parameters of 37 °C and 5 % CO_2.

Variables and measurements

To determine pharmacological susceptibility, the CellTiter-Blue® cell proliferation assay was used to calculate the half-maximal inhibitory concentration (IC_{50}), whose reference values (IC_{50} < 350 nM = sensitive and IC_{50} > 350 nM = resistant), according to Huan et al., allowed estimating drug sensitivity or resistance in the cell models. For this purpose, 10^5 cells/mL of each cell model (Ba/F3 EML4-ALK^{WT}, Ba/F3 EML4-ALK^{L1196M} and Ba/F3 EML4-ALK^{G1202R}) were cultured in 96-well plates and exposed to the drugs crizotinib and alectinib at eight serial concentrations using a 1:3 ratio, with a maximum concentration of 10 µM and a minimum concentration of 0 µM. After 48 hours of pharmacological treatment, the cell models were incubated with 10 % CellTiter-Blue® reagent for three hours, and then monitored using a Multiskan Go spectrophotometer (Thermo Scientific®) at a wavelength of 490 nm. The collected data allowed for the calculation of the half-maximal inhibitory concentration (IC_{50}).

On the other hand, cell apoptosis was determined by flow cytometry using the eBioscience™ Annexin V Apoptosis Detection Kit (Thermo Fisher). Ba/F3 EML4-ALK^{WT}, Ba/F3 EML4-ALK^{L1196M} and Ba/F3 EML4-ALK^{G1202R} cells were cultured in six-well plates, and drugs were added at concentrations of 100 nM crizotinib and 50 nM alectinib. After 48 h of treatment, 500,000 cells were collected, washed once with phosphate-buffered saline (PBS) at 4 °C and then resuspended in 200 µL of binding buffer (1X). Annexin V-FITC and propidium iodide (PI) were added according to the manufacturer's instructions. The flow cytometry analysis was performed with an Attune™ NxT flow cytometer (Thermo Fisher).

Next, protein expression was measured employing the Western blot technique, which consisted of obtaining cell lysates maintained in laemmli buffer (1X), which were loaded onto SDS-polyacrylamide gel wells and transferred to nitrocellulose membrane. Subsequently, the membrane was blocked with 10 % fat-free milk for one hour and then immediately treated with the antibodies anti-BCL2 (mouse) (Ab692, 1:500 dilution - abcam®) and anti-Actin (rabbit) (ab8227, 1:1000 dilution - abcam®), and incubated overnight at -4 °C. The following day, the membrane was exposed to the secondary antibodies anti-mouse (ab190463, 1:5000 dilution - abcam®) and anti-rabbit (ab205718, 1:5000 dilution - abcam®), respectively, for one hour. Finally, it was developed and imaged using the Chemidoc Imaging Instrument (Bio-Rad®), where the expression bands were observed.

As a last step, molecular docking simulations were performed, where in a first stage, the PDB codes 2YFX, 2YHV and 4ANS were imported separately into the YASARA™ program [17] as the substrate template, and the PDB codes 3AOX and/or 6O0K as the ligand template. Next, both structures were superimposed, and all objects were removed, leaving only the ALK^{L1196M} structure as the substrate and alectinib or ABT-199 as the ligand. Subsequently, the energy minimization experiment was selected and the substrate-ligand objects were joined. Finally, the interaction energy estimated by the software was measured in kcal/mol.

Statistical analysis

Statistical analyses were conducted using GraphPad Prism software, version 10.0.3. To minimize statistical bias, the experiments were performed in triplicate and the arithmetic mean ± standard error was used as data for analysis. The IC_{50} was calculated with parametric nonlinear regression, adjusted to a 95% confidence interval. ANOVA and Tukey's test were used for comparison of multiple groups of variables. $P < 0.05$ (* = 0.0332; ** = 0.0021; *** = 0.0002; **** ≤ 0.0001) was considered statistically significant and $p = 0.1234$ not significant.

Ethical considerations

This study was approved by the Institutional Research Ethics Board of the Universidad de Piura (No. PREMED0820219). The participation of human beings or biological samples were not required. It was conducted in the Cell Culture, Immunology and Cell Biology, Protein Analysis and Bioinformatics research laboratories at the Universidad de Piura.

RESULTS

Heterogeneous sensitivity of the EML4-ALK^{L1196M} model to treatment with ALK inhibitors: crizotinib and alectinib

The EML4-ALK^{WT}, EML4-ALK^{L1196M} and EML4-ALK^{G1202R} cell models were exposed to dose-response with the drugs crizotinib and alectinib to assess their sensitivity and resistance (IC_{50} < 350 nM = sensitive and IC_{50} > 350 nM = resistant, reference values according to Huan et al) [18]. The EML4-ALK^{WT} model showed sensitivity to crizotinib (IC_{50}: 46 nM) and alectinib (IC_{50}: 5.07 nM), EML4-ALK^{G1202R} showed resistance to crizotinib (IC_{50}: 616 nM) and alectinib (IC_{50}: 1.22 µM), while EML4-ALK^{L1196M} showed resistance to crizotinib (IC_{50}: 789 nM) and sensitivity to alectinib (IC_{50}: 195 nM); the latter showed heterogeneous sensitivity to ALK inhibitors (see Figure 1a). On the other hand, the apoptosis assay expressed as percentages confirmed the sensitivity of the EML4-ALK^{WT} model (crizotinib: 23 %, alectinib: 74 %), the resistance of the EML4-ALK^{G1202R} model (crizotinib: 5 %, alectinib: 5 %) and the heterogeneous response of the EML4-ALK^{L1196M} model (crizotinib: 12 %, alectinib: 25 %) (Figures 1b and 1c). Our results suggest that these cell models are optimal for the study of NSCLC.

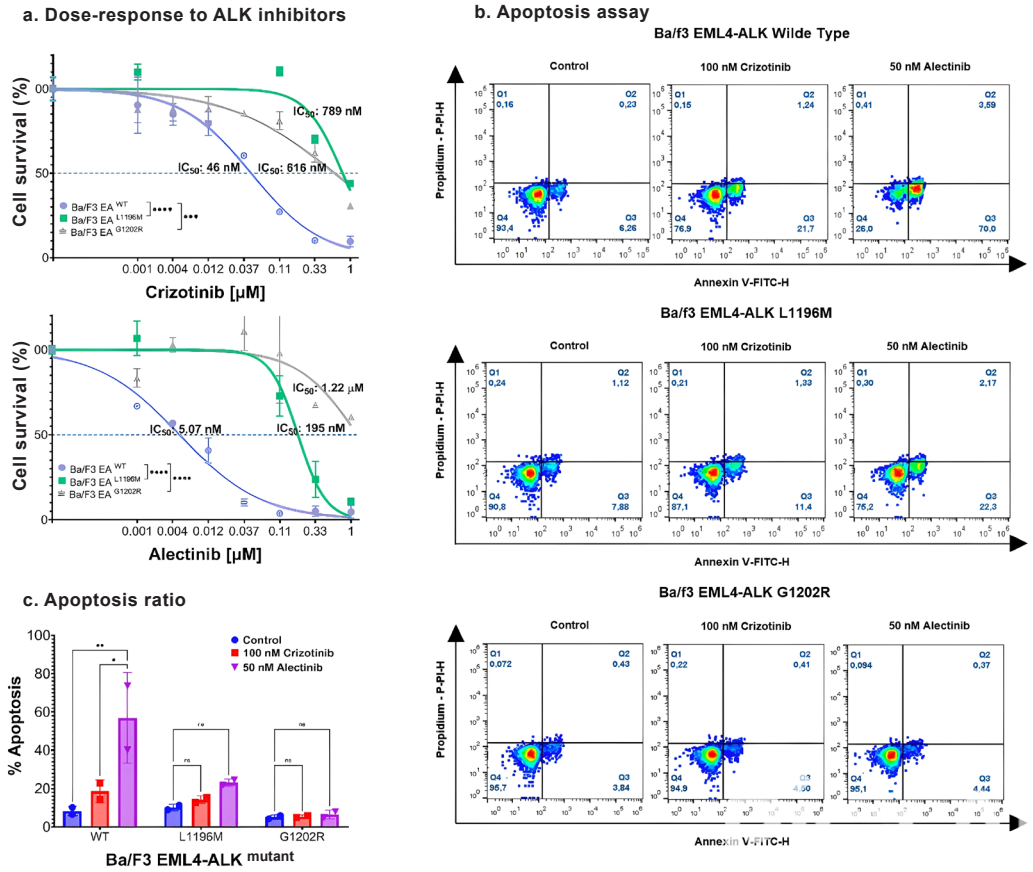

Figure 1. Pharmacological susceptibility and apoptosis assay in cell models. 1a) Dose-response assessment of Ba/F3 EML4-ALK mutant cell models (WT, L1196M, G1202R) to crizotinib and alectinib: WT shows sensitivity to crizotinib (IC_{50}: 46 nM) and alectinib (IC_{50}: 5.07 nM), L1196M shows resistance to crizotinib (IC_{50}: 789 nM) and sensitivity to alectinib (IC_{50}: 195 nM) and G1202R shows resistance to crizotinib (IC_{50}: 616 nM) and alectinib (IC_{50}: 1,220 nM). 1b) Apoptosis assay verifying the pharmacological susceptibility of the cell models to treatments with crizonitib and alectinib. 1c) Bar graph representing the total apoptosis ratio.

Heterogeneous relative BCL2 protein expression in the EML4-ALK cell models

BCL2 protein expression was measured in the EML4-ALK^{WT}, EML4-ALK^{L1196M} and EML4-ALK^{G1202R} models under three different conditions: control = 0 nM (no treatment), crizotinib = 100 nM and alectinib = 50 nM. The results evidenced the normalized overexpression of the BCL2 protein in the EML4-ALK^{WT} model (control: 1.91 ± 1.5; 100 nM crizotinib: 1.63 ± 0.23; 50 nM alectinib: 0.95 ± 0.27) and EML4-ALK^{G1202R} model (control: 0.995 ± 0.12; 100 nM crizotinib: 0.072 ± 0.6; 50 nM alectinib: 0.636 ± 0.446), while the EML4-ALK^{L1196M} model (control: -0.196 ± 0.305; 100 nM crizotinib: -0.301 ± 0.27; 50 nM alectinib: 0.11 ± 0.04) exhibited an expression level relatively close to baseline (Figure 2c). BCL2 expression within each cell group showed no significant difference between the treatment conditions, and the cell group that stood out was EML4-ALK^{L1196M} with a relative expression close to baseline (Figures 2a and 2b).

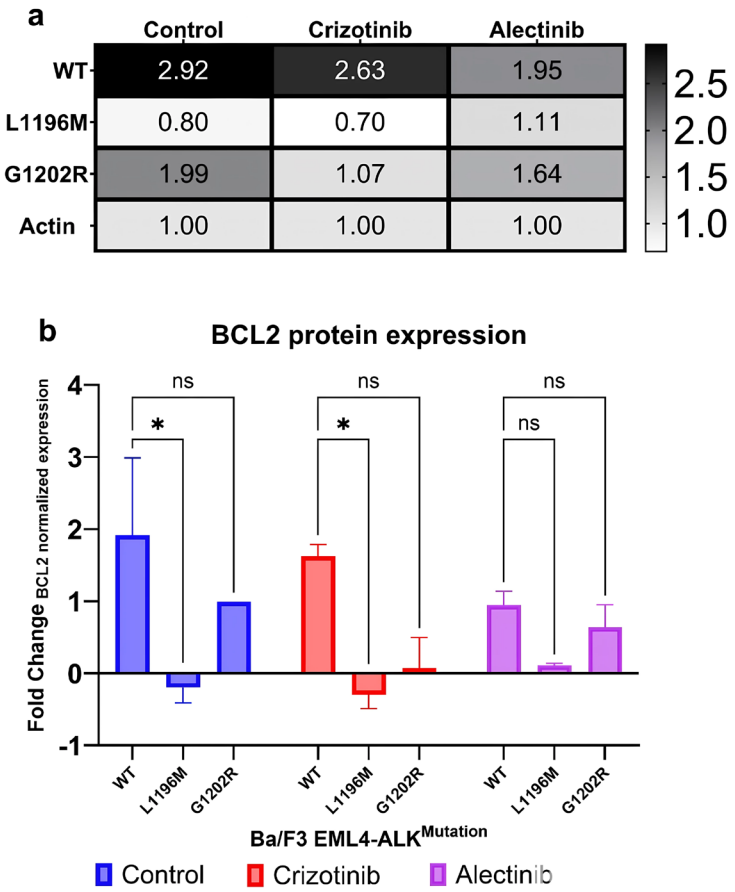

Figure 2. BCL2 protein expression in the cell models. 2a) Heatmap representing the quantification of BCL2 protein expression (values greater than 1: overexpression; values less than 1: inhibition of expression). 2b) Bar graph showing BCL2 expression normalized to actin value. The WT and G1202R models exhibit BCL2 overexpression, while L1196M shows BCL2 expression close to baseline.

Selective activity of the ABT-199 ligand on the BCL2 protein

A search for the best selective ligand for the BCL2 protein was conducted. The protein name was entered in the Uniprot platform using the human search filter. The molecule that met these parameters was identified with the code P10415 [19]. In the structure section, 28 PDB structures were identified (X-ray diffraction: 22 structures; nuclear magnetic resonance: 6 structures), along with one structure predicted by AlphaFold artificial intelligence. The first exclusion criterion was to select those structures that interact with a ligand and do not carry any mutations in the BCL2 protein (*n* = 11) (Figure 3a). The second exclusion criterion was to evaluate the resolution level (high resolution = values close to 1 Å), for which values below 2 Å were considered the cut-off point. The resulting molecules were identified with the PDB codes 6O0K [20], 5VAU and 4LXD with ligands identified as ABT-199 (venetoclax), beclin 1 and navitoclax, respectively. The latter was not taken into account because it was not solely selective for BCL2, and neither was beclin 1 because it plays a role in

autophagy. ABT-199, also known as venetoclax, was the only molecule that met these criteria; therefore, it was proposed as a selective ligand for the BCL2 protein.

a **PDB: BCL2 protein**

PDB	Resolution	Ligand
6O0K	1.62	ABT-199
5VAU	1.75	Beclin 1
4LXD	1.90	Navitoclax
5VAX	2.00	Beclin 1
4MAN	2.07	Navitoclax
5JSN	2.10	BINDI
5FCG	2.10	HBx-BH3 motif
4IEH	2.10	N-heteroaryl sulfonamides
2W3L	2.10	Phenyl Tetrahydroisoquinoline Amide
5AGX	2.24	alpha beta-1 LINEAR
2XA0	2.70	BAX BH3 peptide

b **PDB: 6O0K**

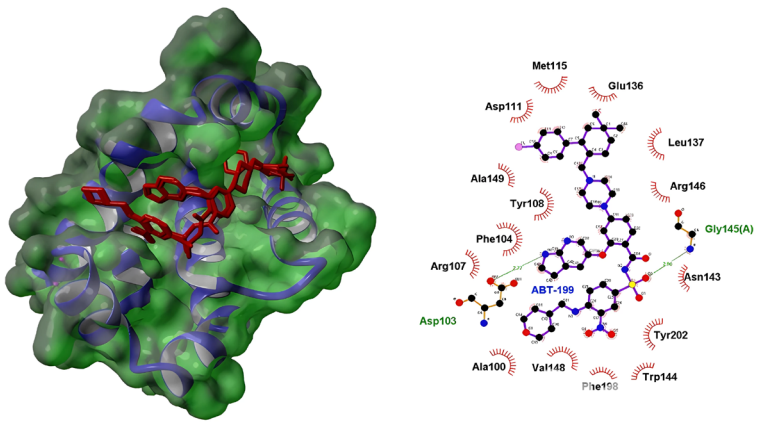

Figure 3. BCL2 protein (PDB: 6O0K) and its ABT-199 ligand. 3a) List of public PDBs downloaded from the RSCB-PDB platform (https://www.rcsb.org), sorted by resolution level. 3b) On the left, the three-dimensional structure of PDB 6O0K (BCL2 in blue-green, interacting with the ABT-199 ligand in red), and on the right, the two-dimensional interaction of the ABT-199 ligand with specific amino acids within the catalytic site of BCL2 (image generated using LigPlot).

Exhibition of higher interaction energy between ABT-199 and ALK with respect to the inhibitors crizotinib and alectinib

The ALK^{L1196M} model was identified with a baseline expression of BCL2; therefore, this model was selected to evaluate its interaction with ABT-199. For this purpose, the ALK protein was searched under the same search protocol applied for BCL2. The ALK molecule with these parameters, identified with the code Q9UM73 [21], showed one structure predicted by AlphaFold and 77 PDB structures (electron microscopy: 1; nuclear magnetic resonance: 5; and by X-ray diffraction: 71 structures). The structures with the L1196M mutation (n = 7 structures) and with the crizotinib ligand (n = 3) were identified, and the following PDB codes with their molecular interaction energies were obtained: 2YFX (31.1677 kcal/mol), 2YHV (31.1674 kcal/mol) [22] and 4ANS (37.0791 kcal/mol). On the other hand, the couplings and measurement of the interaction energy of the described PDBs were predicted when interacting with alectinib (alectinib reference ligand from PDB code 3AOX [23]) and with the ABT-199 ligand (ABT-199 reference ligand from PDB code 6O0K) (Figures 4a and 4b). Our results evidenced significant differences between the interaction of ALK^{L1196M} with ABT-199 with a higher interaction energy than that obtained with crizotinib and alectinib, which are direct ALK inhibitors. Therefore, these findings suggest it is a potential dual ligand targeting both the BCL2 protein and ALK^{L1196M} (Figure 4c).

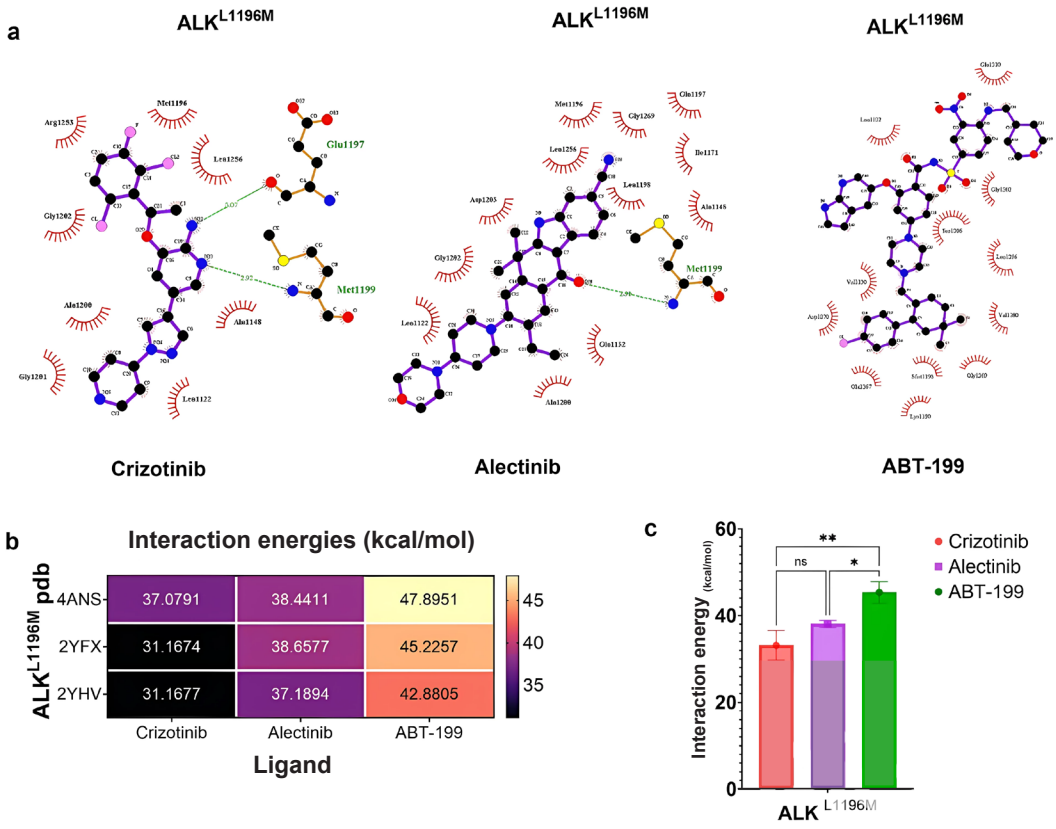

Figure 4. Interaction energies evidence ABT-199 as a candidate for binding to the active site of the ALK^{L1196M} protein. 4a) Crizotinib, alectinib and ABT-199 ligands interact with specific amino acids of the ALK^{L1196M} protein active site (image generated using LigPlot). 4b) The heatmap shows the interaction energies between ALK^{L1196M} structures with crizotinib, alectinib and ABT-199. 4c) Bar graph shows the average interaction energies, where ABT-199 stands out with an interaction energy higher than that of crizotinib and alectinib.

DISCUSSION

Treatments with ALK inhibitors (crizotinib and alectinib) in the EML4-ALK^{WT}, EML4-ALK^{L1196M} and EML4-ALK^{G1202R} cell models demonstrated sensitivity and resistance as reported in the literature, indicating that these models are optimal for characterizing BCL2 protein expression during treatments [16,24,25]. On the other hand, studies have revealed that high BCL2 expression among patients with NSCLC predicts favorable outcomes and could be considered a marker of good prognosis. In the present study, the results evidenced EML4-ALK^{WT}and EML4-ALK^{G1202R} cell models with high BCL2 expression. Thus, according to the foregoing, they would be good candidates to be sensitive to a BCL2 inhibitor, while it could be hypothesized that inhibition in the EML4-ALK^{L1196M} model with baseline BCL2 expression might be more effective [26,27].

Currently, BCL2 overexpression is considered an oncological therapeutic target, with small molecule inhibitors mimicking the mentioned protein, among which ABT-199 (also known as venetoclax) stands out [13,28].

In our study, the search for inhibitors targeting the BCL2 protein also pointed to ABT-199 as the molecule that meets all the conditions with ligand characteristics. Similarly, according to our bioinformatics findings, the interaction energies with ALK^{L1196M} are higher than those with crizotinib and alectinib, respectively [22]. Therefore, it is proposed as a ligand with potential dual inhibition for both the BCL2 protein and ALK. These results are encouraging as ABT-199 has not been described as a dual ligand; in the literature it is referred to as a selective ligand of BCL2 already approved by the FDA [13], while other studies support that ABT-199 could play a leading role in combination therapy [29,30]. Based on this study, the expression of ALK and BCL2 during treatment with ABT-199 is still to be elucidated.

In conclusion, the EML4-ALK^{WT}, EML4-ALK^{L1196M} and EML4-ALK^{G1202R} cell models showed pharmacological susceptibility to crizotinib and alectinib, as described in the literature. This was also confirmed by apoptosis assays. On the other hand, the WT and G1202R cell models demonstrated overexpression of the BCL2 protein, while L1196 exhibited near baseline expression. Furthermore, our bioinformatics studies suggest the potential dual inhibition of ABT-199 targeting BCL2 and ALK as its interaction energies are higher than those calculated for ALK in interaction with crizotinib and alectinib. Finally, we recommend studying the expression of BCL2 and ALK in the various EML4-ALK^{mutant} cell models during treatment with ABT-199 to demonstrate its potential dual inhibition. This would significantly affect its clinical applicability since all three drugs (crizotinib, alectinib and ABT-199) have already been approved by the FDA.

Author contributions: RZD and FST conceptualized the study. JFC and RZD performed data curation and utilized software for the study, while JFC, BMD, SIV, DF, LM, and RZD conducted the formal analysis. Additionally, SIV and RZD obtained funding from Consejo Nacional de Ciencia, Tecnología e Innovación Tecnológica (Concytec – National Council of Science, Technology and Technological Innovation)-ProCiencia. RZD and JFC proposed the research methodology, and BMD was responsible for the management and administrative processes of the project. Furthermore, RZD drafted the original manuscript, and JFC, RZD and SIV supervised the research. All authors contributed to the research, writing, review and editing of the article.

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Conflicts of interest: The authors declare no conflicts of interest.

BIBLIOGRAPHIC REFERENCES

1. American Society of Clinical Oncology. Lung Cancer - Non-Small Cell: Statistics [Internet]. Estados Unidos: ASCO; 2023. Available from: https://www.cancer.net/cancer-types/lung-cancer-non-small-cell/statistics.
2. Siegel RL, Miller KD, Wagle NS, Jemal A. Cancer statistics, 2023. CA Cancer J Clin [Internet]. 2023;73(1):17-48.
3. Chevallier M, Borgeaud M, Addeo A, Friedlaender A. Oncogenic driver mutations in non-small cell lung cancer: past, present and future. World J Clin Oncol [Internet]. 2021;12(4):217-37.
4. Stella GM, Luisetti M, Pozzi E, Comoglio PM. Oncogenes in non-small-cell lung cancer: Emerging connections and novel therapeutic dynamics. Lancet Respir Med [Internet]. 2013;1(3):251-61.
5. Du X, Shao Y, Qin H-F, Tai Y-H, Gao H-J, Yan-Hong Tai C. ALK-rearrangement in non-small-cell lung cancer (NSCLC). Thorac Cancer [Internet]. 2018;9:423-30.
6. Hallberg B, Palmer RH. The role of the ALK receptor in cancer biology. Ann Oncol [Internet]. 2016;27(3):iii4-iii15.
7. Arbour KC, Riely GJ. Diagnosis and treatment of anaplastic lymphoma kinase–positive non–small cell lung cancer. Hematol Oncol Clin North Am [Internet]. 2017;31(1):101-11.
8. Liao BC, Lin CC, Shih JY, Yang JCH. Treating patients with ALK-positive non-small cell lung cancer: latest evidence and management strategy. Ther Adv Med Oncol [Internet].
9. Pan Y, Deng C, Qiu Z, Cao C, Wu F. The Resistance Mechanisms and Treatment Strategies for ALK-Rearranged Non-Small Cell Lung Cancer. Front Oncol [Internet]. 2021;11:1-13.
10. Wang YW, Tu PH, Lin KT, Lin SC, Ko JY, Jou YS. Identification of oncogenic point mutations and hyperphosphorylation of anaplastic lymphoma kinase in lung cancer. Neoplasia [Internet]. 2011;13(8):704-15.
11. Hafezi S, Rahmani M. Targeting bcl-2 in cancer: Advances, challenges, and perspectives. Cancers (Basel) [Internet]. 2021;13(6):1-13.
12. Hanahan D, Weinberg RA. Hallmarks of cancer: The next generation. Cell [Internet]. 2011;144(5):646-74.
13. Cang S, Iragavarapu C, Savooji J, Song Y, Liu D. ABT-199 (venetoclax) and BCL-2 inhibitors in clinical development. J Hematol Oncol [Internet]. 2015;8(1):1-8.
14. Muenchow A, Weller S, Hinterleitner C, Malenke E, Bugl S, Wirths S, et al. The BCL-2 selective inhibitor ABT-199 sensitizes soft tissue sarcomas to proteasome inhibition by a concerted mechanism requiring BAX and NOXA. Cell Death Dis [Internet]. 2020;11(8):701.
15. Instituto Nacional del Cáncer. Terapia de combinación con venetoclax aprobada para LLC - NCI [Internet]. Estados Unidos: NIH; 2019. Available from: https://www.cancer.gov/espanol/noticias/temas-y-relatos-blog/2019/venetoclax-obinutuzumab-fda-aprobacion-llc-llp
16. Fontana D, Ceccon M, Gambacorti-Passerini C, Mologni L. Activity of second-generation ALK inhibitors against crizotinib-resistant mutants in an NPM-ALK model compared to EML4-ALK. Cancer Med [Internet]. 2015;4(7):953-65.
17. Krieger E, Vriend G. YASARA View - molecular graphics for all devices - from smartphones to workstations. Bioinformatics [Internet]. 2014;30(20):2981-2.
18. Huang F, Greer A, Hurlburt W, Han X, Hafezi R, Wittenberg GM, et al. The mechanisms of differential sensitivity to an insulin-like growth factor-1 receptor inhibitor (BMS-536924) and rationale for combining with EGFR/HER2 inhibitors. Cancer Res [Internet]. 2009;69(1):161-70.
19. UniProt. BCL2 - Apoptosis regulator Bcl-2 - Homo sapiens (Human) [Internet]. Suiza: UniProt; 2017. Available from: https://www.uniprot.org/uniprotkb/A0A1L4AQQ5/entry
20. Birkinshaw RW, Gong J nan, Luo CS, Lio D, White CA, Anderson MA, et al. Structures of BCL-2 in complex with venetoclax reveal the molecular basis of resistance mutations. Nat Commun [Internet]. 2019;10(1):2385.
21. UniProt. ALK - ALK tyrosine kinase receptor - Homo sapiens (Human) [Internet]. Suiza: UniProt; 2010. Available from: https://www.uniprot.org/uniprotkb/Q9UM73/entry

22. Huang Q, Johnson TW, Bailey S, Brooun A, Bunker KD, Burke BJ, et al. Design of Potent and Selective Inhibitors to Overcome Clinical Anaplastic Lymphoma Kinase Mutations Resistant to Crizotinib. J Med Chem [Internet]. 2014;57(4):1170-187.
23. Sakamoto H, Tsukaguchi T, Hiroshima S, Kodama T, Kobayashi T, Fukami TA, et al. CH5424802, a Selective ALK Inhibitor Capable of Blocking the Resistant Gatekeeper Mutant. Cancer Cell [Internet]. 2011;19(5):679-90.
24. Bayliss R, Choi J, Fennell DA, Fry AM, Richards MW. Molecular mechanisms that underpin EML4-ALK driven cancers and their response to targeted drugs. Cell Mol Life Sci [Internet]. 2016;73(6):120-24.
25. Elshatlawy M, Sampson J, Clarke K, Bayliss R. EML4-ALK biology and drug resistance in non-small cell lung cancer: a new phase of discoveries. Mol Oncol [Internet]. 2023;17(6):950-63.
26. Anagnostou VK, Lowery FJ, Zolota V, Tzelepi V, Gopinath A, Liceaga C, et al. High expression of BCL-2 predicts favorable outcome in non-small cell lung cancer patients with non squamous histology. BMC Cancer [Internet]. 2010;10(1):1-11.
27. Zhang J, Wang S, Wang L, Wang R, Chen S, Pan B, et al. Prognostic value of Bcl-2 expression in patients with non-small-cell lung cancer: a meta-analysis and systemic review. Onco Targets Ther [Internet]. 2015;8:3361-9.
28. Hanahan D. Hallmarks of Cancer: New Dimensions. Cancer Discov [Internet]. 2022;12(1):31-46.
29. Vogler M. Targeting BCL2-Proteins for the Treatment of Solid Tumours. Adv Med [Internet]. 2014;2014:1-14.
30. Shi Y, Ye J, Yang Y, Zhao Y, Shen H, Ye X, et al. The Basic Research of the Combinatorial Therapy of ABT-199 and Homoharringtonine on Acute Myeloid Leukemia. Front Oncol [Internet]. 2021;11:1-10.

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