ORIGINAL ARTICLE

Proposal of combined therapeutic strategies for KRAS in non-small cell lung cancer based on in silico analysis

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ABSTRACT

Objective: Patients with non-small cell lung cancer positive for the anaplastic lymphoma kinase (ALK+) gene mutation who also have mutations in the Kirsten rat sarcoma (KRAS) gene, such as $KRAS^{G12C}$, are showing resistance to both anaplastic lymphoma kinase (ALK) gene and KRAS inhibitors. Therefore, the interaction between ALK inhibitors and KRAS was analyzed to suggest a synergy between them.
Materials and methods: The study performed homology modeling of the $KRAS^{WT}$, $KRAS^{G12C}$ and ALK^{WT} structures. Subsequently, molecular dockings were carried out to determine the binding energy of ALK and KRAS inhibitors and to evaluate the possible interaction of ALK inhibitors with KRAS and the $KRAS^{G12C}$ structure. Finally, the expression in the RAS/MEK pathway was analyzed using the Western Blot technique.
Results: The binding energy values show the potential interaction of ALK^{WT} inhibitors, such as crizotinib and alectinib, with the $KRAS^{WT}$ and $KRAS^{G12C}$ structures. The binding of crizotinib to $KRAS^{WT}$ and $KRAS^{G12C}$, respectively, indicates interaction energy values (42.77 kcal/mol and 46.20 kcal/mol) which are very similar to those obtained between crizotinib and ALK (42.37 kcal/mol). In turn, alectinib bound to the same site as drugs targeting KRAS and $KRAS^{G12C}$, and showed interaction energy values (51.74 kcal/mol and 54.69 kcal/mol, respectively) higher than those obtained with ALK (44.94 kcal/mol). Finally, a significant decrease in RAS expression within the RAS/MEK pathway was observed in ALK+ and ALK^{I1196M} lung cancer cell lines treated with crizotinib and alectinib.
Conclusions: In silico techniques of this study demonstrate the potential binding of ALK inhibitors (crizotinib and alectinib) to the KRAS structure. In addition, this allows suggesting a possible combination therapy between KRAS and ALK inhibitors for cases of coexistence of both mutations that can be assessed in subsequent trials with cell lines.

Keywords: Carcinoma, Non-Small-Cell Lung; Anaplastic Lymphoma Kinase; Molecular Docking Simulation (Source: MeSH NLM).

INTRODUCTION

Lung cancer (LC) is one of the leading causes of cancer-related deaths globally; thus, it ranked fourth in prevalence among all types of cancer in 2020. Non-small cell lung cancer (NSCLC) is the most prevalent form of LC, accounting for 84 % of the overall diagnoses [1].

Several molecular alterations have been identified in NSCLC, such as gene (EGFR, MET, KRAS, BRAF, ERK) and chromosomal (ALK or ROS1) mutations [2,3]. In patients with anaplastic lymphoma kinase-positive NSCLC (ALK+ NSCLC), a paracentric inversion of the Echinoderm microtubule-associated protein-like 4 (EML4) gene and the ALK gene is observed, leading to the formation of an abnormal fusion protein (EML4-ALK) [4,5], which, in turn, leads to the persistence of catalytic activity in its intracellular domain. Therefore, uncontrolled phosphorylation occurs due to its kinase nature, which triggers the deregulated activation of multiple signaling pathways. These pathways include cell proliferation (through PLC and RAS), cell survival (through PI3K), tumor growth (STAT 3/5 pathway), and the pathway associated with the BCL2 family of anti-apoptotic proteins [6]. Consequently, it is important to highlight the significance of addressing the implications of this mutation in the progression and treatment of NSCLC.

Within the RAS protein family, three crucial proto-oncogenes have been identified: KRAS, NRAS, and HRAS, with KRAS being predominant in solid tumors such as LC [7]. The KRAS protein occurs in two different states: an inactive state bound to guanosine diphosphate (GDP) and an active state bound to guanosine triphosphate (GTP). The inactivation of KRAS is normally induced by RasGTPase-activating proteins (RasGAP) [8]. Nevertheless, the most common mutations in the amino acid residues Gly12, Gly13 and Val61 [9] lead to resistance in RasGAP-mediated GTP hydrolysis [10,11]. This results in a constitutively active form of KRAS, which

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triggers the uncontrolled activation of key mechanisms related to growth, proliferation and survival in oncogenic cells (Figure 1). This thorough understanding of the molecular events related to KRAS underscores its vital role in LC pathogenesis and emphasizes the critical need to develop therapies specifically targeting this signaling pathway to improve clinical outcomes in affected patients.

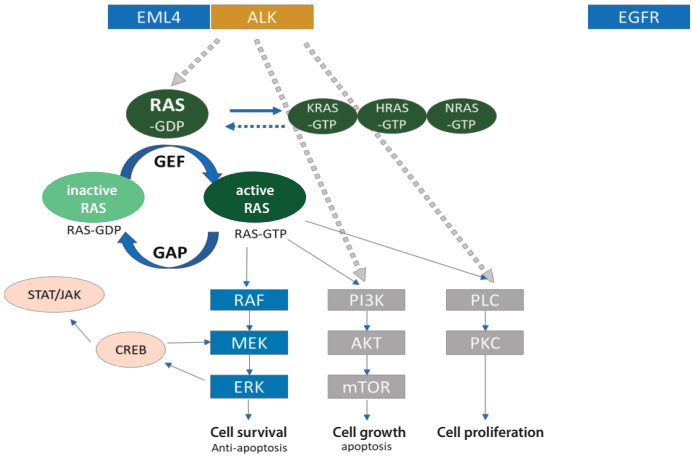

Figure 1. RAS/MEK pathway after KRAS and EML4-ALK alterations in NSCLC. Adapted from Huang L, Guo Z, Wang F, Fu L. KRAS mutation: from undruggable to druggable in cancer. Signal Transduct Target Ther. 2021;6(1):1-20

Conventional targeted therapy strategies focus on the direct competition between ALK inhibitors (ALKinhs) [12,13] and the ATP molecule for the latter's interaction site, which prevents the post-translational phosphorylation process. Nevertheless, in recent years, resistance to ALKinhs— crizotinib, ceritinib, alectinib [14-16] and brigatinib [17]—has been observed in patients with ALK+ NSCLC. This phenomenon is exacerbated by the concomitance of alterations in KRAS, ALK and/or EGFR in these patients, resulting in increased resistance to both ALK and KRAS inhibitors [18,19].

It has been identified that certain fusion proteins incorporating tyrosine kinase receptors, such as EML4-ALK, can associate with GRB2 and SOS to form membrane-free cytoplasmic protein granules, resulting in the activation of KRAS and other downstream signaling [20]. This thorough understanding of the underlying molecular mechanisms highlights the complexity of interactions within signaling pathways in the context of ALK+ NSCLC and suggests the need for more integrated and specific therapeutic approaches to address emerging resistance to conventional treatments.

MATERIALS AND METHODS

Study design and population

The results of this study are based on a descriptive analysis that combined both in silico and in vitro assays. The in silico studies were performed using specialized tools and software, and the study population consisted of the $KRAS^{WT}$, $KRAS^{G12C}$ and ALK structures, from the RCSB-PDB (Research Collaboratory for Structural Bioinformatics: Protein Data Bank) database, while the in vitro assays used the following NSCLC cell lines as the study population: EML4-ALK^{WT}, EML4-$ALKL^{1196M}$ and EML4-ALK^{G1202R}. The experiments were conducted in the research laboratories of the Universidad de Piura. All stages of the research were carried out with the approval of the Institutional Research Ethics Board of the Universidad de Piura.

Variables and measurements

In silico assays presented the variables of molecular docking of crizotinib with $KRAS^{WT}$ and $KRAS^{G12C}$ and molecular docking of alectinib with $KRAS^{WT}$ and $KRAS^{G12C}$, measured in kcal/mol. Experimental assays presented RAS and MEK expression as variables, and such expression was normalized with B-actin expressed in its variation.

Statistical analysis

The half-maximal inhibitory concentration (IC_{50}) was estimated using parametric nonlinear regression adjusted to a 95 % confidence interval. On the other hand, ANOVA and Tukey's tests were used to compare multiple groups of variables; and it was considered statistically significant for $p < 0.05$. Graphs were created using GraphPad Prism, Version 10.0.2.

Ethical considerations

This study was approved by the Institutional Research Ethics Board of the Universidad de Piura (N°: PREMED08202116). 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.

In silico assays

A computational approach was used to obtain a deeper uderstanding of the nature of the interaction at the binding site of the KRAS protein and its specific inhibitory ligands. For this objective, molecular modeling and docking technques were applied, allowing a detailed exploration of the molecular interactions at the atomic level.

Molecular modeling of KRAS

Molecular modeling process is an essential technique to obtain the complete three-dimensional structure of macromolecules, particularly in cases where they are not available or incompletely presented, such as with KRAS and ALK. In the context of this study, the amino acid sequence of the KRAS protein was obtained from UniProt (https://www.uniprot.org/), from which its three-dimensional structure was generated using the YASARA™ homology modeling module (http://www.yasara.org/) Version 22.9.24. In addition, the structure of $KRAS^{G12C}$ was modeled to introduce a mutation into the $KRAS^{WT}$ model. Finally, the ALK model was obtained also using YASARA™ software.

This molecular modeling module facilitates the comparison of the sequence in ".fasta" format with three-dimensional structures stored in RCSB-PDB (https://www.rcsb.org/), which allows generating the corresponding three-dimensional structure. The entire procedure was conducted following a protocol approved by the Critical Assessment of Protein Structure Prediction (CASP), which ensured the accuracy and reliability of the results obtained in this molecular modeling analysis [21].

Docking of KRAS and inhibitors

The molecular docking process was fundamental for generating the complexes formed by the $KRAS^{WT}$, $KRAS^{G12C}$ and ALK^{WT} proteins in interaction with the specific KRAS inhibitors: adagrasib, sotorasib and SML8-73-1, as well as the specific ALK inhibitors: crizotinib and alectinib. The structural characteristics of these drugs were obtained from the ZINC15 database (https://zinc.docking.org/) in MOL format. Thereafter, an optimization process of the complexes formed was carried out, and the missing hydrogens were added using the YASARA™ software.

The interaction energy was calculated in kcal/mol for each complex, thereby allowing the quantitative assessment of the stability and strength of the interaction between the studied macromolecules and the specific inhibitors. This detailed analysis of the molecular interactions provided valuable information on the binding affinity and stability of the complexes formed, significantly contributing to a deeper understanding of the mechanisms underlying drug-protein interaction in the context of NSCLC.

In vitro assays

To evaluate the variation in RAS expression in murine NSCLC cell lines, assays were conducted to determine the IC_{50}, aiming to identify the optimal dose for treatment with ALK inhibitors. Subsequently, the assays used standardized electro-transfer and Western Blot techniques, which enabled a quantitative and qualitative assessment of RAS expression levels in NSCLC cell lines treated with different doses of ALK inhibitors. The analysis of these data provided information on the influence of ALK inhibitors on RAS expression, which contributed to a deeper understanding of the molecular mechanisms involved in the pathogenesis of NSCLC.

Cell lines

To conduct the in vitro assays, three murine NSCLC cell lines were used—Ba/F3 EML4-ALK^{WT}, Ba/F3 EML4-ALK^{L1196M} and Ba/F3 EML4-ALK^{G1202R}—which were provided by researchers Luca Mologni and Diletta Fontana from the Università degli Studi di Milano Bicocca [22]. Each of the experimental assays was performed in three separate replicates, which ensured the robustness and reliability of the data obtained.

The cell lines remained under culture conditions in 1X DMEM Sigma-Aldrich) supplemented with 10 % inactivated bovine serum and 1% penicillin/streptomycin. Cell culture was carried out in an incubation environment at 37° C with a controlled atmosphere of 5 % CO_2, which provided an optimal environment for cell growth and viability during the development of in vitro assays.

Determination of IC_{50}

Cells were cultured at 10^5 cells/mL of each cell line in 96-well plates, followed by treatment with the drugs crizotinib (HY-50878, 877399-52-5) and alectinib (HY-13011, 1256580-46-7) in eight serially diluted concentrations at a 1:3 ratio. The minimum concentration used was 0 µM, while the maximum concentration was 10 µM. The samples were incubated for 48 hours, after which 10 % CellTiter 96® AQueous One Solution Cell Proliferation Assay (MTS) (Promega) was added. Following an additional three hours, absorbance readings at 490 nm were obtained using a Multiskan Go spectrophotometer (TermoScientific®).

The collected data were used to calculate the IC_{50}, which allowed an accurate assessment of the efficacy of the drugs crizotinib and alectinib in inhibiting cell growth in the studied NSCLC cell lines. This quantitative analysis provided crucial information on the cell response to different concentrations of the drugs, contributing to a deeper understanding of their sensitivity and resistance profiles.

Treatment

Cells were cultured at 10^6 cells/ml for each cell line in 12-well plates. Each cell line was subjected to the following experimental conditions: a control group with no treatment, a group treated with an IC_{50} of 50 nM crizotinib

and another group treated with an IC_{50} of 50 nM alectinib. After a 48-hour incubation period, the cells were harvested and proteins were extracted using 1X RIPA Buffer (Thermo Scientific), 100x Halt™ Protease & Phosphatase Inhibitor Cocktail (Thermo Scientific) and 100X 0.5M EDTA Solution (Thermo Scientific).

The extracted proteins were subsequently denatured using Buffer Laemly (Sigma-Aldrich) at 95 °C for 5 min, and subsequently stored at -20 °C for preservation. This procedure ensured optimal conservation of the protein samples and preserved their structural integrity, allowing for subsequent detailed analyses of the proteins of interest.

Protein expression

A Western blot analysis was carried out to determine the expression of RAS and MEK. The extracted proteins were separated on SDS polyacrylamide gels and transferred to 0.2 µm nitrocellulose membranes (Amersham™ Protran™). After transfer, blocking was performed using 5 % fat-free milk for one hour. The membranes were subsequently incubated overnight with the following primary antibodies: anti-RAS (ab52939) at a dilution of 1:5000, anti-MEK (ab178876) at a dilution of 1:20000 and B-actin (ab8227) at a dilution of 1:1000.

Following the incubation with the primary antibodies, the membranes were incubated with the secondary antibody (ab205718) at a dilution of 1:5000 for one hour. After the required washes, the membranes were developed using Clarity™ Western ECL substrate (Bio-Rad). Final images were obtained using the Chemidoc Imaging Instrument imaging system (Bio-Rad).

RESULTS

Molecular modeling of $KRAS^{WT}$, $KRAS^{G12C}$

Modeling of the three-dimensional structures of $KRAS^{WT}$ and $KRAS^{G12C}$ proteins, as well as ALK, was performed using YASARA™ software. For $KRAS^{WT}$, five different models were obtained based on the three-dimensional structures previously stored in RCSB-PDB (codes: 4LDJ, 4QL3, 5XCO, 5E95 and 6MBU). From these models, a hybrid model with a Z-score of 0.586 was generated and identified as the best model for this study. The Z-score describes the number of standard deviations from the mean structure quality obtained from high-resolution X-ray analysis.

A specific mutation was introduced into the previously obtained hybrid model to obtain $KRAS^{G12C}$. This enabled the generation of an accurate representation of this mutational variant in the three-dimensional structure of KRAS.

Finally, the ALK model was generated using the three-dimensional models from the PDB codes—4CLJ, 4FOD, 4ANL, 5FTO—as the structural basis. The hybrid model generated during this process was selected as the final model for the subsequent analysis. The results provided accurate representations of the three-dimensional structures of the KRAS and ALK proteins, which served as a crucial starting point for understanding the relevant molecular interactions in the context of the study.

Molecular docking of $KRAS^{wt}$, $KRAS^{G12C}$ and ALK with specific KRAS and ALK inhibitors

KRAS is a GTPase that switches between an inactive state bound to GDP and an active state bound to GTP. This study includes docking of the structures of $KRAS^{WT}$ and $KRAS^{G12C}$ with their respective specific inhibitors adagrasib, sotorasib and SML8-73-1, as well as with the inhibitors crizotinib and alectinib. The interaction of these proteins with the inhibitors led to obtaining interaction energy values in kcal/mol, as detailed in Table 1.

ALK inhibitors crizotinib and alectinib showed similar binding energy values when interacting with $KRAS^{WT}$, $KRAS^{G12C}$ and ALK. Nevertheless, a decrease in binding energies was observed for the inhibitors adragasib, sotorasib and SML8-73-1 when interacting with ALK compared to $KRAS^{WT}$ and $KRAS^{G12C}$, as indicated in Table 1.

Table 1. Binding energy of $KRAS^{WT}$, $KRAS^{G12C}$, ALK

Drugs	$KRAS^{WT}$ (kcal/mol)	$KRAS^{G12C}$ (kcal/mol)	ALK (kcal/mol)
Adagrasib	55.41	55.71	46.01
Sotorasib	57.75	49.63	35.58
SML	79.22	79.82	44.18
Crizotinib	42.77	46.20	42.37
Alectinib	51.74	54.69	44.94

Since the inhibitors adagrasib and sotorasib are specific for KRAS, they docked at the drug binding site (DBS) of the $KRAS^{WT}$ and KRASG12C proteins. Interaction energies of 55.41 kcal/mol and 55.71 kcal/mol, respectively, were observed for adagrasib, and 57.75 kcal/mol and 49.63 kcal/mol for the complexes formed with sotorasib. Despite these drugs also bound to ALK, the results revealed lower values compared to those obtained when binding to KRAS.

Alectinib, an ALK inhibitor, also docked at the same site as the KRAS-specific drugs and presented interaction energies of 51.74 kcal/mol and 54.69 kcal/mol for the $KRAS^{WT}$-alectinib and $KRAS^{G12C}$-alectinib complexes, respectively (Figures 2A and 2B). These findings point to significant differences in molecular interactions between KRAS- and ALK-specific inhibitors.

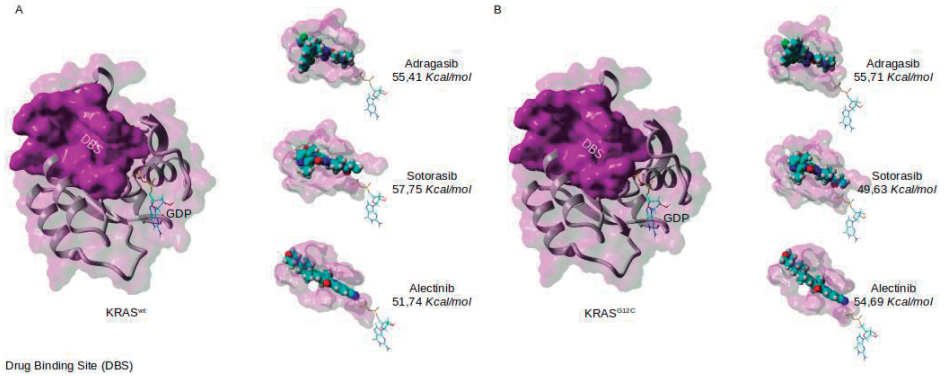

Figure 2A. KRAS and inhibitors that interact at the DBS

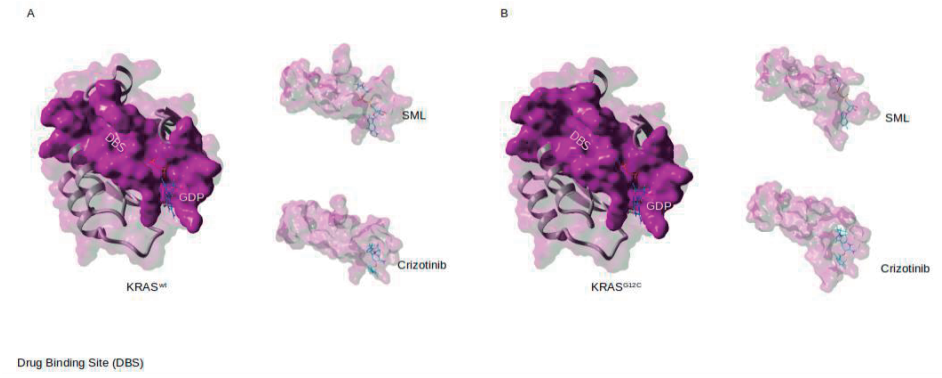

Figure 2B. KRAS and inhibitors that interact at the GDP binding site

SML8-73-1 was selected as a competitive inhibitor of GDP, which targeted the docking towards the GDP-KRAS interaction site. The values obtained for the interaction of $KRAS^{WT}$ and $KRAS^{G12C}$ with SML8-73-1 were 79.22 kcal/mol and 79.82 kcal/mol, respectively. Moreover, the interaction of SML8-73-1 with ALK showed a value of 44.18 kcal/mol, indicating a less energetic interaction compared to those with $KRAS^{WT}$ and $KRAS^{G12C}$.

Concerning the docking of crizotinib with $KRAS^{WT}$ and $KRAS^{G12C}$, interaction energy values of 42.77 kcal/mol and 46.20 kcal/mol, respectively, were observed. Such values turned out to be very similar to the interaction energy obtained between crizotinib and ALK (42.37 kcal/mol), as shown by Figures 3A and 3B.

Expression of the RAS/MEK pathway

The expression of the RAS/MEK pathway was analyzed in Ba/F3 EML4-ALK^{WT}, Ba/F3 EML4-ALK^{L1196M} and Ba/F3 EML4-ALK^{G1202R} cell lines under different conditions in separate assays. These conditions included a control group with no treatment, a group treated with 50 nM crizotinib and another group treated with 50 nM alectinib. The resulting values represent the transformation (Fold Change = FC) of the normalized expression of RAS and MEK relative to ß-actin.

The results revealed a significant decrease in RAS expression in all three cell lines (EML4-ALK^{G1202R} < EML4-ALK^{L1196M} < EML4-ALK^{WT}) compared to ß-actin expression. Furthermore, significantly decreased expressions were observed in Ba/F3 EML4-ALK^{WT} and EML4-ALK^{L1196M} lines treated with crizotinib

and alectinib compared to their respective control groups. In the case of the Ba/F3 EML4-ALK^{G1202R} line, a steady low RAS expression was observed in the control group and the groups treated with crizotinib and alectinib. These findings consistently show a differential regulation of RAS expression in response to treatments in the analyzed cell lines with the inhibitors crizotinib and alectinib (Figure 3A).

As to MEK expression, a significant increase was observed in the Ba/F3 EML4-ALK^{WT} line treated with alectinib compared to its control group and the group treated with crizotinib. Additionally, a sharp increase in MEK expression was detected in the Ba/F3 EML4-ALK^{L1196M} line, with a further increase in the group treated with alectinib. On the other hand, a significant decrease in MEK expression was observed in the Ba/F3 EML4-ALK^{G1202R} line, in its control group and in the groups treated with crizotinib and alectinib (Figure 3B).

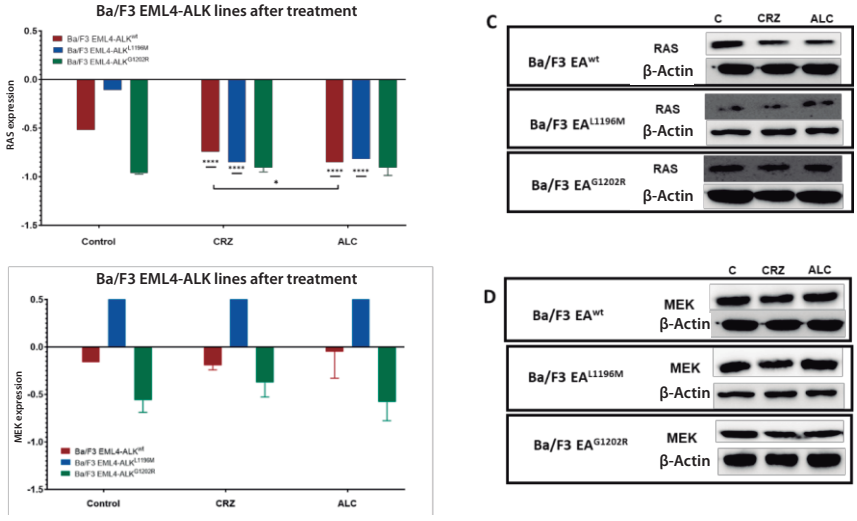

Figure 3. Expression of RAS and MEK in ALK+ NSCLC cell lines treated with ALK inhibitors. A and B. Normalized expression of RAS and MEK in EML4-ALK^{WT}, EML4-ALK^{L1196M}, EML4-ALK^{G1202R} cell lines treated with 50 nM crizotinib (CRZ) and 50 nM alectinib (ALC). C and D. Expression of RAS and MEK by Western Blot: comparison by multiple groups of variables using ANOVA and Tukey's test, considered statistically significant for $p < 0.05$ (* = 0.0332; ** = 0.0021; *** = 0.0002; **** <0.0001) and not significant for $p = 0.1234$.

DISCUSSION

The KRAS gene (Ki-ras2 Kirsten rat sarcoma viral oncogene homolog) is broadly recognized as an oncogene that encodes the GTPase transducer protein KRAS, thus playing a key role in the regulation of cell division and in the transmission of external signals to the cell nucleus [23]. Along with genetic alterations in EGFR and ALK, these represent some of the most frequent abnormalities identified in NSCLC.

Although mutations in the KRAS and ALK genes often occur in a mutually exclusive manner, several studies have revealed the coexistence of both mutations in certain clinical cases [23,24]. Mutations in the KRAS gene have been shown to have a significant impact on cellular transformation, which leads to increased resistance to chemotherapy and biological therapies targeting epidermal growth factor receptors.

In view of the complexity of the uncontrolled activation of signaling pathways, particularly in cell proliferation, this study underscores the importance of seeking novel therapeutic strategies to effectively address these molecular abnormalities. In this regard, both in silico and in vitro experiments were carried out in order to provide a solid foundation for the development of more effective and specific therapies targeting the activated signaling cascade in the context of the KRAS and ALK mutations in NSCLC.

Clinical results from short series have pointed to KRAS mutations as a possible mechanism of secondary resistance to ALK inhibitors, such as crizotinib. This suggests a potential association between the combined alteration of ALK and KRAS and primary resistance to treatment with ALK inhibitors [25]. Consistent with these findings, our results reveal that crizotinib, an ATP-competitive kinase inhibitor, docks at the GDP binding site with similar interaction energy values in KRAS^{WT} and KRAS^{G12C} as well as in ALK.

On the other hand, alectinib showed a preference for the DBS rather than the GDP binding site. The resulting interaction energy values were significantly higher when interacting with $KRAS^{WT}$ and $KRAS^{G12C}$ compared to ALK. These results suggest the possibility of cross-interactions between kinase inhibitors of ALK and $KRAS^{WT}$ as well as $KRAS^{G12C}$. However, tests performed with specific $KRAS^{WT}$ and $KRAS^{G12C}$ inhibitors with ALK revealed lower binding energy values, indicating lower affinity in these interactions.

These findings raise the possibility of exploring combination therapies involving both ALK and KRAS inhibitors since clinical evidence has suggested a limited response to exclusive treatment with tyrosine kinase inhibitors in patients with ALK-KRAS mutations. In this regard, the evaluation of RAS expression in Ba/F3 EML4-ALK^{WT}, L1196M and G1202R cell lines treated with the aforementioned drugs provides important information to better understand cellular responses in the presence of these inhibitors. These results suggest possible more effective combined therapeutic strategies to address the complexity of signaling pathways in NSCLC.

The results obtained using the in silico approach highlight the importance of exploring combination therapies in the treatment of NSCLC. As to our research, previous studies have indicated that patients with ALK+ NSCLC who also have mutations in $KRAS^{G12C}$ may have an improved response to treatment with brigatinib, although efficacy has not yet reached significant levels [18]. Recent research papers have documented resistance towards adagrasib and sotorasib in patients with $KRAS^{G12C}$, though underlying mechanisms are still being studied [9,26,27].

Potential synergistic treatments involving sotorasib and adagrasib in combination with MEK inhibitors, EGFR inhibitors, immune checkpoint inhibitors and tyrosine kinase inhibitors are currently being studied [28], with the aim of achieving improvements in therapeutic responses [9]. Some studies have proposed combinations such as sotorasib + crizotinib, the latter acting as a MEK inhibitor, and have shown promising results in improving response rates [29]. Moreover, recent findings have supported the efficacy of sotorasib in combination with other anticancer drugs [24].

Unlike the results for MEK protein expression obtained in our study, which showed slight but not significant changes, it is possible that MEK is not the main contributor to tumor proliferation in the NSCLC cellular models analyzed. Despite previous research has suggested that MEK inhibitors might be effective in suppressing proliferative activity in NSCLC [30], our findings show that, in this particular context, other pathways such as PI3K/AKT/mTOR might play a more crucial role and could be considered as potential therapeutic targets for future research studies.

In conclusion, in silico studies indicate the possibility of interaction between ALK inhibitors such as crizotinib and alectinib towards $KRAS^{WT}$ and $KRAS^{G12C}$, showing similar or superior docking compared to their interaction with ALK. Nevertheless, $KRAS^{WT}$ and $KRAS^{G12C}$ inhibitors, such as adagrasib and sotorasib, show lower docking values with ALK compared to $KRAS^{WT}$. Finally, after the evaluation of RAS expression—which identified a decrease in its expression in the lines treated with crizotinib and alectinib—the molecular docking detected between KRAS and $KRAS^{WT}$ with ALK inhibitors is confirmed. These results allow suggesting the potential of combination therapy involving KRAS and ALK inhibitors for cases of coexistence of both mutations, which should be evaluated in subsequent assays with cell lines.

Acknowledgment: We express our gratitude to researchers Diletta Fontana and Luca Mologni for providing their murine cellular models of NSCLC.

Author contributions: DCR and SIV conceptualized the study. JFC and RZD performed data curation and managed the software for the study. JFC, BMD, SIV and RZD conducted the formal analysis of the study. SIV and RZD managed funding with Consejo Nacional de Ciencia, Tecnología e Innovación Tecnológica (Concytec - National Council of Science, Technology and Technological Innovation)-ProCiencia. JFC and SIV proposed the research method. BMD was in charge of the administrative aspects of the project. JFC, RZD and SIV supervised the research. DCR wrote the original draft. All authors contributed to the research, writing, review and editing the article.

Funding sources: The research was funded by Concytec through ProCiencia program, by the project *Determinación in vitro de nuevas dianas terapéuticas en modelos celulares de cáncer de pulmón de células no pequeñas positivo para la mutación del gen linfoma anaplásico quinasa (ALK) resistente a inhibidores selectivos de la proteína ALK* (In vitro determination of novel therapeutic targets in cellular models of non-small cell lung cancer positive for the anaplastic lymphoma kinase [ALK] gene mutation and resistant to selective ALK protein inhibitors), with contract number: 375-2019.

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

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Reception date: October 26, 2023
Evaluation date: December 1, 2023
Approval date: December 20, 2023

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