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Liquid biopsy in clinical outcomes and detection of T790M mutation in metastatic non-small cell lung cancer after progression to EGFR-TKI

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Abstract.
BACKGROUND: Liquid biopsy (LB) is used to detect epidermal growth factor receptor (EGFR) mutations in non-small cell lung cancer (NSCLC) and has been demonstrated to have prognostic and predictive value.
OBJECTIVE: To associate the rates of *EGFR* and T790M mutations detected by LB during disease progression after first- or second-generation EGFR-TKIs with clinical characteristics and survival outcomes.
METHODS: From January 2018 to December 2021, 295 patients with advanced EGFR mutant (EGFRm) NSCLC treated with first- or second-generation EGFR-TKIs were retrospectively analyzed. LB was collected at the time of progression. The frequency of $EGFR^{T790M}$ mutations, overall survival (OS), and the clinical characteristics associated with LB positivity were determined.
RESULTS: The prevalence of $EGFR^{T790M}$ mutation detected using LB was 44%. In patients with negative vs. positive LB, the median OS was 45.0 months vs. 25.0 months ($p = 0.0001$), respectively. Patients with a T790M mutation receiving osimertinib had a median OS of 44 months (95% CI [33.05–54.99]). Clinical characteristics associated with positive LB at progression extra-thoracic involvement, >3 metastatic sites, and bone metastases.
CONCLUSIONS: Our findings showed that LB positivity was associated with worse survival outcomes and specific clinical characteristics. This study also confirmed the feasibility and detection rate of T790M mutation in a Latin American population.

Keywords: EGFRm NSCLC, liquid biopsy, T790M mutation, osimertinib, ctDNA

1. Introduction

Non-small cell lung cancer (NSCLC) is the leading cause of cancer death worldwide [1,2]. The majority of NSCLC patients are diagnosed with advanced-stage disease [3,4]. Patients with NSCLC are treated based on a personalized approach, according to genomic profile,

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to ensure better oncological outcomes [5]. The detection of *epidermal growth factor receptor (EGFR)* mutations produces a paradigm shift in the treatment of NSCLC, which varies according to the geographic region in Latin America, and. oscillates between 32–54%. In Mexico, *EGFR* mutations represent around 36% of druggable alterations, which is higher compared with North America and Europe, ranking 10–15% [6,7].

NSCLC patients harboring *EGFR* mutations have improved oncological outcomes with *EGFR*-tyrosine kinase inhibitor (TKI) therapy [8–11]. According to the results of the FLAURA trial, osimertinib is currently the preferred upfront treatment [8]. However, in many other countries, the upfront therapy for patients with *EGFR*m NSCLC still depends on first- and second-generation TKIs followed by osimertinib in patients who developed a positive T790M mutation at disease progression. Among the common mechanism of acquired resistance after this treatment is the acquisition of *EGFR* T790M mutation in approximately 50–60% of cases [12–14].

EGFR T790M resistance mutation can be detected in tumor tissue (surgical biopsy or cytology specimens) or cell-free circulating tumor DNA (ctDNA) extracted from peripheral blood through liquid biopsy (LB) [15]. New tumor tissue biopsy is the most reliable procedure for detecting the T790M mutation at progression. However, this is not always feasible and might be associated with complications due to the invasiveness of tumor biopsy. Other factors, such as tumor heterogeneity and unwillingness, make this method challenging for many patients [16–18].

LB refers to any tumor-derived material circulating through the blood or other body fluid. In lung cancer, circulating tumor cells and ctDNA are the most widely studied substrates [19]. LB has been shown to represent an innovative alternative for patients unable to undergo re-biopsy as a non-invasive method that allows the detection of *EGFR* T790M [20]. Other advantages over tissue samples include the reduced cost, rapid turnaround time, and potential for longitudinal monitoring by serial biopsies, showing a high concordance with standard tissue genotyping [20,21]. In addition, some studies have demonstrated that detection of *EGFR*m ctDNA is associated with worse outcomes and disease burden, which could be predictive of response, increasing ctDNA levels which may anticipate progression to standard imaging progression by RECIST [22,23].

Scarce information is available on the utility of LB to detect T790M in the LATAM population in a real-world context and its association with clinical outcomes. Thus, this study aimed to determine the rate of T790M mutation as a mechanism of resistance to first or second-generation *EGFR*-TKI according to plasma ctDNA detected on LB, associate positiveness to clinical characteristics, and evaluate outcomes in those who received osimertinib.

2. Materials and methods

2.1. Ethics approval

This study was approved by the ethics committee of participating medical institution and was authorized under the number Rev/032/20.

2.2. Experimental subjects

In this retrospective cohort, we evaluated 295 patients with Stage IV NSCLC treated in one institution between January 2018–December 2021. Patients harboring a sensitive *EGFR* mutation (delexon19 or L858R) and disease progression in the first-line setting with one first or second-generation *EGFR*-TKI were included. Patients included had 1) confirmed diagnosis of NSCLC, 2) *EGFR* mutation, 3) disease progression according to RECIST 1.1 criteria, 4) Treatment with first or second-generation *EGFR*-TKI. 5) All patients required standard laboratory workups before starting therapy and during treatment according to local policies to monitor adverse events.

All patients performed an LB at radiological progression with the Biocept Target Selector TM ctDNA platform [24]. Each blood samples consist of 20 ml of peripheral blood collected in the CEE-Sure TM tubes (Biocept, San Diego, California, USA). *EGFR* mutation assay is a quantitative Real-Time PCR-based mutant enrichment assay with selective blocking of *EGFR* wild-type amplification. The amplification products are purified and subject to sequencing to confirm the presence of the *EGFR* mutation.

Two expert medical oncologists extracted all clinical and pathological data from electronic medical records. Collected data included the patient's clinical characteristics at baseline and disease progression, and date, results, and number of LBs before a positive result.

2.3. Statistical analysis

Categorical variables were summarized as frequencies and percentages, while continuous variables were reported as mean or median with their corresponding

Table 1
Baseline characteristics

Characteristics	n^a	%
Sex, female	196	66.4
Age, years		
Mean ± Std. Dev.	62.5 ± 12.31	
Smoking status		
Ever smokers	61	20.6
Nonsmokers	243	79.4
Tobacco index, pack/yrs.		
Mean ± Std. Dev.	10.42 ± 11.12	
Body Mass Index (BMI)		
Mean ± Std. Dev	24.24 ± 4.34	
Comorbidities		
Hypertension	85	28.0
Diabetes Mellitus Type 2	44	15.0
Obesity	19	15.5
Chronic Obstructive pulmonary disease	6	2.0
Heart failure	4	1.3
ECOG PS scale		
0–1	271	92.1
$\geqslant 2$	24	7.9
Adenocarcinoma	285	96.1
Squamous or mixed histology	10	3.9
LADC subtype		
Lepidic	25	8.5
Acinar	84	28.5
Papillary	24	8.1
Solid	76	25.8
Micropapillary	6	2.0
Not otherwise specified	80	27.1
Histological grade		
Well-differentiated	16	5.4
Moderately differentiated	116	39.3
Poor differentiated	113	38.3
Not otherwise specified	50	16.9
Clinical stage IV	295	100
First line treatment		
1st generation TKI (Erlotinib, Gefitinib)	164	55.6
2d generation TKI (Afatinib)	131	44.4
EGFR mutations at diagnosis		
Exon 19 Del	197	66.0
L858R Exon 21	89	30.1
Other mutations	9	3.9

Notes. ECOG: Eastern Cooperative Oncology Group performance status scale. LADC: lung adenocarcinoma. EGFR: epidermal growth factor receptor. [a]Total number of subjects = 295.

dispersion measures. Progression-free survival (PFS), intracranial progression free-survival (icPFS), and Overall survival (OS) were estimated by the Kaplan-Meier method, and statistical differences among groups of interest were calculated with the Log-Rank test method. PFS was defined from the beginning of the second line treatment until disease progression according to RECIST Criteria or death; icPFS was defined from diagnosis to the date of appearance of new brain metastasis. OS was defined as the period from diagnosis to death or loss of follow-up. A Cox proportional hazard model was performed for the multivariate analysis. A significant p-value was < 0.05 in a two-sided test. For these statistical analyses, SPSS version 23 was used.

Table 2
Clinical characteristics at progression

Characteristics	n^a	%
Metastatic sites at progression		
CNS	30	47.6
Bone	28	38.1
Pleura	32	50.8
Adrenal glands	12	19
Liver	13	20.6
Type of progression		
Intrathoracic	89	30.2
Extra-thoracic	206	69.8
Second-line therapy		
Carboplatin Pemetrexed	139	47.1
Other systemic treatment	30	10.2
Other TKI	51	17.3
Osimertinib	22	7.5
TKI + local control	19	6.4
None	26	8.8

Notes. CNS: central nervous system. TKI: tyrosine kinase inhibitor. [a]Total number of subjects = 295.

3. Results

3.1. Clinical characteristics

Two hundred ninety-five patients were analyzed. Of the total, 66.4% were women, 79.4% had never smoked, 92.1% had an ECOG performance status (PS) of 0 to 1, and 96.61% were adenocarcinomas. Regarding mutations at the time of diagnosis, 66% had exon 19 deletions (exon19del), and 30.1% had a point mutation of exon 21 L858R. The most frequent metastatic sites at diagnosis were pleura (50%), followed by the central nervous system (47.6%) and bones (38.1%). All patient demographics and clinical characteristics are summarized in Table 1.

3.2. Clinical characteristics at disease progression

At the time of progression, 69.8% had extra-thoracic progression, whereas 30.2% had exclusively an intrathoracic affection (Table 2). All patients had an initial LB at the first disease progression, 23 underwent a second LB at the next progression, and one underwent a third LB after a third progression.

3.3. Detection of T790M in liquid biopsy

Of the 295 analyzed patients, 122 (41.4%) had positive T790M at the first disease progression. In most

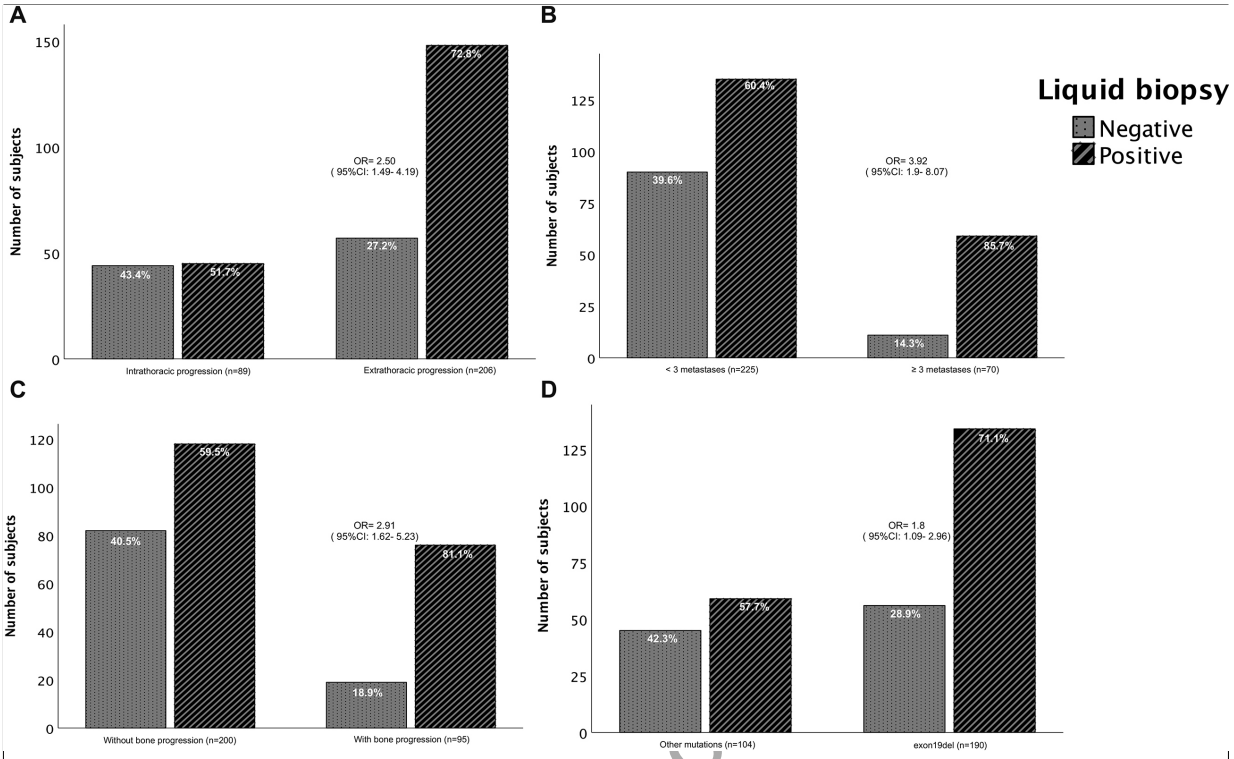

Fig. 1. Detection of ctDNA positivity or negativity by liquid biopsy. Based on clinicopathological characteristics: a) type of thoracic progression ($X_a^{1df} = 12.445$, $p \leqslant 0.001$), b) number of metastases ($X_a^{1df} = 15.290$, $p \leqslant 0.001$), c) bone progression ($z_a = 13.418$, df $= 1$, $p \leqslant 0.001$), and d) type of EGFR mutation ($X_a^{1df} = 5.372$, $p \leqslant 0.020$). Pearson's chi-square test was applied to nominal variables with an expected count of < 5. Odds ratio (OR) with CI: confidence interval at 95 % (95 % CI). Statistical significance was set at $^*p < 0.05$.

Table 3
Results of liquid biopsy

	n^a	%
T790M exon19del	69	56.4
T790M L858R	27	22.3
T790M exon19del + L858R	2	0.7
T790M	24	19.6

Notes. [a]Total number of subjects = 295.

of the cases, T790M was not the only detected alteration; 69 (56.4%) combined with exon19del, 27 (22.3%) with L858R, and 2 (0.7%) with exon19del and L858R. T790M alone was present in 24 (19.6%) patients. In additional nine patients, subsequent LBs were performed, and the T790M was detected for a total of 131 patients (44%) (Table 3).

3.4. Characteristics associated with positivity in liquid biopsy

The patients with exon19del at extra-thoracic progression, more than three sites of metastases involved at baseline, and the presence of bone metastases at progression had a higher LB detection rate. In patients with initial exon19del, LB positivity was 71%. Meanwhile, the positive rate in patients with other mutations was 57.7% (Fig. 1). Similarly, patients with extrathoracic progression had a higher LB detection rate (72.8%) compared with patients with intrathoracic progression (51.7%) ($p = 0.001$). Patients with three or more metastatic sites at disease progression had a higher positivity of LB (85.7%) in comparison with patients with two or less than two metastatic sites (60.4%) ($p = 0.0001$). Also, patients with bone involvement at disease progression had an LB-positive rate of 81.1%, compared with 59.5% in patients without bone affection. No differences in LB positivity were found in patients with or without brain metastases.

3.5. Survival outcomes

The median OS for all patients was 30 months (95% CI: 26.06–33.93). After TKI progression, the median PFS was eight months (95% CI: 6.49–9.50). The median OS was 45.0 months (95% CI: 37.03–52.96) vs.

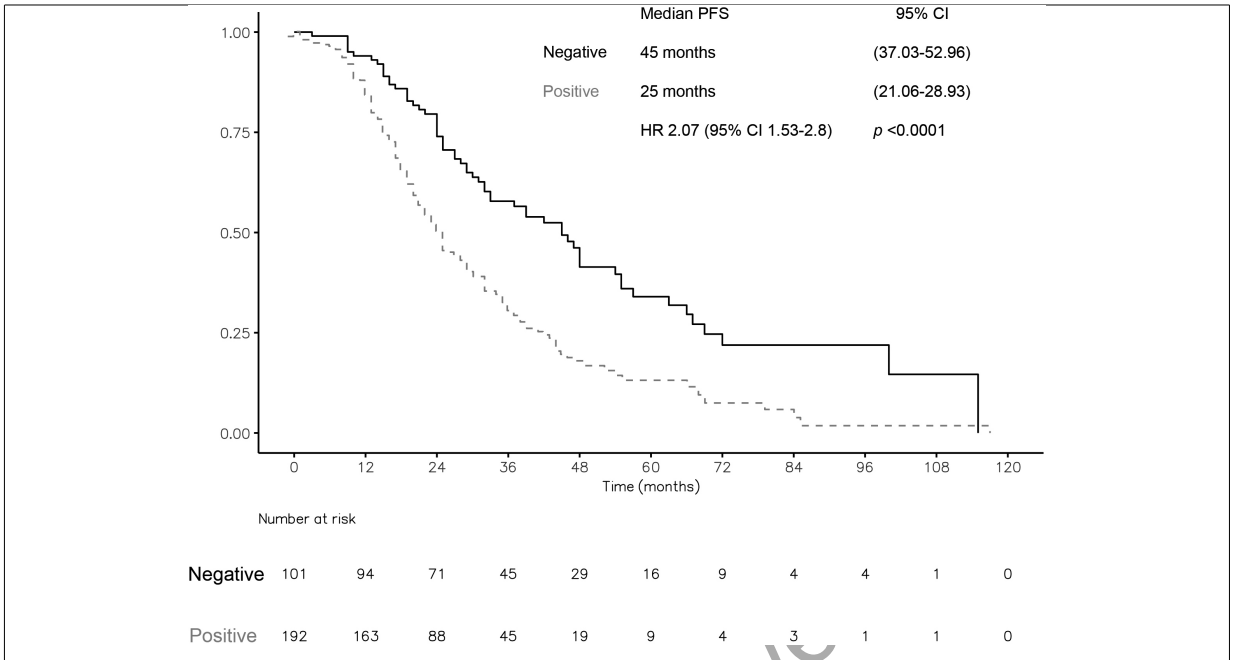

Fig. 2. Overall survival (OS) according to liquid biopsy (LB) results. CI: confidence interval. HR: hazard ratio. Statistical significance was set at $^{*}p$-value < 0.05.

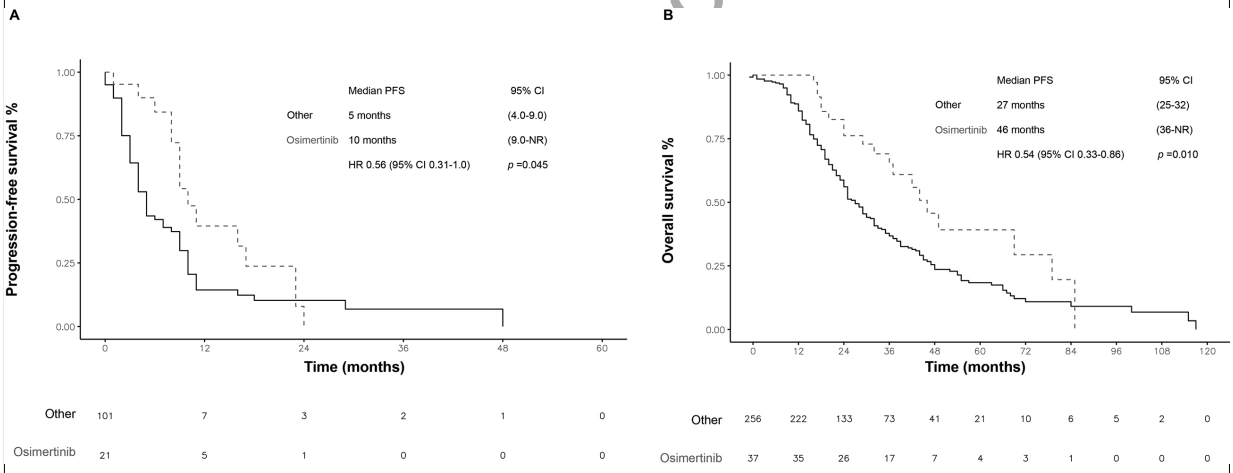

Fig. 3. Survival of patients with T790M mutation at disease progression. A) PFS of patients treated with osimertinib versus those treated with other drugs. B) Overall survival (OS) in patients treated with osimertinib vs. other treatments. PFS: progression-free survival. CI: confidence interval. NR: not rated. HR: hazard ratio. Statistical significance was set at a p-value < 0.05.

25.0 months [95% CI: 21.06–28.93; HR 2.00 (1.48, 2.71); $p = 0.0001$] in patients with negative vs positive LB respectively. Of note, no differences in OS were observed, between EGFR sensitive mutations (exondel19 and L858R) and T790M mutation, 23.0 months (95% CI: 19.27–26.72) vs 27.0 months (95% CI: 21.51–32.49) (Fig. 2). Of note, no differences in OS were observed, between EGFR sensitive mutations (exondel19 and L858R) and T790M mutation, 24.0 months (95% CI: 20.0–32.0) vs 25.0 months (95% CI: 21.0–32.0) (Fig. S1)

The median PFS in patients with a T790M mutation detected at first progression ($n = 122$) by LB and who were treated with second-line osimertinib was 10.0 months (95% CI 9.0–14.24), whereas, in patients treated with other therapy, the median PFS was 5.0 months [95% CI 4.0–9.0; HR 0.56 (0.31.1.00); $p = 0.045$] (Fig. 3A).

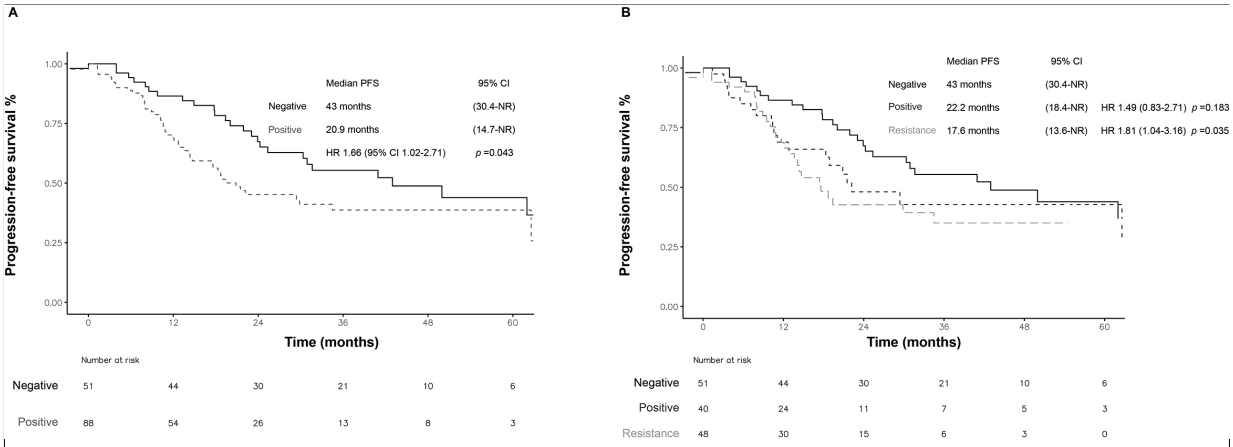

Fig. 4. icPFS according to liquid biopsy results. A) positive and negative. B) Positivity, sensitizing, or resistance mutations. icPFS: intracranial progression-free survival. CI: confidence interval. NR: not rated. HR: hazard ratio. Statistical significance was set at a p-value < 0.05.

In patients treated with osimertinib at any time ($n = 37$), irrespective of the line of treatment, the median OS was 46 months (95% CI 36.0–NR), and in those treated with other therapy was 27 months [95% CI 25.0–32; HR 0.54 (0.33–0.86); $p = 0.010$] (Fig. 3B).

In the case of intracranial disease progression in patients without baseline brain metastases ($n = 139$), the median icPFS was 20.9 months with positive LB compared with a median of 43.0 months in patients with a negative liquid biopsy (HR 1.66; 95% CI 1.02–2.71; $p = 0.043$).

According to the type of mutation detected at progression in the LB, patients with a positive T790M mutation had significantly lower icPFS (17.6 months; HR 1.81 95% CI 1.04–3.16; $p = 0.035$) compared with those with only EGFR sensitive mutations (22.2 months; HR 1.49; 95% CI 0.83–2.71) (Fig. 4).

4. Discussion

In our study, we demonstrated that the positivity of liquid biopsy at disease progression to a first or second-generation TKI was associated with worse survival outcomes including a shorter icPFS, and the detection rate of T790M mutation as the main mechanism of resistance in metastatic *EGFRm* NSCLC was 44% in Hispanic population. Moreover, we could identify that those patients with more than three metastatic extrathoracic diseases, and bone metastasis were associated with a greater probability of a positive result on LB.

A T790M as main resistance mechanism after first-line EGFR-TKI treatment has been reported to be around 47.1% in Hispanic population in tumor specimens [14]. In largest series that assessed mechanisms of acquired resistance to EGFR-TKI therapy, 63% of the patients developed T790M mutations. Of note, biopsies were obtained by the least invasive procedure and typically consisted of either a fine-needle aspiration or image-guided core biopsy, but not employed LB [13].

More complex scenarios have been identified in pretreated patients, in which the presence of T790M and persistence of common *EGFR*-activating mutations (exon 19 del and L858R) has been associated with poor prognosis in patients with advanced *EGFR*m NSCLC [25]. Our study found that in patients with a positive LB, the detection of T790M mutation alone was observed in 19.6%. In contrast, most patients have persistence of *EGFR* sensitive mutations, T790M plus exon 19 del in 56.4%, T790M plus L858R in 22.3%, and more complex combinations (T790M plus exon 19 del plus L858R) were extremely rare ($< 1\%$). In other studies, Liang and colleagues reported similar results. In a first-line post-TKI scenario, 53% of patients had coexistence of T790M mutation and exon19 del, and T790M mutation plus L858R was observed in 36% [26].

One meta-analysis confirmed that the T790M mutation used to coexist more frequently with an L858R than with the exon19del [odds ratio 1.65; 95% CI (1.17 to 2.32)], which potentially might explain the more aggressive biology of L858R tumors [25]. However, this information has been contradictory with further evidence which suggested a stronger association between the emergence of T790M and the persistence of the primary activating alteration, exon19del, among patients with acquired resistance to TKI (53% vs. 36%; OR 1.87; $p < 0.001$) [26]. In our cohort, we found that

exon19del was associated with a higher LB positive rate which is in line with Liang et al. study. Different results in the survival outcomes can be related to subsequent treatments. Patients who develop T790M mutation are candidates to osimertinib at progression to a first-line TKI, which can impact survival outcomes based on the positivity rate. Even so, the type of *EGFR* mutation has been demonstrated to be a factor associated with response to first-line *EGFR*-TKI treatment. In clinical trials, L858R mutation has been related to a shorter median PFS, even for patients receiving osimertinib [27].

Although the feasibility and reliability of LB have been confirmed in clinical practice [28–30], the test's sensitivity is related to the detection limits of the technique and the characteristics of ctDNA. Based on the different platforms, in some assays (BEAMing,) the detection rate of LB is around 70% [31], along with this, a relatively low sensitivity (60%) in comparison with high specificity (80–90%) has been reported in an independent meta-analysis [20,32]. Due to this possible low sensitivity, the current recommendation is that patients with negative LB should undergo tissue testing for T790M [33,34]. In our study, the LB detection rate was 41.4%, then the detection rate went up to 44% with subsequent testing with the same technique. This reinforces that even the LB could be a suitable option for some patients to detect additional resistance mechanisms if tissue is unavailable or the risk of undergoing an invasive procedure is too high. In line with our work, previous evidence has confirmed an additional 12.5% of detection rate in patients with an initial negative LB and then tested positive for T790M at a second LB [35]. In another study that used a median of 3 LB, the prevalence of T790M was 34.5% [36].

In addition to the procedure itself and technique employed in the ctDNA analysis, some clinical characteristics have gained relevance in the performance and sensitivity of the LB. In previous studies, the number of metastatic sites which reflects disease burden was associated with a higher positive rate [37]. We were able to identify the most common metastatic sites of metastases in the present cohort; pleura (47%), central nervous system (47%), and bone (38.1%). Remarkably, most patients had a moderate disease burden between 2 or 3 involved sites. We found that in patients with three or more sites of metastasis, especially when the affection was extra-thoracic and the bone was involved, the detection rate of the LB was significantly higher.

Patterns of progression and sites have gained relevance in clinical practice to consider subsequent strategies and overcome resistance in EGFR NSCLC extra-thoracic disease, as the main site of progression has been associated with a positive LB [38]. However, one-half of patients progressed within the lung as the initial progression after TKI treatment [38,39]. Al Halabil et al., in a retrospective study of patients with NSCLC treated with first and second-generation TKI, found that the main sites of progression were those initially involved in 50% of cases, being the intra-thoracic the most common site [38]. This was confirmed by Patel and colleagues, in which 17.5% had extra-thoracic failure, and 22.2% had intra- and extra-thoracic progression [38]. In contrast with these previous reports, our study found that extra-thoracic progression was the main site of progression (69%), and this finding was associated with a higher detection rate by LB and bone lesions, in line with previous reports [28,35].

ctDNA shedding is related to the disease burden [22, 40] and can help distinguish between residual disease or anticipated imaging changes [41]. This might explain why LB has been associated with worse survival, especially in patients with a persistent EGFR-sensitive mutation. This was replicated in our study in which patients with a positive LB had worse survival independent of the type of mutation [42]. Despite these findings, starting osimertinib at the moment of the T790M mutation detection is not justified, which precedes the radiological progression [43]. In this regard, shedding ctDNA with persistent EGFR-sensitive mutation could be the most essential factor associated with survival.

We found that CNS disease was not associated with LB positivity. However, in patients without baseline brain metastases, the icPFS was significantly shorter in patients with a positive LB at progression. This lower icPFS was statistically significant in patients with a positive T790M mutation. However, a trend to a worse icPFS was also observed for patients with a persistent *EGFR* sensitive mutation (delexon19 and L858R) regardless of the T790M status. The prognostic utility of liquid biopsy for intracranial PFS is particularly interesting, considering the increased risk of brain affection in patients with EGFR alterations. Very limited data have assessed this key point; based on our findings, it will be very attractive to assess prospectively the prognostic and predictive value of liquid biopsy regarding central nervous system efficacy in this subpopulation. Also, the potential utility of liquid biopsy is to guide clinicians to develop strategies with high brain penetration in the subgroup of patients with the highest risk.

Acquired T790M has been associated with indolent growth and a favorable prognosis compared to other ac-

quired resistance mechanisms. In this sense, the absence of T790M after progression probably indicates the presence of alternative resistance mechanisms, which are associated with a higher disease burden and poorer performance status, contributing to the shorter survival of these patients [42]. As demonstrated in our study, patients with better outcomes were those with a positive T790M mutation who received osimertinib treatment, highlighting the importance of detecting T790M and receiving the appropriate treatment.

In clinical trials, such as the AURA3 [44] trial, a low proportion of patients (51.2%) had T790M mutation, as assessed using plasma ctDNA. However, the T790M mutation detected by ctDNA has been demonstrated to be a surrogate marker for T790M in tumor tissues and is predictive of treatment response [27]. In our study, the median PFS of patients with the T790M mutation treated with osimertinib was similar to that reported in the AURA-3 trial [44,45]. In another trial [31,46], no difference in outcomes was found with osimertinib using plasma or tumor tissue to detect T790M, supporting the preferred use of LB and leaving tissue biopsy only for patients with negative LB [31,46].

Some limitations of the present study were the retrospective single-institution design that could not reflect daily practice in other Latin American institutions, and the presence of some bias in our results due to the nature of the research. Even though, our institution is a high-reference center with a complete representation of the entire country, reflecting important real-world evidence. In addition, due to the retrospective design, we could not evaluate the concordance between the tissue and LB because most patients did not have available tissue samples after progression. We recognize that the standard procedure for patients with negative LB results is to perform tissue re-biopsy; however, in real-life, tissue re-biopsy is performed in 26.1% of cases [47]. Tissue confirmation after the first negative LB test was extremely low in our cohort because of the high proportion of patients who had more than one LB, and refused invasive procedures. We consider comparing real-world scenarios paramount in optimizing clinical practice, considering that awareness and knowledge of liquid biopsy use are still limited. This study is of high value because in real life, most patients do not have access to a biopsy at progression as demonstrated previously. With the short turnaround of liquid biopsy, most patients receive adequate treatment with good outcomes, and the rest of the patients can be considered candidates for clinical trials.

5. Conclusions

This study supports a comparable rate of T790M detection by liquid biopsy in the Latin American population, and a higher detection rate was associated with clinical characteristics. Moreover, it emphasizes that the detection of ctDNA by LB is feasible after progression to first-line EGFR therapy and has a prognostic and potentially predictive value in *EGFR*m NSCLC. Patients with positive LB results also had shorter intracranial progression-free intervals, a finding that might be confirmed in a further prospective analysis.

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Author contributions

Conception: D.H., L.L.-M., O.A.
Interpretation and analysis of data: D.H., A.V.-V., E.V.-S., D.C.-F., G.C.-R., M.O.
Preparation of manuscript: D.H., A.V.-V., L.L.-M.
Revision for important intellectual content: O.A., D.H., L.B-G.
Supervision: O.A., D.H., L.L.-M.

Supplementary data

The supplementary files are available to download from http://dx.doi.org/10.3233/CBM-230124.

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