Non-Small Cell Lung Cancer Harboring Concurrent EGFR Genomic Alterations: A Systematic Review and Critical Appraisal of the Double Dilemma

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Abstract: The molecular pathways which promote lung cancer cell features have been broadly explored, leading to significant improvement in prognostic and diagnostic strategies. Epidermal growth factor receptor (EGFR) tyrosine kinase inhibitors (TKIs) have dramatically altered the treatment approach for patients with metastatic non-small cell lung cancer (NSCLC). Latest investigations by using next-generation sequencing (NGS) have shown that other oncogenic driver mutations, believed mutually exclusive for decades, could coexist in EGFR-mutated NSCLC patients. However, the exact clinical and pathological role of concomitant genomic aberrations needs to be investigated. In this systematic review, we aimed to summarize the recent data on the oncogenic role of concurrent genomic alterations, by specifically evaluating the characteristics, the pathological significance, and their potential impact on the treatment approach.

Keywords: NSCLC; NGS; EGFR; concurrent genomic alterations; systematic review

1. Introduction

Lung cancer is the most predominant cancer type and is one of the driving causes of cancer-related death worldwide [1]. Non-small cell lung cancer (NSCLC) accounts for roughly 85–90% of overall cases of lung malignancies and includes different histological subtypes [2–4]. Recently, the treatment landscape of NSCLC has been terrifically changed by the discovery of Epidermal Growth Factor Receptor (EGFR) mutations and their response to the EGFR tyrosine kinase inhibitors (TKIs) [5–7]. EGFR gene aberrations have been defined as oncogenic driver mutations which occurred in 5–17% of lung adenocarcinomas among Caucasian patients, while in approximately 45–55% of the Asian population [8,9]. Nowadays, EGFR-TKIs are the standard of care for patients affected by advanced EGFR-mutated NSCLC considering their established prolonged progression-free survival (PFS) in comparison to the standard chemotherapy approach [10,11]. However, TKIs clinical efficacy remains restricted due to the development of resistance, which has
been hardly clarified. The recent technological breakthrough and the advent of next-generation sequencing (NGS) platforms have enabled comprehensive profiling of the genome, providing novel evidence of co-existing multiple driver alterations. In fact, NGS allows to examine both DNA- and RNA-based aberrations, thus concurrently analyzing significant gene pathogenic variants [12–14]. Additionally, despite oncogenic driver alterations were considered to be mutually exclusive, current findings have called higher attention to the presence of coexisting genomic alterations in EGFR-positive NSCLC patients [15]. The clinical and pathological significance of co-existing driver genomic variants has not been yet elucidated, raising several questions on therapeutic options for these particular subsets of patients. In the current systematic review, we aimed to highlight the updated data on the oncogenic role of concurrent genomic alterations, by specifically evaluating the clinical characteristics, the pathological significance and their potential impact on the treatment approach.

2. Materials and Methods

The systematic review was performed conforming to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses guidelines (PRISMA) (Supplementary Figure S1) [16]. In March 2021, a MEDLINE and Cochrane database systematic literature search was conducted using the following search words “EGFR” OR “ErbB 1 Receptor” AND “concurrent” OR “concomitant” OR “coexisting” AND (“lung” OR “Non-Small-Cell Lung”). We used the terms “EGFR” AND “concurrent” for the significant abstracts published on the American Society of Clinical Oncology (ASCO) and the European Society of Medical Oncology (ESMO) and ClinicalTrials.gov databases. The literature search involved the cited bibliography of the reviewed articles too. The entire search strategy can be found in the supplementary material (Supplementary Figure S2). We searched for clinical trials evaluating patients with histological diagnosis of unresectable or advanced EGFR-positive NSCLC and concurrent genetic alterations, including non-randomized, cohort, cross-sectional, retrospective and case-control studies. Furthermore, we also excluded other reviews (systematic or not) and meta-analyses. Moreover, non-peer-reviewed publications, like abstracts displayed in conferences and meetings were taken into consideration. We excluded research trials conducted on animals, preclinical trials, as well as phase 1 and 2 trials. We reported demographics and clinical information about the included studies, such as concurrent genomic alteration, race of the study population, detection method, sample, variant allele frequency (VAF), treatments, clinical outcomes. The language of the data collected was limited to English. All data collected with the above-mentioned search strategy were reviewed by two authors (M.L.M. and V.G.), who independently screened and selected abstracts and titles according to the aforementioned exclusion and inclusion criteria. Disagreements were discussed and finally solved with a third author (A.G.).

3. Results

The systematic literature search identified a total of 827 records. The literature data collected through the systematic databases search underwent two exclusion steps: The first being based on title and abstract, whereas the second being subject to an exhaustive read-through. Additionally, in the case that an article did not conform to the inclusion and exclusion criteria, it was discarded. Thus, 11 records were excluded because of duplicates, while 56 records were finally ruled out being reviews, letters, commentaries, editorials, or protocols. Moreover, five full text papers were not available in English, thence excluded. After this process, 634 data met the eligibility criteria, whereas 600 were excluded since no data of interest were reported (Figure 1). Finally, a total of 36 studies met our inclusion and exclusion criteria; thus, they were included in the systematic research of the literature (see Table 1). Namely, a total of 11 case reports, two abstracts and 23 original research articles considered have examined concurrent EGFR mutations and their potential impact on NSCLC patients. Particularly, a total of 1313 patients harbored a double concomitant EGFR genomic alteration. Principally, the co-existing mutations identified are on-target
EGFR gene alterations, TP53, PIK3CA, PTEN, RB1 and CDKN2A; whereas concomitant actionable driver aberrations, within anaplastic lymphoma kinase (ALK), c-ros oncogene-1 (ROS-1), v-raf murine sarcoma viral oncogene homolog B1 (BRAF), mesenchymal epithelial transition (MET), Rearranged during transfection (RET) and Kirsten rat sarcoma viral oncogene homolog (KRAS) genes, are less comprehensively represented (Figure 2). Indeed, regarding complex EGFR mutation, we found two case reports and eight original articles reporting complex EGFR mutation in the study population. Concurrent TP53 and EGFR mutations were described in 12 articles and two abstracts, and PI3KCA/EGFR co-alterations were reported in 11 articles, one abstract and a single case report, whereas only three research articles and one abstract included patient harboring co-existing PTEN/EGFR aberrations, and two articles evaluated RB1/EGFR co-existing genomic alterations. Moreover, the systematic review of the literature identified only a single article including a concurrent BRAF/EGFR mutant patient, two works reporting MET concurrent EGFR alterations, a single case report and an original research article evaluating EGFR/RET concurrent alterations, and three papers evaluating ROS-1/EGFR concomitant alterations. Finally, six original research articles and five case reports were conducted on concurrent oncogenic driver ALK and EGFR aberrations, while two reports and five original articles evaluated concomitant EGFR/KRAS mutations. Tables 1 and 2 summarize the demographic characteristics and reported treatment outcomes of patients with NSCLC and double genetic alterations, respectively.

Table 1. Summary of reported demographic characteristics of EGFR-positive NSCLC patients with concomitant genomic alterations.

| Study          | Study Type | Race   | No. of Pts | Concurrent Genomic Alteration | Detection Method                      | Sample       | VAF                  |
|----------------|------------|--------|------------|-------------------------------|--------------------------------------|--------------|----------------------|
| Belardinilli et al. [17] | Case Report | Caucasian | 1          | EGFR complex                | NGS                                   | tumor tissue | 40.30% 41.30% 67.50% |
| Benesova et al. [18]    | Case Series | Caucasian | 4          | EGFR+KRAS EGFR complex       | Sanger                                | tumor tissue | N/A                  |
| Fan et al. [19]         | Case Report | Asian   | 1          | EGFR+ALK                    | NGS                                   | tumor tissue | EGFR 15.58% ALK 6.42% |
| Lammers et al. [20]     | Case Report | Caucasian | 1          | EGFR+PIK3CA                  | SNapShot PCR                          | tumor tissue | N/A                  |
| Lee et al. [21]         | Case Series | Asian   | 12         | EGFR+KRAS EGFR+ALK           | Sanger; Real Time PCR after PNA; FISH and IHC | tumor tissue | N/A                  |
| Miyanaga et al. [22]    | Case Report | Asian   | 1          | EGFR+ALK                    | PNA-LNA PCR clamp method, FISH and IHC | tumor tissue | N/A                  |
| Sweis et al. [23]       | Case Series | Caucasian | 4          | EGFR+ALK                    | N/A                                   | N/A          | N/A                  |
| Thumallapally et al. [24] | Case Report | Caucasian | 1          | EGFR+ALK                    | FISH, direct sequencing                | tumor tissue | N/A                  |
| Zhu et al. [25]         | Case Report | Asian   | 1          | EGFR+ROS-1                  | NGS, PCR and FISH                      | tumor tissue | N/A                  |
| Yang et al. [26]        | Case Series | Asian   | 13         | EGFR+ALK                    | IHC, FISH, Sanger, RT-PCR and RACE-PCR sequencing | tumor tissue | N/A                  |
| Hou et al. [27]         | Retrospective | Asian | 59         | EGFR+TP53 EGFR+RB1          | NGS                                   | tumor tissue | N/A                  |
| Zhu et al. [28]         | Retrospective | Asian  | 2          | EGFR+ALK                    | FISH, RT-PCR                          | tumor tissue | N/A                  |
| Li et al. [29]          | Retrospective | Asian  | 149        | EGFR+PIK3CA EGFR complex EGFR+KRAS EGFR+BRAF | SurPlex® xTAG70plex-EGFR liquidchip | tumor tissue | N/A                  |
| Study          | Study Type | Race     | No. of Pts | Concurrent Genomic Alteration | Detection Method | Sample Description | VAF       |
|---------------|------------|----------|------------|-------------------------------|------------------|---------------------|-----------|
| Liang et al.  | Retrospective | Asian   | 403        | EGFR complex                  | NGS              | tumor tissue + plasma | N/A       |
| Liu et al.    | Retrospective | Asian   | 21         | EGFR+ALK                      | NGS              | tumor tissue + plasma | N/A       |
| Nardo et al.  | Retrospective | Caucasian | 3          | EGFR+KRAS                     | ddPCR            | tumor tissue + plasma | KRAS <0.2 |
| Rachiglio et al. | Retrospective | Caucasian | 38         | EGFR+KRAS, EGFR+MET, EGFR+TP53, EGFR+PIK3CA | NGS, ddPCR       | tumor tissue + plasma | KRAS 2–38% EGFR ≥ 2% |
| Sato et al.   | Retrospective | Asian   | 43         | EGFR complex, EGFR+TP53, EGFR+RB1 | NGS              | tumor tissue         | N/A       |
| VanderLaan et al. | Retrospective | Caucasian | 19         | EGFR+TP53, EGFR+PIK3CA, EGFR+PTEN | NGS, Sanger      | tumor tissue         | N/A       |
| Wu et al.     | Retrospective | Asian   | 12         | EGFR+PIK3CA                    | Sanger, RT-PCR   | tumor tissue         | N/A       |
| Zheng et al.  | Retrospective | Asian   | 11         | EGFR+TP53                      | NGS              | tumor tissue         | N/A       |
| Zhuang et al. | Retrospective | Asian   | 43         | EGFR+ALK, EGFR+ROS-1, EGFR+KRAS, EGFR+BRAF | ARMS             | tumor tissue         | N/A       |
| Huang et al.  | Prospective | Asian   | 18         | EGFR+TP53/PTEN, EGFR+PIK3CA   | N/A              | N/A                 | N/A       |
| Zhang et al.  | Prospective | Asian   | N/A        | EGFR+TP53                      | NGS              | N/A                 | N/A       |
| Canale et al. | Retrospective | Caucasian | 136        | EGFR+TP53                      | Sanger, MassARRAY, NGS | tumor tissue         | N/A       |
| Chang et al.  | Retrospective | Asian   | 26         | EGFR+ALK, EGFR+PIK3CA, EGFR+CDKN2A | NGS, CNV         | tumor tissue         | N/A       |
| Chen et al.   | Retrospective | Asian   | 16         | EGFR complex, EGFR+ALK, EGFR+KRAS, EGFR+PIK3CA, EGFR+TP53 | NGS              | tumor tissue + plasma | N/A       |
| De Marchi et al. | Retrospective | Caucasian | 47         | EGFR complex, EGFR+KRAS, EGFR+PIK3CA | NGS, Sanger, SNP array | tumor tissue         | N/A       |
| Eng et al.    | Retrospective | Caucasian | 13         | EGFR+PIK3CA                    | mutation hotspot testing, FISH, multiplex sizing assays | tumor tissue | N/A       |
| Chevallier et al. | Retrospective | Caucasian | 20         | EGFR+TP53, EGFR+MET, EGFR+KRAS, EGFR+PIK3CA, EGFR+PTEN | NGS              | tumor tissue         | N/A       |
| Hu et al.     | Retrospective | Asian   | 21         | EGFR+ALK, EGFR+PIK3CA, EGFR+KRAS, EGFR+ROS-1, EGFR+RET, EGFR+HER2 | ARMS, adx-RT, mutation detection kit; fusion gene detection kit | tumor tissue | N/A       |
Table 1. Cont.

| Study         | Study Type | Race   | No. of Pts | Concurrent Genomic Alteration | Detection Method | Sample                       | VAF |
|---------------|------------|--------|------------|-------------------------------|------------------|------------------------------|-----|
| Chen et al. [48] | Retrospective | Asian  | 71         | EGFR complex EGFR+TP53 EGFR+ALK EGFR+BRAF EGFR+MET | NGS, ARMS         | tumor tissue + plasma         | N/A |
| Lee et al. [49]  | Retrospective | Asian  | 7          | EGFR+ALK EGFR+MET EGFR+TP53 EGFR complex | FISH, NGS, Sanger | tumor tissue                 | N/A |
| Zhang et al. [50] | Retrospective | Asian  | 9          | EGFR complex EGFR+KRAS EGFR+PIK3CA | FISH, liquid chip platform | tumor tissue                 | N/A |
| Wang et al. [51]  | Retrospective | Asian  | 17         | EGFR+PIK3CA | Sanger, FISH, IHC              | tumor tissue                 | N/A |
| Klempner et al. [52] | Case report | Asian  | 2          | EGFR+RET | NGS                           | tumor tissue | 53% 54% 62% 18% |

Abbreviations: No, number; Pts, patients; VAF, variant allele frequency; NGS, next generation sequencing; N/A, not applicable; FISH, fluorescent in situ hybridization; IHC, immunohistochemistry; PCR, polymerase chain reaction; ARMS, amplification refractory mutation system; CNV, copy number variation; SNP, single nucleotide polymorphism; RT-PCR, real time-PCR; ddPCR, digital droplet PCR; RACE-PCR, rapid amplification cDNA ends PCR; PNA-LNA PCR, peptide nucleic acid-locked nucleic acid PCR.

Figure 1. Preferred reporting items for systematic reviews and meta-analyses (PRISMA) flowchart diagram.
Figure 2. Distribution of the different concurrent EGFR mutations pathways.

Table 2. Summary of reported treatment outcomes of NSCLC patients with double concurrent genetic alterations.

| Study                  | Concurrent Genomic Alteration | TKI        | mPFS (mo.) | mOS (mo.) | Best Response |
|------------------------|------------------------------|------------|------------|-----------|---------------|
| Belardinilli et al.    | EGFR complex                 | Afatinib   | 8          | N/A       | PR            |
| Benesova et al.        | EGFR+K Ras                   | Gefitinib  | 6–12       | 5-23      | 3 PR          |
|                        | EGFR complex                 | Erlotinib  |            |           | 1 CR          |
| Fan et al.             | EGFR+AL K                    | Gefitinib  | 18         | N/A       | PR/SD         |
|                        | Crizotinib                   |            |            |           |               |
| Lammers et al.         | EGFR+PI3KCA                  | Erlotinib  | 1–4        | N/A       | SD/PR         |
|                        | Afatinib                     |            |            |           |               |
|                        | PI3K inhibitor               |            |            |           |               |
| Lee et al.             | EGFR+K Ras                   | Gefitinib  | 4–29       | N/A       | SD/PR         |
|                        | EGFR+AL K                    | Erlotinib  |            |           |               |
|                        | Crizotinib                   |            |            |           |               |
|                        | Crizotinib                   |            |            |           |               |
| Miyanaga et al.        | EGFR+AL K                    | Gefitinib  | 2–7        | N/A       | SD            |
|                        | Erlotinib                    |            |            |           |               |
|                        | Crizotinib                   |            |            |           |               |
| Sweis et al.           | EGFR+AL K                    | Erlotinib  | 2–12       | N/A       | PR/PD         |
|                        | Crizotinib                   |            |            |           |               |
| Thumallapally et al.   | EGFR+AL K                    | Crizotinib | N/A        | 3 wk      | N/A           |
| Zhu et al.             | EGFR+ROS-1                   | Adj CT     | N/A        | N/A       | N/A           |
| Yang et al.            | EGFR+AL K                    | Gefitinib  | 12–27.4    | N/A       | SD/PR/PD      |
|                        | Erlotinib                    |            |            |           |               |
|                        | Crizotinib                   |            |            |           |               |
|                        | Crizotinib                   |            |            |           |               |
| Hou et al.             | EGFR+TP53                    | Erlotinib  | 4–11       | 10–59     | N/A           |
|                        | EGFR+RB1                     | Gefitinib  |            |           |               |
|                        | Icotinib                     |            |            |           |               |
| Zhu et al.             | EGFR+AL K                    | N/A        | N/A        | N/A       | N/A           |
| Study                | Concurrent Genomic Alteration | TKI                          | mPFS (mo.) | mOS (mo.) | Best Response |
|----------------------|------------------------------|------------------------------|------------|-----------|---------------|
| Li et al. [29]       | EGFR+PIK3CA                  | N/A                          | N/A        | N/A       | N/A           |
|                      | EGFR complex                 |                              |            |           |               |
|                      | EGFR+KRAS                    |                              |            |           |               |
|                      | EGFR+BRAF                    |                              |            |           |               |
| Liang et al. [30]    | EGFR Complex                 | N/A                          | N/A        | N/A       | N/A           |
| Liu et al. [31]      | EGFR+ALK                     | Osimertinib Crizotinib Afatinib | 6–15       | N/A       | N/A           |
| Nardo et al. [32]    | EGFR+KRAS                    | Erlotinib Gefitinib Afatinib | 5          | 6         | PR            |
| Rachiglio et al. [33]| EGFR+KRAS EGFR+BRAF EGFR+MET EGFR+TP53 EGFR+PIK3CA | Erlotinib Gefitinib Afatinib | 7          | 15.5      | N/A           |
| Sato et al. [34]     | EGFR complex EGFR+TP53 EGFR+RB1 | Gefitinib Erlotinib | N/A        | N/A       | PR            |
| VanderLaan et al. [35]| EGFR+TP53 EGFR+PIK3CA EGFR+PTEN | Erlotinib Gefitinib Afatinib | 6.5        | 15.5      | N/A           |
| Wu et al. [36]       | EGFR+PIK3CA                  | Erlotinib Gefitinib Afatinib | 12         | 5.1       | PR            |
| Zheng et al. [37]    | EGFR+TP53                    | N/A                          | N/A        | 23.9      | N/A           |
| Zhuang et al. [38]   | EGFR+ALK EGFR+ROS-1 EGFR+KRAS EGFR+BRAF | Gefitinib Erlotinib Afatinib Icotinib Crizotinib Alectinib | 9.6        | N/A       | PR            |
| Huang et al. [39]    | EGFR+TP53/PTEN EGFR+PIK3CA   | Anlotinib Icotinib           | N/A        | N/A       | PR            |
| Zhang et al. [40]    | EGFR+TP53                    | Gefitinib Afatinib           | N/A        | N/A       | N/A           |
| Canale et al. [41]   | EGFR+TP53                    | Erlotinib Gefitinib Afatinib | 5.8–12.9   | 29.7–19.5  | CR            |
| Chang et al. [42]    | EGFR+ALK EGFR+TP53 EGFR+PIK3CA EGFR+CDKN2A | Erlotinib Gefitinib Afatinib | 1–24.2     | 5.4–57.6   | N/A           |
| Chen et al. [43]     | EGFR complex EGFR+ALK EGFR+KRAS EGFR+PIK3CA EGFR+TP53 | Erlotinib Gefitinib Afatinib Osimertinib | 18.7 | N/A | CR |
| De Marchi et al. [44]| EGFR complex EGFR+KRAS EGFR+PIK3CA | N/A                          | N/A        | N/A       | N/A           |
Table 2. Cont.

| Study               | Concurrent Genomic Alteration                      | TKI     | mPFS (mo.) | mOS (mo.) | Best Response |
|---------------------|---------------------------------------------------|---------|------------|-----------|---------------|
| Eng et al. [45]     | EGFR+PI3KCA                                       | Gefitinib | 7.8       | 18        | PR            |
| Chevallier et al. [46] | EGFR+TP53 EGFR+MET EGFR+KRAS EGFR+PI3KCA EGFR+PTEN | Erlotinib Gefitinib Afatinib Osimertinib | 6.8–11.6  | 7.7–16.8  | N/A           |
| Hu et al. [47]      | EGFR+ALK EGFR+PI3KCA EGFR+KRAS EGFR+ROS-1 EGFR+RET EGFR+HER2 | Erlotinib Gefitinib Crizotinib Icotinib | 1–24      | 10–43     | PR/PD         |
| Chen et al. [48]    | EGFR complex EGFR+TP53 EGFR+ALK EGFR+BRAF EGFR+MET | Erlotinib Gefitinib Icotinib | 6–24      | N/A       | N/A           |
| Lee et al. [49]     | EGFR+ALK EGFR+MET EGFR+TP53 EGFR complex          | Erlotinib Gefitinib Crizotinib | 1–2.1     | 1–21.8    | N/A           |
| Zhang et al. [50]   | EGFR complex EGFR+KRAS EGFR+PI3KCA                | N/A     | N/A        | N/A       | N/A           |
| Wang et al. [51]    | EGFR+PI3KCA                                       | Gefitinib | N/A       | N/A       | PR            |
| Klopman et al. [52] | EGFR+RET                                         | Erlotinib | N/A       | N/A       | PR/PD         |

Abbreviations: TKI, tyrosine kinase inhibitor; mPFS, median progression-free survival; mOS, median overall survival; PR, partial response; CR, complete response; SD, stable disease; PD, progressive disease; N/A, not applicable; Adj CT, adjuvant chemotherapy; mo., months; wk, weeks.

3.1. Complex EGFR Mutations

Of note, almost 45% of EGFR gene aberrations are in-frame deletion alterations in exon 19 (19Del) and the p.L858R within exon 21 [8,17]. These activating mutations enhance a better outcome in patients, granting a complete blockade of the EGFR signaling pathway by EGFR-TKIs. Otherwise, EGFR mutations occurring in exons 18 and 20 are correlated with resistance to standard treatments. Uncommonly, complex EGFR alterations could be detected in a single tumor specimen harboring two or more various intra-EGFR mutations [53]. Complex EGFR mutations occur almost in 3–7% of EGFR-mutant patients [54]. Belardinelli et al. described a single clinical case of an NSCLC patient harboring three coexisting aberrations on the EGFR gene, two of which presented on the same allele [17]. In fact, through the use of NGS, the authors detected the simultaneous presence of three missense mutations, a p.L858R and p.L861R both in exon 21 with an allele frequency close to 41%, and a p.R776H in exon 20 with an allele frequency of 67.5%, respectively. Besides, upon therapy with the second-generation EGFR-TKI afatinib, the patient showed a partial response on the target lung lesion with a PFS of eight months. Moreover, a clinical trial conducted by Lee et al. investigated molecular backgrounds of primary resistance to EGFR-TKIs in NSCLC patients harboring sensitive EGFR alterations [49]. The study population included a cohort of 197 patients, out of whom nine individuals had two co-existing EGFR mutations. Additionally, among 11 patients exhibiting de novo resistance to TKI treatment only one patient had a coexisting EGFR complex mutation, particularly p.T790M mutation and 19Del. The authors reported that this patient displayed immediate disease progression involving symptomatic metastasis to the central nervous system (CNS) while receiving EGFR-TKI treatment. Furthermore, a recent analysis by Liang et al. evaluated concomitant
alterations in EGFR 19Del/L858R mutation and their correlation with EGFR-TKIs response in a total of 403 NSCLC patients [30]. This trial included two cohorts and comprehensively analyzed the concomitant mutational profiles of EGFR 19Del and p.L858R in TKI naïve patients. The authors assessed that the existence of somatic p.T790M at baseline was similar in 19Del (120, 73.4%) and p.L858R (160, 72.4%) mutations. Furthermore, Zhang et al. screened 187 patients with complex EGFR mutations out of 5898 EGFR-positive NSCLC patients. Fifty-one of these patients were under first-line treatment with first-generation EGFR-TKIs [54]. Namely, 58 patients were found to carry a concurrent alteration in EGFR exon 20 and 21, while 45 patients harbored a concomitant mutation in exon 19 and 21. Considering the genetic aberrations, simultaneous p.T790M and p.L858R were the most common, followed by 19Del and p.L858R. The median PFS was 9.5 months. The overall response rate (ORR) was 52.2% (95% CI 37.2–67.2%), and the disease control rate (DCR) was 71.7% (95% CI, 58.2–85.3%). Additionally, the authors subdivided patients into four groups: A) patients with 19Del and p.L858R; B) patients harboring a 19Del or p.L858R and atypical mutations; C) double atypical mutations; and D) complex mutations with a primary drug-resistant pattern, such as a primary p.T790M mutation or an exon 20 insertion. As reported by the authors, NSCLC patients with exon 19Del and p.L858R exhibited the best ORR and PFS, 75% and 18.2 months, respectively. On the other hand, patients included in group D displaying complex mutations with a primary drug-resistant pattern, such as a primary p.T790M mutation or an exon 20 insertion, have the worst clinical outcomes. Notably, some of these patients carried a sensitizing EGFR alteration (i.e., 19Del/p.L858R/p.L861Q) plus a p.T790M de novo or an exon 20 insertion. Thus, the worst clinical outcomes achieved by these patients could be explained by the fact that they were treated with first and second-generation EGFR-TKIs. Moreover, Benesova et al. described a single case of a patient with complex EGFR alteration [18]. Of note, the patient exhibited partial response under treatment with gefitinib. Otherwise, Sato et al. reported that 6 patients with double EGFR alterations showed a poorer response to gefitinib treatment [34]. De Marchi et al. found 33 patients with double EGFR genomic aberrations in a cohort of 1006 lung cancer patients, with no data being unfortunately available on their clinical outcomes [44]. Li et al. detected 58/5125 EGFR double mutations, with the highest incidence rate of p.T790M and p.L858R [29]. Chen et al. presented 4/36 patients harboring concurrent 19del and p.L858R with a worse response after TKI treatment [43]. Additionally, Chen et al. reported concurrent EGFR complex genomic alterations in 20 patients with the worst outcome in terms of OS [48].

3.2. Actionable Concomitant Oncogenic Driver Mutations

Although actionable oncogenic gene driver mutations in NSCLC were historically considered mutually exclusive, the recent advent of comprehensive genomic profiling in clinical specimens was able to identify a notable number of concurrent alterations in EGFR-mutated NSCLC. Recently, various original research articles and case reports were conducted on this topic, suggesting that some EGFR-mutant NSCLC patients may carry concomitant genetic aberrations in different oncogenic driver genes.

3.2.1. ALK

ALK is a component of the insulin receptor protein-tyrosine kinase superfamily, formerly reported as a nucleophosmin (NPM)-ALK fusion pattern in cell lines of anaplastic large cell lymphoma (ALCL) [55]. In 2007 ALK fusion was described in lung adenocarcinoma for the first time in a limited cohort of Asian individuals [36]. The most common aberration is an inter-chromosomal inversion in the short arm of chromosome 2, which generates a fusion between the echinoderm microtubule-associated protein like-4 (EML4) gene and the ALK gene [57]. Consequently, the fusion EML4-ALK with tyrosine kinase function stimulates proliferation and cell survival [57]. Chromosomal rearrangements in the ALK gene are detected in approximately 5% of NSCLC patients [58]. Moreover, this driver fusion is predominantly estimated mutually exclusive with other genetic mutations, such
as EGFR [59]. Notwithstanding, with the advent of novel and powerful technologies like NGS the detection rate of concomitant genetic alterations in EGFR and ALK is systematically increased [59,60]. Liu et al. evaluated the efficacy of TKI treatments on 21 co-altered EGFR and ALK patients with advanced NSCLC [60]. Three out of 21 patients received dual blockade TKI treatment with EGFR- and ALK-TKIs, reaching a PFS of 5.2 months with the combination therapy. Furthermore, analyzing the clinical-pathological features of the concomitant mutation patients the authors found that the double genetic alteration was more likely to occur in young females than in males. Additionally, Hu et al. examined the frequency of concurrent genetic alterations in EGFR-positive patients, evaluating the efficacy of EGFR-TKIs treatment in this setting [47]. Out of 320 patients including in the study population, six patients were found harboring a co-alteration in ALK gene and they achieved a mPFS of five months, shorter compared to those with a single EGFR mutation (mPFS 10.9 months). Namely, four out of six patients with concomitant ALK rearrangement were treated with the first-generation ALK-TKI crizotinib and three obtained partial response according to RECIST criteria. Considering the particular subset of patients, a recent report by Zhuang et al. determined that ALK-TKI therapy for the treatment of 20 patients with a co-alteration in ALK fusion was more active as first-line treatment than in later lines of treatment [38]. Yang et al. assessed that 13/977 NSCLC patients screened harbored a concomitant genetic aberration in EGFR and ALK genes [26]. Out of 13 patients, 10 naïve patients received EGFR-TKIs reaching an ORR of 80% and a mPFS of 11.2 months (95%CI 5.6–16.8). Four patients were treated with crizotinib, and three of them in a second-line setting. Considering the clinical outcomes, two patients appeared to respond to EGFR-TKI, yet not to ALK-TKI, whereas one was sensitive to crizotinib. The only patient who received crizotinib as first-line displayed 15.1 months of PFS, still not show response to consecutive EGFR-TKI treatment. Patients with EGFR and ALK coexisting aberrations seemed to better respond to EGFR-TKIs in the first-line setting. Of note, in order to explain the great heterogeneity of clinical outcomes, the authors suggested that different sensitivities to therapies might be correlated with different levels of EGFR or ALK protein phosphorylation. Fan et al. described a single case of a patient harboring EGFR/ALK alteration, who had partial response under ALK-TKI [19]. Besides, Lee et al. described 12 patients with double EGFR/ALK alteration, 11 of which with a partial response to treatments based on gefitinib, erlotinib or crizotinib [21]. Notably, Miyanaga et al. described a single case where the patient showed response both to first-generation EGFR-TKIs and crizotinib [22]. Sweis et al. presented a case series including four patients treated with erlotinib and crizotinib, achieving a stable disease as the best response [23]. Thumallapally et al. reported a single case harboring an ALK translocation together with an EGFR p.L861Q mutation treated with crizotinib reaching a PFS of 3 weeks [24]. In their exploratory study, Lee et al. found two out of 197 EGFR-positive NSCLC patients with a concurrent genomic alteration in ALK [49]. Notably, the patients were treated with gefitinib and consequently with crizotinib, achieving a partial response. Chang et al. did not report the clinical outcome of their single case [42], as well as Zhu et al. who described two patients out of 139 [28]. Chen et al. described a single case of double EGFR/ALK alteration with poor outcomes [48].

3.2.2. KRAS

KRAS alterations are frequently represented by missense mutations occurring in lung adenocarcinomas [61]. Molecular evaluation of KRAS is crucial to predict clinical outcomes and to choose the best therapeutic option, as KRAS-mutant tumors exhibit primary resistance to EGFR-TKIs [61]. Moreover, almost 6–35% of EGFR positive patients harbor a concomitant genetic aberration in the KRAS gene [62]. P.G12C, p.G12V, and p.G12D mutation are the most frequent alteration detected [63]. Several cases have been reported for EGFR and KRAS concurrent alterations. Benesova et al. presented three cases of patients with EGFR mutations combined with KRAS mutation [18]. Despite an initial positive response to EGFR-TKI, the real activity did not last long showing a PFS of three, five, and seven months, respectively. Opposing this report, Zhuang et al. reported a retrospective
study involving 3774 patients with concurrent genetic alterations [38]. Namely, 11 patients of the cohort showed a co-alteration in \textit{EGFR/KRAS} and they were treated EGFR-TKI therapy as first-line treatment, displaying an ORR of 62.5% (5/8). Interestingly, the PFS comparisons between patients with an \textit{EGFR/KRAS} co-mutation and those carrying a single \textit{EGFR} mutation were not statistically significant. Ranchiglio et al. identified 14 patients with concurrent \textit{EGFR} and \textit{KRAS} mutations, among six with a dominant VAF [33]. Notably, their PFS was significantly shorter compared to \textit{EGFR} mutations (2.42 months vs. 11.09 months; \(p = 0.0081\)), and also the ORR was poorer (16.7% vs. 57.1%). Additionally, Nardo et al. analyzed the prevalence of concurrent \textit{KRAS} mutations on 106 patients with \textit{EGFR}-mutant NSCLC focusing on their impact on clinical outcome [32]. Indeed, \textit{KRAS} co-alterations were detected in 3 patients with a VAF of less than 0.2%, which showed poor clinical outcome to first-line EGFR-TKI, in terms of time to treatment failure (TTF), OS and PFS (five, six and five months, respectively). Lee et al. described six patients with \textit{EGFR/KRAS} aberration, not reporting their clinical outcomes [21], as Li et al. who reported 30 patients with double alterations out of a cohort of 5125 individuals [29]. Chevallier et al. described a single case [46], as De Marchi et al. [44]. Moreover, Zhang et al. found two out of 120 patients with double concurrent genomic aberrations [54]. Whereas Hu et al. described a single case of \textit{EGFR/KRAS} out of a cohort including 320 individuals [47], of note the patient showed progression after treatment with erlotinib. Finally, in the trial by Chen et al. [48], seven out of 36 patients displayed a concurrent alteration in \textit{EGFR} and \textit{ALK} with poorer PFS after EGFR-TKI treatment.

3.2.3. ROS-1

\textit{ROS-1} rearrangements has been detected in almost 1–2% of lung adenocarcinoma [25]. The ALK-TKI crizotinib is highly active in \textit{ROS1}-rearranged patients [64]. Patients harboring a concomitant mutation in \textit{EGFR/ROS-1} are very rare, thus we found little data in the current literature. Zhu et al. described a case of a single patient with concurrent \textit{EGFR/ROS-1} alteration [25]. Moreover, in the above-mentioned article by Zhuang et al., two out of 3774 patients harbored a co-alteration in \textit{EGFR/KRAS/ROS-1}. Namely, one patient showed a progression after second-line treatment with crizotinib and partial response to icotinib as third-line treatment (PFS of 27.5 months), while the second patient had a partial response after first-line treatment with gefitinib (PFS of 12.7 months) [38]. Hu et al. reported one out of 320 patients with double \textit{ROS-1/EGFR} genomic alteration and a partial response after erlotinib as first-line treatment [43].

3.2.4. MET

\textit{Mesenchymal–epithelial transition (MET)} encodes a transmembrane tyrosine kinase, which activates downstream signaling pathways by binding to the hepatocyte growth factor. Thusly, it has a crucial role in cell proliferation and survival [65]. \textit{MET} alterations are emerging as important driver aberrations for NSCLCs, particularly \textit{MET} gene amplification and exon 14 skipping mutations are found with a frequency of 1–11% and up to 4% in lung adenocarcinoma [66]. \textit{MET} amplification is a well-known resistance mechanism against EGFR-TKIs, including the third-generation osimertinib [67,68]. Indeed, \textit{MET} amplification is accountable for almost 5–22% of secondary resistance to EGFR-TKIs. Particularly, \textit{MET} amplification induces ErbB3 phosphorylation, hence activating the PI3K/AKT pathway [66]. In line with these data, the treatment combination of EGFR-TKIs and MET-inhibitors has been evaluated in different clinical trials, such as INSIGHT 1 and TATTON [69,70]. Namely, in the phase 1b/2 clinical trial INSIGHT 1, Wu et al. and colleagues evaluated the efficacy of the combination tepotinib/gefitinib in \textit{EGFR}-mutant patients with \textit{MET} amplification and secondary resistance to EGFR-TKIs, reporting better mPFS and mOS in this particular subset of patients (16.6 vs. 4.2, HR 0.13; 37.3 vs. 13.1 HR 0.08, respectively) [69]. Additionally, Oxnard et al. examined the safety of osimertinib in combination with selumetinib/savolitinib/durvalumab [68]. Indeed, only three patients harbored \textit{MET} amplification and p.T790M and they were treated with selumetinib displaying partial
However, osimertinib combination with savolitinib in patients with MET-driven secondary resistance to EGFR-TKIs is under current evaluation in the ongoing trials SAVANNAH (NCT03778229) and ORCHARD (NCT03944772). Whereas MET exon 14 skipping/EGFR mutations are very rare and poorly explored. In preclinical models MET ex14 decrease sensitivity to EGFR-TKIs [70]. As results of our systematic review of the literature, we found only three papers presenting interesting data on this particular setting. In fact, Chevallier et al. reported 15 patients with EGFR/MET alteration known to be non-pathogenic according to international database [46]. Lee et al. described a single patient with MET amplification >15 gene copies in 17% of tumor cells [49]. Chen et al. reported a single case including in the short PFS group (10% vs. 33% p = 0.018) [48]. Finally, there is a strong rationale for the use of combination of EGFR-TKIs and MET inhibitors in this setting, thus larger studies are warranted.

3.2.5. BRAF

BRAF mutations, both p.V600E and non-p.V600E, are detected in 6–8% of NSCLC cases, inducing downstream activation of the MAPK signaling pathway [71]. Over the decades, several BRAF inhibitors have been developed and the combination of trametinib and dabrafenib was the first treatment approved for advanced BRAF p.V600E-mutant NSCLC [72,73]. Concomitant EGFR/BRAF aberrations are found in approximately 11% of EGFR-positive NSCLC patients, with the BRAF p.V600E mutation most frequently identified [74,75]. Chen et al. retrospectively screened 423 NSCLC patients harboring EGFR 19Del or p.L858R mutations reporting only one patient with concurrent BRAF p.V600E [48]. Of note, the patient showed a poor PFS. Furthermore, Li et al. assessed a comprehensive mutation profiling from 5125 Chinese cohorts and they reported 160 concurrent mutations including two EGFR/BRAF concomitant mutations [29]. Moreover, Rachiglio et al. found hotspot mutation in several genes, including BRAF in 14 patients (21.8%) of their cohort [33]. Zhuang et al. described two cases of concomitant EGFR/BRAF alteration, showing better outcomes with EGFR-TKI than with standard chemotherapy [38].

3.2.6. RET

Rearranged during transfection (RET) gene rearrangements are detected in almost 1% of NSCLC patients [76,77]. Recently, FDA has granted accelerated approval to pralsetinib and selpercatinib for lung cancer patients harboring RET fusion based on ARROW and LIBRETTO-001 clinical trials results [78,79]. In up to 10% of NSCLC patients under osimertinib treatment, oncogenic fusions of RET gene have been considered responsible for acquired resistance [78,80]. Taking into account this, the open-label, multicenter, biomarker-guided, phase 2 clinical trial ORCHARD (NCT03944772) is still recruiting NSCLC patients progressed on 1-L osimertinib therapy, and one cohort includes RET rearranged patients which will receive osimertinib in combination with selpercatinib (LOXO-292) [81,82]. Albeit, the co-presence of EGFR mutation and RET rearrangement is rare, we found a single case report and a research article presenting original data on this particular subset of patients. Hu et al. detected one patient with concurrent EGFR and RET genomic alteration out of a cohort including 320 EGFR positive patients [47]. Particularly, the patient was an Asian young female with lung adenocarcinoma with no history of smoking, treated with gefitinib displaying poor OS and PFS (10.2 and 2.2 months, respectively) and PD as best response. Moreover, Klempern et al. and colleagues reported two patients with secondary acquired RET fusion in Asian EGFR-mutant NSCLC patients, both presenting short survival [52]. Of note, none of the patients reported underwent a combination treatment with EGFR-TKIs and RET-inhibitors. These data available from the literature confirmed the fact that RET fusion is a resistance mechanism in EGFR mutated patients and larger clinical trials are warranted in order to evaluate the potential activity of the combo EGFR-TKIs and RET-inhibitors.
3.3. TP53, PTEN, PIK3CA, CDKN2A and RB1

TP53 gene mutations are identified in 35–55% of NSCLC cases, especially in squamous cell carcinoma (SCC) and in smokers or former smokers [83–85]. Inactivating mutations of the TP53 gene affect the normal transcriptional p53 activity leading to tumor susceptibility and hinder patients’ response to chemotherapy treatments [86,87]. Moreover, TP53 alterations might be related to a poor prognosis in NSCLC patients [88]. Almost 55–65% of EGFR-positive NSCLC patients harbor a TP53 coexisting mutation [49,89,90]. Preclinical models have already demonstrated a correlation between TP53 mutation and response to EGFR-TKIs therapy [90–92]; namely apoptosis induced by gefitinib is decreased in p53 mutated cells. Mutation in TP53 gene have been divided into disruptive mutations and non-disruptive ones considering the loss of function of p53 protein. Specifically, disruptive mutations produce a complete loss of function of p53, while non-disruptive alterations result in conservative mutations or non-conservative mutations (excepting stop codons) outside the L2–L3 region [91,93–95]. Comprehensively, the systematic literature review identified a total of 11 reports evaluating the TP53 status in EGFR-mutant patients with lung adenocarcinoma. Namely, Canale et al. conducted an independent retrospective cohort study on a total of 136 EGFR-mutated NSCLC patients under treatment with first or second-generation TKIs as a first line therapy, in order to assess the role of TP53 gene alterations as predictor of survival and response to EGFR-TKIs therapy [41]. Endpoints of the clinical study were DCR, ORR, PFS and OS. TP53 mutations were detected in 42 (30.9%) out of the 136 patients, indeed according to the classification of TP53 aberrations into disruptive and non-disruptive mutations, the authors observed 11 patients harboring a disruptive TP53 mutation, while most of the patients carried a non-disruptive alteration [95,96]. Thusly, the authors found that TP53 mutations in exon 8 are related to a worse PFS regardless to the EGFR-TKIs treatment. Moreover, after a combined analysis the authors confirmed that the worse clinical outcome was independent from the subtype of EGFR mutations reported. Of note, further analysis was conducted on a sub-cohort of lung adenocarcinoma patients who developed a p.T790M resistance mutation and treated with osimertinib. This broadened analysis confirmed worse PFS and OS. These data were consistent with a previous report by Hou et al. [27]. In fact, this clinical trial examined the impact of TP53 gene alterations on the clinical outcomes in a Chinese cohort of 163 patients with NSCLC. By using NGS to establish the mutational status of EGFR and TP53, 43 EGFR-positive patients were found harboring a concurrent TP53 gene alteration. Considering the treatment outcomes, this subset of patients showed shorter median PFS (6.5 vs. 14.0 months) and median OS (28.0 vs. 52.0 months). Notably, differences in outcomes were particularly meaningful in the subset of patients harboring TP53 gene non-missense mutations, non-disruptive mutations, mutations in exon 6 and in exon 7 and mutations in the non-DNA Binding Domain (DBD) region among all TP53 mutations. Interestingly, these data are consistent with the report by VanderLaan et al. [35] who described 10 patients with TP53 concurrent mutation and worse clinical outcomes. Of note, the authors demonstrated a decreased rate of acquired p.T790M mutation as a mechanism of resistance to gefitinib, erlotinib and afatinib in lung adenocarcinomas with concomitant TP53 mutations. This could be explained as genomic complex tumors might trigger different pathways bypassing EGFR as a target. Additionally, an intriguing retrospective research was reported by Chen et al., who validate the number of concurrent mutation and Tumor Mutational Burden (TMB) in 71 patients with EGFR mutation and under treatment with EGFR-TKIs stratified for PSF [48]. Namely, TMB was defined as somatic, coding, base substitution, and indel mutations per megabase of genome analyzed. No significant differences were assessed between the two groups, yet the shorter PSF subgroup revealed a TMB higher than eight. One could guess that an increased TMB is correlated with the existence of resistance pathways, as previous reports suggested [94]. Furthermore, among overall clinical studies, EGFR-TKIs appeared to have less activity in 67 patients harboring concomitant TP53 gene mutations. A novel treatment option for this particular subset of patients is represented by the combination of EGFR-TKIs and antiangiogenic agents. Indeed, the combination of anlotinib plus icotinib...
displayed promising activity in the ALTER-L004 clinical trial for EGFR-positive NSCLC patients. Namely, the intention to treat population (ITT) included 14 patients carrying concomitant TP53 alterations, which showed ORR of 78.5% and DCR of 100% [39]. Additionally, in the ACTIVE study, Zhang et al. reported better PFS in the apatinib plus gefitinib group in naïve patients with EGFR mutations and patients harboring TP53 exon 8 mutations showed significant benefit from the dual blockade (HR 0.24 95%CI 0.06–0.91) [40,97]. Rachiglio et al. described 23 EGFR/TP53 mutant cases, exhibiting a mPFS of 12.3 months and mOS of 18.9 months under EGFR-TKI treatment [33]. Interestingly, Sato et al. reported 12 patients (28%) with EGFR/TP53 alteration [34]. Moreover, Zheng et al. demonstrated that 11 patients with co-existing EGFR and TP53 genomic alteration might have a worse prognosis comparing to EGFR-mutant patients [37]. Lee et al. described three cases out of 197 patients [49]. Chevallier et al. reported 15 cases of double mutation, with no difference of survival [46]. Chang et al. found that TP53 was the most common concomitant alteration detected (10/31 patients) [42], as Chen et al. reported in their study [48].

Phosphatase and tensin homologue deleted on chromosome 10 (PTEN) is a tumor suppressor gene and one of the most important negative regulator of the PI3K/AKT signaling pathway [98,99]. PTEN is deleted in several types of cancers, such as prostate, endometrial, glioblastoma, breast, melanoma and colon [100–102]. Lung cancers are malignant tumors where PTEN deregulation plays a crucial role in tumor cell proliferation, metastasis process, and resistance to treatments. Beyond 40% of NSCLC, cases express loss of PTEN and it is related to poor prognosis, especially for EGFR-positive patients treated with EGFR-TKIs [103]. Various preclinical models have disclosed that PTEN inactivation could alter the pattern of response to EGFR-TKIs [46,104], namely Chevallier et al. reported a retrospective cohort trial of the influence of concurrent mutations on patients with advanced NSCLC treated with TKIs [46]. The authors found five patients harboring a resistance pathogen mutation in PTEN, who showed poor mPFS of 6.8 months. These finding are consistent with a recent report from Huang et al. [39]. Finally, VanderLaan et al. reported 5% (1/19 patients) of PTEN/EGFR altered patients [35].

It has been already proved that the downstream signaling pathway of the HER family phosphatidylinositol-3-kinase (PI3K) is related to carcinogenesis in lung cancer [43]. PIK3CA mutations are detected in almost 3–7% of patients with lung adenocarcinomas and commonly they are located in exons 9 and 20 [105]. These genetic aberrations generate constitutive activation of PI3K, AKT phosphorylation, and mTORC1 downstream which have a crucial role in cell survival and proliferation. In contrast to the mutual exclusivity of various oncogenic aberrations in NSCLCs, the coexistence of PIK3CA mutations with other oncogenic alterations is well established [105,106]. Actually, approximately 3.5% of EGFR-mutant patients harbor PIK3CA gene alterations and this seems to blunt the response to TKIs treatment. In vitro data suggest that EGFR-TKI sensitivity in EGFR-positive NSCLC cell lines has been related to downregulation of the PI3K pathway, and as a matter of fact increased resistance to gefitinib was confirmed after the introduction of the PIK3CA p.E545K mutation into a gefitinib-sensitive lung adenocarcinoma cell line [45]. Eng et al. analyzed the prognostic impact of a concurrent PIK3CA mutation in 13 EGFR-mutant NSCLC patients, finding poor ORR (62% vs. 83%; p = 0.80) and shorter median Time To Progression (TTP) (7.8 vs. 11.1 months; p = 0.84) to EGFR-TKIs [45]. Moreover, Wu et al. examined the significance and the effect of PIK3CA mutations on treatment outcomes to EGFR-TKIs of lung adenocarcinoma [69]. The study population included six PIK3CA mutation-positive patients. In contrast to the analysis by Eng et al., the authors reported similar response (ORR, 66.7 vs. 78.7%; p = 0.476) to EGFR-TKIs as wild-type patients. Notably, PIK3CA-mutant patients displayed a trend toward better PFS (12.0 vs. 8.8 months) and OS (25.1 vs. 21.4 months), still the variations were not statistically significant. Accordingly, Wang et al. investigated a cohort of 1117 NSCLC patients, out of which 17 patients harbored simultaneously a mutation in EGFR and PIK3CA [51]. They found that survival for patients with single PIK3CA mutation was poorer than patients harboring a concurrent double alteration in PIK3CA and EGFR (p = 0.004). Chevallier et al. reported two patients
with double EGFR/PIK3CA alteration and poor survival [46]. De Marchi et al. detected 10/1208 individuals concurrent mutated in EGFR and PIK3CA [44], while Rachiglio et al. identified nine patients with double mutations displaying a mPFS of 5.5 months under EGFR-TKIs treatments [33]. Zhang et al. presented four patients harboring concurrent EGFR/PIK3CA genomic alteration [50], whereas Li et al. reported 64 (3.3%) of their 5125 patients [29]. Additionally, Hu et al. described nine out of 320 patients and of note they reported the longest PFS of 7.6 months, while Chen et al. found three out of 36 patients describing lower ORR (43.75% vs. 80.0%; \(p = 0.024\)) comparing to the population with a single EGFR alteration [43]. Lammers et al. reported three cases among their study population with poor response to erlotinib treatment [20], whereas Huang et al. recently reported better ORR of 72% among the 18 patients harboring double concurrent genomic alteration under icotinib and anlotinib treatment.

CDKN2A gene encodes p16, a tumor suppressor which promotes a cell cycle arrest in G1 phase by inhibiting Rb phosphorylation. In NSCLC patients, the inactivation of CDKN2A is one of the most common genomic alterations detected [101], especially through the mechanisms of homozygous deletions (HDs), presented in up to 29–59% of lung adenocarcinomas regardless of the concurrent EGFR mutation [102]. Jiang et al. studied 127 EGFR-positive patients with NSCLC, identifying 31 out of 127 (24.4%) patients with HDs in CDKN2A, who displayed poor ORR to EGFR-TKIs and shorter mPFS. Of note, these results might justify the use of the combo EGFR-TKI and CDK4/6 inhibitors in this particular subset of patients [104]. Moreover, Chang et al. analyzed 31 NSCLC patients with EGFR alteration revealing copy number variation (CNV) loss in CDKN2A gene in seven patients (22.6%) [42]. Notably, four out of seven patients had an intermediate response (six to 12 months of PFS), while the other three patients presented a poor response (<six months). Finally, Skoulidis et al. and colleagues showed 24.6% of CDKN2A alterations in their cohort, concluding that co-alterations in EGFR and CDKN2A were related to EGFR TKIs acquired resistance [107].

RB1 gene is a regulator of cell cycle and is phosphorylated by CDK4/6 to S-phase entry [108]. The alterations in RB1 pathway have been associated to worse prognosis in NSCLC patients [107]. In their article, Sato et al. and colleagues investigated 43 patients with EGFR mutations revealing 16% (7/43) of RB1 co-alterations [34]. Of note, these patients showed a poor prognosis. Hou et al. examined 71 NSCLC patients with EGFR mutations, of whom seven patients (9.9%) with a concomitant RB1 alteration [109]. Moreover, it is well-established that RB1 loss is a primary event correlated with transformation to Small-Cell Lung Cancer (SCLC) and consequently EGFR-TKIs treatment resistance [110,111]. Additionally, Yu et al. and Kim et al. reported RB1 as one of the most common gene co-altered in NSCLC patients [90]. Particularly, Kim et al. and colleagues identified co-alteration in RB1 as predictor of fast progression to TKI treatment [112].

3.4. Methods of Detection

The mutational analysis should be performed on tissue specimens and the most common methods for EGFR mutation detection with concomitant genomic alterations are reported in Table 1. Generally, the biological material available does not provide an amount of neoplastic cell percentage allowing the use of a Sanger Sequencing method. Conversely, high-sensitivity platforms as digital droplet PCR (ddPCR) (0.1%) [113], or Amplification Refractory Mutation System (ARMS) with a specificity up to 1% [111] should be able to cleverly detect these pathogenetic variants with a specificity running up to wild-type DNA [111]. Nevertheless, the recent development of NGS accomplishes massive parallel gene mutation analysis and requires low amount of tissue, favoring the identification of several targetable molecular alterations until of 5% of VAF [113].

4. Discussion

Over the last decades, our treatment approach to lung cancer patients has been dramatically changed as a result of the development and clinical implementation of an essential
tool as NGS. EGFR mutations represent a molecular target in this particular population. In this context, oncogenic driver mutations in NSCLC were historically considered mutually exclusive, thus the potential association of two or more oncogenic driver aberrations has been poorly explored. Moreover, the advent of comprehensive genomic profiling in clinical samples enabled the detection of a significant number of concurrent alterations in EGFR-mutated NSCLC. Consequently, we perform a systematic review of the literature on concurrent genomic alterations and their oncogenic role to provide a deeper insight into the molecular heterogeneity of EGFR-mutated NSCLCs. Finally, the results of our systematic review of the literature seemed to indicate that EGFR-mutant NSCLC is not a single oncogene driven entity. Most of the results of our systematic review consisted in articles and reports on Asian population. This is consistent with the major prevalence of EGFR genomic alteration in NSCLC Asian patients [114,115]. Notable, Asian patients appeared to display better response comparing to Caucasian cohorts (see Table 2); this finding shows racial differences in genetic pathways and prompts further studies on this field of research.

Notably, the presence of coexisting genetic alteration might likely justify resistance to TKIs treatment [116]. Particularly, different clinical trials assessed that the concurrent presence of mutation provides a worse prognosis in EGFR-positive NSCLC patients treated with first-, second-, and third-generation TKIs. Indeed, these recent findings highlighted that EGFR-mutated tumors have notable intratumor heterogeneity with concomitant evidence of significant oncogenic gene aberrations [54,117]. In fact, through the use of NGS, Belardinilli et al. detected similar VAF of the pair of mutations located in exon 21, likely indicating a co-occurrence within the same tumor cells [17]. Accordingly, it is intriguing to speculate that the above-mentioned particular oncogenetic pattern identified in this lung cancer patient could explain the increased response to TKI treatment with afatinib, generally not found in NSCLC patients harboring complex EGFR alterations. Moreover, this interesting analysis pointed out the urgent need of further investigations in order to clarify the mechanism of differential responses to TKIs. In the study by Zhang et al. [54], patients with a sensitive EGFR alteration such as 19Del/p.L858R/p.L861Q, plus a p.T790M de novo or an exon 20 insertion exhibited the worst clinical outcomes [116], [118]. This could be clarified as they were treated with first- and second-generation EGFR-TKIs. It would have been intriguing evaluating patient’s response after a third-generation EGFR-TKI treatment, like osimertinib. Indeed, based on the results of the FLAURA trial, osimertinib is considered the current standard of care for EGFR p.T790M positive patients. However, NSCLC patients with EGFR exon 20 insertion designate still a crucial unmet need. Recent preliminary data presented on March 2021 at ESMO Targeted Anticancer Therapies (TAT) Virtual Congress by Sacher et al. demonstrated that poziotinib, initially conceived as a HER2-inhibitor, has significant clinical activity on this particular subset of patients [119]. Although further evaluations are warranted, one could speculate that EGFR-positive patients harboring a complex mutation, alike an exon 20 insertion and a sensitive genetic alteration (19Del/p.L858R/p.L861Q) might benefit from a treatment with a potent irreversible TKI, such as poziotinib. Collectively, it is challenging to estimate the efficacy of EGFR-TKIs in NSCLC patients harboring uncommon complex EGFR genetic alterations due to the great heterogeneity of the mutations detected [119,120]. Previous clinical trials have evaluated that first-generation EGFR-TKIs showed poor efficacy for uncommon mutations (alone or plus a compound mutation) [121]. Consequently, afatinib and osimertinib should be taken into consideration for treatment of these patients. In fact, based on the durable responses demonstrated in the multicenter randomized clinical trials LUX-Lung 2, LUX-Lung 3, and LUX-Lung 6, on January 2018, the Food and Drug Administration (FDA) granted approval to afatinib as a first-line treatment option for patients carrying an uncommon mutation [69,122–125]. Additionally, osimertinib confirmed favorable activity in patients with NSCLC harboring uncommon EGFR mutations, as reported by Cho et al. [126,127]. Therefore, a treatment with afatinib or osimertinib seems to be a better strategy for this particular subset of patients; however, because of the high molecular heterogeneity and
low prevalence of this mutational pattern further clinical trials with larger sample size are warranted. Notwithstanding, with the advent of novel and powerful technologies like NGS the detection rate of concomitant genetic alterations in EGFR and ALK is systematically increased [25,44,65]. Zhuang et al. found that ALK-TKI therapy was more active as a first-line treatment than in later lines [39], while Yang et al. detected that patients appeared to better respond to EGFR-TKIs as a first-line setting. The great heterogeneity of clinical outcomes might be correlated with different levels of EGFR or ALK protein phosphorylation. The responses to EGFR- and/or ALK-TKIs appeared to be conflicting, thus it is recommended to fully evaluate by using high-sensitivity molecular techniques and detect the VAF in order to reconstruct the clonal architecture and heterogeneity. Despite further investigations are warranted, a combination of EGFR- and ALK-TKI might be considered a reasoned treatment strategy. Moreover, several cases have been reported EGFR and KRAS concurrent alterations. Given the potential impact of multiclonal characters of NSCLC on treatments, VAF quantitative estimation of both genetic mutations appears to be the best method able to determine who would benefit most from EGFR-TKI treatment. Interestingly, based on our findings, one could speculate that the coexistence of EGFR/ROS-1 alteration is consistent with tumor tissue heterogeneity. Overall, due to the limited data available further larger studies remain mandatory in order to assess the treatment outcomes of patients harboring an EGFR/ROS1 concurrent alteration. The impact of EGFR/MET co-alterations in NSCLC patients still represents an area of active investigation, yet larger clinical trials using uniform criteria to evaluate MET status are required [66]. Methods universally standardized in order to detect MET gene alteration are fluorescence in situ hybridization (FISH), immunohistochemistry (IHC), NGS, and real-time PCR, however the latter technique is not selective for cancels cells [123]. Consequently, the conflicting results in MET positivity detection might be attributed to the lack of data harmonization platform as well as of systematic criteria. Two commonly used scoring systems for assessing MET amplification are the Cappuzzo scoring system and the PathVysion [127–130].

It is well-established that activation of the MET pathway is one of the main acquired resistance mechanisms in EGFR-mutant patients, hence it is conceivable that a combination of EGFR- and MET-TKIs might have some activity in this particular subset of patients [48]. Moreover, several clinical trials have reported a secondary BRAF p.V600E mutation as a potential resistance mechanism to osimertinib treatment in EGFR-mutant patients. Meng et al. reported two patients with a p.T790 M mutation both treated with osimertinib who acquired a BRAF p.V600E mutation at PD [74]. Interestingly, a combination of dabrafenib and trametinib plus osimertinib was administered. One patient showed PR with a PFS of 14 months, whereas the second patient discontinued treatment due to severe pneumonitis. However, combined treatment with dabrafenib, trametinib and osimertinib appeared to be effective. Since literature data on the activity of these combined approaches are limited, further investigation represents an important issue. Furthermore, the worse prognosis of concurrent EGFR/TP53 positive patients could be explained as genomic complex tumors might trigger different pathways bypassing EGFR as a target. Furthermore, among overall clinical studies, EGFR-TKIs appeared to have less activity in patients harboring concomitant TP53 gene mutations. A novel treatment option for this particular subset of patients is represented by the combination of EGFR-TKIs and antiangiogenic agents, as suggested by several trials [39,40,99]. Beyond 40% of NSCLC, cases express loss of PTEN and it is related to poor prognosis, especially for EGFR-positive patients treated with EGFR-TKIs [131]. Collectively, the concurrence of genetic aberrations in different genes might be responsible for the sub clonal heterogeneity, thence it could justify the primary resistance to EGFR-TKI treatments in this particular subset of patients. Based on our findings, the impact of PIK3CA mutations on survival in EGFR-mutant patients is still under debate. On March 2021, Lage et al. presented a retrospective analysis of 1745 NSCLC patients receiving treatment from 2011 to 2020. Out of 1745 patients, 479 patients underwent NGS and 61 (12.7%) patients were identified as having an alteration in the PI3K pathway [132]. Patients harboring a co-altered EGFR was 8% of the study population. The authors concluded that PI3K
pathway alteration was more common in smoker male patients with NSCLC. Notably, this genetic aberration was not mutually exclusive to other mutations, thusly highlights the relationship between molecular pathways. Basically, given the potential importance of PIK3CA concomitant mutations molecular tumor boards are mandatory allowing for individualized therapy in this specific subset of patients. In summary, the limited sample size of these studies prevents us from drawing definitive conclusions. Larger studies with long term follow-up are warranted in order to clarify these controversial results.

5. Conclusions

While for decades NSCLC was considered to be a single disease, it is nowadays becoming more convenient to consider NSCLC as a combination of disease subtypes according to the driver genetic aberration. Concurrence of multiple driver alterations should be considered in order to comprehensively understand tumor mechanisms and therapeutic strategies. Currently, it is possible to identify a larger number of concomitant mutated cancers by using more sensitive and powerful techniques. Considering that the use of large panels of genes might induce to the identification of multiple targeted molecular drivers, multidisciplinary molecular tumor boards (MTBs) are mandatory in order to provide the best treatment strategies in cases of concurrent somatic genomic alterations [130–133]. On the other hand, the determination of the VAF, taking into account the number of cancer cells harboring concomitant genetic aberrations, might be used as a tool to select the correct therapeutic options for this particular subset of patients. In conclusion, co-existing driver gene alterations characterize a small group of NSCLC patients. However, further prospective studies are warranted to examine the treatment outcomes of patients harboring double EGFR mutations.

Supplementary Materials: The following are available online at https://www.mdpi.com/article/10.3390/jmp2020016/s1, Figure S1: PRISMA checklist; Figure S2: Search Strategy.

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References

1. Bray, F.; Ferlay, J.; Soerjomataram, I.; Siegel, R.L.; Torre, L.A.; Jemal, A. Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J. Clin. 2018, 68, 394–424. [CrossRef]
2. Pakkala, S.; Ramalingam, S.S. Personalized therapy for lung cancer: Striking a moving target. JCI Insight 2018, 3. [CrossRef]
3. Nacchio, M.; Sgariglia, R.; Grisantini, V.; Pisapia, P.; Pepe, F.; De Luca, C.; Migliatico, I.; Clery, E.; Greco, L.; Vigliar, E.; et al. KRAS mutations testing in non-small cell lung cancer: The role of Liquid biopsy in the basal setting. J. Thorac. Dis. 2020, 12, 3836–3843. [CrossRef] [PubMed]
4. Passiglia, F.; Galvano, A.; Castiglia, M.; Incorvaia, L.; Calì, V.; Listi, A.; Mazzarisi, S.; Perez, A.; Gallina, G.; Rizzo, S.; et al. Monitoring blood biomarkers to predict nivolumab effectiveness in NSCLC patients. *Ther. Adv. Med Oncol.* 2019, 11, 1758835919839928. [CrossRef] [PubMed]

5. Yang, C.-Y.; Yang, J.-C.-H.; Yang, P.-C. Precision Management of Advanced Non–Small Cell Lung Cancer. *Annu. Rev. Med.* 2020, 71, 117–136. [CrossRef]

6. Shitivelman, E.; Hensing, T.; Simon, G.R.; Dennis, P.A.; Otterson, G.A.; Bueno, R.; Salgia, R. Molecular pathways and therapeutic targets in lung cancer. *Oncoargets 2014*, 5, 1392–1433. [CrossRef] [PubMed]

7. Russo, A.; Russano, M.; Franchina, T.; Migliorini, M.R.; Aprile, G.; Mansueto, G.; Berruti, A.; Falcone, A.; Aietta, M.; Gelibter, A.; et al. Neutrophil-to-Lymphocyte Ratio (NLR), Platelet-to-Lymphocyte Ratio (PLR), and Outcomes with Nivolumab in Pretreated Non-Small Cell Lung Cancer (NSCLC): A Large Retrospective Multicenter Study. *Adv. Ther.* 2020, 37, 1145–1155. [CrossRef]

8. Gristina, V.; Malapelle, U.; Galvano, A.; Pisapia, P.; Pepe, F.; Rolfo, C.; Tortorici, S.; Bazan, V.; Troncone, G.; Russo, A. The significance of epidermal growth factor receptor uncommon mutations in non-small cell lung cancer: A systematic review and critical appraisal. *Cancer Treat. Rev.* 2020, 85, 101994. [CrossRef] [PubMed]

9. Bronte, G.; Passiglia, F.; Galvano, A.; Barraco, N.; Listi, A.; Castiglia, M.; Rizzo, S.; Fiorentino, E.; Bazan, V.; Russo, A. Nintedanib in NSCLC: Evidence to date and place in therapy. *Ther. Adv. Med Oncol.* 2016, 8, 188–197. [CrossRef]

10. Sequist, L.V.; Yang, J.C.-H.; Yamamoto, N.; Obyrne, K.J.; Hirsh, V.; Geater, S.L.; Orlov, S.; Tsai, C.-M.; Boyer, M.; et al. Phase III Study of Afatinib or Cisplatin Plus Pemetrexed in Patients With Metastatic Lungen Adenocarcinoma With EGFR Mutations. *J. Clin. Oncol.* 2013, 31, 3327–3334. [CrossRef]

11. Gray, J.E.; Okamoto, I.; Sriuranpong, V.; Vansteenkiste, J.; Imamura, F.; Lee, J.S.; Pang, Y.-K.; Cobo, M.; Kasahara, K.; Cheng, Y.; et al. Tissue and Plasma EGFR Mutation Analysis in the FLAURA Trial: Osimertinib versus Comparator EGFR Tyrosine Kinase Inhibitor as First-Line Treatment in Patients with EGFR-Mutated Advanced Non–Small Cell Lung Cancer. *Clin. Cancer Res.* 2019, 25, 6644–6652. [CrossRef] [PubMed]

12. De Luca, C.; Pepe, F.; Iaccarino, A.; Pisapia, P.; Righi, L.; Listi, A.; Greco, L.; Gragnano, G.; Campione, S.; De Dominicis, G.; et al. RNA-Based Assay for Next-Generation Sequencing of Clinically Relevant Gene Fusions in Non-Small-Cell Lung Cancer. *Cancers* 2021, 13, 139. [CrossRef]

13. Malapelle, U.; Pepe, F.; Pisapia, P.; Sgariglia, R.; Nacchio, M.; Barberis, M.; Bilh, M.; Bubendorf, L.; Büttner, R.; Cabibi, D.; et al. TargetPlex FFPE-Direct DNA Library Preparation Kit for SiRe NGS panel: An international performance evaluation study. *J. Mol. Pathol.* 2021. [CrossRef]

14. Pepe, F.; Pisapia, P.; Gritsina, V.; Rocco, D.; Bs, M.M.; Micheli, P.; Iaccarino, A.; Tufano, R.; Bs, G.G.; De Luca, C.; et al. Tumor mutational burden on cytological samples: A pilot study. *Cancer Cytopathol.* 2020. [CrossRef]

15. Van Der Steen, N.; Mentens, Y.; Ramael, M.; Leon, L.G.; Germonpré, P.; Ferri, J.; Gandara, D.R.; Giovannetti, E.; Peters, G.J.; Pauwels, P.; et al. Double Trouble: A Case Series on Concomitant Genetic Aberrations in NSCLC. *Clin. Lung Cancer* 2018, 19, 35–41. [CrossRef]

16. Moher, D.; Shamseer, L.; Clarke, M.; Liberati, A.; Petticrew, M.; Shekelle, P.; Stewart, L.A.; PRISMA-P Group. Preferred reporting items for systematic review and meta-analysis protocols (PRISMA-P) 2015 statement. *Syst. Rev.* 2015, 4, 1–9. [CrossRef]

17. Belardinelli, F.; Gradilone, A.; Gelibter, A.; Zani, M.; Occhipinti, M.; Ferraro, S.; Nicolazzo, C.; Coppa, A.; Giannini, G. Coexistence of three EGFR mutations in an NSCLC patient: A brief report. *Int. J. Biol. Markers* 2018, 33, 545–548. [CrossRef]

18. Benesova, L.; Minarik, M.; Jancarikova, D.; Belsanova, B.; Pesek, M. Multiplicity of EGFR and KRAS mutations in non-small cell lung cancer (NSCLC) patients treated with germline T790M, and PIK3CA mutations: The challenge of interpreting results of comprehensive mutational testing in lung cancer. *Diagn. Pathol.* 2020, 15, 42–45. [CrossRef] [PubMed]

19. Lammers, P.E.; Lovly, C.; Horn, L. A patient with metastatic lung adenocarcinoma harboring concurrent EGFR L858R, EGFR germline T790M, and PIK3CA mutations: The challenge of interpreting results of comprehensive mutational testing in lung cancer. *J. Natl. Compr. Cancer Netw.* 2014, 12, 6–11. [CrossRef] [PubMed]

20. Lee, T.; Lee, B.; Choi, Y.-L.; Han, J.; Ahn, M.-J.; Um, S.-W. Non-small Cell Lung Cancer with Concomitant EGFR, KRAS, and ALK Mutation: Clinicopathologic Features of 12 Cases. *J. Pathol. Transl. Med.* 2016, 50, 197–203. [CrossRef]

21. Miyangara, A.; Shimizu, K.; Noro, R.; Seike, M.; Kitamura, K.; Kosaahira, S.; Minegishi, Y.; Shukuya, T.; Yoshimura, A.; Kawamoto, M.; et al. Activity of EGFR-tyrosine kinase and ALK inhibitors for EML4–ALK-rearranged non–small–cell lung cancer harbored coexisting EGFR Mutation. *BMC Cancer* 2013, 13, 262. [CrossRef]

22. Sweis, R.F.; Thomas, S.; Bank, B.; Fishkin, P.; Mooney, C.; Salgia, R. Concurrent EGFR Mutation and ALK Translocation in Non-Small-Cell Lung Cancer. *Cancer* 2016, 8, 513. [CrossRef]

23. Thumallapally, N.; Yu, H.; Farhan, M.; Ibrahim, U.; Odiemi, M. Concomitant Presence of EGFR and ALK Fusion Gene Mutation in Adenocarcinoma of Lung: A Case Report and Review of the Literature. *J. Pharm. Pr.* 2017, 31, 244–248. [CrossRef] [PubMed]

24. Xu, C.-W.; Zhu, Y.-C.; Ye, X.-Q.; Yin, M.-X.; Zhang, J.-X.; Du, K.-Q.; Zhang, Z.-H.; Hu, J. Lung cancer with concurrent EGFR mutation and ROS1 rearrangement: A case report and review of the literature. *OncoTargets Ther.* 2016, 9, 4301–4305. [CrossRef]

25. Yang, J.-J.; Zhang, X.-C.; Su, J.; Xu, C.-R.; Zhou, Q.; Tian, H.-X.; Xie, Z.; Chen, H.-J.; Huang, Y.-S.; Jiang, B.-Y.; et al. Lung Cancers with Concomitant EGFR Mutations and ALK Rearrangements: Diverse Responses to EGFR-TKI and Crizotinib in Relation to Diverse Receptors Phosphorylation. *Clin. Cancer Res.* 2014, 20, 1383–1392. [CrossRef]
27. Hou, H.; Qin, K.; Liang, Y.; Zhang, C.; Liu, D.; Jiang, H.; Liu, K.; Zhu, J.; Lv, H.; Li, T.; et al. Concurrent TP53 mutations predict poor outcomes of EGFR-TKI treatments in Chinese patients with advanced NSCLC. *Cancer Manag. Res.* 2019, *une 11*, 5665–5675. [CrossRef]

28. Zhu, J.; Cai, L.; Yang, H.; Wen, Y.; Wang, J.; Rong, T.; Shao, J.; Zhang, L. Echinoderm microtubule-associated protein-like 4-anaplastic lymphoma kinase rearrangement and epidermal growth factor receptor mutation coexisting in Chinese patients with lung adenocarcinoma. *Thorac. Cancer* 2014, *5*, 411–416. [CrossRef] [PubMed]

29. Li, S.; Li, L.; Zhu, Y.; Huang, C.; Qin, Y.; Liu, H.; Renheidenreich, L.; Shi, B.; Ren, H.; Chu, X.; et al. Coexistence of EGFR with KRAS, or BRAF, or PIK3CA somatic mutations in lung cancer: A comprehensive mutation profiling from 5125 Chinese cohorts. *Br. J. Cancer* 2014, *110*, 2812–2820. [CrossRef] [PubMed]

30. Liang, H.; Li, C.; Zhao, Y.; Zhao, S.; Huang, J.; Cai, X.; Cheng, B.; Xiong, S.; Li, J.; Wang, W.; et al. Concomitant Mutations in EGFR 19Del/L858R Mutation and Their Association with Response to EGFR-TKIs in NSCLC Patients. *Cancer Manag. Res.* 2020, *une 12*, 8653–8662. [CrossRef]

31. Liu, J.; Mu, Z.; Liu, L.; Li, K.; Jiang, R.; Chen, P.; Zhou, Q.; Jin, M.; Ma, Y.; Xie, Y.; et al. Frequency, clinical features and differential response to therapy of concurrent ALK/EGFR alterations in Chinese lung cancer patients. *Drug Des. Dev. Ther.* 2019, *une 13*, 1809–1817. [CrossRef]

32. Nardo, G.; Carlet, J.; Marra, L.; Bonanno, L.; Boscolo, A.; Maso, A.D.; Bragadin, A.B.; Indraccolo, S.; Zulato, E. Detection of Low-Frequency KRAS Mutations in cfDNA From EGFR-Mutated NSCLC Patients After First-Line EGFR Tyrosine Kinase Inhibitors. *Front. Oncol.* 2021, *10*, 3055. [CrossRef]

33. Rachiglio, A.M.; Fenizia, F.; Piccirillo, M.C.; Galetta, D.; Crinò, L.; Vincenzi, B.; Barletta, E.; Pinto, C.; Ferraiu, F.; Lambiase, M.; et al. The Presence of Concomitant Mutations Affects the Activity of EGFR Tyrosine Kinase Inhibitors in EGFR-Mutant Non-Small-Cell Lung Cancer (NSCLC) Patients. *Cancers* 2019, *11*, 341. [CrossRef]

34. Sato, S.; Nagahashi, M.; Koike, T.; Ichikawa, H.; Shimada, Y.; Watanabe, S.; Kikuchi, T.; Takada, K.; Nakanishi, R.; Oki, E.; et al. Impact of Concurrent Genomic Alterations Detected by Comprehensive Genomic Sequencing on Clinical Outcomes in East-Asian Patients with EGFR-Mutated Lung Adenocarcinoma. *Sci. Rep.* 2018, *8*, 1–10. [CrossRef]

35. VanderLaan, P.A.; Rangachari, D.; Mockus, S.M.; Spotlow, V.; Reddi, H.V.; Malcolm, J.; Huberman, M.S.; Joseph, L.J.; Kobayashi, S.S.; Costa, D.B. Mutations in TP53, PIK3CA, PTEN and other genes in EGFR mutated lung cancers: Correlation with clinical outcomes. *Lung Cancer* 2017, *106*, 17–21. [CrossRef]

36. Wu, S.-G.; Chang, Y.-L.; Yu, C.-J.; Yang, P.-C.; Shih, J.-Y. The Role of PIK3CA Mutations among Lung Adenocarcinoma Patients with Primary and Acquired Resistance to EGFR Tyrosine Kinase Inhibition. *Sci. Rep.* 2016, *6*, 35249. [CrossRef] [PubMed]

37. Zheng, C.; Li, X.; Ren, Y.; Yin, Z.; Zhou, B. Coexisting EGFR and TP53 Mutations in Lung Adenocarcinoma Patients Are Associated With COMP and ITGB8 Upregulation and Poor Prognosis. *Front. Mol. Biosci.* 2020, *7*, 30. [CrossRef]

38. Zhuang, X.; Zhao, C.; Li, J.; Su, C.; Chen, X.; Ren, S.; Li, X.; Zhou, C. Clinical features and therapeutic options in non-small cell lung cancer patients with concomitant mutations of EGFR, ALK, ROS1, KRAS or BRAF. *Cancer Med.* 2019, *8*, 2858–2866. [CrossRef]

39. Huang, D.; Zhong, D.; Zhang, C.; Zhang, Y.; Shang, Y.; Wang, L. Study of anlotinib combined with icotinib as the first-line treatment in non-small cell lung cancer (NSCLC) patients harboring activating EGFR mutations (ALTER-L004). *J. Clin. Oncol.* 2020, *38*, 9573. [CrossRef]

40. Zhang, L.; Zhao, H.; Zhang, Z.; Yao, W.; Min, X.; Gu, K.; Yu, G.; Cheng, C.; Cui, J.; Miao, L.; et al. LBA50 ACTIVE: Apatinib plus gefitinib versus placebo plus gefitinib as first-line treatment for advanced epidermal growth factor receptor-mutant (EGFRm) non-small-cell lung cancer (NSCLC): A multicentered, randomized, double-blind, placebo-controlled phase III trial (CTONG1706). *Ann. Oncol.* 2020, *31*, S181. [CrossRef]

41. Canale, M.; Petracci, E.; Delmonte, A.; Bronte, G.; Chiaidi, E.; Ludovini, V.; Dubini, A.; Papi, M.; Baglivo, S.; De Luigi, N.; et al. Concomitant TP53 Mutation Confers Worse Prognosis in EGFR-Mutant Non-Small-Cell Lung Cancer Patients Treated with TKIs. *J. Clin. Med.* 2020, *9*, 1047. [CrossRef] [PubMed]

42. Chang, S.-C.; Lai, Y.-C.; Chang, C.-Y.; Huang, L.-K.; Chen, S.-J.; Tan, K.T.; Yu, P.-N.; Lai, J.-I. Concomitant Genetic Alterations Are Associated with Worse Clinical Outcome in EGFR Mutant NSCLC Patients Treated with Tyrosine Kinase Inhibitors. *Transl. Oncol.* 2019, *12*, 1425–1431. [CrossRef]

43. Chen, H.; Liu, M.; Dai, Z.; Li, S.; Luo, Y.; Wang, Y.; Su, W.; Cai, W.; Yang, D.; Huang, J.; et al. Concomitant genetic alterations are associated with response to therapy targeted therapy in patients with lung adenocarcinoma. *Transl. Lung Cancer Res.* 2020, *9*, 1225–1234. [CrossRef]

44. De Marchi, F.; Haley, L.; Fryer, H.; Ibrahim, J.; Beierl, K.; Zheng, G.; Gocke, C.D.; Eshleman, J.R.; Belchis, D.; Illei, P.; et al. Clinical Validation of Coexisting Activating Mutations Within EGFR, Mitogen-Activated Protein Kinase, and Phosphatidylinositol 3-Kinase Pathways in Lung Cancers. *Arch. Pathol. Lab. Med.* 2019, *143*, 174–182. [CrossRef]

45. Eng, J.; Woo, K.M.; Sima, C.S.; Plodkowski, A.; Hellmann, M.D.; Chaft, J.E.; Kris, M.; Arcila, M.E.; Ladanyi, M.; Drilon, A. Impact of Concurrent PIK3CA Mutations on Response to EGFR Tyrosine Kinase Inhibition in EGFR-Mutant Lung Cancers and on Prognosis in Oncogene-Driven Lung Cancers. *J. Thorac. Oncol.* 2015, *10*, 1713–1719. [CrossRef] [PubMed]

46. Chevallier, M.; Tsantoulis, P.; Addeo, A.; Friedlaender, A. Influence of Concurrent Mutations on Overall Survival in EGFR-mutated Non-small Cell Lung Cancer. *Cancer Genom. Proteoms.* 2020, *17*, 597–603. [CrossRef] [PubMed]
47. Hu, W.; Liu, Y.; Chen, J. Concurrent gene alterations with EGFR mutation and treatment efficacy of EGFR-TKIs in Chinese patients with non-small cell lung cancer. *Oncotarget* **2017**, *8*, 25046–25054. [CrossRef] [PubMed]
48. Chen, M.; Xu, Y.; Zhao, J.; Zhong, W.; Zhang, L.; Bi, Y.; Wang, M. Concurrent Driver Gene Mutations as Negative Predictive Factors in Epidermal Growth Factor Receptor-Positive Non-Small Cell Lung Cancer. *EBioMedicine* **2019**, *42*, 304–310. [CrossRef]
49. Lee, J.-K.; Shin, J.-Y.; Kim, S.; Lee, S.-H.; Park, C.; Kim, J.-Y.; Koh, Y.; Keam, B.; Min, H.S.; Kim, T.M.; et al. Primary resistance to epidermal growth factor receptor (EGFR) tyrosine kinase inhibitors (TKIs) in patients with non-small-cell lung cancer harboring TKI-sensitive EGFR mutations: An exploratory study. *Ann. Oncol.* **2013**, *24*, 2080–2087. [CrossRef]
50. Zhang, Q.; Sun, T.; Kang, P.; Qian, K.; Deng, B.; Zhou, J.; Wang, R.; Jiang, B.; Li, K.; Liu, F.; et al. Combined analysis of rearrangement of ALK, ROS1, somatic mutation of EGFR, KRAS, BRAF, PIK3CA, and mRNA expression of ERCC1, TM45, RRM1, TUBB3, EGFR in patients with non-small cell lung cancer and their clinical significance. *Cancer Chemother. Pharmacol.* **2016**, *77*, 583–593. [CrossRef]
51. Wang, L.; Hu, H.; Pan, Y.; Wang, R.; Li, Y.; Shen, L.; Yu, Y.; Li, H.; Cai, D.; Sun, Y.; et al. PIK3CA Mutations Frequently Coexist with EGFR/KRAS Mutations in Non-Small Cell Lung Cancer and Suggest Poor Prognosis in EGFR/KRAS Wildtype Subgroup. *PLoS ONE* **2014**, *9*, e88291. [CrossRef]
52. Klempern, S.J.; Bazhenova, L.A.; Braiteh, F.S.; Nikolainkos, P.G.; Gowen, K.; Cervantes, C.M.; Chmielecki, J.; Greenbowe, J.R.; Ross, J.S.; Stephens, P.J.; et al. Emergence of RET rearrangement co-existing with activated EGFR mutation in EGFR-mutated NSCLC patients who had progressed on first- or second-generation EGFR TKI. *Lung Cancer* **2015**, *89*, 357–359. [CrossRef]
53. Hsieh, M.-H.; Fang, Y.-F.; Chang, W.-C.; Kuo, H.-P.; Lin, S.-Y.; Liu, H.-P.; Liu, C.-L.; Chen, H.-C.; Ku, Y.-C.; Chen, Y.-T.; et al. Complex mutation patterns of epidermal growth factor receptor gene associated with variable responses to gefitinib treatment in patients with non-small-cell lung cancer. *Lung Cancer* **2006**, *53*, 311–322. [CrossRef]
54. Zhang, B.; Wang, S.; Qian, J.; Yang, W.; Qian, F.; Lu, J.; Zhang, Y.; Qiao, R.; Han, B. Complex epidermal growth factor receptor mutations and their responses to tyrosine kinase inhibitors in previously untreated advanced lung adenocarcinomas. *Cancer* **2018**, *124*, 2399–2406. [CrossRef]
55. Kristina, V.; La Mantia, M.; Iacono, F.; Galvano, A.; Russo, A.; Bazan, V. The Emerging Therapeutic Landscape of ALK Inhibitors in Non-Small Cell Lung Cancer. *Pharmaceuticals* **2020**, *13*, 474. [CrossRef] [PubMed]
56. Russo, A.; Franchina, T.; Ricciardi, G.R.R.; Ferraro, G.; Scimone, A.; Bronte, G.; Russo, A.; Rolfo, C.; Adamo, V. Central nervous system involvement in ALK-rearranged NSCLC: Promising strategies to overcome crizotinib resistance. *Expert Rev. Anticancer Ther.* **2016**, *16*, 615–623. [CrossRef] [PubMed]
57. Hallberg, B.; Palmer, R.H. The role of the ALK receptor in cancer biology. *Ann. Oncol.* **2016**, *27*, iii4–iii5. [CrossRef]
58. Khan, M.; Lin, J.; Liao, G.; Tian, Y.; Liang, Y.; Liu, M.; Yuan, Y. ALK Inhibitors in the Treatment of ALK Positive NSCLC. *Front. Oncol.* **2019**, *8*, 557. [CrossRef] [PubMed]
59. Gainor, J.F.; Varghese, A.M.; Ou, S.-H.I.; Kabraji, S.; Awad, M.M.; Katayama, R.; Pawlak, A.; Mino-Kenudson, M.; Yeap, B.Y.; Riely, G.J.; et al. ALK Rearrangements Are Mutually Exclusive with Mutations in EGFR or KRAS: An Analysis of 1,683 Patients with Non-Small Cell Lung Cancer. *Clin. Cancer Res.* **2013**, *19*, 4273–4281. [CrossRef] [PubMed]
60. Bronte, G.; Incorvaia, L.; Rizzo, S.; Passignola, F.; Galvano, A.; Rizzo, F.; Rolfo, C.; Fanale, D.; Listi, A.; Naioli, C.; et al. The resistance related to targeted therapy in malignant pleural mesothelioma: Why has not the target been hit yet? *Crit. Rev. Oncol. Hematol.* **2016**, *107*, 20–32. [CrossRef] [PubMed]
61. Devarakonda, S.; Morgensztern, D.; Govindan, R. Genomic alterations in lung adenocarcinoma. *Lancet Oncol.* **2015**, *16*, e342–e351. [CrossRef]
62. Zhu, Y.; Liao, X.; Wang, W.; Xu, C.; Zhuang, W.; Wei, J.; Du, K. Dual drive coexistence of EML4-ALK and TPM3-ROS1 fusion in advanced lung adenocarcinoma. *Thoracic Cancer* **2017**, *8*, 324–327. [CrossRef]
63. Vasan, N.; Boyer, J.L.; Herbst, R.S. A RAS Renaissance: Emerging Targeted Therapies for KRAS-Mutated Non–Small Cell Lung Cancer and Suggest Poor Prognosis in EGFR/KRAS Wildtype Subgroup. *Clin. Cancer Res.* **2014**, *20*, 3921–3930. [CrossRef]
64. Shaw, A.T.; Solomon, B. Crizotinib in ROS1-Rearranged Non–Small-Cell Lung Cancer. *New Engl. J. Med.* **2015**, *372*, 683–684. [CrossRef] [PubMed]
65. Lung, J.; Hung, M.-S.; Lin, Y.-C.; Lee, K.-F.; Jiang, Y.Y.; Huang, S.-L.; Fang, Y.H.; Lu, M.-S.; Lin, C.-K.; Yang, T.-M.; et al. MET exon 14 skipping mutations and gene amplification in a Taiwanese lung cancer population. *PLOS ONE* **2019**, *14*, e0220670. [CrossRef]
66. Engelman, J.A.; Zeijnallahu, K.; Mitsudomi, T.; Song, Y.; Hyland, C.; Park, J.O.; Lindeman, N.; Gale, C.-M.; Zhao, X.; Christiansen, J.; et al. MET Amplification Leads to Gefitinib Resistance in Lung Cancer by Activating ERBB3 Signaling. *Science* **2007**, *316*, 1039–1043. [CrossRef] [PubMed]
67. Lu, D.; Li, Y.; Sun, K.D.; Gu, J.; Chen, Z.; Owonikoko, T.K.; Ramalingam, S.S.; Sun, S.-Y. The novel MET inhibitor, HQP8361, possesses single agent activity and enhances therapeutic efficacy of AZD9291 (osimertinib) against AZD9291-resistant NSCLC cells with activated MET. *Am. J. Cancer Res.* **2020**, *10*, 3316–3326. [CrossRef]
68. Sequist, L.V.; Han, J.-Y.; Ahn, M.J.; Cho, B.C.; Yu, H.; Kim, S.-W.; Yang, J.C.-H.; Lee, J.S.; Su, W.-C.; Kowalski, D.; et al. Osimertinib plus savolitinib in patients with EGFR mutation-positive, MET-amplified, non-small-cell lung cancer after progression on EGFR tyrosine kinase inhibitors: Interim results from a multicentre, open-label, phase 1b study. *Lancet Oncol.* **2020**, *21*, 373–386. [CrossRef]
69. Wu, Y.-L.; Cheng, Y.; Zhou, J.; Lu, S.; Zhang, Y.; Zhao, J.; Kim, D.-W.; Soo, R.A.; Kim, S.-W.; Pan, H.; et al. Tepotinib plus gefitinib in patients with EGFR-mutant non-small-cell lung cancer with MET overexpression or MET amplification and acquired resistance
to previous EGFR inhibitor (INSIGHT study): An open-label, phase 1b/2, multicentre, randomised trial. *Lancet Respir. Med.* 2020, 8, 1132–1143. [CrossRef]

70. Helena, A.Y.; Suzawa, K.; Jordan, E.J.; Zehir, A.; Ni, A.; Kim, H.R.; Kris, M.G.; Hellmann, M.D.; Li, B.T.; Somwar, R.; et al. Concurrent Alterations in EGFR-Mutant Lung Cancers Associated with Resistance to EGFR Kinase Inhibitors and Characterization of MTOB as a Biomarker of Resistance. *Clin. Cancer Res.* 2018, 24, 3108–3118. [CrossRef]

71. Lin, L.; Asthana, S.; Chan, E.; Bandyopadhyay, S.; Martins, M.M.; Olivas, V.; Yan, J.J.; Pham, L.; Wang, M.M.; Bollag, G.; et al. Mapping the molecular determinants of BRAF oncogene dependence in human lung cancer. *Proc. Natl. Acad. Sci.* 2014, 111, E748–E757. [CrossRef] [PubMed]

72. Planchard, D.; Smit, E.; Groen, H.; Mazieres, J.; Besse, B.; Helland, Å.; Giannone, V.; D’Amelio, A.; Zhang, P.; Mookerjee, B.; et al. Phase 2 trial (BRF113928) of dabrafenib (D) plus trametinib (T) in patients (pts) with previously untreated BRAF V600E–mutant metastatic non-small cell lung cancer (NSCLC). *Ann. Oncol.* 2017, 28, v637. [CrossRef] [PubMed]

73. Minari, R.; Bordi, P.; La Monica, S.; Squadrilli, A.; Leonetti, A.; Bottarelli, L.; Azzoni, C.; Lagrasta, C.A.M.; Gnetti, L.; Campanini, N.; et al. Concurrent Acquired BRAF V600E Mutation and MET Amplification as Resistance Mechanism of First-Line Osimertinib Treatment in a Patient with EGFR-Mutated NSCLC. *J. Thorac. Oncol.* 2018, 13, e89–e91. [CrossRef] [PubMed]

74. Meng, P.; Koopman, B.; Kok, K.; ter Elst, A.; van Kempen, L.C.; Timens, W.; Hiltermann, T.J.N.; Groen, H.J.; Berg, A.V.D.; et al. Combined osimertinib, dabrafenib and trametinib treatment for advanced non-small-cell lung cancer patients with an osimertinib-induced BRAF V600E mutation. *Lung Cancer* 2020, 146, 358–361. [CrossRef]

75. Kohno, T.; Ichikawa, H.; Totoki, Y.; Yasuda, K.; Hiramoto, M.; Nammo, T.; Sakamoto, H.; Tsuta, K.; Furuta, K.; Shimada, Y.; et al. KIF5B-RET fusions in lung adenocarcinoma. *Nat. Med.* 2012, 18, 375–377. [CrossRef]

76. Lipson, D.; Capelletti, M.; Yelensky, R.; Otto, G.; Parker, A.; Jarosz, M.; A Curran, J.; Balasubramanian, S.; Bloom, T.; Brennan, K.W.; et al. Identification of new ALK and RET gene fusions from colorectal and lung cancer biopsies. *Nat. Med.* 2012, 18, 382–384. [CrossRef]

77. Hu, M.; Subbiah, V.; Wirth, L.; Schuler, M.; Mansfield, A.; Brose, M.; Curigliano, G.; Leboulleux, S.; Zhu, V.; Keam, B.; et al. 1913O Results from the registrational phase I/II ARROW trial of pralsetinib (BLU-667) in patients (pts) with advanced RET mutation-positive medullary thyroid cancer (RET+ MTC). *Ann. Oncol.* 2020, 31, S1084. [CrossRef]

78. Drilon, A.; Oxnard, G.R.; Tan, D.S.; Loong, H.H.; Johnson, M.; Gainor, J.; McCoach, C.E.; Gautschi, O.; Besse, B.; Cho, B.C.; et al. Efficacy of Selipetinib in RET Fusion–Positive Non–Small-Cell Lung Cancer. *New Engl. J. Med.* 2020, 383, 813–824. [CrossRef]

79. Pietrowska, Z.; Hazar-Rethinam, M.; Rizzo, C.; Naredes, B.; Van Severent, E.E.; Shahzade, H.A.; Lennens, I.T.; Iafrate, A.J.; Dias-Santagata, D.; Leshchiner, I.; et al. Heterogeneity and Coexistence of T790M and T790 Wild-Type Resistant Subclones Drive Mixed Response to Third-Generation Epidermal Growth Factor Receptor Inhibitors in Lung Cancer. *JCO Precis. Oncol.* 2018, 2, 1–15. [CrossRef] [PubMed]

80. Le, X.; Puri, S.; Negrao, M.V.; Nilsson, M.B.; Robichaux, J.P.; A Boyle, T.; Hicks, J.K.; Lovinger, K.L.; Roarty, E.B.; Rinsurokgawong, W.; et al. Landscape of EGFR-Mutant and -Independent Resistance Mechanisms to Osimertinib and Continuation Therapy Beyond Progression in EGFR-Mutant NSCLC. *Clin. Cancer Res.* 2018, 24, 6195–6203. [CrossRef]

81. ClinicalTrials.gov. Search Results 05/12/2021. Available online: https://www.clinicaltrials.gov/ct2/show/NCT03944772 (accessed on 25 April 2021).

82. Mogi, A.; Kuwano, H. TP53 Mutations in Nonsmall Cell Lung Cancer. *J. Biomed. Biotechnol.* 2011, 2011, 1–9. [CrossRef] [PubMed]

83. Deben, C.; Deschoolmeester, V.; Lardon, F.; Rolfo, C.; Pauwels, P. TP53 and MDM2 genetic alterations in non-small cell lung cancer: Evaluating their prognostic and predictive value. *Crit. Rev. Oncol.* 2016, 99, 63–73. [CrossRef]

84. Viktorsson, K.; De Petris, L.; Lewensohn, R. The role of p53 in treatment responses of lung cancer. *Biochem. Biophys. Res. Commun.* 2005, 331, 868–880. [CrossRef] [PubMed]

85. Ma, X.; Le Teuff, G.; Lacas, B.; Tsao, M.; Graziano, S.; Pigoni, J.-P.; Douillard, J.-Y.; Le Chevalier, T.; Seymour, L.; Filipits, M.; et al. Prognostic and Predictive Effect of TP53 Mutations in Patients with Non–Small Cell Lung Cancer from Adjuvant Cisplatin–Based Therapy Beyond Randomized Trials: A LACE-Bio Pooled Analysis. *J. Thorac. Oncol.* 2016, 11, 850–861. [CrossRef] [PubMed]

86. Bronte, G.; Ciceri, G.; Casenza, S.; Galvano, A.; Musso, E.; Rizzo, S.; Sortino, G.; Roselli, M.; Bazan, V.; Fiorentino, E.; et al. Monoclonal antibodies in gastrointestinal cancers. *Expert Opin. Biol. Ther.* 2013, 13, 889–900. [CrossRef]

87. Ahrendt, S.A.; Hu, Y.; Buta, M.; McDermott, M.P.; Benoist, N.; Yang, S.C.; Wu, L.; Sidransky, D. p53 Mutations and Survival in Stage I Non-Small-Cell Lung Cancer: Results of a Prospective Study. *J. Natl. Cancer Inst.* 2003, 95, 961–970. [CrossRef]

88. Nahar, R.; Zhai, W.; Zhang, T.; Takano, A.; Khng, A.J.; Lee, Y.Y.; Liu, X.; Lim, C.H.; Koh, T.P.T.; Aung, Z.W.; et al. Elucidating the genomic architecture of Asian EGFR-mutant lung adenocarcinoma through multi-region exome sequencing. *Nat. Commun.* 2018, 9, 1–11. [CrossRef]

89. Galvano, A.; Peri, M.; Guarini, A.A.; Castiglia, M.; Grassadonia, A.; De Tursi, M.; Irtelli, L.; Rizzo, S.; Bertani, A.; Gristina, V.; et al. Analysis of systemic inflammatory biomarkers in neuroendocrine carcinomas of the lung: Prognostic and predictive significance of NLR, LDH, ALI, and LIPI score. *Ther. Adv. Med Oncol.* 2020, 12, 1758835920942378. [CrossRef]

90. Rho, J.K.; Choi, Y.J.; Ryoo, B.-Y.; Na, I.J.; Yang, S.H.; Kim, C.H.; Lee, J.C. p53 Enhances Gefitinib-Induced Growth Inhibition and Apoptosis by Regulation of Fas in Non–Small-Cell Lung Cancer. *Cancer Res.* 2007, 67, 1163–1169. [CrossRef]

91. Molinavila, M.A.; Bertran-Alamillo, J.; Gasco, A.; Mayo-De-Las-Casas, C.; Sanchez-Ronco, M.; Pujantell-Pastor, L.; Bonanno, L.; Favaretto, A.G.; Cardona, A.F.; Vergnenegre, A.; et al. Nondisruptive p53 Mutations Are Associated with Shorter Survival in Patients with Advanced Non–Small Cell Lung Cancer. *Clin. Cancer Res.* 2014, 20, 4647–4659. [CrossRef] [PubMed]
115. Yi, Q.-Q.; Yang, R.; Shi, J.-F.; Zeng, N.-Y.; Liang, D.-Y.; Sha, S.; Chang, Q. Effect of preservation time of formalin-fixed paraffin-embedded tissues on extractable DNA and RNA quantity. *J. Int. Med. Res.* 2020, 48, 300060520931259. [CrossRef] [PubMed]

116. Cheng, Y.-W.; Stefanucci, C.; Jakubowski, M.A. Real-time PCR and targeted next-generation sequencing in the detection of low level EGFR mutations: Instructive case analyses. *Respir. Med. Case Rep.* 2019, 28, 100901. [CrossRef] [PubMed]

117. Zhang, B.; Xu, C.-W.; Shao, Y.; Wang, H.-T.; Wu, Y.-F.; Song, Y.-Y.; Li, X.-B.; Zhang, Z.; Wang, W.-J.; Li, L.-Q.; et al. Comparison of droplet digital PCR and conventional quantitative PCR for measuring EGFR gene mutation. *Exp. Ther. Med.* 2015, 9, 1383–1388. [CrossRef]

118. Bell, D.W.; Brannigan, B.W.; Matsuo, K.; Finkelstein, D.M.; Sordella, R.; Settleman, J.; Mitsudomi, T.; Haber, D.A. Increased Prevalence of EGFR-Mutant Lung Cancer in Women and in East Asian Populations: Analysis of Estrogen-Related Polymorphisms. *Clin. Cancer Res.* 2008, 14, 4079–4084. [CrossRef]

119. Sacher, A.; Le, X.; Cornelissen, R.; Shum, E.; Suga, J.; Socinski, M.; Molina, J.R.; Haura, E.; Clarke, J.; Bhat, G.; et al. 36MO Safety, tolerability and preliminary efficacy of poziotinib with twice daily strategy in EGFR/HER2 Exon 20 mutant non-small cell lung cancer. *Ann. Oncol.* 2021, 32, S15. [CrossRef]

120. Ramalingam, S.S.; Vansteenkiste, J.; Planchard, D.; Cho, B.C.; Gray, J.E.; Ohe, Y.; Zhou, C.; Reungwetwattana, T.; Cheng, Y.; Chewaskulyong, B.; et al. Overall Survival with Osimertinib in Untreated, EGFR-Mutated Advanced NSCLC. *N. Engl. J. Med.* 2020, 382, 41–50. [CrossRef]

121. Shen, Y.-C.; Tseng, G.-C.; Tu, C.-Y.; Chen, W.-C.; Liao, W.-C.; Li, C.-H.; Chen, H.-J.; Hsia, T.-C. Comparing the effects of afatinib with gefitinib or Erlotinib in patients with advanced-stage lung adenocarcinoma harboring non-classical epidermal growth factor receptor mutations. *Lung Cancer* 2017, 110, 56–62. [CrossRef]

122. Yang, J.C.-H.; Schuler, M.H.; Yamamoto, N.; O’Byrne, K.J.; Hirsh, V.; Mok, T.; Geater, S.L.; Orlov, S.V.; Tsai, C.-M.; Boyer, M.J.; et al. LUX-Lung 3: A randomized, open-label, phase III study of afatinib versus pemetrexed and cisplatin as first-line treatment for patients with advanced adenocarcinoma of the lung harboring EGFR-activating mutations. *J. Clin. Oncol.* 2012, 30, LBA7500. [CrossRef]

123. Lin, Y.-T.; Tsai, T.-H.; Wu, S.-G.; Liu, Y.-N.; Yu, C.-J.; Shih, J.-Y. Complex EGFR mutations with secondary T790M mutation confer shorter osimertinib progression-free survival and overall survival in advanced non-small cell lung cancer. *Lung Cancer* 2020, 145, 1–9. [CrossRef]

124. Yang, J.C.-H.; Shih, J.-Y.; Su, W.-C.; Hsia, T.-C.; Tsai, C.-M.; Ou, S.-H.I.; Yu, C.-J.; Chang, G.-C.; Ho, C.-L.; Sequist, L.V.; et al. Afatinib for patients with lung adenocarcinoma and epidermal growth factor receptor mutations (LUX-Lung 2): A phase 2 trial. *Lancet Oncol.* 2012, 13, 539–548. [CrossRef]

125. Cho, J.H.; Lim, S.H.; An, H.J.; Kim, K.H.; Park, K.U.; Kang, E.J.; Choi, Y.H.; Ahn, M.S.; Lee, M.H.; Sun, J.-M.; et al. Osimertinib for Patients With Non–Small-Cell Lung Cancer Harboring Uncommon EGFR Mutations: A Multicenter, Open-Label, Phase II Trial (KCSG-LU15-09). *J. Clin. Oncol.* 2020, 38, 488–495. [CrossRef]

126. Ramalingam, S.S.; Vansteenkiste, J.; Planchard, D.;Cho, B.C.;Gray,J.E.;Ohe,Y.;Zhou,C.;Reungwetwattana,T.;Cheng,Y.;Chewaskulyong,B.;et al. Overall Survival with Osimertinib in Untreated, EGFR-Mutated Advanced NSCLC. *N. Engl. J. Med.* 2020, 382, 41–50. [CrossRef]

127. Kobayashi, N.; Aragane, N.; Nakamura, T.; Sato, A.; Takeda, Y.; Mitsuoka, F.; Yamasaki, F.; Hayashi, S.; Sueoka, E.; Kimura, S. Co-existence of positive MET FISH status with EGFR mutations signifies poor prognosis in lung adenocarcinoma patients. *Lung Cancer* 2012, 75, 89–94. [CrossRef]

128. Kobayashi, N.; Aragane, N.; Nakamura, T.; Sato, A.; Takeda, Y.; Mitsuoka, M.; Yamasaki, F.; Hayashi, S.; Sueoka, E.; Kimura, S. Abstract 1737: Co-existence of positiveMETFISH status withEGFRmutations signifies poor prognosis in lung adenocarcinoma. *Clinical Trials* 2012, 72, 1737. [CrossRef]

129. Lai, G.G.; Lim, T.H.; Lim, J.; Liew, P.J.; Kwang, X.L.; Nahar, R.; Aung, Z.W.; Takano, A.; Lee, Y.Y.; Lau, D.P.; et al. Clonal MET Amplification as a Determinant of Tyrosine Kinase Inhibitor Resistance in Epidermal Growth Factor Receptor–Mutant Non–Small-Cell Lung Cancer. *J. Clin. Oncol.* 2019, 37, 876–884. [CrossRef]

130. Drilon, A.; Cappuzzo, F.; Ou, S.-H.I.; Camidge, D.R. Targeting MET in Lung Cancer: Will Expectations Finally Be Met? *J. Thorac. Oncol.* 2017, 12, 15–26. [CrossRef]

131. Bronkhorst, A.J.; Ungerer, V.; Holdenrieder, S. The emerging role of cell-free DNA as a molecular marker for cancer management. *BioMed. Detect. Quantif.* 2019, 17, 100087. [CrossRef]

132. Wang, F.; Diao, X.-Y.; Zhang, X.; Shao, Q.; Feng, Y.-F.; An, X.; Wang, H.-Y. Identification of genetic alterations associated with primary resistance to EGFR-TKIs in patients with advanced-stage lung cancer patients with EGFR sensitive mutations. *Cancer Commun.* 2019, 39, 7–15. [CrossRef]

133. Lage, Y.; Ballesteros, P.; Álvarez, García, M.O.; Rueda, A.G.; de la Fuente, E.C.; Jiménez, J.T.; Navas, E.V.; Castillo, J.S.; Lario, M.; Berlínchez, A.B.; et al. 6P Clinical and molecular characteristics in non-small cell lung cancer patients with alteration in PIK3 pathway. *J. Thorac. Oncol.* 2021, 16, S701. [CrossRef]

134. Passiglia, F.; Rizzo, S.; Rolfo, C.; Galvano, A.; Bronte, E.; Incorvaia, L.; Listi, A.; Barraco, N.; Castiglia, M.; Calò, V.; et al. Metastatic Site Location Influences the Diagnostic Accuracy of ctDNA EGFR- Mutation Testing in NSCLC Patients: A Pooled Analysis. *Curr. Cancer Drug Targets* 2018, 18, 697–705. [CrossRef] [PubMed]