International Journal of
Molecular Sciences

MDPI

Review

Understanding the Mechanisms of Resistance in *EGFR*-Positive NSCLC: From Tissue to Liquid Biopsy to Guide Treatment Strategy

Marzia Del Re [1,*], Stefania Crucitta [1], Giulia Gianfilippo [1], Antonio Passaro [2], Iacopo Petrini [3], Giuliana Restante [1], Angela Michelucci [4], Stefano Fogli [1], Filippo de Marinis [2], Camillo Porta [5,6], Antonio Chella [7] and Romano Danesi [1]

1. Unit of Clinical Pharmacology and Pharmacogenetics, Department of Clinical and Experimental Medicine, University of Pisa, 56126 Pisa, Italy
2. Division of Thoracic Oncology, IEO, European Institute of Oncology, IRCCS, 20141 Milan, Italy
3. General Pathology, Department of Translational Research & New Technologies in Surgery and Medicine, University of Pisa, 56126 Pisa, Italy
4. Unit of Molecular Genetics, Department of Laboratory Medicine, University Hospital, 56126 Pisa, Italy
5. Department of Internal Medicine, University of Pavia, 27100 Pavia, Italy
6. Division of Translational Oncology, I.R.C.C.S. Istituti Clinici Scientifici Maugeri, 27100 Pavia, Italy
7. Unit of Respiratory Medicine, Department of Critical Area and Surgical, Medical and Molecular Pathology, University Hospital, 56126 Pisa, Italy

* Correspondence: marzia.delre@gmail.com; Tel.: +39-050-992632; Fax: +39-050-2218758

Received: 29 June 2019; Accepted: 9 August 2019; Published: 14 August 2019

Abstract: Liquid biopsy has emerged as an alternative source of nucleic acids for the management of Epidermal Growth Factor Receptor (*EGFR*)-mutant non-Small Cell Lung Cancer (NSCLC). The use of circulating cell-free DNA (cfDNA) has been recently introduced in clinical practice, resulting in the improvement of the identification of druggable *EGFR* mutations for the diagnosis and monitoring of response to targeted therapy. *EGFR*-dependent (T790M and C797S mutations) and independent (Mesenchymal Epithelial Transition [*MET*] gene amplification, Kirsten Rat Sarcoma [*KRAS*], Phosphatidyl-Inositol 4,5-bisphosphate 3-Kinase Catalytic subunit Alpha isoform [*PI3KCA*], and RAF murine sarcoma viral oncogene homolog B1 [*BRAF*] gene mutations) mechanisms of resistance to EGFR tyrosine kinase inhibitors (TKIs) have been evaluated in plasma samples from NSCLC patients using highly sensitive methods (i.e., digital droplet PCR, Next Generation Sequencing), allowing for the switch to other therapies. Therefore, liquid biopsy is a non-invasive method able to detect the molecular dynamic changes that occur under the pressure of treatment, and to capture tumor heterogeneity more efficiently than is allowed by tissue biopsy. This review addresses how liquid biopsy may be used to guide the choice of treatment strategy in *EGFR*-mutant NSCLC.

Keywords: liquid biopsy; epidermal growth factor receptor; non-Small Cell Lung Cancer; circulating cell-free DNA; tyrosine kinase inhibitors; mechanisms of resistance

---

1. Introduction

The *Epidermal Growth Factor Receptor* mutant ($EGFR^{mut}$) is an important molecular subtype of non-small cell lung cancer (NSCLC) and is highly sensitive to anti-EGFR tyrosine kinase inhibitors (TKIs). The $EGFR^{mut}$ NSCLC is a good model of the "oncogene addiction" theory, in which a specific oncogenic signaling pathway drives the transformation and proliferation of cancer cells [1–3]. The identification of the *EGFR* mutations and the related targeted agents allowed an important

paradigm shift in the treatment and prognosis of patients with NSCLC harboring these alterations [4–6]. At present, several EGFR TKIs are approved for the treatment of NSCLC carrying activating *EGFR*^{mut}.

In particular, three different generations of EGFR TKIs are available and have been approved: the first-generation gefitinib and erlotinib; the second-generation afatinib and dacomitinib; and the third-generation osimertinib. The use of EGFR TKIs significantly improved the clinical outcome, i.e., progression-free survival (PFS) and overall response rate (ORR), when compared with standard platinum-based chemotherapy [7–11]. However, the major challenge now is to overcome primary or acquired resistance in NSCLC patients treated with targeted therapy [12]. In fact, even though treatment with EGFR TKIs allows for a durable response, the majority of patients develop progressive disease (PD) after 10–12 months of treatment. In addition, acquired resistance arises and restricts the long-term efficacy of these EGFR TKIs [12].

Since the initial therapeutic choice depends on the genetic identification of individual tumor profiles, tissue biopsy is the gold standard for molecular analysis [13,14]. Nowadays, the introduction into clinical practice of the minimally invasive liquid biopsy, i.e., the analysis of circulating cell-free DNA (cfDNA), allows for a better management of NSCLC patients and the optimization of their therapy [15], especially for the early identification of the increasing number of resistance mutations that may arise during treatment. Several studies have already highlighted the importance of liquid biopsy to detect molecular alterations responsible for the resistance mechanism [16,17]. Dai et al. conducted a study in which the choice of the targeted therapy was made on the basis of molecular analysis of tissue and liquid biopsy; the authors demonstrated a high consistency in *EGFR*^{mut} status between plasma and tissue, supporting the use of liquid biopsy to select patients for TKI therapy [18].

Moreover, in a retrospective study, blood samples were collected from 1138 advanced NSCLC patients at presentation and during the progression of the disease. The authors detected sensitizing *EGFR*^{mut} in cfDNA of 113 patients, showing a difference between plasma and serum samples. Specifically, the *EGFR*^{mut} was detected in cfDNA isolated from plasma of 31 patients, and the *EGFR*^{mut} was detected in cfDNA isolated from serum of only 11 patients [19]. Therefore, even though plasma is considered to be a better source of ctDNA for molecular analysis, the results of this study highlight the need to increase our capability to detect druggable mutations by testing serum when plasma is negative.

In a recent study, actionable genomic alterations were analyzed on cfDNA of 116 NSCLC patients due to the lack of tissue samples or a negative molecular tissue analysis. A treatment decision was established in 23% of patients before the first-line therapy and was changed in 32% of patients who progressed to EGFR TKIs, demonstrating that an analysis of cfDNA by Next Generation Sequencing (NGS) improves genetic profiling of advanced NSCLC patients and the use of targeted therapy [20].

The focus of this article is to show how and when liquid biopsy may be used in the choice of treatment strategy in *EGFR*-mutant NSCLC.

2. Mechanisms of Resistance to TKIs in *EGFR*^{mut} NSCLC and Treatment Strategies

2.1. Liquid Biopsy to Track EGFR-Dependent Mechanisms of Primary and Acquired Resistance

The first use of liquid biopsy is to discover the appearance of new point mutations and, since the most commonly acquired resistance mechanism to first/second-generation TKIs is the expansion of clones bearing the T790M mutation in the *EGFR* exon 20, a liquid biopsy can satisfactorily meet this need [21,22]. Due to its steric hindrance, T790M confers resistance to gefitinib, erlotinib, and afatinib, and its detection allows for the use of the third-generation EGFR-TKI osimertinib as a second-line therapy [23]. Several studies have investigated the feasibility of plasma genotyping using digital droplet PCR (ddPCR) or NGS platforms to select patients who progressed during first-line EGFR-TKIs therapy for treatment with osimertinib, demonstrating an overall objective response rate of 70–75% with plasma analysis [24–26]. In this context, the phase II APPLE Trial (AZD9291 Treatment on Positive PLasma T790M in EGFR-mutant NSCLC Patients; NCT02856893), a study evaluating osimertinib

treatment in T790M positive plasma *EGFR*^{mut} NSCLC patients, might provide additional data on this issue [27].

Interestingly, the disappearance of *EGFR* mutations such as T790M or the LREAT747del/T790M double mutant clone at progression to osimertinib has been demonstrated, suggesting the loss of the drug target as a mechanism of resistance [28]. Similarly, an association has been demonstrated between the loss of the T790M mutation and a shorter time to treatment discontinuation (6.1 vs. 15.2 months), suggesting the emergence of pre-existing resistant clones and a range of competing resistance mechanisms [29]. Recently, the *EGFR* C797S mutation has been reported to be an acquired mechanism of resistance to osimertinib [30]. Moreover, it has been reported that if C797S and T790M mutations are detected in *trans*, a combination of first- and third-generation EGFR TKIs may be more effective [31,32].

On the contrary, if mutations are in *cis*, the EGFR TKIs alone or in combination are not able to suppress the EGFR activity. In addition, when osimertinib is administered in a first-line setting and the C797S mutation develops without the presence of the T790M mutation, a re-challenge with first-generation TKIs has been proposed as an effective strategy [33]. To overcome the resistance caused by the C797S mutation, the efficacy of brigatinib in combination with osimertinib has been demonstrated in an in vitro study [34]. In the last few years, a number of studies have been published that use cfDNA to identify the presence of the T790M/C797S mutations or to monitor their amount in order to predict tumor response to treatment [29,35,36]. Overall, considering the use of different platforms (e.g., ddPCR, Real Time PCR, NGS), liquid biopsy has demonstrated a good sensitivity and specificity (>80%) in both the discovery of acquired mutations and monitoring tumor dynamics [37,38].

2.2. Using Liquid Biopsy to Track EGFR-Independent Mechanisms of Primary and Acquired Resistance

Considering the *EGFR*-independent mechanism of resistance, multiple primary or secondary mutations have been reported in the following genes: Kirsten rat sarcoma (*KRAS*), phosphatidylinositol 4,5-bisphosphate 3-kinase catalytic subunit alpha isoform (*PI3KCA*), human epidermal growth factor receptor 2 (*HER2*), and RAF murine sarcoma viral oncogene homolog B1 (*BRAF*) [39–41]. It is well-known that the occurrence of mutations in the *RAS* gene promotes cell proliferation and drug resistance, by-passing the blockade of EGFR signaling [39]. *KRAS*, a member of the *RAS* family, is highly mutated in NSCLC (15–30%) and is associated with a lack of response to EGFR inhibitors [42]. A recent study investigated the role of concomitant driver mutations (e.g., *KRAS*, *NRAS*, *BRAF*, *PIK3CA*) on the outcome of 133 *EGFR*^{mut} NSCLC patients treated with EGFR TKIs [43]. In particular, PFS was significantly shorter in patients with concomitant driver mutations than it was in patients with *EGFR*^{mut} only (7 vs. 11.3 months; $p = 0.04$), suggesting that patients with clonal *EGFR* and other sub-clonal driver mutations benefit less from treatment with EGFR TKIs [43].

Interestingly, in cases carrying both *EGFR* and *KRAS* mutations, patients with a *KRAS* allele fraction that was higher than that of the *EGFR*^{mut} had a significantly shorter PFS (2.42 vs. 11.09 months; $p = 0.0081$) and a lower response rate (16.7 vs. 57.1%) [43]. Similarly, a study on cfDNA showed that 48.5% of plasma samples were positive for *KRAS* mutation after progression to EGFR TKIs and 39.4% of those had a *KRAS* and *EGFR* co-mutation [44]. Many other studies, on both plasma and tissue samples, detected *KRAS* mutations in *EGFR*^{mut} patients with EGFR-TKI resistance, confirming its predictive role in resistance to treatment [29,45,46]. *NRAS* mutations are detected in a low number of NSCLC patients (~1%), and Q61K, E63K, G12V, and G12R mutations were found in patients with acquired resistance to EGFR TKIs in pre-clinical and clinical studies as a mechanism of resistance to first- and third-generation EGFR TKIs [29,47–50].

PI3KCA mutations may coexist with the *EGFR*^{mut} and play a role in resistance to TKIs [51,52]. *BRAF* mutation in NSCLC refractory to EGFR TKIs occurs in ~5–7% of NSCLC patients [53,54], and V600E and G469A mutations seem to co-exist with *EGFR* T790M, mediating acquired resistance in 1% and 10% of patients who progressed to first-generation EGFR-TKIs or osimertinib, respectively [53,55].

Interestingly, a case report showed that a patient positive for *BRAF* V600E mutation at progression to osimertinib benefitted from the combination of a BRAF inhibitor (encorafenib) and osimertinib [56].

Other well-known mechanisms of resistance include the dysregulation of Mesenchymal Epithelial Transition (*MET*) gene signaling, which is involved in the control of cell differentiation [57], proliferation [58,59], and angiogenesis [60,61]. Of note, high *MET* expression or amplification are connected with poor outcomes in patients with NSCLC [62,63]. The *MET* signaling pathway is linked to the EGFR network through the *PI3K/Protein kinase B* (*Akt*) and Mitogen-activated Protein Kinases (*MAPK*) nodes, showing mutual compensation [64]. For these reasons, *MET* activation is one of the potential mechanisms of resistance to EGFR TKIs in NSCLC. As a matter of fact, *MET* amplification is frequently reported as a mechanism of loss of efficacy of EGFR TKI therapy among *EGFR^{mut}* patients [65–67].

Several studies report the presence of *MET* amplification in NSCLC treated with anti-EGFR TKIs, with or without the T790M mutation [67,68]. A study of 34 NSCLC patients evaluated the amount of *EGFR* mutation in cfDNA as a marker of response/resistance to osimertinib. Eight patients showed early progression during treatment, and a tumor re-biopsy revealed the presence of *MET* amplification in one case [69]. Similarly, the FLAURA trial (AZD9291 Versus Gefitinib or Erlotinib in Patients With Locally Advanced or Metastatic Non-small Cell Lung Cancer, NCT02296125) showed *MET* amplification and *BRAF* mutation as mechanisms of resistance in patients treated with osimertinib, opening up the possibility of future combinations to overcome resistance [70].

A study showed a plasma tissue correlation using NGS in 13 NSCLC patients with acquired resistance to osimertinib. Four patients were found to be positive for *MET* amplification in both plasma and tissue samples. In addition, a survival analysis showed a better PFS/overall survival (OS) in patients without *MET* alterations, confirming *MET* to be a mechanism of resistance to third-generation EGFR TKIs. To overcome this resistance, an exploratory evaluation with a treatment combination of first/third-generation EGFR TKIs and the *MET* inhibitor crizotinib was conducted; partial responses were clinically and radiographically achieved, and a cfDNA analysis was negative for common cancer-related mutations, suggesting the efficacy of the treatment combination [71]. Similarly, a combination of full-dose osimertinib and crizotinib was administered to two patients with emergent *MET* amplification in a liquid biopsy after progression to erlotinib [72,73]. A partial response was achieved without experiencing serious adverse events in one patient [72], while a dose-reduction of crizotinib due to hematological toxicity was needed in the other patient [73].

These cases demonstrate that combination therapy with osimertinib and crizotinib can be effective in patients with *EGFR^{mut}* and *MET* amplification detected by liquid biopsy [72,73]. Moreover, cfDNA may help us to understand the molecular response to pharmacological treatment and provide information on clonal heterogeneity, showing the correlation between dynamic changes in the *EGFR* activating mutation (L858R) and *MET* amplification in treatment response [74]. In the last update of the Phase III AURA3 trial (AZD9291 Versus Platinum-Based Doublet-Chemotherapy in Locally Advanced or Metastatic Non-Small Cell Lung Cancer, NCT02151981), an analysis of the ctDNA genomic profile was also carried out in patients with the T790M mutation who progressed on osimertinib during the study, and several resistance mechanisms were observed, including *MET* amplification [75]. In order to overcome resistance, several agents have been developed to target *MET* or its ligand Hepatocyte Growth Factor (*HGF)* [66,72,76–79], such as small molecules (e.g., capmatinib, tepotinib, and tivantinib) [80,81] or monoclonal antibodies (e.g., onartuzumab and emibetuzumab) [82] and anti-HGF antibodies (e.g., ficlatuzumab and rilotumumab) [83,84]. Moreover, several *MET* inhibitors have been investigated in combination with EGFR TKIs or cytotoxic agents in NSCLC patients who acquired resistance to TKIs due to the appearance of *MET* amplification [66,85,86].

2.3. Small-Cell Lung Cancer (SCLC) Transformation: Still a Challenge for Liquid Biopsy?

Transformation to SCLC is reported to be one of the mechanisms of resistance to treatment and has been observed after both first- and subsequent generation of EGFR TKIs [87,88], occurring in

approximately 5–14% of patient biopsies at the time of TKI resistance [21,89]. Tumor heterogeneity in *EGFR*^{mut} NSCLC has been widely described, and concurrent SCLC transformation and *EGFR* T790M mutation have been reported [90–92]. Clinical cases of *EGFR*^{mut} NSCLC patients who received TKIs and developed SCLC transformation at progression have been published [91,93]. In one case, SCLC developed after treatment with gefitinib; cisplatin and etoposide were used as a second-line therapy, followed by chemotherapy and immunotherapy with amrubicin, irinotecan, and nivolumab. At this point, the primary lesion that had transformed into SCLC reconverted into an adenocarcinoma with *EGFR* L858R and T790M mutations. Thus, the patient was treated with osimertinib, showing a clinical remission [93].

Similarly, another study presented an *EGFR*^{mut} lung adenocarcinoma, which was treated with erlotinib and chemotherapy and was later found to have transformed into SCLC. The patient was treated with cisplatin and irinotecan and then developed resistance to the therapy; the cfDNA revealed the presence of the *EGFR* T790M mutation, allowing for treatment with osimertinib, which resulted in a good clinical response [94].

Two case reports described the transformation into SCLC as a possible mechanism of resistance to afatinib. The first report described a case of a lung adenocarcinoma harboring *EGFR* exon 19 deletion that, after seven months of treatment with afatinib, progressed and showed SCLC transformation at re-biopsy with concomitant *EGFR* exon 19 deletion. Afatinib was discontinued and chemotherapy was administered with a cisplatin and irinotecan regimen, with no disease progression after four cycles of chemotherapy [95]. Similarly, the second case report described a switch of tumor histotype to SCLC with features of a G3 neuroendocrine carcinoma and positivity for exon 19 deletion of *EGFR*. Interestingly, the switch occurred during hepatic progression, which was the only site not responsive to afatinib. Thus, the patient was treated with a carboplatin plus etoposide chemotherapy, showing a complete response [96]. Transformation into SCLC has also been described as a mechanism of acquired resistance to osimertinib [97,98]. Two case reports showed that the transformation into SCLC occurred after 13–18 months of treatment with osimertinib, and a molecular analysis showed the presence of the EGFR^{mut} exon 19 deletion and L858R without the T790M mutation. Patients were treated with a chemotherapy regimen, showing a complete or partial response [97,98].

Despite the fact that transformation into SCLC is difficult to detect by liquid biopsy, a recent publication demonstrated that ctDNA may be analyzed in terms of changes in global copy number to monitor its dynamics in patients with a histological transformation into SCLC. In particular, *TP53* mutation levels change in accordance with the clinical status of the patient and their response to chemotherapy. Moreover, copy number alterations in the avian myelocytomatosis viral oncogene lung carcinoma derived homolog 1 [*MYCL1*], Sry-related HMG box 2 [*SOX2*], and *SOX4* genes and a gain/loss of cancer genes were associated with transformation into SCLC and were linked to genomic instability due to *TP53* mutant clones [99].

3. The Clinical Utility of a Liquid Biopsy in Guiding Treatment with EGFR TKIs

The development of sensitive molecular diagnostic tests has increased our knowledge of the genomic landscape of NSCLC, which shows a complex pattern of molecular abnormalities [100]. While in *EGFR*^{mut} NSCLC both tumor growth and response to therapies are driven by *EGFR* signaling, the co-occurrence of genomic alterations has been described and used to identify several biological subsets of NSCLC patients with different outcomes to TKI treatments [101]. While, on the one hand, TKIs are effective against cell clones harboring *EGFR*-activating mutations, on the other hand the drug treatment is able to select cell clones carrying different molecular subtypes that are often resistant to TKI treatment [102]. In fact, tumor heterogeneity promotes different mechanisms of resistance at multiple metastatic sites [103,104].

Loss of sensitivity to EGFR TKIs may be divided into three groups, based on the mechanisms of selection by treatment: 1) mutations acquired by the target (EGFR), e.g., the T790M or C797S mutations, which reduce the activity of the drug because of a steric hindrance between the target and the drug

without diminishing the kinase activity of the receptor [105,106]; 2) activation of a bypass signaling pathway, e.g., *RAS* mutations or *MET* amplification, in the presence of the $EGFR^{mut}$ [43,69]; and 3) histologic transformation to SCLC [107] (Figure 1A,B). In this context, the ideal approach to monitor tumor dynamics and comprehensively understanding NSCLC's heterogeneity would be a non-invasive one that is able to capture the molecular events that occur at different tumor sites. For these reasons, liquid biopsy is a useful instrument for following tumor dynamics and heterogeneity [108].

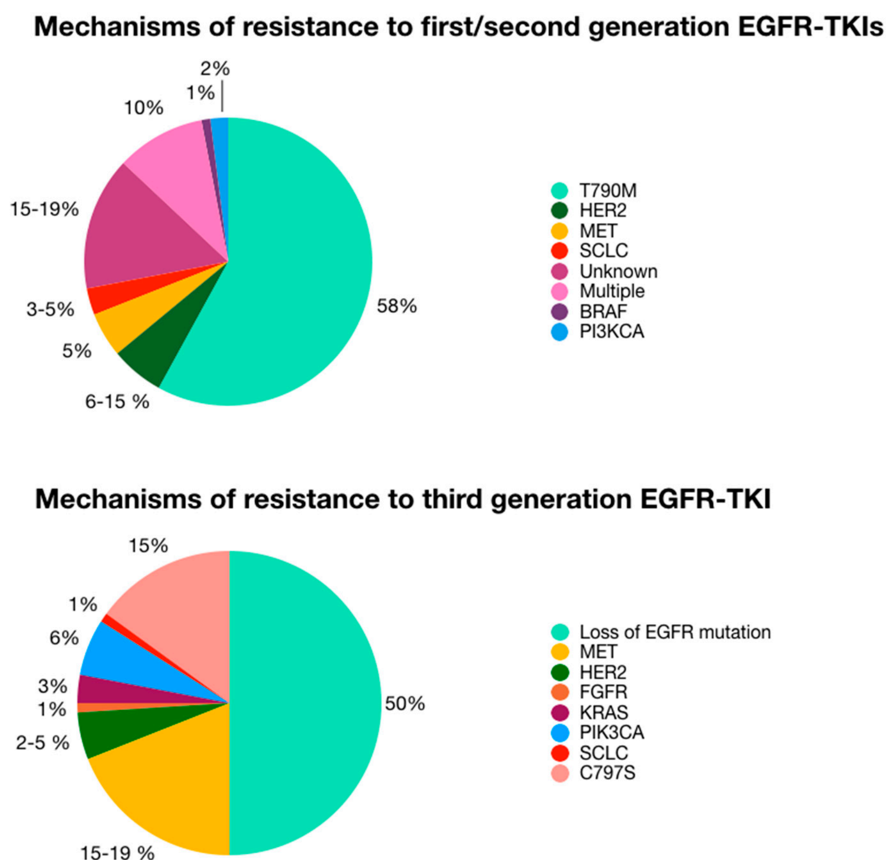

Figure 1. Mechanisms of resistance to EGFR-TKIs and their frequencies. Human Epidermal Growth Factor Receptor 2 (*HER2*), Mesenchymal Epithelial Transition [*MET*], Small Cell Lung Cancer (SCLC), RAF murine sarcoma viral oncogene homolog B1 (*BRAF*), Phosphatidyl-Inositol 4,5-bisphosphate 3-Kinase Catalytic subunit Alpha (*PI3KCA*), Kirsten rat sarcoma (*KRAS*), Fibroblast Growth Factor Receptor (*FGFR*).

Based on the evidence from the AURA trial, in which an analysis of cfDNA was demonstrated to be comparable to a tissue biopsy for the identification of *EGFR* mutational events, many other studies investigated the predictive potential of liquid biopsy and its advantages for the longitudinal monitoring of tumors, providing results with great relevance to the clinical setting [69,109–115]. A liquid biopsy, obtained from a routine blood draw of 6–20 mL, can overcome most of the limitations of a tissue biopsy, such as its invasive nature and its inability to represent the tumor's heterogeneity [116–119].

Although a liquid biopsy includes an analysis of the circulating free and tumor nucleic acids (DNA and RNA), exosomes, and circulating tumor cells (CTCs) in body fluids, only the analysis of $EGFR^{mut}$ in cfDNA has been approved for NSCLC patients [15]. Due to the low amount of DNA that can be obtained from CTCs [120], it seems that the major role of CTCs may be that of a prognostic biomarker both in lung cancer and in other solid tumors [121]. However, the lack of Federal Drug Administration (FDA) approval and the cost of CTC isolation remain major issues. Exosomes are micro-vesicles released by cells and contain a wide variety of molecules, such as DNA, RNA, proteins, and lipids,

and seem to be implicated in intercellular communication and tumor–host interactions [122]. Exosomes have been described in the literature as markers for the monitoring of tumor dynamics; however, despite their importance, methods to detect and analyze exosomes require further development before they can be introduced into clinical practice [123–128].

cfDNA has clearly demonstrated advantages over other markers that make it appropriate for use in clinical practice as a predictive biomarker to monitor response to treatment. In particular, it provides a minimally invasive approach to the early detection of disease recurrence and information about the molecular profile underpinning drug resistance [35]. However, the use of cfDNA presents some limitations and challenges, especially considering the occurrence of false-negative results depending on the following reasons: 1) shedding of cfDNA differs among different tumor types [120]; 2) detection of cfDNA depends on tumor location and volume [129]; 3) detection of cfDNA is lower in patients without progression or who are responding to therapy [130]; and 4) cfDNA shedding is variable [111,131,132].

False-negative results are related to the abovementioned factors; however, the technical limits of detection may also define the level of "false-negatives", being strictly related to the analytical platform. While it is quite difficult to obtain a technical false-negative result when using very sensitive techniques, such as ddPCR or NGS (which have a lower limit of detection that ranges from 0.001% to 1%) [133,134], technical false-negative results may occur more frequently with less-sensitive techniques, such as the Scorpion Amplified-Refractory Mutation System (SARMS) or Peptide Nucleic Acid-Locked Nucleic Acid (PNA-LNA PCR clamp) [135].

On the other hand, the detection of somatic mutations in cfDNA released from non-cancer cells should be taken into consideration. It is known that clonal hematopoiesis of an indeterminate potential (CHIP) can lead to the development of such mutations [136,137]. Finally, in order to use liquid biopsy in an overall diagnostic/therapeutic strategy for *EGFR*^{mut} lung cancers, the analysis of cfDNA should be able to identify *EGFR*-dependent and independent mechanisms of resistance to help us understand the relationship between baseline and resistance mutations.

A liquid biopsy holds the promise to help us understand the biology and heterogeneity of the tumor and the characteristics of drug-tolerant cells, and has the considerable advantage of short laboratory turn-around time, low cost, mini-invasiveness and the potential of being repeated over time. Such a test may help directing targeted therapies against EGFR and other druggable mutations to prevent the emergence of resistant clones. Figure 2 describes the potential applications of cfDNA in diagnosis, monitoring response and progression of disease and provides suggestions on how a liquid biopsy may be implemented in clinical practice.

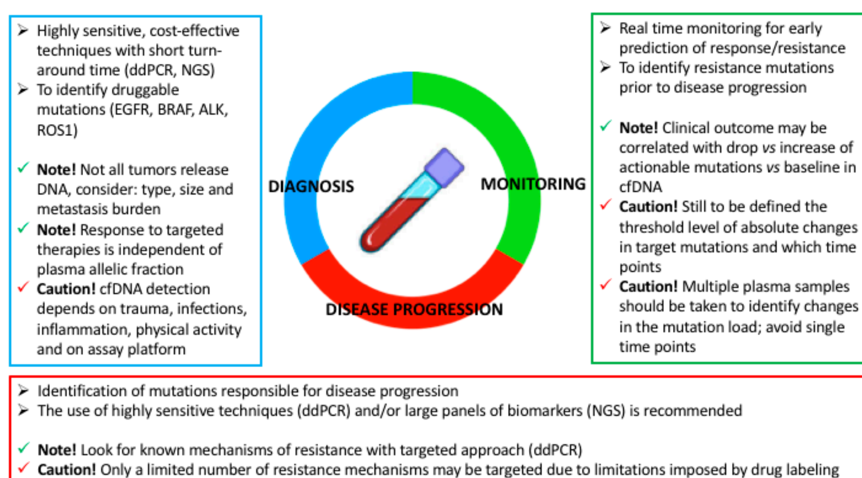

Figure 2. The use of liquid biopsy and circulating cell-free DNA (cfDNA) in clinical practice to guide the choice of treatment strategy. Digital droplet PCR (ddPCR), next generation sequencing (NGS), Epidermal Growth factor Receptor (EGFR), Anaplastic Lymphoma Kinase (ALK), avian UR2 sarcoma virus oncogene homolog 1 ROS1.

In conclusion, a liquid biopsy may be implemented in the clinical management of patients at diagnosis, during treatment (repeated serial liquid biopsies may identify mechanisms of resistance even after transformation into SCLC [93,94]), or during disease progression in order to select an appropriate treatment according to the therapy-dependent clonal selection.

Author Contributions: M.D.R., S.C., G.G., A.P., and R.D. made substantial contributions to the conception and the creation of the review; M.D.R., S.C., G.G., A.P., I.P., G.R., A.M., S.F., F.d.M., C.P., A.C., and R.D. critically revised and improved the manuscript.

Funding: This research received no external funding.

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

References

1. Graham, R.P.; Treece, A.L.; Lindeman, N.I.; Vasalos, P.; Shan, M.; Jennings, L.J.; Rimm, D.L. Worldwide Frequency of Commonly Detected EGFR Mutations. *Arch. Pathol. Lab. Med.* **2018**, *142*, 163–167. [CrossRef]
2. Fogli, S.; Polini, B.; Del Re, M.; Petrini, I.; Passaro, A.; Crucitta, S.; Rofi, E.; Danesi, R. EGFR-TKIs in non-small-cell lung cancer: Focus on clinical pharmacology and mechanisms of resistance. *Pharmacogenomics* **2018**, *19*, 727–740. [CrossRef]
3. Weinstein, I.B. Cancer. Addiction to oncogenes–the Achilles heal of cancer. *Science* **2002**, *297*, 63–64. [CrossRef]
4. Passaro, A.; Guerini-Rocco, E.; Pochesci, A.; Vacirca, D.; Spitaleri, G.; Catania, C.M.; Rappa, A.; Barberis, M.; de Marinis, F. Targeting EGFR T790M mutation in NSCLC: From biology to evaluation and treatment. *Pharmacol. Res.* **2017**, *117*, 406–415. [CrossRef]
5. Passaro, A.; Pochesci, A.; Spitaleri, G.; Catania, C.; Noberasco, C.; Del Signore, E.; de Marinis, F. Afatinib in first-line setting for NSCLC harbouring common EGFR mutations: New light after the preliminary results of LUX-Lung 7? *J. Thorac. Dis.* **2016**, *8*, E217–E220. [CrossRef]
6. Passaro, A.; Palazzo, A.; Trenta, P.; Mancini, M.L.; Morano, F.; Cortesi, E. Molecular and clinical analysis of predictive biomarkers in non-small-cell lung cancer. *Curr. Med. Chem.* **2012**, *19*, 3689–3700. [CrossRef]
7. Mok, T.S.; Wu, Y.L.; Thongprasert, S.; Yang, C.-H.; Chu, D.-T.; Saijo, N.; Patrapim Sunpaweravong, P.; Han, B.; Margono, B.; Ichinose, Y.; et al. Gefitinib of carboplatin-paclitaxel in pulmonary adenocarcinoma. *N. Engl. J. Med.* **2009**, *361*, 947–957. [CrossRef]
8. Rosell, R.; Carcereny, E.; Gervais, R.; Vergnenegre, A.; Massuti, B.; Felip, E.; Palmero, R.; Garcia-Gomez, R.; Pallares, C.; Sanchez, J.M.; et al. Erlotinib versus standard chemotherapy as first-line treatment for European patients with advanced non-small-cell lung cancer (EURTAC): A multicentre, open-label, randomised phase 3 trial. *Lancet Oncol.* **2012**, *13*, 239–246. [CrossRef]
9. Yang, J.C.; Wu, Y.L.; Schuler, M.; Sebastian, M.; Popat, S.; Yamamoto, N.; Zhou, C.; Hu, C.P.; O'Byrne, K.; Feng, J.; et al. Afatinib versus cisplatin-based chemotherapy for EGFR mutation-positive lung adenocarcinoma (LUX-Lung 3 and LUX-Lung 6): Analysis of overall survival data from two randomised, phase III trials. *Lancet Oncol.* **2015**, *16*, 141–151. [CrossRef]
10. Wu, Y.L.; Cheng, Y.; Zhou, X.; Lee, K.H.; Nakagawa, K.; Niho, S.; Tsuji, F.; Linke, R.; Rosell, R.; Corral, J.; et al. Dacomitinib versus gefitinib as first-line treatment for patients with EGFR-mutation-positive non-small-cell lung cancer (ARCHER 1050): A randomised, open-label, phase 3 trial. *Lancet Oncol.* **2017**, *18*, 1454–1466. [CrossRef]
11. Soria, J.C.; Ohe, Y.; Vansteenkiste, J.; Reungwetwattana, T.; Chewaskulyong, B.; Lee, K.H.; Dechaphunkul, A.; Imamura, F.; Nogami, N.; Kurata, T.; et al. Osimertinib in untreated EGFR-mutated advanced non-small-cell lung cancer. *N. Engl. J. Med.* **2018**, *378*, 113–125. [CrossRef]
12. Kobayashi, S.; Boggon, T.J.; Dayaram, T.; Janne, P.A.; Kocher, O.; Meyerson, M.; Johnson, B.E.; Eck, M.J.; Tenen, D.G.; Halmos, B. EGFR mutation and resistance of non-small-cell lung cancer to gefitinib. *N. Engl. J. Med.* **2005**, *352*, 786–792. [CrossRef]
13. Pasic, M.D.; Samaan, S.; Yousef, G.M. Genomic medicine: New frontiers and new challenges. *Clin. Chem.* **2013**, *59*, 158–167. [CrossRef]
14. Heitzer, E.; Ulz, P.; Geigl, J.B. Circulating tumor DNA as a liquid biopsy for cancer. *Clin. Chem.* **2015**, *61*, 112–123. [CrossRef]

15. Novello, S.; Barlesi, F.; Califano, R.; Cufer, T.; Ekman, S.; Levra, M.G.; Kerr, K.; Popat, S.; Reck, M.; Senan, S.; et al. Metastatic non-small-cell lung cancer: ESMO Clinical Practice Guidelines for diagnosis, treatment and follow-up. *Ann. Oncol.* **2016**, *27*, v1–v27. [CrossRef]
16. Lee, J.Y.; Qing, X.; Xiumin, W.; Yali, B.; Chi, S.; Bak, S.H.; Lee, H.Y.; Sun, J.M.; Lee, S.H.; Ahn, J.S.; et al. Longitudinal monitoring of EGFR mutations in plasma predicts outcomes of NSCLC patients treated with EGFR TKIs: Korean Lung Cancer Consortium (KLCC-12-02). *Oncotarget* **2016**, *7*, 6984–6993. [CrossRef]
17. Hu, Y.; Alden, R.S.; Odegaard, J.I.; Fairclough, S.R.; Chen, R.; Heng, J.; Feeney, N.; Nagy, R.J.; Shah, J.; Ulrich, B.; et al. Discrimination of Germline EGFR T790M Mutations in Plasma Cell-Free DNA Allows Study of Prevalence Across 31,414 Cancer Patients. *Clin. Cancer Res.* **2017**, *23*, 7351–7359. [CrossRef]
18. Dai, L.J.; Wang, C.; Ding, Z.Y. A Case-control Study Supporting the Use of Liquid Biopsy in the Targeted Therapy for Lung Cancer. *Asian Pac. J. Cancer Prev.* **2018**, *19*, 1761–1766. [CrossRef]
19. Mayo-de-Las-Casas, C.; Jordana-Ariza, N.; Garzon-Ibanez, M.; Balada-Bel, A.; Bertran-Alamillo, J.; Viteri-Ramirez, S.; Reguart, N.; Munoz-Quintana, M.A.; Lianes-Barragan, P.; Camps, C.; et al. Large scale, prospective screening of EGFR mutations in the blood of advanced NSCLC patients to guide treatment decisions. *Ann. Oncol.* **2017**, *28*, 2248–2255. [CrossRef]
20. Laufer-Geva, S.; Rozenblum, A.B.; Twito, T.; Grinberg, R.; Dvir, A.; Soussan-Gutman, L.; Ilouze, M.; Roisman, L.C.; Dudnik, E.; Zer, A.; et al. The Clinical Impact of Comprehensive Genomic Testing of Circulating Cell-Free DNA in Advanced Lung Cancer. *J. Thorac. Oncol.* **2018**, *13*, 1705–1716. [CrossRef]
21. Yu, H.A.; Arcila, M.E.; Rekhtman, N.; Sima, C.S.; Zakowski, M.F.; Pao, W.; Kris, M.G.; Miller, V.A.; Ladanyi, M.; Riely, G.J. Analysis of tumor specimens at the time of acquired resistance to EGFR-TKI therapy in 155 patients with EGFR-mutant lung cancers. *Clin. Cancer Res.* **2013**, *19*, 2240–2247. [CrossRef]
22. Sequist, L.V.; Waltman, B.A.; Dias-Santagata, D.; Digumarthy, S.; Turke, A.B.; Fidias, P.; Bergethon, K.; Shaw, A.T.; Gettinger, S.; Cosper, A.K.; et al. Genotypic and histological evolution of lung cancers acquiring resistance to EGFR inhibitors. *Sci. Transl. Med.* **2011**, *3*, 75ra26. [CrossRef]
23. Skrzypski, M.; Szymanowska-Narloch, A.; Dziadziuszko, R. Osimertinib - effective treatment of NSCLC with activating EGFR mutations after progression on EGFR tyrosine kinase inhibitors. *Contemp. Oncol. (Pozn.)* **2017**, *21*, 254–258. [CrossRef]
24. Hochmair, M.J.; Buder, A.; Schwab, S.; Burghuber, O.C.; Prosch, H.; Hilbe, W.; Cseh, A.; Fritz, R.; Filipits, M. Liquid-Biopsy-Based Identification of EGFR T790M Mutation-Mediated Resistance to Afatinib Treatment in Patients with Advanced EGFR Mutation-Positive NSCLC, and Subsequent Response to Osimertinib. *Target. Oncol.* **2019**, *14*, 75–83. [CrossRef]
25. Buder, A.; Hochmair, M.J.; Schwab, S.; Bundalo, T.; Schenk, P.; Errhalt, P.; Mikes, R.E.; Absenger, G.; Patocka, K.; Baumgartner, B.; et al. Cell-Free Plasma DNA-Guided Treatment With Osimertinib in Patients With Advanced EGFR-Mutated NSCLC. *J. Thorac. Oncol.* **2018**, *13*, 821–830. [CrossRef]
26. Mok, T.S.; Wu, Y.L.; Ahn, M.J.; Garassino, M.C.; Kim, H.R.; Ramalingam, S.S.; Shepherd, F.A.; He, Y.; Akamatsu, H.; Theelen, W.S.; et al. Osimertinib or Platinum-Pemetrexed in EGFR T790M-Positive Lung Cancer. *N. Engl. J. Med.* **2017**, *376*, 629–640. [CrossRef]
27. Remon, J.; Menis, J.; Hasan, B.; Peric, A.; De Maio, E.; Novello, S.; Reck, M.; Berghmans, T.; Wasag, B.; Besse, B.; et al. The APPLE Trial: Feasibility and Activity of AZD9291 (Osimertinib) Treatment on Positive PLasma T790M in EGFR-mutant NSCLC Patients. EORTC 1613. *Clin. Lung Cancer* **2017**, *18*, 583–588. [CrossRef]
28. Kim, T.M.; Song, A.; Kim, D.W.; Kim, S.; Ahn, Y.O.; Keam, B.; Jeon, Y.K.; Lee, S.H.; Chung, D.H.; Heo, D.S. Mechanisms of Acquired Resistance to AZD9291: A Mutation-Selective, Irreversible EGFR Inhibitor. *J. Thorac. Oncol.* **2015**, *10*, 1736–1744. [CrossRef]
29. Oxnard, G.R.; Hu, Y.; Mileham, K.F.; Husain, H.; Costa, D.B.; Tracy, P.; Feeney, N.; Sholl, L.M.; Dahlberg, S.E.; Redig, A.J.; et al. Assessment of Resistance Mechanisms and Clinical Implications in Patients With EGFR T790M-Positive Lung Cancer and Acquired Resistance to Osimertinib. *JAMA Oncol.* **2018**, *4*, 1527–1534. [CrossRef]
30. Thress, K.S.; Paweletz, C.P.; Felip, E.; Cho, B.C.; Stetson, D.; Dougherty, B.; Lai, Z.; Markovets, A.; Vivancos, A.; Kuang, Y.; et al. Acquired EGFR C797S mutation mediates resistance to AZD9291 in non-small cell lung cancer harboring EGFR T790M. *Nat. Med.* **2015**, *21*, 560–562. [CrossRef]

31. Wang, Z.; Yang, J.J.; Huang, J.; Ye, J.Y.; Zhang, X.C.; Tu, H.Y.; Han-Zhang, H.; Wu, Y.L. Lung Adenocarcinoma Harboring EGFR T790M and In Trans C797S Responds to Combination Therapy of First- and Third-Generation EGFR TKIs and Shifts Allelic Configuration at Resistance. *J. Thorac. Oncol.* **2017**, *12*, 1723–1727. [CrossRef] [PubMed]
32. Arulananda, S.; Do, H.; Musafer, A.; Mitchell, P.; Dobrovic, A.; John, T. Combination Osimertinib and Gefitinib in C797S and T790M EGFR-Mutated Non-Small Cell Lung Cancer. *J. Thorac. Oncol.* **2017**, *12*, 1728–1732. [CrossRef] [PubMed]
33. Niederst, M.J.; Hu, H.; Mulvey, H.E.; Lockerman, E.L.; Garcia, A.R.; Piotrowska, Z.; Sequist, L.V.; Engelman, J.A. The Allelic Context of the C797S Mutation Acquired upon Treatment with Third-Generation EGFR Inhibitors Impacts Sensitivity to Subsequent Treatment Strategies. *Clin. Cancer Res.* **2015**, *21*, 3924–3933. [CrossRef] [PubMed]
34. Uchibori, K.; Inase, N.; Araki, M.; Kamada, M.; Sato, S.; Okuno, Y.; Fujita, N.; Katayama, R. Brigatinib combined with anti-EGFR antibody overcomes osimertinib resistance in EGFR-mutated non-small-cell lung cancer. *Nat. Commun.* **2017**, *8*, 14768. [CrossRef] [PubMed]
35. Del Re, M.; Rofi, E.; Cappelli, C.; Puppo, G.; Crucitta, S.; Valeggi, S.; Chella, A.; Danesi, R.; Petrini, I. The increase in activating EGFR mutation in plasma is an early biomarker to monitor response to osimertinib: A case report. *BMC Cancer* **2019**, *19*, 410. [CrossRef] [PubMed]
36. Marchetti, A.; Palma, J.F.; Felicioni, L.; De Pas, T.M.; Chiari, R.; Del Grammastro, M.; Filice, G.; Ludovini, V.; Brandes, A.A.; Chella, A.; et al. Early Prediction of Response to Tyrosine Kinase Inhibitors by Quantification of EGFR Mutations in Plasma of NSCLC Patients. *J. Thorac. Oncol.* **2015**, *10*, 1437–1443. [CrossRef] [PubMed]
37. Gale, D.; Lawson, A.R.J.; Howarth, K.; Madi, M.; Durham, B.; Smalley, S.; Calaway, J.; Blais, S.; Jones, G.; Clark, J.; et al. Development of a highly sensitive liquid biopsy platform to detect clinically-relevant cancer mutations at low allele fractions in cell-free DNA. *PLoS ONE* **2018**, *13*, e0194630. [CrossRef]
38. Esposito Abate, R.; Pasquale, R.; Fenizia, F.; Rachiglio, A.M.; Roma, C.; Bergantino, F.; Forgione, L.; Lambiase, M.; Sacco, A.; Piccirillo, M.C.; et al. The role of circulating free DNA in the management of NSCLC. *Expert Rev. Anticancer Ther.* **2019**, *19*, 19–28. [CrossRef]
39. Xu, J.; Wang, J.; Zhang, S. Mechanisms of resistance to irreversible epidermal growth factor receptor tyrosine kinase inhibitors and therapeutic strategies in non-small cell lung cancer. *Oncotarget* **2017**, *8*, 90557–90578. [CrossRef]
40. Hong, S.; Gao, F.; Fu, S.; Wang, Y.; Fang, W.; Huang, Y.; Zhang, L. Concomitant Genetic Alterations With Response to Treatment and Epidermal Growth Factor Receptor Tyrosine Kinase Inhibitors in Patients With EGFR-Mutant Advanced Non-Small Cell Lung Cancer. *JAMA Oncol.* **2018**, *4*, 739–742. [CrossRef]
41. Jakobsen, J.N.; Santoni-Rugiu, E.; Grauslund, M.; Melchior, L.; Sorensen, J.B. Concomitant driver mutations in advanced EGFR-mutated non-small-cell lung cancer and their impact on erlotinib treatment. *Oncotarget* **2018**, *9*, 26195–26208. [CrossRef] [PubMed]
42. Pao, W.; Wang, T.Y.; Riely, G.J.; Miller, V.A.; Pan, Q.; Ladanyi, M.; Zakowski, M.F.; Heelan, R.T.; Kris, M.G.; Varmus, H.E. KRAS mutations and primary resistance of lung adenocarcinomas to gefitinib or erlotinib. *PLoS Med.* **2005**, *2*, e17. [CrossRef] [PubMed]
43. Rachiglio, A.M.; Fenizia, F.; Piccirillo, M.C.; Galetta, D.; Crino, L.; Vincenzi, B.; Barletta, E.; Pinto, C.; Ferrau, 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*. [CrossRef] [PubMed]
44. Del Re, M.; Tiseo, M.; Bordi, P.; D'Incecco, A.; Camerini, A.; Petrini, I.; Lucchesi, M.; Inno, A.; Spada, D.; Vasile, E.; et al. Contribution of KRAS mutations and c.2369C > T (p.T790M) EGFR to acquired resistance to EGFR-TKIs in EGFR mutant NSCLC: A study on circulating tumor DNA. *Oncotarget* **2017**, *8*, 13611–13619. [CrossRef]
45. Kitazono, S.; Sakai, K.; Yanagitani, N.; Ariyasu, R.; Yoshizawa, T.; Dotsu, Y.; Koyama, J.; Saiki, M.; Sonoda, T.; Nishikawa, S.; et al. Barcode sequencing identifies resistant mechanisms to EGFR-inhibitors in circulating tumor DNA of lung cancer patients. *Cancer Sci.* **2019**. [CrossRef] [PubMed]
46. Nakatani, K.; Yamaoka, T.; Ohba, M.; Fujita, K.I.; Arata, S.; Kusumoto, S.; Taki-Takemoto, I.; Kamei, D.; Iwai, S.; Tsurutani, J.; et al. KRAS and EGFR Amplifications Mediate Resistance to Rociletinib and Osimertinib in Acquired Afatinib-Resistant NSCLC Harboring Exon 19 Deletion/T790M in EGFR. *Mol. Cancer Ther.* **2019**, *18*, 112–126. [CrossRef] [PubMed]

47. Huang, M.H.; Lee, J.H.; Chang, Y.J.; Tsai, H.H.; Lin, Y.L.; Lin, A.M.; Yang, J.C. MEK inhibitors reverse resistance in epidermal growth factor receptor mutation lung cancer cells with acquired resistance to gefitinib. *Mol. Oncol.* **2013**, *7*, 112–120. [CrossRef]
48. Eberlein, C.A.; Stetson, D.; Markovets, A.A.; Al-Kadhimi, K.J.; Lai, Z.; Fisher, P.R.; Meador, C.B.; Spitzler, P.; Ichihara, E.; Ross, S.J.; et al. Acquired Resistance to the Mutant-Selective EGFR Inhibitor AZD9291 Is Associated with Increased Dependence on RAS Signaling in Preclinical Models. *Cancer Res.* **2015**, *75*, 2489–2500. [CrossRef]
49. Cui, Y.; Xu, J.; Xin, L.; Tian, Y.; Zhan, Z.; Qi, D. Gene mutation characteristics of nonsmall-cell lung carcinoma patients with wild-type epidermal growth factor receptor and sensitivity to Tarceva therapy. *J. Cancer Res. Ther.* **2015**, *11* (Suppl 1), C80–C83. [CrossRef]
50. Hah, J.H.; Zhao, M.; Pickering, C.R.; Frederick, M.J.; Andrews, G.A.; Jasser, S.A.; Fooshee, D.R.; Milas, Z.L.; Galer, C.; Sano, D.; et al. HRAS mutations and resistance to the epidermal growth factor receptor tyrosine kinase inhibitor erlotinib in head and neck squamous cell carcinoma cells. *Head Neck* **2014**, *36*, 1547–1554. [CrossRef]
51. Lammers, P.E.; Lovly, C.M.; 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. Canc. Netw.* **2014**, *12*, 6–11. [CrossRef] [PubMed]
52. 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]
53. Ohashi, K.; Sequist, L.V.; Arcila, M.E.; Moran, T.; Chmielecki, J.; Lin, Y.L.; Pan, Y.; Wang, L.; de Stanchina, E.; Shien, K.; et al. Lung cancers with acquired resistance to EGFR inhibitors occasionally harbor BRAF gene mutations but lack mutations in KRAS, NRAS, or MEK1. *Proc. Natl. Acad. Sci. USA* **2012**, *109*, E2127–E2133. [CrossRef] [PubMed]
54. Paik, P.K.; Arcila, M.E.; Fara, M.; Sima, C.S.; Miller, V.A.; Kris, M.G.; Ladanyi, M.; Riely, G.J. Clinical characteristics of patients with lung adenocarcinomas harboring BRAF mutations. *J. Clin. Oncol.* **2011**, *29*, 2046–2051. [CrossRef] [PubMed]
55. Guibert, N.M.; Paweletz, C.; Hu, Y.; Feeney, N.B.; Plagnol, V.; Poole, V.; Jones, G.; Oxnard, G.R. Early detection of competing resistance mutations using plasma next-generation sequencing (NGS) in patients (pts) with EGFR-mutant NSCLC treated with osimertinib. *J. Clin. Oncol.* **2017**, *35*, 11529. [CrossRef]
56. Ho, C.C.; Liao, W.Y.; Lin, C.A.; Shih, J.Y.; Yu, C.J.; Chih-Hsin Yang, J. Acquired BRAF V600E Mutation as Resistant Mechanism after Treatment with Osimertinib. *J. Thorac. Oncol.* **2017**, *12*, 567–572. [CrossRef] [PubMed]
57. Furge, K.A.; Zhang, Y.W.; Vande Woude, G.F. Met receptor tyrosine kinase: Enhanced signaling through adapter proteins. *Oncogene* **2000**, *19*, 5582–5589. [CrossRef]
58. Blumenschein, G.R., Jr.; Mills, G.B.; Gonzalez-Angulo, A.M. Targeting the hepatocyte growth factor-cMET axis in cancer therapy. *J. Clin. Oncol.* **2012**, *30*, 3287–3296. [CrossRef]
59. Rosario, M.; Birchmeier, W. How to make tubes: Signaling by the Met receptor tyrosine kinase. *Trends Cell Biol.* **2003**, *13*, 328–335. [CrossRef]
60. Gentile, A.; Trusolino, L.; Comoglio, P.M. The Met tyrosine kinase receptor in development and cancer. *Cancer Metastasis Rev.* **2008**, *27*, 85–94. [CrossRef]
61. Zhang, Y.W.; Su, Y.; Volpert, O.V.; Vande Woude, G.F. Hepatocyte growth factor/scatter factor mediates angiogenesis through positive VEGF and negative thrombospondin 1 regulation. *Proc. Natl. Acad. Sci. USA* **2003**, *100*, 12718–12723. [CrossRef] [PubMed]
62. Beau-Faller, M.; Ruppert, A.M.; Voegeli, A.C.; Neuville, A.; Meyer, N.; Guerin, E.; Legrain, M.; Mennecier, B.; Wihlm, J.M.; Massard, G.; et al. MET gene copy number in non-small cell lung cancer: Molecular analysis in a targeted tyrosine kinase inhibitor naive cohort. *J. Thorac. Oncol.* **2008**, *3*, 331–339. [CrossRef] [PubMed]
63. Cappuzzo, F.; Marchetti, A.; Skokan, M.; Rossi, E.; Gajapathy, S.; Felicioni, L.; Del Grammastro, M.; Sciarrotta, M.G.; Buttitta, F.; Incarbone, M.; et al. Increased MET gene copy number negatively affects survival of surgically resected non-small-cell lung cancer patients. *J. Clin. Oncol.* **2009**, *27*, 1667–1674. [CrossRef] [PubMed]

64. Lai, A.Z.; Abella, J.V.; Park, M. Crosstalk in Met receptor oncogenesis. *Trends Cell Biol.* **2009**, *19*, 542–551. [CrossRef] [PubMed]
65. Turke, A.B.; Zejnullahu, K.; Wu, Y.L.; Song, Y.; Dias-Santagata, D.; Lifshits, E.; Toschi, L.; Rogers, A.; Mok, T.; Sequist, L.; et al. Preexistence and clonal selection of MET amplification in EGFR mutant NSCLC. *Cancer Cell* **2010**, *17*, 77–88. [CrossRef] [PubMed]
66. Engelman, J.A.; Zejnullahu, K.; Mitsudomi, T.; Song, Y.; Hyland, C.; Park, J.O.; Lindeman, N.; Gale, C.M.; Zhao, X.; Christensen, J.; et al. MET amplification leads to gefitinib resistance in lung cancer by activating ERBB3 signaling. *Science* **2007**, *316*, 1039–1043. [CrossRef] [PubMed]
67. Planchard, D.; Loriot, Y.; Andre, F.; Gobert, A.; Auger, N.; Lacroix, L.; Soria, J.C. EGFR-independent mechanisms of acquired resistance to AZD9291 in EGFR T790M-positive NSCLC patients. *Ann. Oncol.* **2015**, *26*, 2073–2078. [CrossRef] [PubMed]
68. Le, X.; Puri, S.; Negrao, M.V.; Nilsson, M.B.; Robichaux, J.; Boyle, T.; Hicks, J.K.; Lovinger, K.L.; Roarty, E.; Rinsurongkawong, W.; et al. Landscape of EGFR-Dependent and -Independent Resistance Mechanisms to Osimertinib and Continuation Therapy Beyond Progression in EGFR-Mutant NSCLC. *Clin. Cancer Res.* **2018**, *24*, 6195–6203. [CrossRef]
69. Del Re, M.; Bordi, P.; Rofi, E.; Restante, G.; Valleggi, S.; Minari, R.; Crucitta, S.; Arrigoni, E.; Chella, A.; Morganti, R.; et al. The amount of activating EGFR mutations in circulating cell-free DNA is a marker to monitor osimertinib response. *Br. J. Cancer* **2018**, *119*, 1252–1258. [CrossRef]
70. 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]
71. Wang, Y.; Li, L.; Han, R.; Jiao, L.; Zheng, J.; He, Y. Clinical analysis by next-generation sequencing for NSCLC patients with MET amplification resistant to osimertinib. *Lung Cancer* **2018**, *118*, 105–110. [CrossRef] [PubMed]
72. Zhu, V.W.; Schrock, A.B.; Ali, S.M.; Ou, S.I. Differential response to a combination of full-dose osimertinib and crizotinib in a patient with EGFR-mutant non-small cell lung cancer and emergent MET amplification. *Lung Cancer* **2019**, *10*, 21–26. [CrossRef] [PubMed]
73. Deng, L.; Kiedrowski, L.A.; Ravera, E.; Cheng, H.; Halmos, B. Response to Dual Crizotinib and Osimertinib Treatment in a Lung Cancer Patient with MET Amplification Detected by Liquid Biopsy Who Acquired Secondary Resistance to EGFR Tyrosine Kinase Inhibition. *J. Thorac. Oncol.* **2018**, *13*, e169–e172. [CrossRef] [PubMed]
74. Zheng, X.; Zhang, G.; Li, P.; Zhang, M.; Yan, X.; Zhang, X.; Yang, J.; Li, H.; Liu, X.; Ma, Z.; et al. Mutation tracking of a patient with EGFR-mutant lung cancer harboring de novo MET amplification: Successful treatment with gefitinib and crizotinib. *Lung Cancer* **2019**, *129*, 72–74. [CrossRef] [PubMed]
75. Papadimitrakopoulou, V.A.; Wu, Y.-L.; Han, J.-Y.; Ahn, M.-J.; Ramalingam, S.S.; John, T.; Okamoto, I.; Yang, J.C.-H.; Bulusu, K.C.; Laus, G.; et al. LBA51Analysis of resistance mechanisms to osimertinib in patients with EGFR T790M advanced NSCLC from the AURA3 study. *Ann. Oncol.* **2018**, *29*. [CrossRef]
76. Seki, N.; Natsume, M.; Ochiai, R.; Haruyama, T.; Ishihara, M.; Fukasawa, Y.; Sakamoto, T.; Tanzawa, S.; Usui, R.; Honda, T.; et al. Promising Combination Therapy with Bevacizumab and Erlotinib in an EGFR-Mutated NSCLC Patient with MET Amplification Who Showed Intrinsic Resistance to Initial EGFR-TKI Therapy. *Case Rep. Oncol.* **2019**, *12*, 91–97. [CrossRef] [PubMed]
77. Giroux-Leprieur, E.; Dumenil, C.; Chinet, T. Combination of Crizotinib and Osimertinib or Erlotinib Might Overcome MET-Mediated Resistance to EGFR Tyrosine Kinase Inhibitor in EGFR-Mutated Adenocarcinoma. *J. Thorac. Oncol.* **2018**, *13*, e232–e234. [CrossRef] [PubMed]
78. Zeng, L.; Xia, C.; Zhang, Y.; Yang, N. Identification of a Novel MET Exon 14 Skipping Variant Coexistent with EGFR Mutation in Lung Adenocarcinoma Sensitive to Combined Treatment with Afatinib and Crizotinib. *J. Thorac. Oncol.* **2019**, *14*, e70–e72. [CrossRef] [PubMed]
79. Ninomiya, K.; Ohashi, K.; Makimoto, G.; Tomida, S.; Higo, H.; Kayatani, H.; Ninomiya, T.; Kubo, T.; Ichihara, E.; Hotta, K.; et al. MET or NRAS amplification is an acquired resistance mechanism to the third-generation EGFR inhibitor naquotinib. *Sci. Rep.* **2018**, *8*, 1955. [CrossRef]
80. Bladt, F.; Friese-Hamim, M.; Ihling, C.; Wilm, C.; Blaukat, A. The c-Met Inhibitor MSC2156119J Effectively Inhibits Tumor Growth in Liver Cancer Models. *Cancers* **2014**, *6*, 1736–1752. [CrossRef]

81. Yoshioka, H.; Azuma, K.; Yamamoto, N.; Takahashi, T.; Nishio, M.; Katakami, N.; Ahn, M.J.; Hirashima, T.; Maemondo, M.; Kim, S.W.; et al. A randomized, double-blind, placebo-controlled, phase III trial of erlotinib with or without a c-Met inhibitor tivantinib (ARQ 197) in Asian patients with previously treated stage IIIB/IV nonsquamous nonsmall-cell lung cancer harboring wild-type epidermal growth factor receptor (ATTENTION study). *Ann. Oncol.* **2015**, *26*, 2066–2072. [CrossRef] [PubMed]
82. Spigel, D.R.; Ervin, T.J.; Ramlau, R.A.; Daniel, D.B.; Goldschmidt, J.H., Jr.; Blumenschein, G.R., Jr.; Krzakowski, M.J.; Robinet, G.; Godbert, B.; Barlesi, F.; et al. Randomized phase II trial of Onartuzumab in combination with erlotinib in patients with advanced non-small-cell lung cancer. *J. Clin. Oncol.* **2013**, *31*, 4105–4114. [CrossRef] [PubMed]
83. Comoglio, P.M.; Giordano, S.; Trusolino, L. Drug development of MET inhibitors: Targeting oncogene addiction and expedience. *Nat. Rev. Drug Discov.* **2008**, *7*, 504–516. [CrossRef] [PubMed]
84. Mok, T.S.; Geater, S.L.; Su, W.C.; Tan, E.H.; Yang, J.C.; Chang, G.C.; Han, M.; Komarnitsky, P.; Payumo, F.; Garrus, J.E.; et al. A Randomized Phase 2 Study Comparing the Combination of Ficlatuzumab and Gefitinib with Gefitinib Alone in Asian Patients with Advanced Stage Pulmonary Adenocarcinoma. *J. Thorac. Oncol.* **2016**, *11*, 1736–1744. [CrossRef] [PubMed]
85. Matsubara, D.; Ishikawa, S.; Sachiko, O.; Aburatani, H.; Fukayama, M.; Niki, T. Co-activation of epidermal growth factor receptor and c-MET defines a distinct subset of lung adenocarcinomas. *Am. J. Pathol.* **2010**, *177*, 2191–2204. [CrossRef] [PubMed]
86. Wu, Y.L.; Zhang, L.; Kim, D.W.; Liu, X.; Lee, D.H.; Yang, J.C.; Ahn, M.J.; Vansteenkiste, J.F.; Su, W.C.; Felip, E.; et al. Phase Ib/II Study of Capmatinib (INC280) Plus Gefitinib After Failure of Epidermal Growth Factor Receptor (EGFR) Inhibitor Therapy in Patients With EGFR-Mutated, MET Factor-Dysregulated Non-Small-Cell Lung Cancer. *J. Clin. Oncol.* **2018**, *36*, 3101–3109. [CrossRef]
87. Oxnard, G.R.; Arcila, M.E.; Chmielecki, J.; Ladanyi, M.; Miller, V.A.; Pao, W. New strategies in overcoming acquired resistance to epidermal growth factor receptor tyrosine kinase inhibitors in lung cancer. *Clin. Cancer Res.* **2011**, *17*, 5530–5537. [CrossRef]
88. Piotrowska, Z.; Niederst, M.J.; Karlovich, C.A.; Wakelee, H.A.; Neal, J.W.; Mino-Kenudson, M.; Fulton, L.; Hata, A.N.; Lockerman, E.L.; Kalsy, A.; et al. Heterogeneity Underlies the Emergence of EGFRT790 Wild-Type Clones Following Treatment of T790M-Positive Cancers with a Third-Generation EGFR Inhibitor. *Cancer Discov.* **2015**, *5*, 713–722. [CrossRef]
89. Oser, M.G.; Niederst, M.J.; Sequist, L.V.; Engelman, J.A. Transformation from non-small-cell lung cancer to small-cell lung cancer: Molecular drivers and cells of origin. *Lancet Oncol.* **2015**, *16*, e165–e172. [CrossRef]
90. Fallet, V.; Ruppert, A.M.; Poulot, V.; Lacave, R.; Belmont, L.; Antoine, M.; Cadranel, J.; Wislez, M.; Lavole, A. Secondary resistance to erlotinib: Acquired T790M mutation and small-cell lung cancer transformation in the same patient. *J. Thorac. Oncol.* **2012**, *7*, 1061–1063. [CrossRef]
91. Alì, G.; Bruno, R.; Giordano, M.; Prediletto, I.; Marconi, L.; Zupo, S.; Fedeli, F.; Ribechini, A.; Chella, A.; Fontanini, G. Small cell lung cancer transformation and the T790M mutation: A case report of two acquired mechanisms of TKI resistance detected in a tumor rebiopsy and plasma sample of EGFR-mutant lung adenocarcinoma. *Oncol. Lett.* **2016**, *12*, 4009–4012. [CrossRef] [PubMed]
92. Suda, K.; Murakami, I.; Sakai, K.; Mizuuchi, H.; Shimizu, S.; Sato, K.; Tomizawa, K.; Tomida, S.; Yatabe, Y.; Nishio, K.; et al. Small cell lung cancer transformation and T790M mutation: Complimentary roles in acquired resistance to kinase inhibitors in lung cancer. *Sci. Rep.* **2015**, *5*, 14447. [CrossRef] [PubMed]
93. Sonoda, T.; Nishikawa, S.; Sakakibara, R.; Saiki, M.; Ariyasu, R.; Koyama, J.; Kitazono, S.; Yanagitani, N.; Horiike, A.; Ohyanagi, F.; et al. EGFR T790M mutation after chemotherapy for small cell lung cancer transformation of EGFR-positive non-small cell lung cancer. *Respir. Med. Case Rep.* **2018**, *24*, 19–21. [CrossRef] [PubMed]
94. Nishioka, N.; Yamada, T.; Harita, S.; Hirai, S.; Katayama, Y.; Nakano, T.; Okura, N.; Tamiya, N.; Kaneko, Y.; Uchino, J.; et al. Successful sequential treatment of refractory tumors caused by small cell carcinoma transformation and EGFR-T790M mutation diagnosed by repeated genetic testing in a patient with lung adenocarcinoma harboring epidermal growth factor receptor mutations: A case report. *Respir. Med. Case Rep.* **2018**, *25*, 261–263. [CrossRef] [PubMed]
95. Shiroyama, T.; Nasu, S.; Tanaka, A.; Takata, S.; Masuhiro, K.; Takada, H.; Morita, S.; Morishita, N.; Suzuki, H.; Okamoto, N.; et al. Transformation to small cell lung cancer after first-line afatinib treatment. *Respir. Med. Case Rep.* **2018**, *23*, 188–190. [CrossRef] [PubMed]

96. Manca, P.; Russano, M.; Pantano, F.; Tonini, G.; Santini, D. Change from lung adenocarcinoma to small cell lung cancer as a mechanism of resistance to afatinib. *Oncotarget* **2017**, *8*, 59986–59990. [CrossRef] [PubMed]
97. Taniguchi, Y.; Horiuchi, H.; Morikawa, T.; Usui, K. Small-Cell Carcinoma Transformation of Pulmonary Adenocarcinoma after Osimertinib Treatment: A Case Report. *Case Rep. Oncol.* **2018**, *11*, 323–329. [CrossRef] [PubMed]
98. Ham, J.S.; Kim, S.; Kim, H.K.; Byeon, S.; Sun, J.M.; Lee, S.H.; Ahn, J.S.; Park, K.; Choi, Y.L.; Han, J.; et al. Two Cases of Small Cell Lung Cancer Transformation from EGFR Mutant Adenocarcinoma During AZD9291 Treatment. *J. Thorac. Oncol.* **2016**, *11*, e1–e4. [CrossRef] [PubMed]
99. Tsui, D.W.Y.; Murtaza, M.; Wong, A.S.C.; Rueda, O.M.; Smith, C.G.; Chandrananda, D.; Soo, R.A.; Lim, H.L.; Goh, B.C.; Caldas, C.; et al. Dynamics of multiple resistance mechanisms in plasma DNA during EGFR-targeted therapies in non-small cell lung cancer. *EMBO Mol. Med.* **2018**, *10*. [CrossRef]
100. Aggarwal, C.; Thompson, J.C.; Black, T.A.; Katz, S.I.; Fan, R.; Yee, S.S.; Chien, A.L.; Evans, T.L.; Bauml, J.M.; Alley, E.W.; et al. Clinical Implications of Plasma-Based Genotyping With the Delivery of Personalized Therapy in Metastatic Non-Small Cell Lung Cancer. *JAMA Oncol.* **2018**. [CrossRef]
101. Kim, Y.; Lee, B.; Shim, J.H.; Lee, S.H.; Park, W.Y.; Choi, Y.L.; Sun, J.M.; Ahn, J.S.; Ahn, M.J.; Park, K. Concurrent Genetic Alterations Predict the Progression to Target Therapy in EGFR-Mutated Advanced NSCLC. *J. Thorac. Oncol.* **2019**, *14*, 193–202. [CrossRef] [PubMed]
102. Soucheray, M.; Capelletti, M.; Pulido, I.; Kuang, Y.; Paweletz, C.P.; Becker, J.H.; Kikuchi, E.; Xu, C.; Patel, T.B.; Al-Shahrour, F.; et al. Intratumoral Heterogeneity in EGFR-Mutant NSCLC Results in Divergent Resistance Mechanisms in Response to EGFR Tyrosine Kinase Inhibition. *Cancer Res.* **2015**, *75*, 4372–4383. [CrossRef] [PubMed]
103. Chong, C.R.; Janne, P.A. The quest to overcome resistance to EGFR-targeted therapies in cancer. *Nat. Med.* **2013**, *19*, 1389–1400. [CrossRef] [PubMed]
104. Piotrowska, Z.; Isozaki, H.; Lennerz, J.K.; Gainor, J.F.; Lennes, I.T.; Zhu, V.W.; Marcoux, N.; Banwait, M.K.; Digumarthy, S.R.; Su, W.; et al. Landscape of Acquired Resistance to Osimertinib in EGFR-Mutant NSCLC and Clinical Validation of Combined EGFR and RET Inhibition with Osimertinib and BLU-667 for Acquired RET Fusion. *Cancer Discov.* **2018**, *8*, 1529–1539. [CrossRef] [PubMed]
105. Zou, B.; Lee, V.H.F.; Chen, L.; Ma, L.; Wang, D.D.; Yan, H. Deciphering mechanisms of acquired T790M mutation after EGFR inhibitors for NSCLC by computational simulations. *Sci. Rep.* **2017**, *7*, 6595. [CrossRef] [PubMed]
106. Grabe, T.; Lategahn, J.; Rauh, D. C797S Resistance: The Undruggable EGFR Mutation in Non-Small Cell Lung Cancer? *ACS Med. Chem. Lett.* **2018**, *9*, 779–782. [CrossRef] [PubMed]
107. Minari, R.; Bordi, P.; Del Re, M.; Facchinetti, F.; Mazzoni, F.; Barbieri, F.; Camerini, A.; Comin, C.E.; Gnetti, L.; Azzoni, C.; et al. Primary resistance to osimertinib due to SCLC transformation: Issue of T790M determination on liquid re-biopsy. *Lung Cancer* **2018**, *115*, 21–27. [CrossRef]
108. Del Re, M.; Vasile, E.; Falcone, A.; Danesi, R.; Petrini, I. Molecular analysis of cell-free circulating DNA for the diagnosis of somatic mutations associated with resistance to tyrosine kinase inhibitors in non-small-cell lung cancer. *Expert Rev. Mol. Diagn.* **2014**, *14*, 453–468. [CrossRef]
109. Oxnard, G.R.; Thress, K.S.; Alden, R.S.; Lawrance, R.; Paweletz, C.P.; Cantarini, M.; Yang, J.C.; Barrett, J.C.; Janne, P.A. Association Between Plasma Genotyping and Outcomes of Treatment With Osimertinib (AZD9291) in Advanced Non-Small-Cell Lung Cancer. *J. Clin. Oncol.* **2016**, *34*, 3375–3382. [CrossRef]
110. Thress, K.S.; Brant, R.; Carr, T.H.; Dearden, S.; Jenkins, S.; Brown, H.; Hammett, T.; Cantarini, M.; Barrett, J.C. EGFR mutation detection in ctDNA from NSCLC patient plasma: A cross-platform comparison of leading technologies to support the clinical development of AZD9291. *Lung Cancer* **2015**, *90*, 509–515. [CrossRef]
111. Sacher, A.G.; Paweletz, C.; Dahlberg, S.E.; Alden, R.S.; O'Connell, A.; Feeney, N.; Mach, S.L.; Janne, P.A.; Oxnard, G.R. Prospective Validation of Rapid Plasma Genotyping for the Detection of EGFR and KRAS Mutations in Advanced Lung Cancer. *JAMA Oncol.* **2016**, *2*, 1014–1022. [CrossRef] [PubMed]
112. Sundaresan, T.K.; Sequist, L.V.; Heymach, J.V.; Riely, G.J.; Janne, P.A.; Koch, W.H.; Sullivan, J.P.; Fox, D.B.; Maher, R.; Muzikansky, A.; et al. Detection of T790M, the Acquired Resistance EGFR Mutation, by Tumor Biopsy versus Noninvasive Blood-Based Analyses. *Clin. Cancer Res.* **2016**, *22*, 1103–1110. [CrossRef] [PubMed]

113. Paweletz, C.P.; Sacher, A.G.; Raymond, C.K.; Alden, R.S.; O'Connell, A.; Mach, S.L.; Kuang, Y.; Gandhi, L.; Kirschmeier, P.; English, J.M.; et al. Bias-Corrected Targeted Next-Generation Sequencing for Rapid, Multiplexed Detection of Actionable Alterations in Cell-Free DNA from Advanced Lung Cancer Patients. *Clin. Cancer Res.* **2016**, *22*, 915–922. [CrossRef] [PubMed]
114. Thompson, J.C.; Yee, S.S.; Troxel, A.B.; Savitch, S.L.; Fan, R.; Balli, D.; Lieberman, D.B.; Morrissette, J.D.; Evans, T.L.; Bauml, J.; et al. Detection of Therapeutically Targetable Driver and Resistance Mutations in Lung Cancer Patients by Next-Generation Sequencing of Cell-Free Circulating Tumor DNA. *Clin. Cancer Res.* **2016**, *22*, 5772–5782. [CrossRef] [PubMed]
115. Jenkins, S.; Yang, J.C.; Ramalingam, S.S.; Yu, K.; Patel, S.; Weston, S.; Hodge, R.; Cantarini, M.; Janne, P.A.; Mitsudomi, T.; et al. Plasma ctDNA Analysis for Detection of the EGFR T790M Mutation in Patients with Advanced Non-Small Cell Lung Cancer. *J. Thorac. Oncol.* **2017**, *12*, 1061–1070. [CrossRef]
116. Janku, F. Tumor heterogeneity in the clinic: Is it a real problem? *Ther. Adv. Med. Oncol.* **2014**, *6*, 43–51. [CrossRef] [PubMed]
117. Wong, S.Q.; Li, J.; Tan, A.Y.; Vedururu, R.; Pang, J.M.; Do, H.; Ellul, J.; Doig, K.; Bell, A.; MacArthur, G.A.; et al. Sequence artefacts in a prospective series of formalin-fixed tumours tested for mutations in hotspot regions by massively parallel sequencing. *BMC Med. Genom.* **2014**, *7*, 23. [CrossRef]
118. Ilie, M.; Hofman, P. Pros: Can tissue biopsy be replaced by liquid biopsy? *Transl. Lung Cancer Res.* **2016**, *5*, 420–423. [CrossRef]
119. Jung, A.; Kirchner, T. Liquid Biopsy in Tumor Genetic Diagnosis. *Dtsch. Arztebl. Int.* **2018**, *115*, 169–174. [CrossRef]
120. Bettegowda, C.; Sausen, M.; Leary, R.J.; Kinde, I.; Wang, Y.; Agrawal, N.; Bartlett, B.R.; Wang, H.; Luber, B.; Alani, R.M.; et al. Detection of circulating tumor DNA in early- and late-stage human malignancies. *Sci. Transl. Med.* **2014**, *6*, 224ra224. [CrossRef]
121. Tong, B.; Xu, Y.; Zhao, J.; Chen, M.; Zhong, W.; Xing, J.; Wang, M. Prognostic role of circulating tumor cells in patients with EGFR-mutated or ALK-rearranged non-small cell lung cancer. *Thorac. Cancer* **2018**, *9*, 640–645. [CrossRef] [PubMed]
122. Tickner, J.A.; Urquhart, A.J.; Stephenson, S.A.; Richard, D.J.; O'Byrne, K.J. Functions and therapeutic roles of exosomes in cancer. *Front. Oncol.* **2014**, *4*, 127. [CrossRef] [PubMed]
123. Masaoutis, C.; Mihailidou, C.; Tsourouflis, G.; Theocharis, S. Exosomes in lung cancer diagnosis and treatment. From the translating research into future clinical practice. *Biochimie* **2018**, *151*, 27–36. [CrossRef] [PubMed]
124. Zheng, H.; Zhan, Y.; Liu, S.; Lu, J.; Luo, J.; Feng, J.; Fan, S. The roles of tumor-derived exosomes in non-small cell lung cancer and their clinical implications. *J. Exp. Clin. Cancer Res.* **2018**, *37*, 226. [CrossRef] [PubMed]
125. Niu, L.; Song, X.; Wang, N.; Xue, L.; Song, X.; Xie, L. Tumor-derived exosomal proteins as diagnostic biomarkers in non-small cell lung cancer. *Cancer Sci.* **2019**, *110*, 433–442. [CrossRef] [PubMed]
126. Jing, C.; Cao, H.; Qin, X.; Yu, S.; Wu, J.; Wang, Z.; Ma, R.; Feng, J. Exosome-mediated gefitinib resistance in lung cancer HCC827 cells via delivery of miR-21. *Oncol. Lett.* **2018**, *15*, 9811–9817. [CrossRef] [PubMed]
127. Liu, Q.; Xiang, Y.; Yuan, S.; Xie, W.; Li, C.; Hu, Z.; Wu, N.; Wu, L.; Yu, Z.; Bai, L.; et al. Plasma exosome levels in non-small-cell lung cancer: Correlation with clinicopathological features and prognostic implications. *Cancer Biomark.* **2018**, *22*, 267–274. [CrossRef] [PubMed]
128. Del Re, M.; Marconcini, R.; Pasquini, G.; Rofi, E.; Vivaldi, C.; Bloise, F.; Restante, G.; Arrigoni, E.; Caparello, C.; Bianco, M.G.; et al. PD-L1 mRNA expression in plasma-derived exosomes is associated with response to anti-PD-1 antibodies in melanoma and NSCLC. *Br. J. Cancer* **2018**, *118*, 820–824. [CrossRef]
129. Passiglia, F.; Rizzo, S.; Di Maio, M.; Galvano, A.; Badalamenti, G.; Listi, A.; Gulotta, L.; Castiglia, M.; Fulfaro, F.; Bazan, V.; et al. The diagnostic accuracy of circulating tumor DNA for the detection of EGFR-T790M mutation in NSCLC: A systematic review and meta-analysis. *Sci. Rep.* **2018**, *8*, 13379. [CrossRef]
130. Liu, L.; Toung, J.M.; Jassowicz, A.F.; Vijayaraghavan, R.; Kang, H.; Zhang, R.; Kruglyak, K.M.; Huang, H.J.; Hinoue, T.; Shen, H.; et al. Targeted methylation sequencing of plasma cell-free DNA for cancer detection and classification. *Ann. Oncol.* **2018**, *29*, 1445–1453. [CrossRef]
131. Abbosh, C.; Birkbak, N.J.; Wilson, G.A.; Jamal-Hanjani, M.; Constantin, T.; Salari, R.; Le Quesne, J.; Moore, D.A.; Veeriah, S.; Rosenthal, R.; et al. Phylogenetic ctDNA analysis depicts early-stage lung cancer evolution. *Nature* **2017**, *545*, 446–451. [CrossRef] [PubMed]

132. Chen, K.Z.; Lou, F.; Yang, F.; Zhang, J.B.; Ye, H.; Chen, W.; Guan, T.; Zhao, M.Y.; Su, X.X.; Shi, R.; et al. Circulating Tumor DNA Detection in Early-Stage Non-Small Cell Lung Cancer Patients by Targeted Sequencing. *Sci. Rep.* **2016**, *6*, 31985. [CrossRef] [PubMed]
133. Milbury, C.A.; Zhong, Q.; Lin, J.; Williams, M.; Olson, J.; Link, D.R.; Hutchison, B. Determining lower limits of detection of digital PCR assays for cancer-related gene mutations. *Biomol. Detect. Quantif.* **2014**, *1*, 8–22. [CrossRef] [PubMed]
134. Diaz, L.A.; Bardelli, A., Jr. Liquid biopsies: Genotyping circulating tumor DNA. *J. Clin. Oncol.* **2014**, *32*, 579–586. [CrossRef] [PubMed]
135. Normanno, N.; Denis, M.G.; Thress, K.S.; Ratcliffe, M.; Reck, M. Guide to detecting epidermal growth factor receptor (EGFR) mutations in ctDNA of patients with advanced non-small-cell lung cancer. *Oncotarget* **2017**, *8*, 12501–12516. [CrossRef] [PubMed]
136. Zink, F.; Stacey, S.N.; Norddahl, G.L.; Frigge, M.L.; Magnusson, O.T.; Jonsdottir, I.; Thorgeirsson, T.E.; Sigurdsson, A.; Gudjonsson, S.A.; Gudmundsson, J.; et al. Clonal hematopoiesis, with and without candidate driver mutations, is common in the elderly. *Blood* **2017**, *130*, 742–752. [CrossRef] [PubMed]
137. Xie, M.; Lu, C.; Wang, J.; McLellan, M.D.; Johnson, K.J.; Wendl, M.C.; McMichael, J.F.; Schmidt, H.K.; Yellapantula, V.; Miller, C.A.; et al. Age-related mutations associated with clonal hematopoietic expansion and malignancies. *Nat. Med.* **2014**, *20*, 1472–1478. [CrossRef]

© 2019 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (http://creativecommons.org/licenses/by/4.0/).