The Validity and Predictive Value of Blood-Based Biomarkers in Prediction of Response in the Treatment of Metastatic Non-Small Cell Lung Cancer: A Systematic Review

Frederik van Delft [1], **Hendrik Koffijberg** [1], **Valesca Retèl** [1,2], **Michel van den Heuvel** [3] **and Maarten IJzerman** [1,4,5,*]

1   Health Technology and Services Research Department, Technical Medical Centre, University of Twente, Hallenweg 5, 7522 NH Enschede, The Netherlands; f.a.vandelft@utwente.nl (F.v.D.); h.koffijberg@utwente.nl (H.K.); v.retel@nki.nl (V.R.)
2   Division of Psychosocial Research and Epidemiology, Netherlands Cancer Institute, Plesmanlaan 121, 1066 CX Amsterdam, The Netherlands
3   Respiratory Diseases, Radboud University Medical Center, Geert Grooteplein Zuid 10, 6525 GA Nijmegen, The Netherlands; Michel.vandenHeuvel@radboudumc.nl
4   Centre for Cancer Research and Centre for Health Policy, University of Melbourne, Parkville, VIC 3000, Australia
5   Peter MacCallum Cancer Centre, Parkville, VIC 3000, Australia
*   Correspondence: maarten.ijzerman@unimelb.edu.au; Tel.: +61-3-8559-8585

check for updates

Abstract: With the introduction of targeted therapies and immunotherapy, molecular diagnostics gained a more profound role in the management of non-small cell lung cancer (NSCLC). This study aimed to systematically search for studies reporting on the use of liquid biopsies (LB), the correlation between LBs and tissue biopsies, and finally the predictive value in the management of NSCLC. A systematic literature search was performed, including results published after 1 January 2014. Articles studying the predictive value or validity of a LB were included. The search (up to 1 September 2019) retrieved 1704 articles, 1323 articles were excluded after title and abstract screening. Remaining articles were assessed for eligibility by full-text review. After full-text review, 64 articles investigating the predictive value and 78 articles describing the validity were included. The majority of studies investigated the predictive value of LBs in relation to therapies targeting the epidermal growth factor receptor (EGFR) or anaplastic lymphoma kinase (ALK) receptor (n = 38). Of studies describing the validity of a biomarker, 55 articles report on one or more EGFR mutations. Although a variety of blood-based biomarkers are currently under investigation, most studies evaluated the validity of LBs to determine EGFR mutation status and the subsequent targeting of EGFR tyrosine kinase inhibitors based on the mutation status found in LBs of NSCLC patients.

Keywords: liquid biopsy; non-small cell lung cancer; biomarkers

1. Introduction

Lung cancer is the leading cause of cancer-related deaths worldwide and is known for its high incidence and mortality rates [1,2]. The current treatment standard for early-stage non-small cell lung cancer (NSCLC) (stage I–II) is resection. In addition to resection, patients are offered stereotactic radiotherapy, adjuvant chemotherapy, or a combination of both depending on the tumor stage. Patients diagnosed with stage III–IV are eligible for systemic therapy such as chemotherapy, chemoradiotherapy,

immunotherapy, combinations, and targeted therapy based on the presence of specific genetic druggable mutations [3].

With the introduction of targeted therapies and immunotherapy, molecular testing has become more important, as the effectiveness of selected therapies depends on the presence of specific molecular or genomic alterations [4]. Furthermore, patients tend to develop treatment resistance leading to disease progression and requiring the use of second- and third-line targeted therapies. Moreover, tumor heterogeneity and clonal evolution possibly play a role in the development of drug resistance, increasing the need for repeat biopsies to guide treatment decisions [5–7]. Currently, oncogenic mutations are derived from tumor tissue samples obtained by means of invasive biopsies. However, (re-)biopsies can be highly complex and sometimes require an invasive procedure, therefore unfeasible in a substantial proportion of patients, due to location, tumor size, or general health status. Besides being performed in case of an inadequate first sample, re-biopsies can be performed to track tumor progression and clonal evolution. Since a re-biopsy does also not always provide sufficient tissue for molecular testing, liquid biopsies (LBs) might help overcome this issue [8–10]. LBs provide an alternative way of obtaining genetic information without the need for an invasive procedure, the low patient burden allows for more frequent biopsies to guide treatment decisions. LBs are also expected to have a health economic benefit by better treatment targeting and by earlier identification of non-response [11].

Liquid biopsies obtained through blood samples contain different types of tumor-related genetic or protein markers, e.g., circulating tumor cells (CTCs), RNA, exosomes, carcinoembryonic antigen, cytokeratin, and cell-free DNA (cfDNA), which can provide valuable information with regard to prognostics, early disease detection, treatment response monitoring, identification of emerging treatment resistance, and recurrence monitoring [12,13].

Despite the potential benefits of liquid biopsies, not many LBs are routinely used nor are they reimbursed. This partly has to do with the challenges of genome backward development of new biomarkers compared to biomarker and drug co-development, as the evidence of clinical validity needs to be established post-hoc for the latter. While clinical utility to change patient management is the holy grail in such studies, an essential and intermediate requirement is to demonstrate diagnostic validity and predictive validity. Existing reviews focus on a specific mutational pathway or biomarker and not on the evidence of validity obtained through prospective studies. This review aims to identify papers investigating a rage of liquid biopsy-based biomarkers for patients with NSCLC and, second, to extract and compare evidence of clinical and predictive validity. It primarily focuses on the diagnostic and predictive validity, where diagnostic validity is defined as the ability of LBs to identify mutations or biomarkers for which tissue analysis currently is the gold standard. Predictive validity on the other hand is defined as if the LB can predict response to a particular treatment, which is a requirement for a test to have utility.
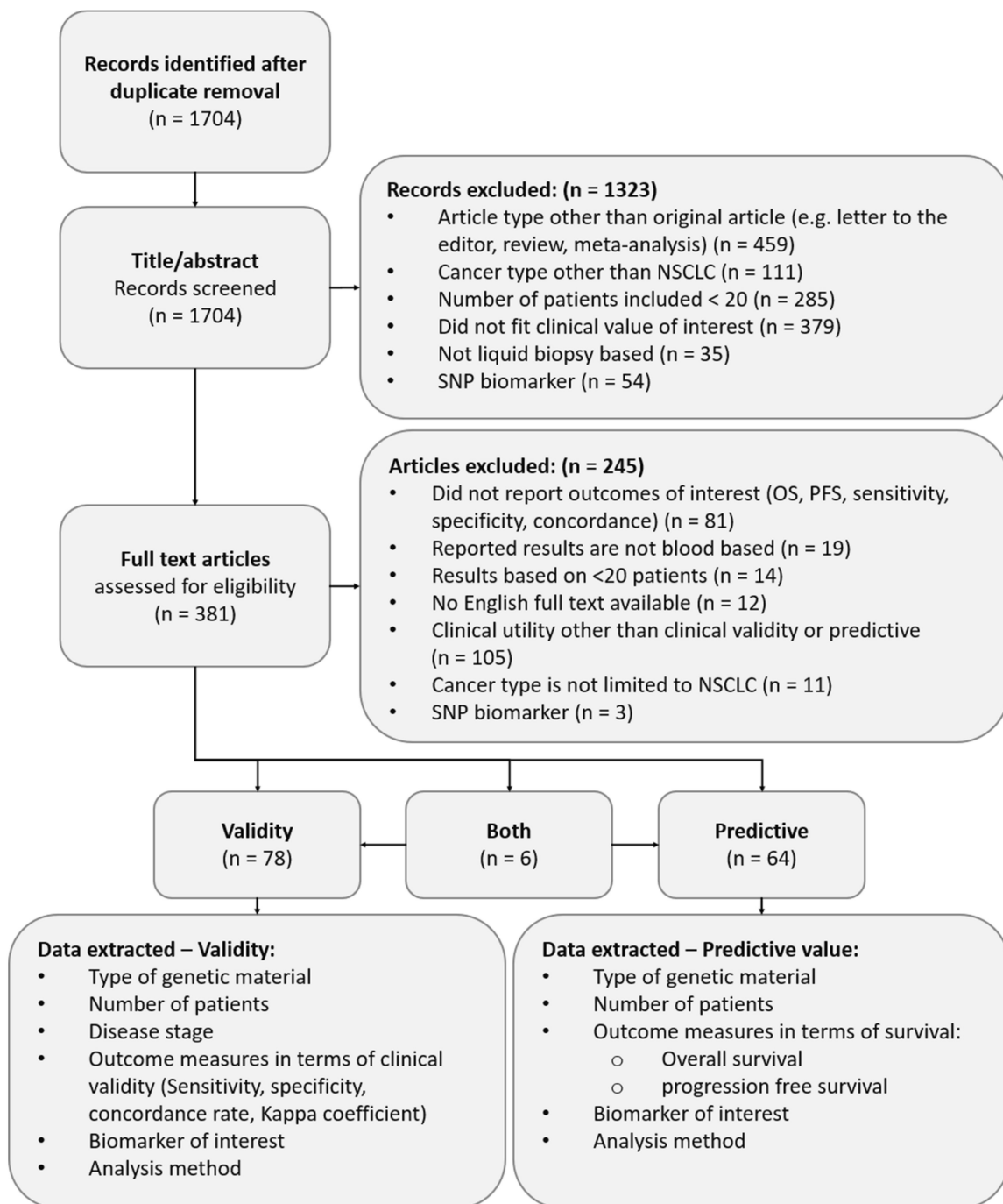
2. Results

The initial search in Scopus (*n* = 1489) and PubMed (*n* = 1037) returned a total of 2526 studies, and after duplicate removal, 1704 unique records were identified. Based on the screening of all abstracts of the 1704 identified records, 1323 records (78%) were excluded from the full-text review. The full study selection process is depicted in Figure 1, and a detailed description is provided in Tables 1 and 2 for presenting the full reference to the selected studies.

2.1. Extracted Data on the Validity of the Biomarker

A description of all studies included in the validity group is provided in Table 1.

Figure 2 depicts the reported sensitivity, specificity, and concordance rate of the different biomarkers identified. Results were stratified according to the analysis method used. For each study, the sensitivity, specificity, and concordance rate of LBs compared to tissue biopsies (TBs) were extracted from the included papers. Data were only extracted if the study had included at least 10 patients for whom

the biomarker was also detected in a matched tissue sample. The number of included patients ranged from 10 to 989 with a mean of 55 patients.

```
┌─────────────────────────┐
│ Records identified after │
│     duplicate removal    │
│        (n = 1704)        │
└─────────────────────────┘
```

```
┌─────────────────────────┐      ┌──────────────────────────────────────────────┐
│      Title/abstract      │      │ Records excluded: (n = 1323)                   │
│     Records screened     │─────▶│ • Article type other than original article     │
│        (n = 1704)        │      │   (e.g. letter to the editor, review,          │
└─────────────────────────┘      │   meta-analysis) (n = 459)                     │
                                  │ • Cancer type other than NSCLC (n = 111)       │
                                  │ • Number of patients included < 20 (n = 285)   │
                                  │ • Did not fit clinical value of interest       │
                                  │   (n = 379)                                    │
                                  │ • Not liquid biopsy based (n = 35)             │
                                  │ • SNP biomarker (n = 54)                       │
                                  └──────────────────────────────────────────────┘
```

```
┌─────────────────────────┐      ┌──────────────────────────────────────────────┐
│    Full text articles    │      │ Articles excluded: (n = 245)                   │
│  assessed for eligibility│─────▶│ • Did not report outcomes of interest          │
│        (n = 381)         │      │   (OS, PFS, sensitivity, specificity,          │
└─────────────────────────┘      │   concordance) (n = 81)                        │
                                  │ • Reported results are not blood based (n = 19)│
                                  │ • Results based on <20 patients (n = 14)       │
                                  │ • No English full text available (n = 12)      │
                                  │ • Clinical utility other than clinical validity │
                                  │   or predictive (n = 105)                      │
                                  │ • Cancer type is not limited to NSCLC (n = 11) │
                                  │ • SNP biomarker (n = 3)                        │
                                  └──────────────────────────────────────────────┘
```

```
┌──────────────┐   ┌──────────────┐   ┌──────────────┐
│   Validity   │◀──│     Both     │──▶│  Predictive  │
│   (n = 78)   │   │   (n = 6)    │   │   (n = 64)   │
└──────────────┘   └──────────────┘   └──────────────┘
```

```
┌───────────────────────────────────┐   ┌───────────────────────────────────┐
│ Data extracted – Validity:        │   │ Data extracted – Predictive value: │
│ • Type of genetic material        │   │ • Type of genetic material        │
│ • Number of patients              │   │ • Number of patients              │
│ • Disease stage                   │   │ • Outcome measures in terms of     │
│ • Outcome measures in terms of    │   │   survival:                        │
│   clinical validity (Sensitivity, │   │     o Overall survival             │
│   specificity, concordance rate,  │   │     o progression free survival    │
│   Kappa coefficient)              │   │ • Biomarker of interest           │
│ • Biomarker of interest           │   │ • Analysis method                 │
│ • Analysis method                 │   │                                    │
└───────────────────────────────────┘   └───────────────────────────────────┘
```

Figure 1. Study selection flow chart. (Non-small cell lung cancer: NSCLC, single nucleotide polymorphism: SNP, Overall survival: OS, Progression free survival: PFS).

Table 1. Description of included studies describing the validity of a liquid biopsy-based biomarker.

1st Author	Publication Year	Evaluated Biomarker(s)	Analysis Method	Reference Number
Alegre et al.	2016	L858R, del E746-A750	PCR	[14]
Arriola et al.	2018	EGFR	PCR	[15]
Balgkouranidou et al.	2014	BRMS1	PCR	[16]
Chai et al.	2016	Exon 19 deletion, L858R, L861Q, T790M, exon 20 insertions	cSMART	[17]
Chen et al.	2017	Gene panel	NGS	[18]
Chen et al.	2016	Gene panel	NGS	[19]
Cui et al.	2017	ALK	NGS	[20]
Douillard et al.	2014	EGFR	PCR	[21]
Duan et al.	2015	EGFR	PCR	[22]
Guibert et al.	2018	PD-L1	Fluorescence	[23]
Guibert et al.	2018	EGFR, T790M	NGS & PCR	[24]
Guibert et al.	2016	KRAS	NGS & PCR	[25]
Guo et al.	2016	Gene panel	NGS	[26]
Han et al.	2016	EGFR, KRAS	PCR	[27]
He et al.	2017	L858R, exon 19 deletion, EGFR	PCR	[28]
Ilie et al.	2018	PD-L1	Fluorescence	[29]
Ilie et al.	2017	MET	Fluorescence	[30]
Jenkins et al.	2017	T790M, L858R, exon 19 deletion	PCR	[31]
Kasahara et al.	2017	Exon 19 deletion, L858R	PCR	[32]
Krug et al.	2018	EGFR, T790M	NGS & PCR	[33]
Lam et al.	2015	EGFR	PCR	[34]
Lee et al.	2016	Exon 19 deletion, L858R	PCR	[35]
Li et al.	2017	Exon 19 deletion, L858R, gene panel	PCR	[36]
Li et al.	2014	EGFR	PCR	[37]
Liu et al.	2018	Gene panel, L858R, exon 19 deletion, KRAS, ALK	NGS	[38]
Ma	2016	EGFR, exon 19 deletion, L858R	PCR	[39]
Mayo-de-las-Casas et al.	2017	EGFR	PCR	[40]
Mok et al.	2015	EGFR, exon 19 deletion, L858R, G719X, L861Q	PCR	[41]
Muller et al.	2017	Gene panel	NGS	[42]
Nilsson et al.	2016	ALK	PCR	[43]
Oxnard et al.	2016	Exon 19 deletion, L858R, T790M	PCR	[44]
Que et al.	2016	EGFR	Chromatograpy	[45]
Reck et al.	2016	EGFR	PCR	[46]
Reckamp et al.	2016	T790M, L858R, exon 19 deletion	NGS	[47]
Sacher et al.	2016	Exon 19 deletion, L858R, T790M, KRAS	PCR	[48]
Schwaederle et al.	2017	Gene panel, EGFR	NGS	[49]
Shi et al.	2018	EGFR, exon 19 deletion, L858R	cSMART	[50]
Sim et al.	2018	EGFR	PCR	[51]
Sundaresan	2016	T790M	PCR	[52]
Sung et al.	2017	Exon 19 deletion, L858R	NGS	[53]
Tompson et al.	2016	Gene panel, EGFR	NGS	[54]
Thress et al.	2015	Exon 19 deletion, L858R, T790M	PCR	[55]
Uchida et al.	2015	L858R, EGFR, gene panel	NGS	[56]
Vazquez et al.	2016	EGFR	NGS	[57]
Wan et al.	2017	EGFR	PCR	[58]
Wang et al.	2014	EGFR	PCR	[59]
Wang et al.	2016	ALK	NGS	[60]
Watanabe et al.	2016	EGFR, exon 19 deletion	PCR	[61]
Wei et al.	2018	Gene panel, L858R, exon 19 deletion	EFIRM	[62]
Wei et al.	2017	Gene panel, L858R, exon 19 deletion	PCR	[63]
Wu et al.	2018	EGFR	PCR	[64]
Wu et al.	2017	Exon 19 deletion, L858R	PCR	[65]
Xu et al.	2016	Gene panel	NGS	[66]
Yang et al.	2017	BRAF, EGFR, exon 19 deletion, L858R, T790M	PCR	[67]
Yao et al.	2017	Gene panel	NGS	[68]
Yu et al.	2019	EGFR	PCR	[69]
Yu et al.	2017	Exon 19 deletion, L858R, T790M	PCR	[70]

Table 1. *Cont.*

1st Author	Publication Year	Evaluated Biomarker(s)	Analysis Method	Reference Number
Zhang et al.	2018	EGFR, L858R, exon 19 deletion	PCR	[71]
Zhang et al.	2017	L858R, exon 19 deletion	PCR	[72]
Zheng et al.	2016	T790M	PCR	[73]
Zhu et al.	2015	Exon 19 deletion, L858R	PCR	[74]
Zhu et al.	2017	Exon 19 deletion, L858R	PCR	[75]
Zhu et al.	2017	Exon 19 deletion, L858R	PCR	[76]
Chen et al.	2019	PD-L1	Fluorescence	[77]
Ding et al.	2019	Exon 19 deletion, L858R, S768I, L861Q	PCR	[78]
Garcia et al.	2019	EGFR	NGS	[79]
He et al.	2019	ALK	Fluorescence	[80]
Li et al.	2019	ALK, KRAS, EGFR, MET, ERBB2, BRAF, ROS1, RET, T790M	NGS	[81]
O'kane et al.	2019	T790M	NGS	[82]
Park et al.	2019	ALK	PCR	[83]
Soria-Comes et al.	2019	EGFR	PCR	[84]
Usui et al.	2019	T790M	NGS	[85]
Wang et al.	2019	EGFR	NGS	[86]
Yang et al.	2018	T790M	PCR	[87]
Ye et al.	2019	KRAS	PCR	[88]
Zhang et al.	2019	EGFR	NGS	[89]
Zhang et al.	2019	Exon 19 deletion, L858R	PCR	[90]
Yoshida et al.	2017	Exon 19 deletion, L858R, T790M	PCR	[91]

Polymerase chain reaction: PCR, Next generation sequencing: NGS, Circulating single molecule amplification and re-sequencing technology: cSMART, Epidermal Growth Factor Receptor: EGFR, Breast Cancer Metastasis Supressor-1: BRMS1, Anaplastic Lymphoma Kinase: ALK, Programmed Death Ligand 1: PD-L1, Kirsten Rat Sarcoma: KRAS, MET proto-oncogene: MET, B-Raf proto-oncogene: BRAF, erb-b2 receptor tyrosine kinase 2: ERBB2, ROS proto-oncogene 1: ROS1, ret proto-oncogene: RET.

The majority of studies (72%, *n* = 56) reported validity of EGFR mutations, including exon 19 deletion, L858R, and T790M mutations. Reported sensitivity values for identified biomarkers ranged from 19.6% to a perfect 100%. In these studies, the sensitivity was reported for EGFR, exon 19 deletion, L858R, and T790M in 23, 21, 23, and 10 studies. The results indicate that next generation sequencing (NGS) is more sensitive than polymerase chain reaction (PCR) in the detection of EGFR and T790M mutations, but less for L858R mutations. Figure 2 depicts the sensitivity, specificity, and concordance reported by each. As shown in Figure 2, the average sensitivity of NGS in the detection of EGFR and T790M mutations was 81% and 87%, respectively. While the average sensitivity of PCR in the detection of EGFR and T790M mutations was 62% and 64%, respectively. A slightly higher sensitivity of PCR compared to NGS was reported for exon 19 deletions (NGS 67%, PCR 76%).

Specificity was reported in 21, 20, 20, and 8 studies for L858R, exon 19 deletion, EGFR, and T790M mutations respectively. A specificity of >90% was seen in most of the studies, despite a few exceptions like a study reporting a specificity of 47% in a 50-gene panel including EGFR, ALK, and KRAS [19]. The specificity of L858R mutation detection was 97.8% and 98.2% for PCR and NGS-based methods respectively. While the average specificity for PCR- and NGS-based methods in the detection of exon 19 deletion was 98% and 97%, respectively. In the detection of T790M mutations with an average reported specificity of 94% and 82% for NGS- and PCR-based methods.

Finally, the concordance between LBs and TBs is reported as a percentage agreement. Concordance rates of EGFR mutation detection were reported in 14, 15, 14, and 6 studies for L858R, exon 19 deletion, EGFR, and T790M mutations, respectively. Concordance ranged from 40% for detection of the T790M mutation to 98.7% for the detection of EGFR mutations. On average reported concordance rates were higher for NGS-based methods compared to PCR-based methods for all EGFR mutations. With an average concordance rate for NGS and PCR of 91% vs. 88% in L858R mutations, 90% vs. 87% for exon 19 deletions, 89% vs. 84% for EGFR mutations, and 69% vs. 68% for T790M mutations.
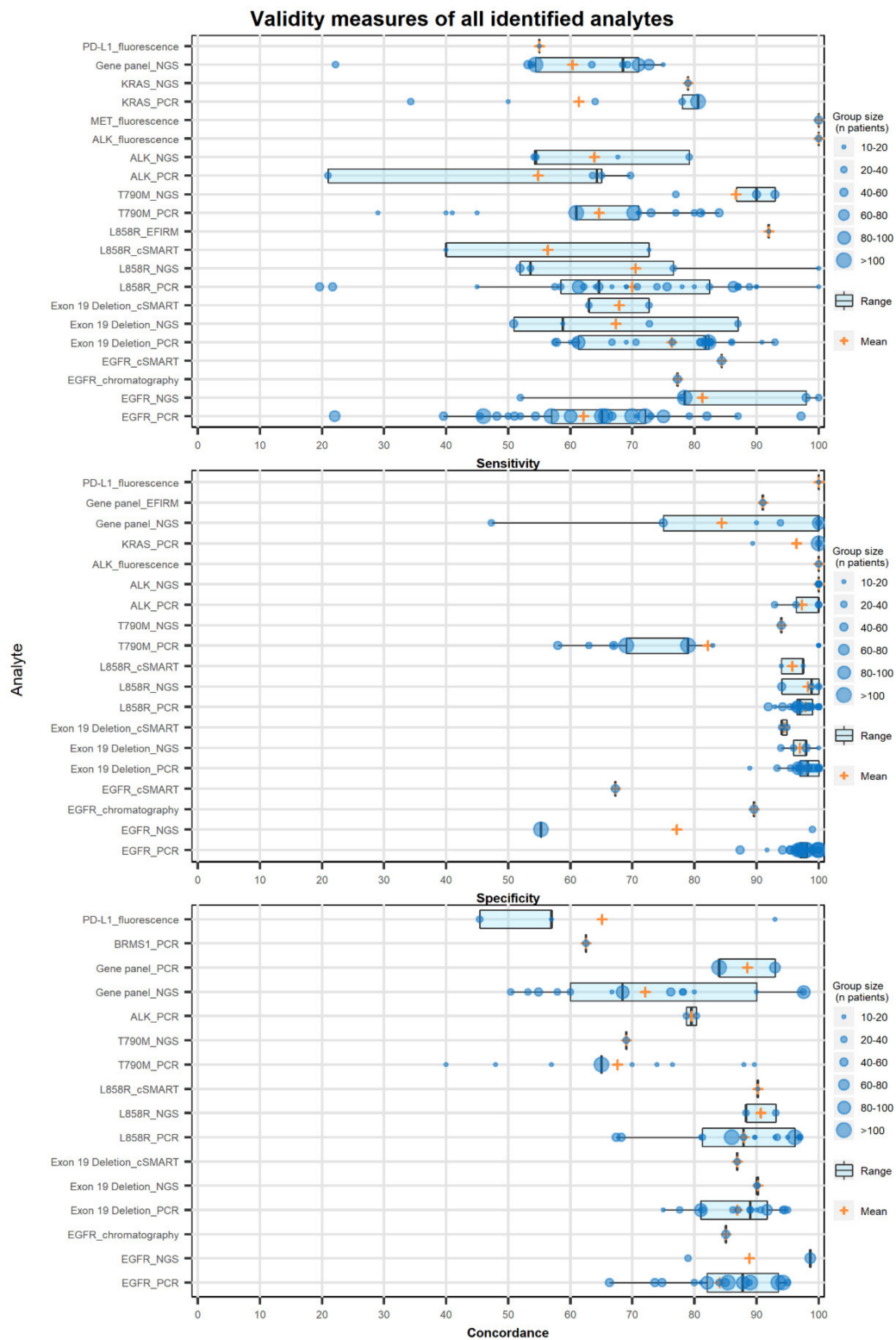
Figure 2. Validity measures of all identified analytes, including the sensitivity, specificity, and concordance of liquid biopsy results compared to matched tissue samples. The y-axis presents each of the reported biomarkers with analysis platform used and separated through an underscore (e.g., EGFR_NGS). The size of the "circle" (see caption right of the figure) depicts the number of patients in whom the biomarker was detected in the tissue sample. Likewise, the "plus" shaped marker depicts the average of the reported values. A boxplot is used to present the range of the reported values, the box represents the 25th and 75th percentiles, while the whiskers extend to a maximum of 1.5 times the inter-quartile range.

2.2. Study Evidence Levels for Predictive Biomarkers

A description of all studies included as describing the predictive value of biomarkers is listed in Table 2.

Table 2. Description of included studies describing the predictive value of a liquid biopsy-based biomarker.

1st Author	Publication Year	Evaluated Biomarker(s)	Treatment	Reference Number
Arrieta et al.	2014	Ck18, Ck19, CEA	Chemotherapy	[92]
Azuma et al.	2016	c-MET	Erlotinib, Tivantinib	[93]
Chen et al.	2016	has-miR-98-5p, has-miR-302e, has-miR-495-3p, has-miR-613	Radiotherapy	[94]
Costantini et al.	2018	sGranB	Nivolumab	[95]
Del Re et al.	2017	KRAS	Erlotinib, Gefitinib	[96]
Dowler Nygaard et al.	2014	cfDNA level, KRAS	Chemotherapy, bevacizumab	[97]
Fiala et al.	2014	NSE, TK	Erlotinib, Gefitinib	[98]
Fiala et al.	2014	CYFRA, CEA	Erlotinib	[99]
Fiala et al.	2015	CRP	Erlotinib	[100]
Haghgoo et al.	2017	TGF-aplha, soluble EGFR, amphiregulin	EGFR TKI	[101]
He et al.	2017	L858R, exon 19 deletion, T790M	Afatinib	[28]
Jiang et al.	2017	MMB	Chemotherapy	[102]
Jiang et al.	2018	CTC count	Afatinib, Erlotinib, Gefitinib, Icotinib	[103]
Juan et al.	2014	CTC count	Chemotherapy	[104]
Karachaliou et al.	2015	Exon 19 deletion, L858R	Erlotinib, chemotherapy	[105]
Lee et al.	2014	BIM	Chemotherapy	[106]
Li et al.	2014	EGFR	EGFR TKI	[37]
Li et al.	2014	RRM1, ERCC1, BRCA1	Chemotherapy	[107]
Ma et al.	2016	EGFR	EGFR TKI	[39]
Mai et al.	2017	CD8/CD4	Montanide ISA 51	[108]
Mok et al.	2015	EGFR	Chemotherapy, Erlotinib	[41]
Muinelo-Romay et al.	2014	CTC count	Chemotherapy	[109]
Nel et al.	2014	CD133/pan-CK, N-cadherin	Chemotherapy	[110]
Nilsson et al.	2016	ALK	Crizotinib	[43]
Nygaard et al.	2014	cfDNA level	Chemotherapy	[111]
Ostheimer et al.	2017	OPN plasma level	Radiotherapy, Chemotherapy	[112]
Oxnard et al.	2016	T790M	Osimertinib	[44]
Paz-Ares et al.	2015	EGFR	Sorafenib	[113]
Qi et al.	2017	CTC count	Chemotherapy	[114]
Que et al.	2016	EGFR	EGFR TKI	[45]
Quoix et al.	2016	TrPAL	TG4010+chemotherapy, chemotherapy	[115]
Shi et al.	2014	survivin mRNA, EGFR	Gefitinib, Erlotinib	[116]
Sun et al.	2017	BIM	Gefitinib, Erlotinib	[117]
Svaton et al.	2014	Natrium level	Erlotinib	[118]
Tissot et al.	2015	cfDNA level	Chemotherapy	[119]
Tu et al.	2017	CD4/CD8, NK expression	EGFR TKI	[120]
Uchibori et al.	2018	Exon 19 deletion, L858R, T790M	Gefitinib+ chemotherapy	[121]
Wang et al.	2017	T790M	EGFR TKI, chemotherapy	[122]
Wang et al.	2018	T790M	EGFR TKI	[123]
Wang et al.	2014	T790M	Gefitinib, erlotinib	[124]
Winther-Larsen et al.	2017	cfDNA mutation	Erlotinib	[125]
Wu et al.	2017	EGFR	Chemotherapy, Afatinib	[126]
Yanagita et al.	2016	CTC count, cfDNA level	Erlotinib	[127]
Yang et al.	2017	CTC count	Gefitinib, Erlotinib	[128]
Yang et al.	2018	CTC count	AZD9291	[129]
Yonesaka et al.	2017	Soluble HRG	Patritumab + Erlotinib	[130]
Zhang et al.	2015	Pokemon	Chemotherapy	[131]
Zhang et al.	2016	sAPE1	Chemotherapy	[132]
Zhou et al.	2017	CTC count	Chemotherapy	[133]
Zhu et al.	2017	EGFR, L858R, exon 19 deletion	EGFR TKI	[134]

Table 2. *Cont.*

1st Author	Publication Year	Evaluated Biomarker(s)	Treatment	Reference Number
Akamatsu et al.	2019	EGFR	Afatinib	[135]
Alama et al.	2019	CTC count, cfDNA level	Nivolumab	[136]
Bordi et al.	2019	Mutation level, EGFR	Osimertinib	[137]
Hojbjerg et al.	2019	miR-30b, miR-30c- miR-211, miR-222	Erlotinib	[138]
Kotsakis et al.	2019	CD4, T-cells, PD-1, PD-L1, B-cells, DC/monocytes	Chemotherapy	[139]
Navarro et al.	2019	Exosome seize	Surgery	[140]
O'Kane et al.	2019	EGFR, T790M	EGFR TKI	[82]
Park et al.	2019	ALK	Crizotinib	[83]
Passiglia et al.	2019	cfDNA level, neutrophil to lymphocyte ratio	Nivolumab	[141]
Tamminga et al.	2019	CTC count	Checkpoint inhibitors	[142]
Wang et al.	2019	TMB	Anti PD-(L)1	[143]
Zhang et al.	2018	Lymphocyte to monocyte ratio, neutrophil to lymphocyte ratio	EGFR TKI	[144]
Yang et al.	2018	MiR-10b	Chemotherapy	[145]
Chea et al.	2019	TMB, MAF	Checkpoint inhibitors	[146]

Cytokeratin 18: CK18, Cytokeratin 19: CK19, Carcinoembryonic antigen: CEA, MET proto-oncogene: c-MET, Granzyme B: sGranB, Kirsten Rat Sarcoma: KRAS, cell-free DNA: cfDNA, Neuron specific enolase: NSE, Thymidine kinase: TK, Cytokeratin-19 fragments: CYFRA, C-reactive protein: CRP, Transforming Growth Factor-alpha: TGF alpha, Epidermal Growth Factor Receptor: EGFR, molecular mutational burden: MMB, circulating tumor cells: CTC, Bcl-2-like protein: BIM, M1 subunit of ribonucleotide reductase: RRM1, excision repair cross-complementation 1 gene: ERCC1, breast cancer susceptibility gene 1: BRCA1, pan-cytokeratin: pan-CK, anaplastic lymphoma kinase: ALK, osteopontin: OPN, triple-positive activated lymphocytes: TrPAL, heregulin: HRG, programmed cell death 1: PD-1, dendritic cells: DC, tumor mutational burden: TMB, mutant allele frequency: MAF.

Studies were classified according to the evidence framework as proposed by Rao et al. [147]. Six different evidence levels were identified, ranging from retrospective non-case/control studies, to post-hoc biomarker correlative analysis of a prospective randomized clinical trial. The majority of studies were classified as III B, a prospective observational study (*n* = 38.59%). Other classes included I D post-hoc biomarker correlative analysis of a prospective randomized controlled trial (*n* = 6.10%), II B prospective biomarker driven non-randomized clinical trial (*n* = 5.8%), II C a post-hoc biomarker correlative analysis of non-randomized clinical trial (*n* = 3.5%), III C a case-control study (*n* = 1.2%), III E a retrospective non-case-control study (*n* = 11.17%)(Figure 3.).

Evidence levels of studies in catagory: predictive value

Description of evidence levels:

I D: Post-hoc biomarker correlative analysis of a prospective randomized clinical trial

II B: Prospective biomarker driven non-randomized clinical trial

II C: Post-hoc biomarker correlative analysis of a non-randomized clinical trial

III B: Prospective observational study

III C: Case-control study

III E: Retrospective non-case/control study

Figure 3. Evidence levels of studies in category: predictive value. Evidence levels identified in studies classified as describing the predictive value of liquid biopsies.

2.3. Evidence of Predictive Value of a Biomarker Based on LBs

A total of 64 studies were identified reporting on the predictive value of a LB to guide a specific treatment. The included studies tested 67 different analytes for 24 different treatments or treatment combinations. EGFR mutations (including exon 19 deletion, T790M, and L858R) were described in 18 studies (28%), while 10 studies described the predictive value of CTC count (16%). Nineteen studies (30%) evaluated the LB to indicate chemotherapy, either a single, doublet or combination therapy. Targeted therapy agents (e.g., erlotinib, gefitinib, icotinib, afatinib) were subject of evaluation in 31 (48%) of the identified studies, while immunotherapy agents (e.g., patritumab, nivolumab, bevacizumab) were described by 9 (14%) studies.

2.4. Evidence Level Per Analyte and Therapy

Figure 4 depicts the analytes and therapies described in the different studies, stratified according to the evidence level. As previously shown in Figure 3, the majority of studies were classified as class III B, a prospective observational study. In this category, CTC count, EGFR mutations, and cfDNA level were identified most frequently. While CTC count and cfDNA level were researched in combination with several types of treatments including chemotherapy, immunotherapy, and targeted therapies. EGFR mutations in this category were exclusively researched in combination with targeted therapies. Looking at the class with the highest evidence level (I D, post-hoc biomarker correlative analysis of a prospective randomized clinical trial), we see that majority of studies in this class evaluate EGFR mutations including exon 19 deletion and L858R.

3. Discussion

Our results provide a clear overview of the current developments within the field and the potential clinical utility of the biomarkers identified in our study. More specific, our findings suggest that in the diverse and active landscape of biomarker research, many studies focus on EGFR mutation detection in LBs. The review also concludes that the EGFR is a valid marker in comparison to tissue analysis. It was shown that using these LB markers it is possible to indicate the treatment likely to be effective.
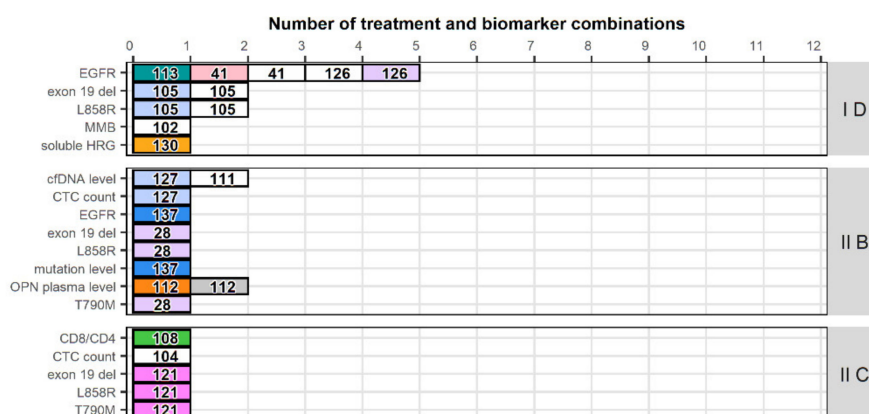
Figure 4. *Cont.*
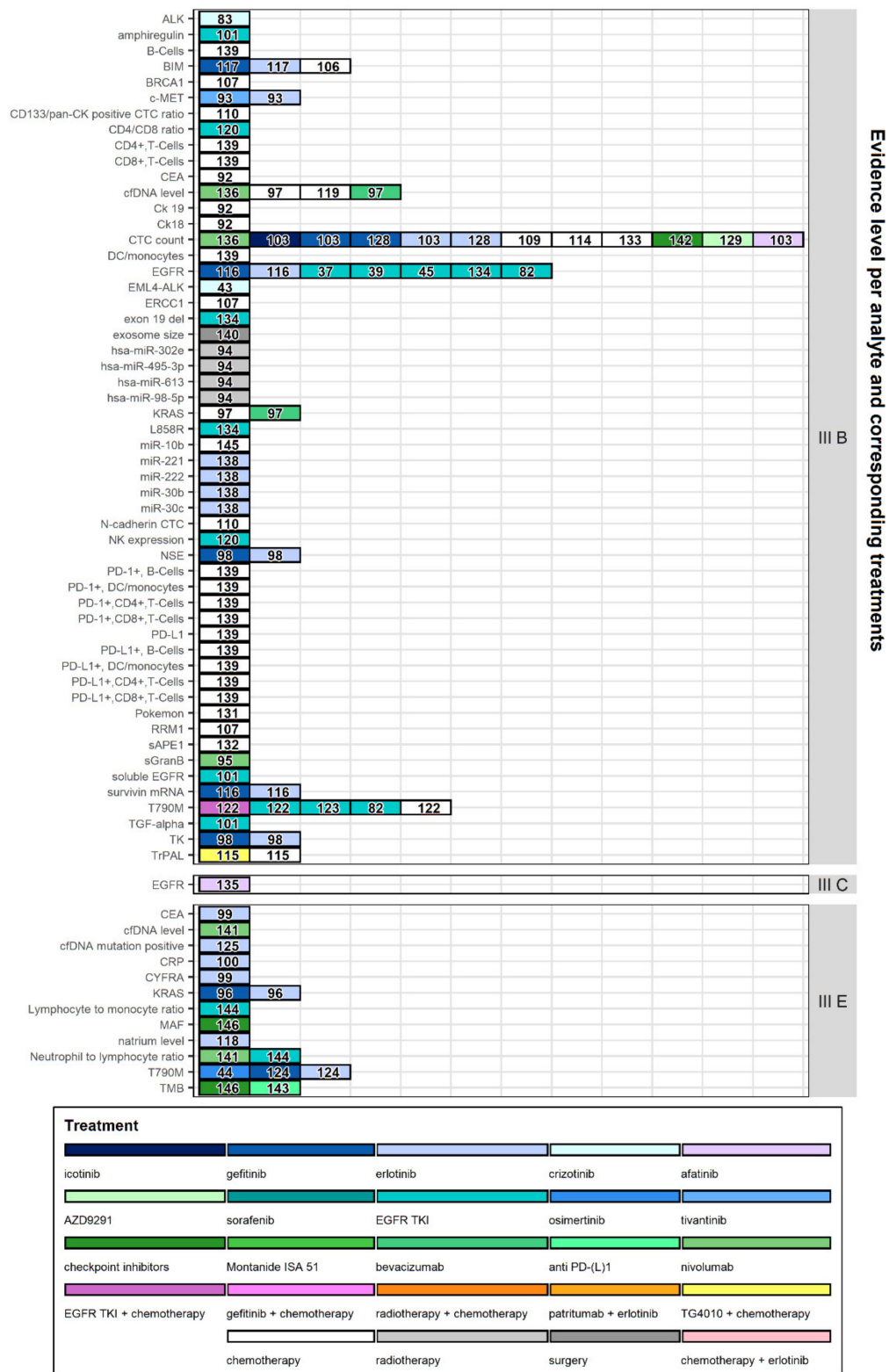
Figure 4. Evidence level per analyte and with reference to the companion therapies. The data is presented for each evidence level (levels I-III; right y-axis) and studies were categorized based on the biomarker of interest. Different colors are used to indicate the treatment these biomarkers were compared to and numbers within the bars refer to the corresponding reference number. The evidence levels were adopted from Rao et al. [147]. I D: Post-hoc biomarker correlative analysis of a prospective randomized clinical trial. II B: Prospective biomarker driven non-randomized clinical trial. II C: Post-hoc biomarker correlative analysis of a non-randomized clinical trial. III B: Prospective observational study. III C: Case-control study. III E: Retrospective non-case/control study.

Results show a significant variety in reported sensitivity, specificity, and concordance values for LB results compared to matched tissue samples. The variation in results might be explained by differences in sample preparation, sample volume, used assay, previous lines of treatment prior to study inclusion, disease stage, amount of tumor shedding, and the number of patients included in the study. The difference in sensitivity and specificity of the platform used in mutation detection was also shown in a review by Li et al. [148]. In this review, the authors compared the performance of multiple platforms in the detection of T790M mutations. In a review, Kim et al. [149] reported on the sensitivity, specificity, and concordance rate in the detection of EGFR mutations. In this review, authors reported a variation in outcomes depending on the technology and the genomic mutation of interest. Variations in the sensitivity of mutation detection might indicate that at this moment, LBs are not ready to replace TBs in practice; however, LBs might be a good alternative in patients in whom a TB was deemed unfeasible. Moreover, as shown in Figure 2, there are studies reporting sensitivity values exceeding 90%, indicating that by selecting the correct analysis method and patient group, LBs might provide a satisfactory sensitivity for clinical applications. The variation in concordance and specificity might be an indication of tumor heterogeneity missed by TB and would indicate that there might be an added value of performing LBs alongside tissue analysis. In a report, the International Association for the Study of Lung Cancer recommended the use of LB techniques to detect EGFR mutations in treatment naïve patients. However, a negative result should be considered uninformative and should be followed by a TB [150]. Moreover, the dominant presence of studies reporting on the clinical validity of LBs in the detection of EGFR mutations found in this review is in line with the view of the International Association for the Study of Lung Cancer, and it is to be expected that the first role of LBs in the management of NSCLC will involve detection of EGFR mutations. Our results are potentially biased towards the evaluation of the validity of LBs in the detection of EGFR mutations. Considering the potential of LBs in the detection of acquired resistance to 1st and 2nd generation EGFR tyrosine kinase inhibitors (TKIs) attracted considerable attention; however, the introduction of osimertinib, a 3rd generation EGFR TKI, lessens the need for detection of the T790M resistance mechanism, for which the FDA approved the use of plasma ctDNA analysis. Moreover, current guidelines now recommend the use of osimertinib in the first-line setting, further reducing the need for the detection of acquired resistance to 1st and 2nd generation EGFR TKIs [151]. Although the necessity for LBs in the detection of T790M mutations was diminished by the introduction of osimertinib, a more comprehensive frame of reference seems appropriate, since only 12% to 45% of NSCLC patients present with EGFR mutations, depending on geography, histology, and smoking status [152,153]. While more driver mutations, targetable pathways, drugs, and companion diagnostics are being discovered [154]. Indicating that there is a lot of potential for LBs beyond the detection of EGFR mutations and resistance mechanisms to provide clinical benefit in the future. Looking at Figure 4 it becomes apparent that a lot of biomarkers are being investigated at this moment in relation to a large variety of treatments and treatment combinations. This indicates that this is an active field in which multiple research groups try to identify the most beneficial treatment for patients based on genomic mutations or other biomarkers identified by LBs. In this review, we looked at studies reporting on treatment outcomes based on biomarker analysis prior to initiation of the study related treatment. In a number of studies, patients did receive previous lines of treatment before study inclusion. Response monitoring could also be considered a predictive value of LBs; however, response monitoring was not taken into account in this review.

Currently, most targeted treatments requiring a companion diagnostic focused on tissue-based analyses for treatment selection, as indicated by Bernabé et al. [155] and also supported by the classification of studies according to their evidence level in this review (Figures 3 and 4). The preliminary nature of the evidence makes it difficult to access the clinical benefit of mutation detection using LBs since the beneficial effect of the treatment is unclear in tissue negative, plasma positive patients. Therefore, more studies should aim to include LB analysis in the study design to build on the currently available preliminary evidence. In our review, we found that 59% of identified

studies were of prospective observational nature, while only 10% of the identified studies reported on a randomized clinical trial with post-hoc biomarker analysis. Future directions towards implementation might include large registry studies, which include matched tissue and LB results, and repeated LB measurements to possibly evaluate the predictive value of a LB in response monitoring.

Like every review, this review has potential limitations. Despite the generally accepted problems of selection of studies, A more fundamental problem might be the decision not to report the specific methodology used in sample preparation and analysis. This was deliberately chosen as our focus was to review evidence levels for each of the analytes. However, it is acknowledged that specific analytic issues (such as DNA extraction) will potentially impact the clinical validity and predictive value. One of the reasons for this restriction, was that more than half of the included studies in the validity group did not provide detailed information regarding the applied methodology (e.g., DNA input quantity), referred back to previous work, only listed the test kits used, the authors stated that DNA purification or library preparation was performed according to manufacturers' instructions, or sample analysis was performed in an external laboratory. This lack of information makes it difficult to compare different test accuracies, even within biomarkers analyzed using similar methodologies (e.g., NGS or PCR). Second, TBs are regarded as the gold standard in determining the sensitivity, specificity, and concordance rate of LBs. In this review we did not collect information on the methods used to detect biomarkers in tissue samples, the accuracy of methodologies used in the analysis of tissue samples directly influences the accuracy of LB results, e.g., mutations missed in the analysis of tissue samples potentially lead to a reduction in the specificity and concordance rate of the LB analysis. However, it was expected that all studies included in this review applied generally accepted methods or used commercially available equipment in the analysis of tissue samples.

4. Materials and Methods

4.1. Eligibility Criteria

Studies included in this review could cover a wide range of LBs, but had to present results of either the clinical validity or predictive validity. Original full-text articles published in English were selected for review.

4.2. Search

A systematic literature search was performed in September 2019 using Scopus and PubMed databases to identify relevant studies published between 1 January 2014 and 1 September, 2019. The time span was selected to cover all recent developments in LB development while maintaining a amenable amount of search results. The search included the following keywords and allowed for different conjugations: NSCLC, non-small cell, ctDNA, microRNA (miRNAs), CTCs, extracellular vesicles, blood, and serum. The full search queries used to perform the literature search are depicted in Supplementary Materials I (S1). All article types were included in the initial search. This systematic review was conducted according to the Preferred Reporting Items for Systematic Reviews and Meta-Analysis (PRISMA) guidelines [156].

4.3. Study Selection

After removing duplicate records, a review protocol (Figure 1) was used towards the selection of relevant articles. Prior to conducting a full-text review, one author (F.v.D.) reviewed the title and abstract of all records to determine their relevance. Exclusion of records from full-text review was based on article type (e.g., review, letter to the editor, short communication, meta-analysis), cancer type (other than NSCLC), number of patients included in the study (<20), clinical utility (the absence of a relation between the biomarker and treatment outcome or a comparison between the LB results and matched tissue samples), and biomarker type (single nucleotide polymorphisms (SNPs) were excluded from this review). Inclusion criteria were checked in a fixed order (as depicted in Figure 1),

inclusion criteria were not mutually exclusive and exclusion of articles was based on the first unmet criteria. All reviewed abstracts were discussed with two co-authors (V.R. and H.K.) in case of doubt, until a consensus was made on the inclusion of the paper. The two co-authors independently reviewed 70 randomly selected abstracts (~ 4% of all records identified). Results were compared to check for disagreement between reviewers.

Articles were excluded if (1) the described study included less than 20 patients, (2) the intended use of the biomarker was not categorized in terms of being prognostic, predictive, or diagnostic, (3) the study did not report overall survival (OS), progression free survival (PFS), sensitivity, specificity, and/or concordance rate, (4) full English text was unavailable.

Studies were excluded if they only reported on a biomarker of interest that could be classified as an SNP. Reported sensitivity, specificity, and concordance were only extracted in case the study included >10 patients in whom the biomarker was detected in matched tissue samples. Thresholds were chosen to ensure a minimal evidence base.

4.4. Data Extraction

A full-text review was conducted on all records selected by title and abstract screening to determine the eligibility of the articles for data extraction.

Full-text articles were screened for relevant outcomes, including Overall Survival (in months or days, OS), Progression-Free Survival (PFS, in months or days), Sensitivity, Specificity, and Concordance rate (percentage of identical measurement outcomes).

Records included after full-text screening were classified into two categories, namely validity and predictive value. Articles describing a direct comparison between LB and tissue-based molecular analysis were categorized in the category validity. From these papers we extracted the sensitivity, specificity, and concordance rate. The sensitivity and specificity reflect the true positive and true negative rate, respectively. While the concordance rate should reflect the overlap between LB and TB outcomes. The category predictive value was assigned to articles describing differences in clinical outcomes from study treatments based on the presence of a biomarker detected by LB analysis.

4.5. Evidence Classification

To gain an insight into the stage of biomarker research, we classified the level of evidence for all articles included after full-text review and categorized as describing the predictive validity of a biomarker. For this purpose, the evidence framework as proposed by Rao et al. was adopted [147]. evidence levels were classified from level I A (high-quality meta-analysis) to level IV E (expert opinion). All records were classified by the first author (F.v.D.) and discussed with co-authors (V.R. and H.K.) in case of doubt. Records were classified according to the highest applicable evidence level.

4.6. Data Interpretation

Information provided by included studies was summarized to provide a comprehensible overview. Meaning, in studies classified as predictive all mentioned chemotherapy agents in single, doublet, and combination therapies were labeled as chemotherapy. The therapy of interest in the study was labeled as EGFR TKI in case the study included multiple comparable EGFR therapies, e.g., erlotinib and gefitinib without stratification of results based on the prescribed therapy. In studies describing the validity of a biomarker, the detailed description of the biomarker analysis method was reduced to the principal technique or method. The distribution and average of the reported sensitivity, specificity, and concordance values were estimated using a weighted approach based on the study size.

5. Conclusions

Current literature shows that the field is moving towards the use of LBs in the detection of EGFR mutations and the prescription of EGFR TKI inhibitors. Moreover, the first adoption of LBs in practice is expected to involve the detection of EGFR mutations as an addition to currently

employed TBs. The currently available evidence for most analytes is limited to observational studies, and the sensitivity, specificity, and concordance rates of LBs showed a strong variation between studies. Although the diagnostic accuracy of LB compared to TB results is not perfect, it should be noted that LBs might detect mutations missed in TBs, and further research is needed to evaluate the clinical benefit of adopting LBs in practice.

Supplementary Materials: The following are available online at http://www.mdpi.com/2072-6694/12/5/1120/s1,S1: Search queries used in the systematic literature review.

References

1. Global Cancer Observatory: Cancer Today; International Agency for Research on Cancer. Estimated Number of New Cases in 2018. Available online: http://gco.iarc.fr/ (accessed on 18 January 2019).
2. Global Cancer Observatory: Cancer Today; International Agency for Research on Cancer. Estimated Number of Deaths in 2018. Available online: http://gco.iarc.fr (accessed on 18 January 2019).
3. Organisation, N.C.C. *Guideline Non Small Cell Lung Carcinoma*; IKNL Oncoline: Utrecht, The Netherlands, 2015; Available online: www.oncoline.nl.
4. Pakkala, S.; Ramalingam, S.S. Personalized therapy for lung cancer: Striking a moving target. *JCI Insight* **2018**, *3*, 15. [CrossRef] [PubMed]
5. Cheng, X.; Chen, H. Tumor heterogeneity and resistance to EGFR-targeted therapy in advanced nonsmall cell lung cancer: Challenges and perspectives. *OncoTargets Ther.* **2014**, *7*, 1689–1704. [CrossRef] [PubMed]
6. Deng, Q.; Xie, B.; Wu, L.; Ji, X.; Li, C.; Feng, L.; Fang, Q.; Bao, Y.; Li, J.; Jin, S.; et al. Competitive evolution of NSCLC tumor clones and the drug resistance mechanism of first-generation EGFR-TKIs in Chinese NSCLC patients. *Heliyon* **2018**, *4*, e01031. [CrossRef] [PubMed]
7. Ricordel, C.; Friboulet, L.; Facchinetti, F.; Soria, J.C. Molecular mechanisms of acquired resistance to third-generation EGFR-TKIs in EGFR T790M-mutant lung cancer. *Ann. Oncol.* **2018**, *29*, i28–i37. [CrossRef] [PubMed]
8. Nosaki, K.; Satouchi, M.; Kurata, T.; Yoshida, T.; Okamoto, I.; Katakami, N.; Imamura, F.; Tanaka, K.; Yamane, Y.; Yamamoto, N.; et al. Re-biopsy status among non-small cell lung cancer patients in Japan: A retrospective study. *Lung Cancer* **2016**, *101*, 1–8. [CrossRef]
9. Uozu, S.; Imaizumi, K.; Yamaguchi, T.; Goto, Y.; Kawada, K.; Minezawa, T.; Okamura, T.; Akao, K.; Hayashi, M.; Isogai, S.; et al. Feasibility of tissue re-biopsy in non-small cell lung cancers resistant to previous epidermal growth factor receptor tyrosine kinase inhibitor therapies. *BMC Pulm. Med.* **2017**, *17*, 175. [CrossRef]
10. Zanwar, S.; Noronha, V.; Joshi, A.; Patil, V.M.; Chougule, A.; Kumar, R.; More, S.; Goud, S.; Janu, A.; Mahajan, A.; et al. Repeat biopsy in epidermal growth factor receptor mutation-positive nonsmall cell lung cancer: Feasibility, limitations, and clinical utility in Indian patients. *Indian J. Cancer* **2017**, *54*, 280–284. [CrossRef]
11. Ijzerman, M.J.; Berghuis, A.M.S.; De Bono, J.S.; Terstappen, L.W.M.M. Health economic impact of liquid biopsies in cancer management. *Expert Rev. Pharm. Outcomes Res.* **2018**, *18*, 593–599. [CrossRef]
12. Buder, A.; Tomuta, C.; Filipits, M. The potential of liquid biopsies. *Curr. Opin. Oncol.* **2016**, *28*, 130–134. [CrossRef]
13. Siravegna, G.; Marsoni, S.; Siena, S.; Bardelli, A. Integrating liquid biopsies into the management of cancer. *Nat. Rev. Clin. Oncol.* **2017**, *14*, 531–548. [CrossRef]
14. Alegre, E.; Fusco, J.P.; Restituto, P.; Salas-Benito, D.; Rodriguez-Ruiz, M.E.; Andueza, M.P.; Pajares, M.J.; Patino-Garcia, A.; Pio, R.; Lozano, M.D.; et al. Total and mutated EGFR quantification in cell-free DNA from

non-small cell lung cancer patients detects tumor heterogeneity and presents prognostic value. *Tumour Biol.* **2016**, *37*, 13687–13694. [CrossRef] [PubMed]

15. Arriola, E.; Paredes-Lario, A.; Garcia-Gomez, R.; Diz-Tain, P.; Constenla, M.; Garcia-Giron, C.; Marquez, G.; Reck, M.; Lopez-Vivanco, G. Comparison of plasma ctDNA and tissue/cytology-based techniques for the detection of EGFR mutation status in advanced NSCLC: Spanish data subset from ASSESS. *Clin. Transl. Oncol.* **2018**, *20*, 1261–1267. [CrossRef] [PubMed]

16. Balgkouranidou, I.; Chimonidou, M.; Milaki, G.; Tsarouxa, E.G.; Kakolyris, S.; Welch, D.R.; Georgoulias, V.; Lianidou, E.S. Breast cancer metastasis suppressor-1 promoter methylation in cell-free DNA provides prognostic information in non-small cell lung cancer. *Br. J. Cancer* **2014**, *110*, 2054–2062. [CrossRef] [PubMed]

17. Chai, X.; Ren, P.; Wei, B.; Ma, J.; Mai, L.; Cram, D.S.; Song, Y.; Guo, Y. A comparative study of EGFR oncogenic mutations in matching tissue and plasma samples from patients with advanced non-small cell lung carcinoma. *Clin. Chim. Acta* **2016**, *457*, 106–111. [CrossRef] [PubMed]

18. Chen, K.; Zhang, J.; Guan, T.; Yang, F.; Lou, F.; Chen, W.; Zhao, M.; Zhang, J.; Chen, S.; Wang, J. Comparison of plasma to tissue DNA mutations in surgical patients with non–small cell lung cancer. *J. Thorac. Cardiovasc. Surg.* **2017**, *154*, 1123–1131.e1122. [CrossRef] [PubMed]

19. 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*, 1–8. [CrossRef] [PubMed]

20. Cui, S.; Zhang, W.; Xiong, L.; Pan, F.; Niu, Y.; Chu, T.; Wang, H.; Zhao, Y.; Jiang, L. Use of capture-based next-generation sequencing to detect ALK fusion in plasma cell-free DNA of patients with non-small-cell lung cancer. *Oncotarget* **2017**, *8*, 2771–2780. [CrossRef]

21. Douillard, J.Y.; Ostoros, G.; Cobo, M.; Ciuleanu, T.; McCormack, R.; Webster, A.; Milenkova, T. First-line gefitinib in Caucasian EGFR mutation-positive NSCLC patients: A phase-IV, open-label, single-arm study. *Br. J. Cancer* **2014**, *110*, 55–62. [CrossRef]

22. Duan, H.; Lu, J.; Lu, T.; Gao, J.; Zhang, J.; Xu, Y.; Wang, M.; Wu, H.; Liang, Z.; Liu, T. Comparison of EGFR mutation status between plasma and tumor tissue in non-small cell lung cancer using the Scorpion ARMS method and the possible prognostic significance of plasma EGFR mutation status. *Int. J. Clin. Exp. Pathol.* **2015**, *8*, 13136–13145.

23. Guibert, N.; Delaunay, M.; Lusque, A.; Boubekeur, N.; Rouquette, I.; Clermont, E.; Mourlanette, J.; Gouin, S.; Dormoy, I.; Favre, G.; et al. PD-L1 expression in circulating tumor cells of advanced non-small cell lung cancer patients treated with nivolumab. *Lung Cancer* **2018**, *120*, 108–112. [CrossRef]

24. Guibert, N.; Hu, Y.; Feeney, N.; Kuang, Y.; Plagnol, V.; Jones, G.; Howarth, K.; Beeler, J.F.; Paweletz, C.P.; Oxnard, G.R. Amplicon-based next-generation sequencing of plasma cell-free DNA for detection of driver and resistance mutations in advanced non-small cell lung cancer. *Ann. Oncol.* **2018**, *29*, 1049–1055. [CrossRef] [PubMed]

25. Guibert, N.; Pradines, A.; Farella, M.; Casanova, A.; Gouin, S.; Keller, L.; Favre, G.; Mazieres, J. Monitoring KRAS mutations in circulating DNA and tumor cells using digital droplet PCR during treatment of KRAS-mutated lung adenocarcinoma. *Lung Cancer* **2016**, *100*, 1–4. [CrossRef] [PubMed]

26. Guo, N.; Lou, F.; Ma, Y.; Li, J.; Yang, B.; Chen, W.; Ye, H.; Zhang, J.B.; Zhao, M.Y.; Wu, W.J.; et al. Circulating tumor DNA detection in lung cancer patients before and after surgery. *Sci. Rep.* **2016**, *6*, 33519. [CrossRef] [PubMed]

27. Han, J.Y.; Choi, J.J.; Kim, J.Y.; Han, Y.L.; Lee, G.K. PNA clamping-assisted fluorescence melting curve analysis for detecting EGFR and KRAS mutations in the circulating tumor DNA of patients with advanced non-small cell lung cancer. *BMC Cancer* **2016**, *16*, 627. [CrossRef]

28. He, J.; Tan, W.; Tang, X.; Ma, J. Variations in EGFR ctDNA Correlates to the Clinical Efficacy of Afatinib in Non Small Cell Lung Cancer with Acquired Resistance. *Pathol. Oncol. Res.* **2017**, *23*, 307–315. [CrossRef]

29. Ilie, M.; Szafer-Glusman, E.; Hofman, V.; Chamorey, E.; Lalvee, S.; Selva, E.; Leroy, S.; Marquette, C.H.; Kowanetz, M.; Hedge, P.; et al. Detection of PD-L1 in circulating tumor cells and white blood cells from patients with advanced non-small-cell lung cancer. *Ann. Oncol.* **2018**, *29*, 193–199. [CrossRef]

30. Ilie, M.; Szafer-Glusman, E.; Hofman, V.; Long-Mira, E.; Suttmann, R.; Darbonne, W.; Butori, C.; Lalvee, S.; Fayada, J.; Selva, E.; et al. Expression of MET in circulating tumor cells correlates with expression in tumor tissue from advanced-stage lung cancer patients. *Oncotarget* **2017**, *8*, 26112–26121. [CrossRef]

31. 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]

32. Kasahara, N.; Kenmotsu, H.; Serizawa, M.; Umehara, R.; Ono, A.; Hisamatsu, Y.; Wakuda, K.; Omori, S.; Nakashima, K.; Taira, T.; et al. Plasma epidermal growth factor receptor mutation testing with a chip-based digital PCR system in patients with advanced non-small cell lung cancer. *Lung Cancer* **2017**, *106*, 138–144. [CrossRef]

33. Krug, A.K.; Enderle, D.; Karlovich, C.; Priewasser, T.; Bentink, S.; Spiel, A.; Brinkmann, K.; Emenegger, J.; Grimm, D.G.; Castellanos-Rizaldos, E.; et al. Improved EGFR mutation detection using combined exosomal RNA and circulating tumor DNA in NSCLC patient plasma. *Ann. Oncol.* **2018**, *29*, 700–706. [CrossRef]

34. Lam, D.C.L.; Tam, T.C.C.; Lau, K.M.K.; Wong, W.M.; Hui, C.K.M.; Lam, J.C.M.; Wang, J.K.L.; Lui, M.M.S.; Ho, J.C.M.; Ip, M.S.M. Plasma EGFR mutation detection associated with survival outcomes in advanced-stage lung cancer. *Clin. Lung Cancer* **2015**, *16*, 507–513. [CrossRef]

35. 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]

36. Li, F.; Huang, J.; Ji, D.; Meng, Q.; Wang, C.; Chen, S.; Wang, X.; Zhu, Z.; Jiang, C.; Shi, Y.; et al. Utility of urinary circulating tumor DNA for EGFR mutation detection in different stages of non-small cell lung cancer patients. *Clin. Transl. Oncol.* **2017**, *19*, 1283–1291. [CrossRef]

37. Li, X.; Ren, R.; Ren, S.; Chen, X.; Cai, W.; Zhou, F.; Zhang, Y.; Su, C.; Zhao, C.; Li, J.; et al. Peripheral blood for epidermal growth factor receptor mutati detection in non-small cell lung cancer patients. *Transl. Oncol.* **2014**, *7*, 341–348. [CrossRef] [PubMed]

38. Liu, L.; Liu, H.; Shao, D.; Liu, Z.; Wang, J.; Deng, Q.; Tang, H.; Yang, H.; Zhang, Y.; Qiu, Y.; et al. Development and clinical validation of a circulating tumor DNA test for the identification of clinically actionable mutations in nonsmall cell lung cancer. *Genes Chromosomes Cancer* **2018**, *57*, 211–220. [CrossRef] [PubMed]

39. Ma, M.; Shi, C.; Qian, J.; Teng, J.; Zhong, H.; Han, B. Comparison of plasma and tissue samples in epidermal growth factor receptor mutation by ARMS in advanced non-small cell lung cancer. *Gene* **2016**, *592*, 58–64. [CrossRef] [PubMed]

40. 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]

41. Mok, T.; Wu, Y.L.; Lee, J.S.; Yu, C.J.; Sriuranpong, V.; Sandoval-Tan, J.; Ladrera, G.; Thongprasert, S.; Srimuninnimit, V.; Liao, M.; et al. Detection and dynamic changes of EGFR mutations from circulating tumor DNA as a predictor of survival outcomes in NSCLC Patients treated with first-line intercalated erlotinib and chemotherapy. *Clin. Cancer Res.* **2015**, *21*, 3196–3203. [CrossRef]

42. Muller, J.N.; Falk, M.; Talwar, J.; Neemann, N.; Mariotti, E.; Bertrand, M.; Zacherle, T.; Lakis, S.; Menon, R.; Gloeckner, C.; et al. Concordance between Comprehensive Cancer Genome Profiling in Plasma and Tumor Specimens. *J. Thorac. Oncol.* **2017**, *12*, 1503–1511. [CrossRef]

43. Nilsson, R.J.; Karachaliou, N.; Berenguer, J.; Gimenez-Capitan, A.; Schellen, P.; Teixido, C.; Tannous, J.; Kuiper, J.L.; Drees, E.; Grabowska, M.; et al. Rearranged EML4-ALK fusion transcripts sequester in circulating blood platelets and enable blood-based crizotinib response monitoring in non-small-cell lung cancer. *Oncotarget* **2016**, *7*, 1066–1075. [CrossRef]

44. 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] [PubMed]

45. Que, D.; Xiao, H.; Zhao, B.; Zhang, X.; Wang, Q.; Xiao, H.; Wang, G. EGFR mutation status in plasma and tumor tissues in non-small cell lung cancer serves as a predictor of response to EGFR-TKI treatment. *Cancer Biol. Ther.* **2016**, *17*, 320–327. [CrossRef] [PubMed]

46. Reck, M.; Hagiwara, K.; Han, B.; Tjulandin, S.; Grohe, C.; Yokoi, T.; Morabito, A.; Novello, S.; Arriola, E.; Molinier, O.; et al. ctDNA Determination of EGFR Mutation Status in European and Japanese Patients with Advanced NSCLC: The ASSESS Study. *J. Thorac. Oncol.* **2016**, *11*, 1682–1689. [CrossRef] [PubMed]

47. Reckamp, K.L.; Melnikova, V.O.; Karlovich, C.; Sequist, L.V.; Camidge, D.R.; Wakelee, H.; Perol, M.; Oxnard, G.R.; Kosco, K.; Croucher, P.; et al. A highly sensitive and quantitative test platform for detection of NSCLC EGFR mutations in urine and plasma. *J. Thorac. Oncol.* **2016**, *11*, 1690–1700. [CrossRef] [PubMed]

48. 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]

49. Schwaederle, M.C.; Patel, S.P.; Husain, H.; Ikeda, M.; Lanman, R.B.; Banks, K.C.; Talasaz, A.; Bazhenova, L.; Kurzrock, R. Utility of Genomic Assessment of Blood-Derived Circulating Tumor DNA (ctDNA) in Patients with Advanced Lung Adenocarcinoma. *Clin. Cancer Res.* **2017**, *23*, 5101–5111. [CrossRef]

50. Shi, C.; Zheng, Y.; Li, Y.; Sun, H.; Liu, S. Association between clinical characteristics and the diagnostic accuracy of circulating single-molecule amplification and resequencing technology on detection epidermal growth factor receptor mutation status in plasma of lung adenocarcinoma. *J. Clin. Lab. Anal.* **2018**, *32*, e22271. [CrossRef]

51. Sim, W.C.; Loh, C.H.; Toh, G.L.X.; Lim, C.W.; Chopra, A.; Chang, A.Y.C.; Goh, L.L. Non-invasive detection of actionable mutations in advanced non-small-cell lung cancer using targeted sequencing of circulating tumor DNA. *Lung Cancer* **2018**, *124*, 154–159. [CrossRef]

52. 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]

53. Sung, J.S.; Chong, H.Y.; Kwon, N.J.; Kim, H.M.; Lee, J.W.; Kim, B.; Lee, S.B.; Park, C.W.; Choi, J.Y.; Chang, W.J.; et al. Detection of somatic variants and EGFR mutations in cell-free DNA from non-small cell lung cancer patients by ultra-deep sequencing using the ion ampliseq cancer hotspot panel and droplet digital polymerase chain reaction. *Oncotarget* **2017**, *8*, 106901–106912. [CrossRef]

54. 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]

55. 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] [PubMed]

56. Uchida, J.; Kato, K.; Kukita, Y.; Kumagai, T.; Nishino, K.; Daga, H.; Nagatomo, I.; Inoue, T.; Kimura, M.; Oba, S.; et al. Diagnostic accuracy of noninvasive genotyping of EGFR in lung cancer patients by deep sequencing of plasma cell-free DNA. *Clin. Chem.* **2015**, *61*, 1191–1196. [CrossRef] [PubMed]

57. Vazquez, S.; Casal, J.; Afonso Afonso, F.J.; Firvida, J.L.; Santome, L.; Baron, F.; Lazaro, M.; Pena, C.; Amenedo, M.; Abdulkader, I.; et al. EGFR testing and clinical management of advanced NSCLC: A Galician Lung Cancer Group study (GGCP 048-10). *Cancer Manag. Res.* **2016**, *8*, 11–20. [CrossRef]

58. Wan, R.; Wang, Z.; Lee, J.J.; Wang, S.; Li, Q.; Tang, F.; Wang, J.; Sun, Y.; Bai, H.; Wang, D.; et al. Comprehensive Analysis of the Discordance of EGFR Mutation Status between Tumor Tissues and Matched Circulating Tumor DNA in Advanced Non–Small Cell Lung Cancer. *J. Thorac. Oncol.* **2017**, *12*, 1376–1387. [CrossRef]

59. Wang, S.; Han, X.; Hu, X.; Wang, X.; Zhao, L.; Tang, L.; Feng, Y.; Wu, D.; Sun, Y.; Shi, Y. Clinical significance of pretreatment plasma biomarkers in advanced non-small cell lung cancer patients. *Clin. Chim. Acta* **2014**, *430*, 63–70. [CrossRef]

60. Wang, Y.; Tian, P.W.; Wang, W.Y.; Wang, K.; Zhang, Z.; Chen, B.J.; He, Y.Q.; Li, L.; Liu, H.; Chuai, S.; et al. Noninvasive genotyping and monitoring of anaplastic lymphoma kinase (ALK) rearranged non-small cell lung cancer by capture-based next-generation sequencing. *Oncotarget* **2016**, *7*, 65208–65217. [CrossRef]

61. Watanabe, K.; Fukuhara, T.; Tsukita, Y.; Morita, M.; Suzuki, A.; Tanaka, N.; Terasaki, H.; Nukiwa, T.; Maemondo, M. EGFR Mutation Analysis of Circulating Tumor DNA Using an Improved PNA-LNA PCR Clamp Method. *Can. Respir. J.* **2016**, *1–7*. [CrossRef]

62. Wei, F.; Strom, C.M.; Cheng, J.; Lin, C.C.; Hsu, C.Y.; Soo Hoo, G.W.; Chia, D.; Kim, Y.; Li, F.; Elashoff, D.; et al. Electric Field–Induced Release and Measurement Liquid Biopsy for Noninvasive Early Lung Cancer Assessment. *J. Mol. Diagn.* **2018**, *20*, 738–742. [CrossRef]

63. Wei, Z.; Wang, W.; Shu, Z.; Zhou, X.; Zhang, Y. Correlation between circulating tumor dna levels and response to tyrosine kinase inhibitors (TKI) treatment in non-small cell lung cancer. *Med Sci. Monit.* **2017**, *23*, 3627–3634. [CrossRef]

64. Wu, Y.L.; Lee, V.; Liam, C.K.; Lu, S.; Park, K.; Srimuninnimit, V.; Wang, J.; Zhou, C.; Appius, A.; Button, P.; et al. Clinical utility of a blood-based EGFR mutation test in patients receiving first-line erlotinib therapy in the ENSURE, FASTACT-2, and ASPIRATION studies. *Lung Cancer* **2018**, *126*, 1–8. [CrossRef] [PubMed]

65. Wu, Y.L.; Tong, R.Z.; Zhang, Y.; Hu, B.B.; Zheng, K.; Ding, Z.Y.; Peng, F.; Gong, Y.L.; Liu, Y.M.; Lu, Y. Conventional real-time PCR-based detection of T790M using tumor tissue or blood in patients with EGFR TKI-resistant NSCLC. *OncoTargets Ther.* **2017**, *10*, 3307–3312. [CrossRef] [PubMed]

66. Xu, S.; Lou, F.; Wu, Y.; Sun, D.Q.; Zhang, J.B.; Chen, W.; Ye, H.; Liu, J.H.; Wei, S.; Zhao, M.Y.; et al. Circulating tumor DNA identified by targeted sequencing in advanced-stage non-small cell lung cancer patients. *Cancer Lett.* **2016**, *370*, 324–331. [CrossRef] [PubMed]

67. Yang, Y.; Shen, X.; Li, R.; Shen, J.; Zhang, H.; Yu, L.; Liu, B.; Wang, L. The detection and significance of EGFR and BRAF in cell-free DNA of peripheral blood in NSCLC. *Oncotarget* **2017**, *8*, 49773–49782. [CrossRef] [PubMed]

68. Yao, Y.; Liu, J.; Li, L.; Yuan, Y.; Nan, K.; Wu, X.; Zhang, Z.; Wu, Y.; Li, X.; Zhu, J.; et al. Detection of circulating tumor DNA in patients with advanced non-small cell lung cancer. *Oncotarget* **2017**, *8*, 2130–2140. [CrossRef]

69. Yu, H.; Liu, M.; Qiu, H.; Yang, K. Urinary and Plasma Cell-Free DNA Comparison for Lung Cancer Patients Treated With Epidermal Growth Factor Receptor-Thyroxine Kinase Inhibitors. *Am. J. Med. Sci.* **2019**, *357*, 29–36. [CrossRef]

70. Yu, Q.; Huang, F.; Zhang, M.; Ji, H.; Wu, S.; Zhao, Y.; Zhang, C.; Wu, J.; Wang, B.; Pan, B.; et al. Multiplex picoliter-droplet digital PCR for quantitative assessment of EGFR mutations in circulating cell-free DNA derived from advanced non-small cell lung cancer patients. *Mol. Med. Rep.* **2017**, *16*, 1157–1166. [CrossRef]

71. Zhang, H.; He, B.; Cui, J.; Zhao, M.; Zhang, Z. Comparison of circulating DNA from plasma and urine for EGFR mutations in NSCLC patients. *Cancer Biomark.* **2018**, *23*, 427–436. [CrossRef]

72. Zhang, Y.; Xu, Y.; Zhong, W.; Zhao, J.; Chen, M.; Zhang, L.; Li, L.; Wang, M. Total DNA input is a crucial determinant of the sensitivity of plasma cell-free DNA EGFR mutation detection using droplet digital PCR. *Oncotarget* **2017**, *8*, 5861–5873. [CrossRef]

73. Zheng, D.; Ye, X.; Zhang, M.Z.; Sun, Y.; Wang, J.Y.; Ni, J.; Zhang, H.P.; Zhang, L.; Luo, J.; Zhang, J.; et al. Plasma EGFR T790M ctDNA status is associated with clinical outcome in advanced NSCLC patients with acquired EGFR-TKI resistance. *Sci. Rep.* **2016**, *6*. [CrossRef]

74. Zhu, G.; Ye, X.; Dong, Z.; Lu, Y.C.; Sun, Y.; Liu, Y.; McCormack, R.; Gu, Y.; Liu, X. Highly sensitive droplet digital PCR method for detection of EGFR-activating mutations in plasma cell-free DNA from patients with advanced non-small cell lung cancer. *J. Mol. Diagn.* **2015**, *17*, 265–272. [CrossRef] [PubMed]

75. Zhu, Y.J.; Zhang, H.B.; Liu, Y.H.; Zhang, F.L.; Zhu, Y.Z.; Li, Y.; Bai, J.P.; Liu, L.R.; Qu, Y.C.; Qu, X.; et al. Quantitative cell-free circulating EGFR mutation concentration is correlated with tumor burden in advanced NSCLC patients. *Lung Cancer* **2017**, *109*, 124–127. [CrossRef] [PubMed]

76. Zhu, Y.J.; Zhang, H.B.; Liu, Y.H.; Zhu, Y.Z.; Chen, J.; Li, Y.; Bai, J.P.; Liu, L.R.; Qu, Y.C.; Qu, X.; et al. Association of mutant EGFR L858R and exon 19 concentration in circulating cell-free DNA using droplet digital PCR with response to EGFR-TKIs in NSCLC. *Oncol. Lett.* **2017**, *14*, 2537–2579. [CrossRef] [PubMed]

77. Chen, Y.L.; Huang, W.C.; Lin, F.M.; Hsieh, H.B.; Hsieh, C.H.; Hsieh, R.K.; Chen, K.W.; Yen, M.H.; Lee, J.; Su, S.; et al. Novel circulating tumor cell-based blood test for the assessment of PD-L1 protein expression in treatment-naive, newly diagnosed patients with non-small cell lung cancer. *Cancer Immunol. Immunother. CII* **2019**, *68*, 1087–1094. [CrossRef]

78. Ding, P.N.; Becker, T.M.; Bray, V.J.; Chua, W.; Ma, Y.F.; Lynch, D.; Po, J.; Luk, A.W.S.; Caixeiro, N.; de Souza, P.; et al. The predictive and prognostic significance of liquid biopsy in advanced epidermal growth factor receptor-mutated non-small cell lung cancer: A prospective study. *Lung Cancer* **2019**, *134*, 187–193. [CrossRef]

79. Garcia, J.; Wozny, A.S.; Geiguer, F.; Delherme, A.; Barthelemy, D.; Merle, P.; Tissot, C.; Jones, F.S.; Johnson, C.; Xing, X.; et al. Profiling of circulating tumor DNA in plasma of non-small cell lung cancer patients, monitoring of epidermal growth factor receptor p.T790M mutated allelic fraction using beads, emulsion, amplification, and magnetics companion assay and evaluation in future application in mimicking circulating tumor cells. *Cancer Med.* **2019**, *8*, 3685–3697. [CrossRef]

80. He, W.; Xu, D.; Wang, Z.; Wu, H.; Xiang, X.; Tang, B.; Jiang, W.; Cui, Y.; Wang, H.; Jiang, N.; et al. Three-dimensional nanostructured substrates enable dynamic detection of ALK-rearrangement in circulating tumor cells from treatment-naive patients with stage III/IV lung adenocarcinoma. *J. Transl. Med.* **2019**, *17*, 32. [CrossRef]

81. Li, B.T.; Janku, F.; Jung, B.; Hou, C.; Madwani, K.; Alden, R.; Razavi, P.; Reis-Filho, J.S.; Shen, R.; Isbell, J.M.; et al. Ultra-deep next-generation sequencing of plasma cell-free DNA in patients with advanced lung cancers: Results from the actionable genome consortium. *Ann. Oncol.* **2019**, *30*, 597–603. [CrossRef]

82. O'Kane, G.M.; Liu, G.; Stockley, T.L.; Shabir, M.; Zhang, T.; Law, J.H.; Le, L.W.; Sacher, A.; Shepherd, F.A.; Bradbury, P.A.; et al. The presence and variant allele fraction of EGFR mutations in ctDNA and development of resistance. *Lung Cancer* **2019**, *131*, 86–89. [CrossRef]

83. Park, C.K.; Kim, J.E.; Kim, M.S.; Kho, B.G.; Park, H.Y.; Kim, T.O.; Shin, H.J.; Cho, H.J.; Choi, Y.D.; Oh, I.J.; et al. Feasibility of liquid biopsy using plasma and platelets for detection of anaplastic lymphoma kinase rearrangements in non-small cell lung cancer. *J. Cancer Res. Clin. Oncol.* **2019**, *145*, 2071–2082. [CrossRef]

84. Soria-Comes, T.; Palomar-Abril, V.; Ureste, M.M.; Guerola, M.T.; Maiques, I.C.M. Real-World Data of the Correlation between EGFR Determination by Liquid Biopsy in Non-squamous Non-small Cell Lung Cancer (NSCLC) and the EGFR Profile in Tumor Biopsy. *Pathol. Oncol. Res.* **2019**, 1–7. [CrossRef]

85. Usui, K.; Yokoyama, T.; Naka, G.; Ishida, H.; Kishi, K.; Uemura, K.; Ohashi, Y.; Kunitoh, H. Plasma ctDNA monitoring during epidermal growth factor receptor (EGFR)-Tyrosine kinase inhibitor treatment in patients with EGFR-mutant non-small cell lung cancer (JP-CLEAR trial). *Jpn. J. Clin. Oncol.* **2019**, *49*, 554–558. [CrossRef]

86. Wang, L.; Hu, X.; Guo, Q.; Huang, X.; Lin, C.H.; Chen, X.; Li, M.; Yao, Q.; Zhou, Q.; Wang, J.; et al. CLAmp-seq: A Novel Amplicon-Based NGS Assay with Concatemer Error Correction for Improved Detection of Actionable Mutations in Plasma cfDNA from Patients with NSCLC. *Small Methods* **2019**, *4*, 1900357. [CrossRef]

87. Yang, K.; Li, J.; Zhao, J.; Ren, P.; Wang, Z.; Wei, B.; Dong, B.; Sun, R.; Wang, X.; Groen, H.J.M.; et al. Developing Ultrasensitive Library-Aliquot-Based Droplet Digital PCR for Detecting T790M in Plasma-Circulating Tumor DNA of Non-small-Cell-Lung-Cancer Patients. *Anal. Chem.* **2018**, *90*, 11203–11209. [CrossRef] [PubMed]

88. Ye, Y.; Luo, Z.; Shi, D. Use of cell free DNA as a prognostic biomarker in non-small cell lung cancer patients with bone metastasis. *Int. J. Biol. Markers* **2019**, *34*, 381–388. [CrossRef] [PubMed]

89. Zhang, M.; Li, L.; Wang, A.; Zhang, L.; Xu, J.; Liu, H. The clinical significance of detection of EGFR mutation in peripheral blood of patients with advanced non-small cell lung cancer. *Acta Med. Mediterr.* **2019**, *35*, 333–338. [CrossRef]

90. Zhang, S.; Chen, Z.; Huang, C.; Ding, C.; Li, C.; Chen, J.; Zhao, J.; Miao, L. Ultrasensitive and quantitative detection of: EGFR mutations in plasma samples from patients with non-small-cell lung cancer using a dual PNA clamping-mediated LNA-PNA PCR clamp. *Analyst* **2019**, *144*, 1718–1724. [CrossRef]

91. Yoshida, H.; Kim, Y.H.; Ozasa, H.; Nagai, H.; Sakamori, Y.; Tsuji, T.; Nomizo, T.; Funazo, T.; Yasuda, Y.; Hirai, T. EGFR T790M detection in circulating tumor DNA from non-small cell lung cancer patients using the PNA-LNA clamp method. *Anticancer Res.* **2017**, *37*, 2721–2725. [CrossRef]

92. Arrieta, O.; Pineda, B.; Muniz-Hernandez, S.; Flores, D.; Ordonez, G.; Borbolla-Escoboza, J.R.; Orta, D. Molecular detection and prognostic value of epithelial markers mRNA expression in peripheral blood of advanced non-small cell lung cancer patients. *Cancer Biomark. Sect. A Dis. Markers* **2014**, *14*, 215–223. [CrossRef]

93. Azuma, K.; Hirashima, T.; Yamamoto, N.; Okamoto, I.; Takahashi, T.; Nishio, M.; Hirata, T.; Kubota, K.; Kasahara, K.; Hida, T.; et al. Phase II study of erlotinib plus tivantinib (ARQ 197) in patients with locally advanced or metastatic EGFR mutation-positive non-small-cell lung cancer just after progression on EGFR-TKI, gefitinib or erlotinib. *ESMO Open* **2016**, *1*, e000063. [CrossRef]

94. Chen, X.; Xu, Y.; Liao, X.; Liao, R.; Zhang, L.; Niu, K.; Li, T.; Li, D.; Chen, Z.; Duan, Y.; et al. Plasma miRNAs in predicting radiosensitivity in non-small cell lung cancer. *Tumor Biol.* **2016**, *37*, 11927–11936. [CrossRef] [PubMed]

95. Costantini, A.; Julie, C.; Dumenil, C.; Helias-Rodzewicz, Z.; Tisserand, J.; Dumoulin, J.; Giraud, V.; Labrune, S.; Chinet, T.; Emile, J.F.; et al. Predictive role of plasmatic biomarkers in advanced non-small cell lung cancer treated by nivolumab. *Oncoimmunology* **2018**, *7*, e1452581. [CrossRef] [PubMed]

96. 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] [PubMed]

97. Dowler Nygaard, A.; Spindler, K.L.; Pallisgaard, N.; Andersen, R.F.; Jakobsen, A. Levels of cell-free DNA and plasma KRAS during treatment of advanced NSCLC. *Oncol. Rep.* **2014**, *31*, 969–974. [CrossRef]

98. Fiala, O.; Pesek, M.; Finek, J.; Benesova, L.; Minarik, M.; Bortlicek, Z.; Topolcan, O. The role of neuron-specific enolase (NSE) and thymidine kinase (TK) levels in prediction of efficacy ofEGFR-TKIs in patients with advanced-stage NSCLC [corrected]. *Anticancer Res.* **2014**, *34*, 5193–5198.

99. Fiala, O.; Pesek, M.; Finek, J.; Benesova, L.; Minarik, M.; Bortlicek, Z.; Topolcan, O. Predictive role of CEA and CYFRA 21-1 in patients with advanced-stage NSCLC treated with erlotinib. *Anticancer Res.* **2014**, *34*, 3205–3210.

100. Fiala, O.; Pesek, M.; Finek, J.; Topolcan, O.; Racek, J.; Minarik, M.; Benesova, L.; Bortlicek, Z.; Poprach, A.; Buchler, T. High serum level of C-reactive protein is associated with worse outcome of patients with advanced-stage NSCLC treated with erlotinib. *Tumour Biol.* **2015**, *36*, 9215–9222. [CrossRef]

101. Haghgoo, S.M.; Khosravi, A.; Mortaz, E.; Pourabdollah-Toutkaboni, M.; Seifi, S.; Sabour, S.; Allameh, A. Prognostic value of rare and complex mutations in EGFR and serum levels of soluble EGFR and its ligands in non-small cell lung carcinoma patients. *Clin. Biochem.* **2017**, *50*, 293–300. [CrossRef]

102. Jiang, T.; Li, X.; Wang, J.; Su, C.; Han, W.; Zhao, C.; Wu, F.; Gao, G.; Li, W.; Chen, X.; et al. Mutational Landscape of cfDNA Identifies Distinct Molecular Features Associated With Therapeutic Response to First-Line Platinum-Based Doublet Chemotherapy in Patients with Advanced NSCLC. *Theranostics* **2017**, *7*, 4753–4762. [CrossRef]

103. Jiang, T.; Zhao, J.; Zhao, C.; Li, X.; Shen, J.; Zhou, J.; Ren, S.; Su, C.; Zhou, C.; O'Brien, M. Dynamic Monitoring and Predictive Value of Circulating Tumor Cells in EGFR-Mutated Advanced Non-Small-Cell Lung Cancer Patients Treated With First-Line EGFR Tyrosine Kinase Inhibitors. *Clin. Lung Cancer* **2018**, *20*, 124–133. [CrossRef]

104. Juan, O.; Vidal, J.; Gisbert, R.; Munoz, J.; Macia, S.; Gomez-Codina, J. Prognostic significance of circulating tumor cells in advanced non-small cell lung cancer patients treated with docetaxel and gemcitabine. *Clin. Transl. Oncol.* **2014**, *16*, 637–643. [CrossRef] [PubMed]

105. Karachaliou, N.; Mayo-de las Casas, C.; Queralt, C.; De Aguirre, I.; Melloni, B.; Cardenal, F.; Garcia-Gomez, R.; Massuti, B.; Sanchez, J.M.; Porta, R.; et al. Association of EGFR L858R Mutation in Circulating Free DNA With Survival in the EURTAC Trial. *JAMA Oncol.* **2015**, *1*, 149–157. [CrossRef] [PubMed]

106. Lee, J.H.; Lin, Y.L.; Hsu, W.H.; Chen, H.Y.; Chang, Y.C.; Yu, C.J.; Shih, J.Y.; Lin, C.C.; Chen, K.Y.; Ho, C.C.; et al. Bcl-2-like protein 11 deletion polymorphism predicts survival in advanced non-small-cell lung cancer. *J. Thorac. Oncol.* **2014**, *9*, 1385–1392. [CrossRef]

107. Li, Y.; Wang, L.R.; Chen, J.; Lou, Y.; Zhang, G.B. First-line gemcitabine plus cisplatin in nonsmall cell lung cancer patients. *Dis. Markers* **2014**, *2014*, 960458. [CrossRef] [PubMed]

108. Mai, T.; Takano, A.; Suzuki, H.; Hirose, T.; Mori, T.; Teramoto, K.; Kiyotani, K.; Nakamura, Y.; Daigo, Y. Quantitative analysis and clonal characterization of T-cell receptor β repertoires in patients with advanced non-small cell lung cancer treated with cancer vaccine. *Oncol. Lett.* **2017**, *14*, 283–292. [CrossRef] [PubMed]

109. Muinelo-Romay, L.; Vieito, M.; Abalo, A.; Nocelo, M.A.; Baron, F.; Anido, U.; Brozos, E.; Vazquez, F.; Aguin, S.; Abal, M.; et al. Evaluation of Circulating Tumor Cells and Related Events as Prognostic Factors and Surrogate Biomarkers in Advanced NSCLC Patients Receiving First-Line Systemic Treatment. *Cancers (Basel)* **2014**, *6*, 153–165. [CrossRef] [PubMed]

110. Nel, I.; Jehn, U.; Gauler, T.; Hoffmann, A.C. Individual profiling of circulating tumor cell composition in patients with non-small cell lung cancer receiving platinum based treatment. *Transl. Lung Cancer Res.* **2014**, *3*, 100–106. [CrossRef] [PubMed]

111. Nygaard, A.D.; Holdgaard, P.C.; Spindler, K.L.; Pallisgaard, N.; Jakobsen, A. The correlation between cell-free DNA and tumour burden was estimated by PET/CT in patients with advanced NSCLC. *Br. J. Cancer* **2014**, *110*, 363–368. [CrossRef]

112. Ostheimer, C.; Gunther, S.; Bache, M.; Vordermark, D.; Multhoff, G. Dynamics of heat shock protein 70 serum levels as a predictor of clinical response in non-small-cell lung cancer and correlation with the hypoxia-related marker osteopontin. *Front. Immunol.* **2017**, *8*, 1305. [CrossRef]

113. Paz-Ares, L.; Hirsh, V.; Zhang, L.; de Marinis, F.; Yang, J.C.; Wakelee, H.A.; Seto, T.; Wu, Y.L.; Novello, S.; Juhasz, E.; et al. Monotherapy Administration of Sorafenib in Patients With Non-Small Cell Lung Cancer

(MISSION) Trial: A Phase III, Multicenter, Placebo-Controlled Trial of Sorafenib in Patients with Relapsed or Refractory Predominantly Nonsquamous Non-Small-Cell Lung Cancer after 2 or 3 Previous Treatment Regimens. *J. Thorac. Oncol.* **2015**, *10*, 1745–1753. [CrossRef]

114. Qi, Y.; Wang, W. Clinical significance of circulating tumor cells in squamous cell lung cancer patients. *Cancer Biomark.* **2017**, *18*, 161–167. [CrossRef] [PubMed]

115. Quoix, E.; Lena, H.; Losonczy, G.; Forget, F.; Chouaid, C.; Papai, Z.; Gervais, R.; Ottensmeier, C.; Szczesna, A.; Kazarnowicz, A.; et al. TG4010 immunotherapy and first-line chemotherapy for advanced non-small-cell lung cancer (TIME): Results from the phase 2b part of a randomised, double-blind, placebo-controlled, phase 2b/3 trial. *Lancet Oncol.* **2016**, *17*, 212–223. [CrossRef]

116. Shi, W.L.; Li, J.; Bao, Q.L.; Wu, J.N.; Ge, L.P.; Zhu, L.R.; Wang, Y.; Zhu, W.F. Survivin mRNA expression in blood as a predictor of the response to EGFR-tyrosine kinase inhibitors and prognosis in patients with non-small cell lung cancer. *Med. Oncol.* **2014**, *31*, 893. [CrossRef] [PubMed]

117. Sun, S.; Yu, H.; Wang, H.; Zhao, X.; Zhao, X.; Wu, X.; Qiao, J.; Chang, J.; Wang, J. Exploratory cohort study and meta-analysis of BIM deletion polymorphism in patients with epidermal growth factor receptor-mutant nonsmall-cell lung cancer treated with epidermal growth factor receptor tyrosine kinase inhibitors. *OncoTargets Ther.* **2017**, *10*, 1955–1967. [CrossRef]

118. Svaton, M.; Fiala, O.; Pesek, M.; Bruha, F.; Mukensnabl, P.; Racek, J.; Minarik, M.; Bortlicek, Z. Predictive and prognostic significance of sodium levels in patients with NSCLC treated by erlotinib. *Anticancer Res.* **2014**, *34*, 7461–7465.

119. Tissot, C.; Toffart, A.C.; Villar, S.; Souquet, P.J.; Merle, P.; Moro-Sibilot, D.; Pérol, M.; Zavadil, J.; Brambilla, C.; Olivier, M.; et al. Circulating free DNA concentration is an independent prognostic biomarker in lung cancer. *Eur. Respir. J.* **2015**, *46*, 1773–1780. [CrossRef]

120. Tu, C.; Zhu, Y.; Jiang, B.; He, W.; Jin, C. Correlation between circulating tumor cells EGFR expression and t cell subsets in advanced non-small cell lung cancer patients after tyrosine kinase inhibitor treatment. *Neoplasma* **2017**, *64*, 619–625. [CrossRef]

121. Uchibori, K.; Satouchi, M.; Sueoka-Aragane, N.; Urata, Y.; Sato, A.; Imamura, F.; Inoue, T.; Tachihara, M.; Kobayashi, K.; Katakami, N.; et al. Phase II trial of gefitinib plus pemetrexed after relapse using first-line gefitinib in patients with non-small cell lung cancer harboring EGFR gene mutations. *Lung Cancer* **2018**, *124*, 65–70. [CrossRef]

122. Wang, W.; Song, Z.; Zhang, Y. A Comparison of ddPCR and ARMS for detecting EGFR T790M status in ctDNA from advanced NSCLC patients with acquired EGFR-TKI resistance. *Cancer Med.* **2017**, *6*, 154–162. [CrossRef]

123. Wang, Y.; Wei, Y.; Ma, X.; Ma, X.; Gong, P. Association between advanced NSCLC T790 M EGFR-TKI secondary resistance and prognosis: A observational study. *Medicine* **2018**, *97*, e11346. [CrossRef]

124. Wang, Z.; Chen, R.; Wang, S.; Zhong, J.; Wu, M.; Zhao, J.; Duan, J.; Zhuo, M.; An, T.; Wang, Y.; et al. Quantification and dynamic monitoring of EGFR T790M in plasma cell-free DNA by digital PCR for prognosis of EGFR-TKI treatment in advanced NSCLC. *PLoS ONE* **2014**, *9*, e110780. [CrossRef]

125. Winther-Larsen, A.; Demuth, C.; Fledelius, J.; Madsen, A.T.; Hjorthaug, K.; Meldgaard, P.; Sorensen, B.S. Correlation between circulating mutant DNA and metabolic tumour burden in advanced non-small cell lung cancer patients. *Br. J. Cancer* **2017**, *117*, 704–709. [CrossRef]

126. Wu, Y.L.; Sequist, L.V.; Hu, C.P.; Feng, J.; Lu, S.; Huang, Y.; Li, W.; Hou, M.; Schuler, M.; Mok, T.; et al. EGFR mutation detection in circulating cell-free DNA of lung adenocarcinoma patients: Analysis of LUX-Lung 3 and 6. *Br. J. Cancer* **2017**, *116*, 175–185. [CrossRef]

127. Yanagita, M.; Redig, A.J.; Paweletz, C.P.; Dahlberg, S.E.; O'Connell, A.; Feeney, N.; Taibi, M.; Boucher, D.; Oxnard, G.R.; Johnson, B.E.; et al. A Prospective Evaluation of Circulating Tumor Cells and Cell-Free DNA in EGFR-Mutant Non-Small Cell Lung Cancer Patients Treated with Erlotinib on a Phase II Trial. *Clin. Cancer Res.* **2016**, *22*, 6010–6020. [CrossRef]

128. Yang, B.; Qin, A.; Zhang, K.; Ren, H.; Liu, S.; Liu, X.; Pan, X.; Yu, G. Circulating tumor cells predict prognosis following tyrosine kinase inhibitor treatment in EGFR-mutant non-small cell lung cancer patients. *Oncol. Res.* **2017**, *25*, 1601–1606. [CrossRef]

129. Yang, B.; Zheng, D.; Zeng, U.; Qin, A.; Gao, J.; Yu, G. Circulating tumor cells predict prognosis following secondline AZD 9291 treatment in EGFR-T790M mutant non-small cell lung cancer patients. *J. BUON.* **2018**, *23*, 1077–1081.

130. Yonesaka, K.; Hirotani, K.; von Pawel, J.; Dediu, M.; Chen, S.; Copigneaux, C.; Nakagawa, K. Circulating heregulin level is associated with the efficacy of patritumab combined with erlotinib in patients with non-small cell lung cancer. *Lung Cancer* **2017**, *105*, 1–6. [CrossRef]

131. Zhang, Q.L.; Xing, X.Z.; Li, F.Y.; Xing, Y.J.; Li, J. Pretreatment Pokemon Level as a Predictor of Response to Cisplatin and Paclitaxel in Patients with Unresectable Non-Small Cell Lung Cancer. *Oncol. Res. Treat.* **2015**, *38*, 496–502. [CrossRef]

132. Zhang, S.; He, L.; Dai, N.; Guan, W.; Shan, J.; Yang, X.; Zhong, Z.; Qing, Y.; Jin, F.; Chen, C.; et al. Serum APE1 as a predictive marker for platinum-based chemotherapy of non-small cell lung cancer patients. *Oncotarget* **2016**, *7*, 77482–77494. [CrossRef]

133. Zhou, J.; Dong, F.; Cui, F.; Xu, R.; Tang, X. The role of circulating tumor cells in evaluation of prognosis and treatment response in advanced non-small-cell lung cancer. *Cancer Chemother. Pharmacol.* **2017**, *79*, 825–833. [CrossRef]

134. Zhu, Y.J.; Zhang, H.B.; Liu, Y.H.; Zhang, F.L.; Zhu, Y.Z.; Li, Y.; Bai, J.P.; Liu, L.R.; Qu, Y.C.; Qu, X.; et al. Estimation of cell-free circulating EGFR mutation concentration predicts outcomes in NSCLC patients treated with EGFR-TKIs. *Oncotarget* **2017**, *8*, 13195–13205. [CrossRef] [PubMed]

135. Akamatsu, H.; Koh, Y.; Okamoto, I.; Fujimoto, D.; Bessho, A.; Azuma, K.; Morita, S.; Yamamoto, N.; Nakagawa, K. Clinical significance of monitoring EGFR mutation in plasma using multiplexed digital PCR in EGFR mutated patients treated with afatinib (West Japan Oncology Group 8114LTR study). *Lung Cancer* **2019**, *131*, 128–133. [CrossRef] [PubMed]

136. Alama, A.; Coco, S.; Genova, C.; Rossi, G.; Fontana, V.; Tagliamento, M.; Giovanna Dal Bello, M.; Rosa, A.; Boccardo, S.; Rijavec, E.; et al. Prognostic Relevance of Circulating Tumor Cells and Circulating Cell-Free DNA Association in Metastatic Non-Small Cell Lung Cancer Treated with Nivolumab. *J. Clin. Med.* **2019**, *8*, 1011. [CrossRef] [PubMed]

137. Bordi, P.; Del Re, M.; Minari, R.; Rofi, E.; Buti, S.; Restante, G.; Squadrilli, A.; Crucitta, S.; Casartelli, C.; Gnetti, L.; et al. From the beginning to resistance: Study of plasma monitoring and resistance mechanisms in a cohort of patients treated with osimertinib for advanced T790M-positive NSCLC. *Lung Cancer* **2019**, *131*, 78–85. [CrossRef] [PubMed]

138. Hojbjerg, J.A.; Ebert, E.B.F.; Clement, M.S.; Winther-Larsen, A.; Meldgaard, P.; Sorensen, B. Circulating miR-30b and miR-30c predict erlotinib response in EGFR-mutated non-small cell lung cancer patients. *Lung Cancer* **2019**, *135*, 92–96. [CrossRef]

139. Kotsakis, A.; Kallergi, G.; Aggouraki, D.; Lyristi, Z.; Koinis, F.; Lagoudaki, E.; Koutsopoulos, A.; Georgoulias, V.; Vetsika, E.K. CD8(+) PD-1(+) T-cells and PD-L1(+) circulating tumor cells in chemotherapy-naive non-small cell lung cancer: Towards their clinical relevance? *Ther. Adv. Med. Oncol.* **2019**, *11*, 1758835919853193. [CrossRef]

140. Navarro, A.; Molins, L.; Marrades, R.M.; Moises, J.; Vinolas, N.; Morales, S.; Canals, J.; Castellano, J.J.; Ramirez, J.; Monzo, M. Exosome Analysis in Tumor-Draining Pulmonary Vein Identifies NSCLC Patients with Higher Risk of Relapse after Curative Surgery. *Cancers (Basel)* **2019**, *11*, 249. [CrossRef]

141. Passiglia, F.; Galvano, A.; Castiglia, M.; Incorvaia, L.; Calo, 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]

142. Tamminga, M.; De Wit, S.; Hiltermann, T.J.N.; Timens, W.; Schuuring, E.; Terstappen, L.; Groen, H.J.M. Circulating tumor cells in advanced non-small cell lung cancer patients are associated with worse tumor response to checkpoint inhibitors. *J. Immunother. Cancer* **2019**, *7*, 173. [CrossRef] [PubMed]

143. Wang, Z.; Duan, J.; Cai, S.; Han, M.; Dong, H.; Zhao, J.; Zhu, B.; Wang, S.; Zhuo, M.; Sun, J.; et al. Assessment of Blood Tumor Mutational Burden as a Potential Biomarker for Immunotherapy in Patients with Non-Small Cell Lung Cancer with Use of a Next-Generation Sequencing Cancer Gene Panel. *JAMA Oncol.* **2019**, *5*, 696–702. [CrossRef] [PubMed]

144. Zhang, Y.; Feng, Y.C.; Zhu, H.G.; Xiong, T.C.; Hou, Y.S.; Song, J.; Jiang, W.; Zhu, C.J. The peripheral blood neutrophil-to-lymphocyte ratio is a prognostic predictor for survival of EGFR-mutant nonsmall cell lung cancer patients treated with EGFR-TKIs. *Medicine (United States)* **2018**, *97*, 30. [CrossRef]

145. Yang, Y.L.; Wang, W.; Xu, L.P. Predictive value of microRNA-10b expression in peripheral blood mononuclear cells in evaluating short- and long-term efficacy of chemotherapy for patients with advanced non-small-cell lung cancer. *Neoplasma* **2018**, *65*, 610–619. [CrossRef]

146. Chae, Y.K.; Davis, A.A.; Agte, S.; Pan, A.; Simon, N.I.; Iams, W.T.; Cruz, M.R.; Tamragouri, K.; Rhee, K.; Mohindra, N.; et al. Clinical Implications of Circulating Tumor DNA Tumor Mutational Burden (ctDNA TMB) in Non-Small Cell Lung Cancer. *Oncologist* **2019**, *24*, 820–828. [CrossRef]

147. Rao, S.; Beckman, R.A.; Riazi, S.; Yabar, C.S.; Boca, S.M.; Marshall, J.L.; Pishvaian, M.J.; Brody, J.R.; Madhavan, S. Quantification and expert evaluation of evidence for chemopredictive biomarkers to personalize cancer treatment. *Oncotarget* **2017**, *8*, 37923–37934. [CrossRef]

148. Li, X.; Zhou, C. Comparison of cross-platform technologies for EGFR T790M testing in patients with non-small cell lung cancer. *Oncotarget* **2017**, *8*, 100801. [CrossRef]

149. Kim, E.; Feldman, R.; Wistuba, I.I. Update on EGFR Mutational Testing and the Potential of Noninvasive Liquid Biopsy in Non–Small-cell Lung Cancer. *Clin. Lung Cancer* **2018**, *19*, 105–114. [CrossRef]

150. Rolfo, C.; Mack, P.C.; Scagliotti, G.V.; Baas, P.; Barlesi, F.; Bivona, T.G.; Herbst, R.S.; Mok, T.S.; Peled, N.; Pirker, R.; et al. Liquid Biopsy for Advanced Non-Small Cell Lung Cancer (NSCLC): A Statement Paper from the IASLC. *J. Thorac. Oncol.* **2018**, *13*, 1248–1268. [CrossRef]

151. Network, N.C.C. Non-Small Cell Lung Cancer (Version 3.2020). Available online: Nccn.org (accessed on 17 April 2020).

152. Midha, A.; Dearden, S.; McCormack, R. EGFR mutation incidence in non-small-cell lung cancer of adenocarcinoma histology: A systematic review and global map by ethnicity (mutMapII). *Am. J. Cancer Res.* **2015**, *5*, 2892–2911.

153. Zhang, Y.-L.; Yuan, J.-Q.; Wang, K.-F.; Fu, X.-H.; Han, X.-R.; Threapleton, D.; Yang, Z.-Y.; Mao, C.; Tang, J.-L. The prevalence of EGFR mutation in patients with non-small cell lung cancer: A systematic review and meta-analysis. *Oncotarget* **2016**, *7*, 78985–78993. [CrossRef]

154. Yuan, M.; Huang, L.-L.; Chen, J.-H.; Wu, J.; Xu, Q. The emerging treatment landscape of targeted therapy in non-small-cell lung cancer. *Signal Transduct. Target. Ther.* **2019**, *4*, 61. [CrossRef]

155. Bernabé, R.; Hickson, N.; Wallace, A.; Blackhall, F.H. What do we need to make circulating tumour DNA (ctDNA) a routine diagnostic test in lung cancer? *Eur. J. Cancer* **2017**, *81*, 66–73. [CrossRef]

156. Moher, D.; Liberati, A.; Tetzlaff, J.; Altman, D.G. Preferred reporting items for systematic reviews and meta-analyses: The PRISMA statement. *BMJ* **2009**, *339*, b2535. [CrossRef]