Diagnostic, Predictive, and Prognostic Biomarkers in Non-Small Cell Lung Cancer (NSCLC) Management

Šutić, Maja; Vukić, Ana; Baranašić, Jurica; Försti, Asta; Džubur, Feđa; Samaržija, Miroslav; Jakopović, Marko; Brčić, Luka; Knežević, Jelena

Journal of *Personalized Medicine*

MDPI

Review

Diagnostic, Predictive, and Prognostic Biomarkers in Non-Small Cell Lung Cancer (NSCLC) Management

Maja Šutić [1,†], Ana Vukić [1,†], Jurica Baranašić [1], Asta Försti [2,3], Feđa Džubur [4,5], Miroslav Samaržija [4,5], Marko Jakopović [4,5], Luka Brčić [6,‡] and Jelena Knežević [1,7,*,‡]

1 Division of Molecular Medicine, Ruđer Bošković Institute, 10000 Zagreb, Croatia; Maja.Sutic@irb.hr (M.Š.); ana.vukic259@gmail.com (A.V.); Jurica.Baranasic@irb.hr (J.B.)
2 Hopp Children's Cancer Center (KiTZ), 69120 Heidelberg, Germany; a.foersti@kitz-heidelberg.de
3 Division of Pediatric Neurooncology, German Cancer Research Center (DKFZ), 69120 Heidelberg, Germany
4 German Cancer Consortium (DKTK), 69120 Heidelberg, Germany; fedja1104@gmail.com (F.D.); miroslav.samarzija@gmail.com (M.S.); marko.jakopovic@kbc-zagreb.hr (M.J.)
5 Clinical Department for Respiratory Diseases Jordanovac, University Hospital Centre Zagreb, School of Medicine, University of Zagreb, 10000 Zagreb, Croatia
6 Diagnostic and Research Institute of Pathology, Medical University of Graz, 8010 Graz, Austria; luka.brcic@medunigraz.at
7 Faculties for Dental Medicine and Health, University of Osijek, 31000 Osijek, Croatia
* Correspondence: jknezev@irb.hr; Tel.: +385-14560-964
† These authors equally contributed.
‡ These authors share senior authorship.

Abstract: Lung cancer is the leading cause of cancer-related deaths worldwide. Despite growing efforts for its early detection by screening populations at risk, the majority of lung cancer patients are still diagnosed in an advanced stage. The management of lung cancer has dramatically improved in the last decade and is no longer based on the "one-fits-all" paradigm or the general histological classification of non-small cell versus small cell lung cancer. Emerging options of targeted therapies and immunotherapies have shifted the management of lung cancer to a more personalized treatment approach, significantly influencing the clinical course and outcome of the disease. Molecular biomarkers have emerged as valuable tools in the prognosis and prediction of therapy response. In this review, we discuss the relevant biomarkers used in the clinical management of lung tumors, from diagnosis to prognosis. We also discuss promising new biomarkers, focusing on non-small cell lung cancer as the most abundant type of lung cancer.

Keywords: lung cancer; adenocarcinoma; squamous cell lung cancer; biomarker; diagnosis; prognosis; targeted therapy; immunotherapy

Citation: Šutić, M.; Vukić, A.; Baranašić, J.; Försti, A.; Džubur, F.; Samaržija, M.; Jakopović, M.; Brčić, L.; Knežević, J. Diagnostic, Predictive, and Prognostic Biomarkers in Non-Small Cell Lung Cancer (NSCLC) Management. *J. Pers. Med.* **2021**, *11*, 1102. https://doi.org/10.3390/jpm11111102

Academic Editor: Alessandra Pulliero

Received: 24 August 2021
Accepted: 25 October 2021
Published: 27 October 2021

1. Introduction

Lung cancer (LC) is the leading cause of cancer-related mortality worldwide, responsible for 18.4% of all cancer deaths. The latest GLOBOCAN database estimates that 2.2 million new lung cancer cases emerged in 2020 worldwide [1]. The American Cancer Society estimated the appearance of 230,000 newly diagnosed lung carcinoma cases in 2020 in the United States of America, with an anticipated mortality of 22–23% of the total cancer deaths [2]. Traditionally, lung cancer is classified into two major groups—non-small cell lung cancer (NSCLC; 80–85% of LC cases) and small cell lung cancer (SCLC; 15–20% of LC cases). The most common histologic subtype of NSCLC is adenocarcinoma (ADC), with an incidence of 40% [3]. Squamous cell lung carcinoma (SQC) comprises 20–30% of all LC cases [3]. Unfortunately, only a small proportion of NSCLC patients (<20%) are diagnosed at the early stage of the disease, while the tumor is localized and does not involve regional lymph nodes. However, the majority of NSCLC patients (47%) are still diagnosed at later stages (stages III/IV), when the tumor has already spread to multiple lymph nodes and/or

to distant organs [4], which consequently impacts the median survival time that barely exceeds 18 months [5,6]. The 5-year relative survival for patients diagnosed at an advanced stage is approximately 6%, compared to patients diagnosed at early stages that are expected to surpass this survival timeframe in 61% of cases [4]. It is also important to note that the 5-year survival rate varies across countries. Japan has the highest 5-year survival rate of 30%, most likely due to a higher relative proportion of EGFR mutation-positive patients and efforts to improve personalized cancer care [7]. Most countries, including 21 countries from Europe, have a 5-year survival rate of 10–19%, while the lowest 5-year survival rate was recorded in India, Brazil, Thailand, and Bulgaria (<10%) [8].

1.1. Lung Cancer Classification

Accurate histologic classification is crucial in the management of lung tumors because it is known that some therapeutics have potentially harmful side effects (such as bevacizumab [9]) or are inefficient (such as pemetrexed [10]) for the treatment of SQC. Until recently, guidelines were lacking for more specific sub-classifications of lung tumors from small biopsies and cytological samples. The World Health Organization Classification of Lung Tumors of 2015 addressed this issue and incorporated relevant genetic and immunohistochemical (IHC) aspects of different tumor subtypes [11]. Major revisions in the approach to adenocarcinoma, based on the 2011 IASLC/ATS/ERS Classification of lung adenocarcinoma [12], were accepted in the 2015 and 2021 WHO classification [13]. Furthermore, diagnosis of large cell carcinoma was restricted to the resected tumors lacking morphological and immunohistochemical signs of differentiation. SQC tumors are now classified as keratinizing, non-keratinizing, and basaloid. Lymphoepithelial carcinoma is also included in the SQC type. SCLC is a member of the neuroendocrine carcinomas, under neuroendocrine neoplasms of the lung. The group of sarcomatoid carcinomas comprises pleomorphic carcinoma (encompassing spindle cell and giant cell carcinoma), pulmonary blastoma, and carcinosarcoma. It is clearly stated that, in small biopsy and cytology samples, diagnoses of large cell and adenosquamous carcinoma should not be made, but in these cases, not-otherwise-specified NSCLC should be used [13]. The summary of this classification is shown in Figure 1.

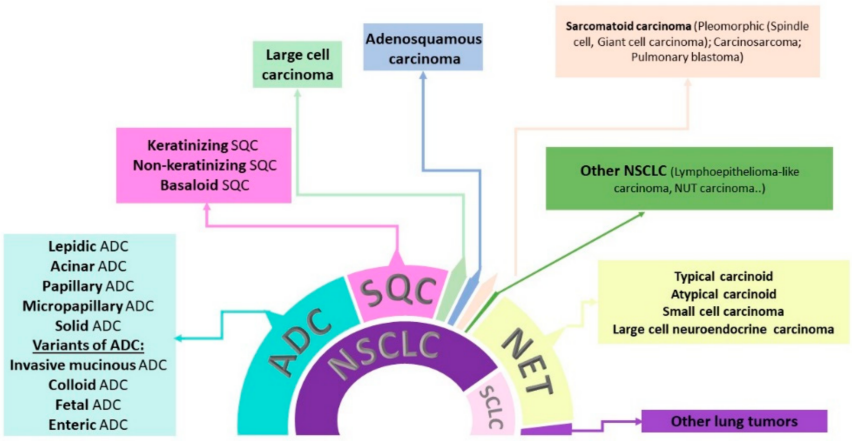

Figure 1. Classification of lung tumors based on resection specimens. The inner circle represents the traditional classification of lung tumors into non-small cell lung cancer (NSCLC) and small cell lung cancer (SCLC). The outer circle represents the WHO 2021 classification of lung tumors, in which SCLC is grouped with other types in the category of neuroendocrine tumors. ADC—adenocarcinoma; SQC—squamous cell carcinoma; NET—neuroendocrine tumors.

1.2. Diagnosis of Lung Cancer

The initial evaluation of patients with susceptibility to LC is usually made with a chest X-ray, CT scan, and/or PET-CT scan [14]. Due to differences in management

options for various LC subtypes, the accurate identification of specific histologic subtypes needs to be performed on tissue samples collected in various ways, such as bronchoscopy, transbronchial needle aspiration, transthoracic fine-needle aspiration, core biopsy, among others [15]. The general differentiation between LC subtypes is based on the morphological features of tumor samples stained with hematoxylin and eosin. Tumors with morphological evidence of keratinization or intercellular bridges are classified as SQC, while tumors with mucin production or glandular architecture are classified as ADCs [16]. However, morphology can be insufficient for the proper classification of tumor types in some cases, especially when the tumor is poorly differentiated or when it lacks specific morphologic or phenotypic features [17]. In this case, it is recommended to use immunohistochemistry for the classification of LC, but saving enough tumor tissue for predictive biomarker testing [11,18].

It is also worth mentioning that, in addition to tumor tissues, liquid biopsy, relatively recently, has became a valuable source of material for diagnostic purposes, not only of lung cancer, but also of many other types of cancer. This arose from a paradoxical situation: advances in technology and the accumulation of the new knowledge has brought us into a position in which we need to obtain significant amounts of samples for multiple analyses of a growing number of different biomarkers, with minimally invasive approaches. The problem is that traditionally obtained cytological samples are often insufficient for comprehensive molecular examinations, leading us to need new tissue sources. In principle, liquid biopsy is defined as the sampling of the non-solid biological materials/tissues. Liquid biopsy is any tumor-derived material circulating through the blood or any other body fluid. The most frequently studied, or used, materials from the blood, in NSCLC diagnosis, are circulating tumor cells (CTCs) or circulating tumor DNA (ctDNA). It has also been shown that exosomes, which contain RNAs derived from the patient's tumor, can be found in the blood of patients [19,20]. Later, in this article, it will be explained in more detail how micro RNAs (miRNAs), single-stranded noncoding RNAs, could be used as a potential diagnostic biomarker in the management of NSCLC.

1.3. Treatment Options

The successful treatment of LC depends on several important factors: stage at diagnosis (defined with tumor size, regional lymph nodes involvement, and the presence of metastasis), histologic subtype, and molecular characterization. The therapy of non-metastatic NSCLC usually consists of surgical resection, adjuvant and neoadjuvant chemotherapy, as well as immunotherapy in the unresectable stage III of NSCLC [21]. The successful treatment of LC in the advanced stages depends on the histologic subtype, the presence of targetable mutations, and the patient's clinical status and comorbidities [15]. The approval of targeted therapeutics, such as epidermal growth factor receptor (EGFR)—tyrosine kinase inhibitors (TKIs) [22,23] or anaplastic lymphoma kinase (ALK) inhibitors [24], improved survival in patients positive for these driving somatic mutations. Similar benefits for patients' survival were shown with immune checkpoint inhibitors that target programmed-death 1 receptor (PD-1) or its ligand (PD-L1) [25,26]. However, most NSCLC patients are EGFR or ALK negative [27], and less than one-third of advanced lung tumors express PD-L1 in more than 50% of tumor cells [26]. Finally, patients not eligible for targeted or immune therapy are treated with platinum-based chemotherapy as the first line, and eligible patients will receive it after the failure of targeted or immune therapy [15,28]. We would also like to mention that a relatively new strategy in NSCLC treatment is to combine different approaches, including targeted therapy, immunotherapy, radiotherapy, and chemotherapy. The fact that the tumors develop resistance to any form of therapeutic strategy has forced us to look at the problem from a different angle. There are numerous different examples of combined therapy approaches. For example, in 2018, The FDA approved the addition of the PD-1 inhibitor pembrolizumab to platinum-based chemotherapy. The results of this study showed improved outcomes in patients with squamous NSCLC of any level of PD-L1 expression, when compared with the use of chemotherapy treatment

only (KEYNOTE-407/NCT02775435) [29]. Another example is the CheckMate 012 study that combined nivolumab with erlotinib in patients with advanced, EGFR-mutant NSCLC, who were EGFR tyrosine kinase inhibitor (TKI)–naive or TKI-treated, but had not received chemotherapy. Previous studies in mouse models have reported that activation of the oncogenic EGFR pathway enhances the susceptibility of lung cancer to PD-1 blockade, suggesting that the combination of PD1 blockade with EGFR TKIs may be a promising therapeutic strategy. The results of this study revealed that treatment with nivolumab and erlotinib was tolerable, with durable responses in patients with EGFR-mutant, TKI-treated NSCLC [30]. Trials, such as this one, will definitely improve the treatment of lung cancer. However, the search for predictive biomarkers is the only option to lead us to better treatment strategies.

In this review, we discuss well-defined biomarkers for the management of patients with NSCLC, including diagnostic, predictive, and prognostic biomarkers. We also point to some novel and exciting molecular biomarkers that have not yet been included in clinical practice, but show potential for translation to the clinics in the future. The key definitions for the biomarkers are summarized in Box 1.

Box 1. A summary of the key definitions.

Biomarkers are measurable characteristics that indicate biological processes of patients or their tumors or can indicate responses to treatment intervention [31]. **Diagnostic biomarkers** should be able to detect and differentiate specific diseases from other conditions or identify a relevant subtype of a particular disease [32]. **Predictive biomarkers** are used to identify individuals most likely to benefit from certain treatments [31]. **Prognostic biomarkers** can indicate the likelihood of a clinical outcome or the pace of disease recurrence and progression [6]. The most informative characteristics of biomarkers are specificity and sensitivity. **Sensitivity** is a percentage of true positive cases in the analyzed group of patients, and **specificity** is a percentage of truly negative cases in the control group [31].

2. Diagnostic Biomarkers Used in NSCLC Clinical Management

Approved drugs for patients with NSCLCs are especially beneficial for patients with ADCs carrying driver alterations due to the higher rate of targetable mutations present compared to SQCs [31]. The delineation of the histology is therefore essential for optimal treatment decisions. In this section, we discuss the biomarkers used in daily clinical practice, such as immunohistochemical and blood/serum diagnostic biomarkers. We also point to novel biomarkers that have not yet been included in routine clinical practice, but show promising diagnostic potential.

2.1. Immunohistochemical Biomarkers

The primary technique for the diagnosis and classification of lung cancer histology in clinical practice is immunohistochemistry (IHC). Based on the recent publication about best practice recommendations for the usage of IHC in lung cancer diagnostic, TTF-1 (Thyroid Transcription Factor 1) (for ADC) and p40 (for SQC) are designated as the best markers for the subtyping of NSCLC, especially when the 8G7G3/1 monoclonal antibody is used for TTF-1 detection. Napsin A is the second best marker for ADC, while p63 can be positive both in lung ADC and some other tumors. However, while for the TTF-1 only the focal positive nuclear reaction is considered as a valid positive, for p40 more than 50% of tumor cells must demonstrate nuclear positivity [32]. In the case of a TTF-1 negative ADC, one should always think about the possibility of metastasis and apply additional IHC. Rare lung tumors and undifferentiated neoplasms always require additional sets of antibodies. As discussed above, in the case of clinical suspicion of a primary lung tumor and a negative reaction with p40/TTF-1, one should diagnose the tumor as a non-small cell carcinoma-not otherwise specified (NSCC-NOS) and send it for additional testing for predictive biomarkers, without "wasting" tumor tissue for definitive diagnosis/classification [3].

2.2. Circulating Tumor Protein Biomarkers from the Blood and Serum

In comparison to IHC, which requires tumor samples obtained by biopsy or resection, blood/serum samples are more easily obtained and a helpful tool in clinical settings. Cytokeratin 19 fragment (CYFRA 21-1), carcinoembryonic antigen (CEA), squamous cell carcinoma antigen (SCCA), and carbohydrate antigen 125 (CA125) are well established and the most commonly used blood/serum biomarkers for the detection of LC, used either as a single biomarker [33] or in panels of several combined biomarkers [34,35]. Several studies propose different combinations of well-established biomarkers and novel markers, such as cancer/testis antigen 1B (CTAG1B/NY-ESO-1) [36], prolactin (PRL) [37], retinol binding protein (RBP), 1-antitrypsin (ATT) [38], thymidine kinase 1 (TK1), neuron specific enolase (NSE) [39], or autoantibodies Annexin A1-Ab and α-enolase-Ab (ENO1) [40]. All mentioned studies report that specificity and sensitivity of assays are increased with the addition of novel markers compared to the performance of a single marker. Testing autoantibodies together with established biomarkers could also increase usefulness of established biomarkers in early cancer detection, as autoantibodies are produced early in tumorigenesis and can be detected in the serum sooner than tumor-associated antigens [41,42].

2.3. miRNAs—Potential Diagnostic Biomarkers

Micro RNAs (miRNAs) are single-stranded noncoding RNAs, 20–25 nucleotides in length that can alter gene expression post-transcriptionally through direct degradation of mRNA or repression of translation. They have an important role in numerous biological processes, including cell growth, apoptosis, inflammation, and cancer [43]. Since miRNAs are stable and can be detected in various biological fluids, such as in serum, plasma, pleural fluid, urine, or cerebrospinal fluid, miRNAs could be ideal non-invasive diagnostic biomarkers [44].

Expression levels of certain miRNAs vary between pathological conditions and healthy controls, and these differences might enable new strategies in the diagnosis of many diseases, including LC. For example, miR-33a-5p and miR-128-3p are down-regulated in LC tissue compared to adjacent normal tissue and a combination of these miRNAs shows good diagnostic characteristics [45]. Some miRNAs are shown to be NSCLC subtype-specific, such as miR-205 for squamous cell LC [46,47] and miR-375 for adenocarcinoma [48]. Additionally, miR-93 and miR-221 have increased expression in squamous cell lung cancer compared to adjacent non-malignant tissue, while high levels of miR-100 are correlated with adenocarcinoma in smokers [49]. A miRview lung assay was developed for expression analysis of eight miRNAs (miR-106a, miR-125a-5p, miR-129-3p, miR-205, miR-21, miR-29b, miR-375, and miR-7). Based on the miRNA expression profile, the assay can differentiate between SCLC and NSCLC, as well as SCLC from carcinoid lung tumor or squamous from non-squamous NSCLC with high accuracy, showing a great diagnostic potential [50].

Interestingly, several studies indicated that miRNAs could also be used as circulating diagnostic biomarkers of early stage NSCLC. It has been shown that miR-324-3p is significantly up-regulated, while miR-1285 was significantly down regulated, in plasma samples of patients with stage 1 squamous cell LC, compared to healthy controls [51]. Wang et al. identified a panel of five serum miRNA for NSCLC diagnosis in patients of different races. The panel can discriminate NSCLC from controls and differentiate between malignant lesions and benign nodules. The panel includes miR-483-5p, miR-193a-3p, miR-25, miR-214, and miR-7. In both testing and validation cohorts, these miRNAs were significantly elevated in NSCLC compared to controls [52].

Sozzi et al. investigated the diagnostic potential of the miRNA signature classifier (MSC) assay on plasma samples collected within the Multicentric Italian Lung Detection (MILD) trial that included patients with low-dose computed tomography (LDCT) screening results. The authors used an expression ratio of 24 miRNAs and reported promising assay characteristics for LC detection. The LDCT alone showed similar sensitivity as the MSC assay, but the reported false–positive rate was high (19.4%). However, when used in

combination with the miRNA expression ratio, the LDCT false–positive ratio was reduced to 3.7% [53]. Montani et al. also proposed miR-Test as a first-line screening tool [54].

Based on the literature, it seems that only the combination of several miRNAs will significantly raise diagnostic accuracy. However, there are some limitations and inconsistencies in the reported studies. For example, there is only a partial concordance in the miRNAs repertoire used in different studies. Moreover, only a few panels were validated on bigger cohorts [53,54], which implies the need for further validations. Furthermore, the type of samples used for diagnosis (plasma vs. serum) and a good normalization control for the RT-qPCR approach are still not well defined. To translate miRNAs into routine clinical practice, scientists should first agree on solving the aforementioned inconsistencies and establish models that could be validated in large-scale clinical trials.

3. Predictive Biomarkers in NSCLC Management

The histologic type of NSCLC is still used as a predictive factor for chemotherapy treatment. For instance, pemetrexed treatment was demonstrated as beneficial for patients with non-squamous NSCLC, while patients with squamous NSCLC had a similar overall survival (OS) in both pemetrexed and placebo groups. Therefore, non-squamous histology is a predictive factor for pemetrexed-based chemotherapy [55]. In addition to histology, specific genetic alterations are also predictive biomarkers for NSCLC treatment. At present, there are several predictive genetic biomarkers used in clinical settings, which will be described in this section, together with promising new biomarkers.

The identification of the predictive markers has a great impact on treatment choice. Therefore, the detection of the known genetic alterations is a prerequisite for treatment. When testing for predictive biomarkers, two important factors need to be considered: obtaining an adequate specimen and choosing the right method of testing [56,57]. One of the problems in biomarker testing is tissue exhaustion due to series of single-gene tests for assessing multiple types of genetic alterations [56]. The College of American Pathologists (CAP), the International Association for the Study of Lung Cancer (IASLC), and the Association for Molecular Pathology (AMP) issued updated guidelines for LC testing in 2018. These guidelines recommend routine multigene testing of all advanced NSCLC with an adenocarcinoma component for EGFR mutations and ALK and ROS1 rearrangements, together with additional genes (RET, MET, Her2, KRAS, and BRAF). They also recommended to test samples of SQC in younger patients (<50 years of age) and who have never smoked. Testing for T790M is recommended in all patients with sensitizing EGFR mutations who have progressed after treatment with EGFR-TKIs [58]. The current Pan-Asian guidelines recommend the testing mentioned above and PD-L1 immunohistochemistry to be performed in all patients with advanced non-squamous NSCLC [59]. Some local guidelines, such as those in Austria, recommend reflex testing for all non-squamous carcinoma regardless of the stage, using multigene testing and reflex testing for PD-L1 in both SQC and AC [60]. In this section, we focus on the most commonly used biomarkers that predict response to available targeted therapies and immunotherapy. Molecular alterations used as predictive biomarkers are summarized in Figure 2. Approved targeted therapeutics are summarized in Table 1. Additionally, we discuss new predictive biomarkers reported in the literature that might become relevant for routine use in the future.

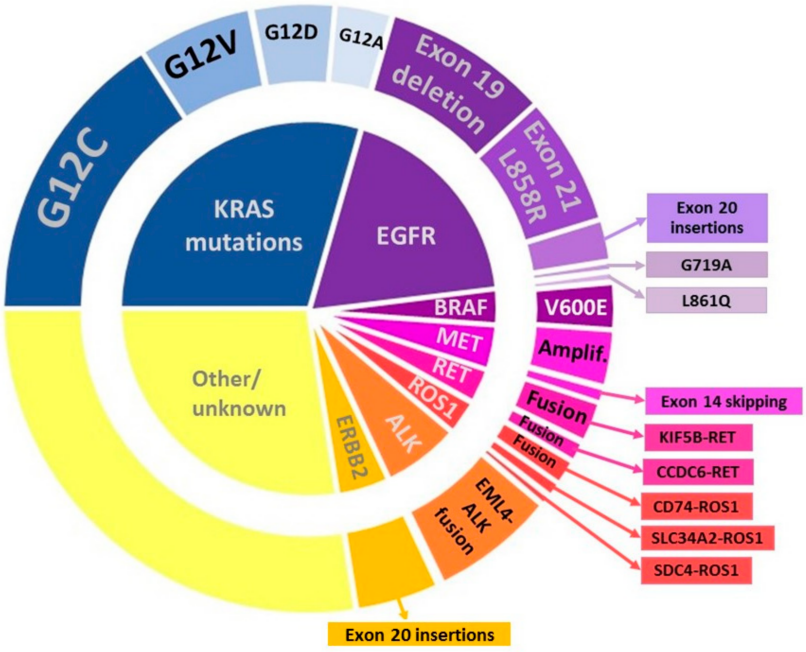

Figure 2. Molecular alterations in lung adenocarcinoma.

Table 1. Summary of targeted therapeutics approved for NSCLC treatment. The table was created based on the FDA and EMA database of approved therapeutics for the treatment of NSCLCs positive for genetic alterations. FDA—The Food and Drug Administration; EMA—European Medicines Agency.

Biomarker	Alteration	Targeted Therapy	Year of FDA Approval	Year of EMA Approval
EGFR	Exon 19 deletion	Erlotinib	2013	2011
		Gefitinib	2015	2009
		Afatinib	2013	2013
	Exon 21 (L858R) substitution mutation	Osimertinib	2018	2018
		Dacomitinib	2018	2019
		Erlotinib + Ramucirumab	2020	
	T790M	Osimertinib	2015	2016
	L861Q, G719X, S768I	Afatinib	2018	
ALK	ALK rearrangement	Crizotinib	2011	2012/2015
		Ceritinib	2014/2017	2015/2017
		Alectinib	2017	2017
		Brigatinib	2017	2018/2020
		Lorlatinib	2018	2019
ROS1	ROS1 rearrangement	Crizotinib	2016	
		Entrectinib	2019	
		Ceritinib	2019	
BRAF	V600E mutation	Dabrafenib + Trametinib	2017	
NTRK 1/2/3	Gene fusion	Larotrectinib	2018	2019
		Entrectinib	2019	2020
MET	Exon 14 skipping	Capmatinib	2020	
		Tepotinib	2021	
RET	RET rearrangement	Selpercatinib	2020	
		Praseltinib	2020	

3.1. Predictive Biomarkers for Targeted Therapy in NSCLC Patients

Epidermal growth factor receptor (EGFR): also known as HER1, it is a member of the protein kinase superfamily. The activated EGFR is involved in different biological processes, such as cell proliferation, differentiation, and apoptosis [61]. However, specific EGFR mutations, also known as activating or sensitizing mutations, can cause constitutive activation of the receptor, leading to uncontrolled cell division and tumor pathogenesis. These mutations are more common in ADC female, non-smoking patients from East Asia [62]. The two most common EGFR mutations are exon 19 deletion (45–50% of all mutations) and exon 21 (L858R) substitution (35–45% of all mutations) [62]. Although these mutations are more common in ADCs, they do appear in SQC as well, but at a lower rate (3.3% in Western and 4.6% in Asian populations). The aforementioned EGFR mutations are predictive for their response to drugs called tyrosine kinase inhibitors (TKIs) that bind to tyrosine kinase receptors, reversibly or irreversibly, and inhibit downstream signaling [63]. To date, the US Food and Drug Administration (FDA) has approved five TKIs for the treatment of NSCLC. Erlotinib and gefitinib are first-generation reversible tyrosine kinase inhibitors that have been proven beneficial to ADC patients [64,65]. Afatinib is a second-generation TKI that binds to EGFR and other members of the ERBB family irreversibly [66]. Osimertinib is a third-generation EGFR TKI, used to treat patients harboring EGFR T790M mutation, the main cause of drug resistance to the first-generation TKIs [67].

Anaplastic Lymphoma Kinase (ALK): the ALK gene encodes an enzymatic protein known as ALK tyrosine kinase receptor or CD246 [68], a member of the insulin receptor superfamily of tyrosine kinases. ALK activation leads to the activation of downstream signaling pathways, such as PI3K/AKT, RAS/MAPK, and JAK/STAT [69]. Approximately 2–7% of NSCLC patients have alterations in the ALK gene. ALK alterations include rearrangements, amplifications, and point mutations [70]. These alterations can cause constitutive expression and activation of the ALK protein, leading to oncogenic phenotype and tumor pathogenesis [69]. The most common rearrangement is an echinoderm microtubule-associated protein-like 4 (EML4) inversion rearrangement with ALK, resulting in an EML4–ALK fusion oncogene [5,70]. Many different variants of the EML4–ALK fusion oncogene have been described, as well as rearrangements of ALK with other genes, such as KIF5B, KLC1, TFG, TPR, HIP1, STRN, DCTN1, SQSTM1, NPM1, BCL11A, and BIRC6 [70]. ALK rearrangements are more common in younger, ADC patients who have never smoked with a median age of 55 years [70,71]. ALK rearrangements are almost always mutually exclusive with EGFR and KRAS mutations [71]. The first drug to be approved for ALK rearrangements was crizotinib, an inhibitor of ALK and ROS1 [72]. However, patients tend to develop resistance to the treatment due to either a new ALK point mutation (like L1196M) or as a result of the activation of EGFR or KRAS signaling pathways [71]. Therefore, new drugs were developed for patients resistant to crizotinib, such as ceritinib, alectinib, brigatinib, and lorlatinib [5].

ROS Proto-Oncogene 1, Receptor Tyrosine Kinase (ROS1): ROS1 is a tyrosine kinase receptor, involved in cell growth and differentiation [73]. Although it is mainly expressed during embryonic development, ROS1 is also expressed in limited amounts in adults, especially in lung tissue. In 1–3% of lung adenocarcinomas, rearrangements of the ROS1 gene are observed [74]. These rearrangements cause the constitutive activation of the ROS1 gene, leading to cell proliferation, survival, and migration. Many ROS1 fusion partners have been identified, and CD74–ROS1 is the most commonly found in NSCLC [73]. In a similar manner to ALK, ROS1 rearrangements are more commonly found in younger patients who have never smoked, and are almost exclusively observed in ADCs [74,75]. Crizotinib is also approved for ROS-rearranged NSCLC [76], but resistance can occur as in treatment of ALK rearrangements. Many studies and trials are currently testing the effectiveness of multi-kinase inhibitors, such as ceritinib [77], brigatinib [78], alectinib [79], cabozantinib [80,81], and lorlatinib [82], in the treatment of ROS1-rearranged NSCLC [73].

V-raf murine sarcoma viral oncogene homolog B (BRAF): the BRAF gene encodes a cytosolic serine/threonine protein kinase B-Raf, a member of the Raf kinase family. It

is downstream of KRAS (Kirsten rat sarcoma oncogene) and is involved in the mitogen-activated protein kinase (MAPK) signaling pathway [83]. The constitutive activation of the mutated BRAF gene activates the MAPK signaling pathway, leading to increased cell proliferation and cell growth. These mutations are observed in 2–4% of NSCLC, primarily in ADC [83]. A V600E mutation is the most common and occurs in approximately 50% of BRAF-positive NSCLC cases. V600E constitutively activates BRAF, which then phosphorylates the downstream effector MEK [84–86]. It seems that V600E mutation is mutually exclusive with KRAS mutations, while BRAF non-V600 positive patients might harbor KRAS mutations as well [83]. Non-V600 mutations might be associated with patients with a smoking history and of male gender [84–86]. The FDA approved a combination of dabrafenib (BRAF inhibitor) and trametinib (MEK inhibitor) to treat NSCLC patients with advanced or metastatic tumor carrying BRAF V600E mutation.

Mesenchymal–epithelial transition tyrosine kinase receptor (MET): upon ligand binding, MET mediates the activation of several signaling pathways, such as PI3K/AKT, STAT3, SRC/FAK, and MAPK/ERK. Reported MET mutations in NSCLC include amplification, an exon 14-skipping mutation, and mutations in the kinase domain [87]. It is reported that NSCLC patients harboring MET exon 14-skipping mutation and MET overexpression have a better response to crizotinib [88] and tivantinib [89,90]. The FDA also approved capmatinib for patients with metastatic NSCLC that harbor the MET exon 14-skipping mutation, based on results from the GEOMETRY mono-1 trial [91]. Most recently, in 2021, the FDA approved tepotinib for metastatic NSCLC with MET exon 14 skipping alterations (VISION trial, NCT02864992).

Rearranged During Transfection (RET): RET fusions are found in 1–2% of NSCLC, mainly in ADC [92,93]. The most common fusion partner of RET is KIF5B [94]. Coiled-coil domains of RET fusion partner proteins foster dimerization of RET fusion proteins, leading to the constitutive activation of RET kinase [27] and the activation of several kinases, including MAPK, (PI3K)/AKT, and JNK [93]. Various trials and case reports have shown the benefit of cabozantinib [93,95] and vandetanib [96] treatment in RET-rearranged NSCLC patients. In 2020. The FDA approved selpercatinib and pralestinib for the treatment of NSCLS with RET gene alterations.

Neurotrophic receptor tyrosine kinase (NTRK): NTRKs are involved in the regulation, growth, differentiation, and programmed cell death of neurons in both peripheral and central nervous system. Upon binding with respective ligands, they activate different downstream signaling pathways, such as the Ras/Raf/MAPK pathway, PI3K/Akt/mTOR pathway, and PLCγ/PKC pathway [97]. Most studied NTRK1 fusions are MPRIP–NTRK1 and CD74–NTRK1, found in 3% of lung ADCs [98]. TRIM24–NTRK2 fusion has also been found in some lung adenocarcinomas [99]. The FDA approved larotrectinib (pan-NTRK inhibitor) for the treatment of solid tumors that have NTRK gene fusions based on the LOXO-TRK-14001 (NCT02122913), SCOUT (NCT02637687), and NAVIGATE (NCT02576431) clinical trials. The FDA also approved entrectinib for the treatment of ROS1-positive and NTRK-positive solid tumors based on the ALKA, STARTRK-1 (NCT02097810), and STARTRK-2 (NCT02568267) clinical trials. Some other multi-kinase inhibitors are being tested in ongoing trials, such as cabozantinib, which is in a phase II study (NCT01639508), and MGCD516, which is in phase I/Ib (NCT02219711).

Neuregulin-1 (NRG1): NRGs are growth factors for the ErbB family of receptor tyrosine kinases. NRG1 is the most studied member of the NRGs group and is a ligand of HER3/4 [100]. Its primary role is in normal physiology during neural development [101], but it can also have a pathologic role in several types of cancer, including NSCLC. Fusions of NRG1 with several identified partners (to date), of which the CD74–NRG1 fusion is the most common, are relatively rare. The estimated incidence of NRG1 fusions in NSCLC is 0,3% [102]. Afatinib, an inhibitor of ErbB receptors, is a treatment option for NSCLC patients harboring NRG1 fusions [103–105]. An ongoing phase I/II trial study (NCT02912949) is evaluating the activity and safety of Zenocutuzumab (MCLA-128), an anti-HER2/3 antibody, in patients with solid tumors, including NSCLC, harboring NRG1

fusion. Another ongoing phase 2 trial study, CRESTONE (NCT04383210), is investigating Seribantumab, an anti-HER3 monoclonal antibody, for treatment of NRG1 fusion-positive advanced/metastatic solid tumors [106]. The summary of currently approved and available therapies is shown in Table 1.

3.2. Predictive Biomarkers for Immunotherapy in NSCLC Patients

The immune evasion of cancer cells is considered as a hallmark of cancer [107]. It is well established that tumor cells can express or produce immune-suppressive molecules that inhibit the function of T lymphocytes, which helps them to evade immune surveillance. One of the known immune evasion mechanisms that cancer cells exploit is through immune-inhibitory pathways called immune checkpoints. Immune checkpoints are proteins expressed on the surface of immune cells that recognize the corresponding ligand and transmit stimulatory or inhibitory signals that modulate immune response [108]. In this Section, we will discuss the programmed death receptor1 (PD-1) and its ligand (PD-L1) in the context of NSCLC immunotherapy.

The programmed death-ligand 1 (PD-L1) protein, also called B7-H1, is encoded by the CD274 gene. PD-L1 is constitutively expressed on the surface of many immune cells, such as macrophages, antigen-presenting cells, B cells, and T lymphocytes. PD-L1 binds to a programmed death receptor (PD-1) predominantly expressed on the surface of activated cytotoxic T cells. This binding leads to the suppression of the immune system and is important in preventing an autoimmune response during inflammation [109]. However, PD-L1 is also expressed by many different tumor cells, including lung cancer, and its expression enables their evasion from immune response [110]. Higher expression of PD-L1, at both mRNA and protein level, was observed in NSCLC compared to healthy lung tissue, regardless of NSCLC type [111]. Currently, four FDA-approved monoclonal antibodies targeting the PD-1/PD-L1 interaction are used for the treatment of patients with NSCLC: nivolumab, pembrolizumab, atezolizumab, and durvalumab [112]. Summary of immune checkpoint inhibitors approved by FDA and EMA is shown in Table 2. However, the validity of PD-L1 expression as a predictive biomarker is questionable, because it is has been shown that patients with low PD-L1 expression, less than 1%, responded exceptionally to anti-PD-1/PD-L1 treatment. It is possible that PD-L1 expression changes over time and is inconsistent throughout the tumor tissue. Furthermore, there are different clones of PD-L1 antibodies, with different cut-off points in the immunohistochemical analysis of PD-L1 expression, and they are not identical [113].

Table 2. Summary of immune checkpoint inhibitors approved for NSCLC treatment. The table was created based on the FDA and EMA database of approved anti-PD-1/PD-L1 therapeutics for the treatment of NSCLCs. FDA—The Food and Drug Administration; EMA—European Medicines Agency; mNSCLC—metastatic non-small cell lung cancer; TPS-tumor proportional score.

Antibody	Target	Therapeutic Indication	Line of Therapy	Year of FDA Approval	Year of EMA Approval
Pembrolizumab	PD-1	Advanced/mNSCLC that express PD-L1 (TPS $\geq$ 1%)	Second-line	2015	2016
		mNSCLC with high PD-L1 expression (TPS $\geq$ 50%) [1]	First-line	2016	2017
		Metastatic non-squamous NSCLC, regardless of PD-L1 expression	First-line (+ Carboplatin & Pemetrexed)	2017/2018	2018
		Metastatic SQC	First-line (+ Carboplatin and Paclitaxel or Nabpaclitaxel)	2018	2019
		Stage III NSCLC with PD-L1 expression (TPS $\geq$ 1%) [2]	First-line	2019	

Table 2. *Cont.*

Antibody	Target	Therapeutic Indication	Line of Therapy	Year of FDA Approval	Year of EMA Approval
Nivolumab	PD-1	Advanced/mSQC	Second-line	2015	2015
		Advanced/mNSCLC [3]	Second-line	2015	2016
		Recurrent/mNSCLC [4]	First-line (+ Ipilimumab and 2 cycles of platinum-based chemotherapy)	2020	
		mNSCLC with PD-L1 expression ($\geq$ 1%)	First-line	2020	
Atezolizumab	PD-L1	mNSCLC [5]	Second-line	2016	2017
		Metastatic non-SQC NSCLC [6]	First-line (+ Bevacizumab, Carboplatin, and Paclitaxel)	2018	2019
		Metastatic non-SQC NSCLC (PD-L1 $\geq$ 5%) [7]	First-line (+ Nab-paclitaxel and Carboplatin)	2019	2019
		mNSCLC with high PD-L1 expression ($\geq$ 50%) [8]	First-line	2020	
Durvalumab	PD-L1	Stage III NSCLC [9]	Maintenance therapy	2018	2018

[1] Approved for mNSCLC with no EGFR or ALK genomic aberration and no prior systemic therapy. [2] Approved for patients with stage III NSCLC who are not candidates for surgical resection, definitive chemoradiation, or mNSCLC, with no EGFR or ALK genomic tumor aberrations. [3] Approved for progression on or after platinum-based chemotherapy. [4] Approved for recurrent/mNSCLC without ALK aberrations determined by the FDA approved PD-L1 IHC 28-8 pharmDx diagnostic device, approved for mNSCLS (PD-L1 $\geq$ 1%) with no EGFR or ALK genomic tumor aberrations. [5] Approved for mNSCLC, whose disease progressed during/after platinum-based chemotherapy. mNSCLC with EGFR or ALK genomic tumor aberration should receive atezolizumab only after the failing of the targeted therapy. [6] Approved for metastatic NSCLC, with no EGFR or ALK genomic tumor aberrations. [7] EMA approved as first-line therapy for metastatic NSCLC with PD-L1 expression of at least 5% FDA approval regardless of PD-L1 expression. [8] Approved for metastatic NSCLC with high PD-L1 expression (PD-L1 stained $\geq$ 50% of tumor cells) or PD-L1 stained tumor-infiltrating immune cells covering $\geq$ 10% of the tumor area, with no EGFR or ALK genomic tumor aberrations, determined by the FDA approved VENTANA PD-L1 (SP142) diagnostic assay. [9] Approved for patients with unresectable stage III NSCLC, whose disease has not progressed following concurrent platinum-based chemotherapy and radiation therapy.

3.3. Novel Predictive Biomarkers for Targeted Therapy

Even though current biomarkers notably improved NSCLC treatment, there is still an ongoing need for novel predictors and targeted therapeutics that could help to achieve better outcomes and cost-effectiveness in treating patients with NSCLC, especially those with a squamous subtype diagnosis. In this Section, we summarize the literature for reported potential biomarkers that are already being tested in several clinical trials (summarized in Table 3) and might become relevant in the future.

Kirsten rat sarcoma viral oncogene homolog (KRAS): the KRAS gene encodes for RAS protein, a GTPase crucial for the activation of several pathways, including the Raf/MEK/ERK, PI3K/AKT/mTOR, and RalGDS/RalAIB pathways [6]. Mutations in KRAS cause the constitutive activation of KRAS signaling, leading to cell proliferation and survival. Activating KRAS mutations are almost exclusive to ADCs and are more frequent in Western populations (~30% in Western and ~10% in Asian populations), making them the most common mutations in Western NSCLC cases [114]. Considering the mutual exclusivity of KRAS and EGFR mutations, as well as the downstream role of RAS proteins in the EGFR signaling pathway, KRAS status could be used to determine whether a certain patient would benefit from an EGFR inhibitor treatment [115]. There are also several ongoing clinical trials investigating therapeutics for KRAS G12C mutation, the most common KRAS mutation in NSCLC.

Fibroblast growth factor receptor 1 (FGFR1): Fibroblast growth factor receptor 1 (FGFR1) is a cell surface tyrosine kinase involved in the regulation of proliferation, dif-

ferentiation, cell migration, and survival [116]. FGFR1 amplification, mutations, and rearrangements can cause the constitutive activation of the receptor and contribute to tumor promotion [117]. Amplification of FGFR1 is identified in 9–20% of SQCs and up to 15% in lung ADCs, and seems to be more common in male patients with a smoking history [118,119]. It has been shown that the aberrant expression of FGF or FGFR family reduces the sensitivity of mesenchymal-like NSCLC cells to EGFR inhibitors [120]. Numerous nonselective FGFR inhibitors were evaluated in NSCLCs over the years, such as dovitinib [121], lenvatinib [122], pazopanib [123], nintedanib [124], brivanib [125], ponatinib [126], lucitanib [127], and regorafenib [128]. Unfortunately, most of the studies observed limited antitumor activity and high drug toxicity. However, assessing the validity of FGFR as a predictive biomarker is still an ongoing endeavor, and the list of novel FGFR inhibitors is still expanding [129].

Discoidin domain receptor 2 (DDR2): Discoidin domain receptors, DDR1 and DDR2, are tyrosine kinases involved in mammary gland development, long bone growth, and the occurrence of many types of diseases, including arthritis, atherosclerosis, and cancer [130]. The deregulation of DDR pathways, due to somatic mutations or the altered expression of receptors, can cause tumor growth and promote cell migration and invasion [131]. Mutations in the DDR2 gene are observed in 2–4% of lung SQCs and approximately 30% of SQC cases have elevated levels of the DDR2 protein [132–134]. Currently, clinical activity of multi-kinase inhibitor MGCD516 is being evaluated in NSCLCs and head and neck cancer populations with DDR2 mutations and/or other activating mutations (MET, NTRK2, NTRK3), rearrangements (MET, RET, AXL, NTRK1, or NTRK3), or amplifications (MET or KIT/PDGFRA/KDR) (NCT02219711).

Human epidermal growth factor receptor 2 (HER2/ERBB2): HER2 is an important member of the epidermal growth factor receptor family (ERBB) involved in the activation of PI3K-AKT and MEK-ERK proliferation pathways. HER2 is activated by dimerization with other ERBB family receptors, ligand-activated EGFR and HER3, or homodimerization when it is overexpressed. This usually happens in cancer, which leads to increased cell proliferation and the promotion of cell cycle progression. Other types of HER2 aberrations found in cancer include gene amplification and mutations [135–137]. The overexpression of the HER2 protein is observed in 2–38% of lung ADCs and in 1–16% of SQCs [138–140]. Mutations in the HER2 gene are found in approximately 2% of NSCLCs, and the most common is the exon 20 HER2 in-frame insertion. These mutations are more frequently observed in female patients who have never smoked [141]. HER2 amplification was detected in 10–20% of lung ADCs [140].

Table 3. Summary of ongoing clinical trials for novel predictive biomarkers for targeted therapy.

Gene	Alteration	Drug	Eligible Patients	Trial Name	Treatment	Ref.
KRAS	G12C	AMG 510 (Sotorasib)	Previously treated, locally advanced, unresectable, or metastatic NSCLC	CodeBreak 200 NCT04303780 Phase 3	AMG 510 vs. Docetaxel	[142]
		MRTX849 (Adagrasib)	Previously treated for metastatic NSCLC	KRYSTAL-12 NCT04685135 Phase 3	MRTX849 vs. Docetaxel	[143]
	KRAS mutation in codons 12 or 13	Selumetinib + Docetaxel	Locally advanced or metastatic NSCLC	SELECT-1 NCT01933932 Phase 3	Selumetinib + Docetaxel vs. Placebo + Docetaxel	[144]
		Abemaciclib (LY2835219)	Stage IV NSCLC patients who have progressed after platinum-based chemotherapy	JUNIPER NCT02152631 Phase 3	Abemaciclib vs. Erlotinib	[145]

Table 3. *Cont.*

Gene	Alteration	Drug	Eligible Patients	Trial Name	Treatment	Ref.
	G12V, G12C	Carboplatin + paclitaxel + bevacizumab	IIIB or stage IV NSCLC patients eligible for platinum-based chemotherapy and are chemotherapy naïve	NCT02743923 Phase 3	Carboplatin-paclitaxel-bevacizumab vs. Cisplatin-pemetrexed	[146]
FGFR1	FGFR1 amplification (>5 copies)	Dovitinib	Pretreated advanced SQC	NCT01861197 Phase 2	Dovitinib	[121]
	Aberrant signaling	E7080 + Carboplatin + Paclitaxel	Advanced or metastatic NSCLC	NCT0083281 9Phase 1	E7080 + Carboplatin+ Paclitaxel	[122]
		Pazopanib	Resectable stage I/II NSCLC	NCT0036767 9Phase 2	Pazopanib	[123]
		Nintedanib BIBF 1120	Stage IIIB/IV or recurrent NSCLC after the failure of first-fine chemotherapy	LUME-Lung 1 NCT00805194 Phase 3	Nintedanib + Docetaxel vs. Placebo + Docetaxel	[124]
DDR2	DDR2 mutations	MGCD516	Advanced solid tumor, including NSCLC	NCT02219711 Phase 1	MGCD516	
HER2	exon 20 mutations	Afatinib	Pretreated patients with advanced NSCLC	NICHE NCT02369484 Phase 2	Afatinib	[147]
		Pyrotinib	Advanced non-squamous NSCLC patients who failed platinum-based chemotherapy	PYRAMID-1 NCT04447118 Phase 3	Pyrotinib vs. Docetaxel	
		Pertuzumab+ Trastuzumab + Docetaxel	NSCLC patients harboring HER2 exon 20 mutation or insertion	NCT03845270 Phase 2	Pertuzumab+ Trastuzumab + Docetaxel	
	HER2 mutations	Neratinib, temsirolimus	Advanced (stage IIIB) or metastatic (stage IV) NSCLC	NCT01827267 Phase 2	Neratinib or Neratinib + Temsirolimus	[148]
	HER2 mutations or overexpression	Trastuzumab deruxtecan (DS-8201a)	Unresectable and/or metastatic NSCLC	DESTINY-Lung01 NCT03505710 Phase 2	Trastuzumab deruxtecan (DS-8201a)	[149]

3.4. Novel Predictive Biomarkers for Immunotherapy

In this Section, we summarize the literature for reported predictive biomarkers that are already being tested in several clinical trials (e.g., dMMR, MSI, or TMB). We also report potentially novel biomarkers that are reported by only a few studies, but which we believe might become relevant to the clinics in the future. Ongoing clinical trials for novel immunotherapy biomarkers are summarized in Table 4.

Deficient mismatch repair (dMMR) and microsatellite instability (MSI): DNA mismatch repair system (MMR) is a highly conserved repair mechanism in cellular evolution. The MMR system maintains integrity and stability of the genome by overlooking genetic recombination and repairing the identified mismatched nucleotides while avoiding deletions or insertions of DNA microsatellites [150]. Deficiency in the MMR system (dMMR) is caused by germline mutations or in the case of the occurrence of sporadic tumors, most commonly due to epigenetic alterations, such as the methylation status of the four key genes MLH1, MSH2, MSH6, and PMS2 [151], which are active as DNA MMR enzymes in heterodimeric form, usually as MLH1/PMS2 and MSH2/MSH6. In the dMMR status, one or more of the MMR proteins are dysfunctional or not expressed [150]. That leads to genetic

hypermutability most frequently at the sites of microsatellites, the so called microsatellite instability (MSI) [152]. Standard sites in testing panels for MSI are BAT25, BAT26, D5S346, D2S123, and D17S250. A tumor is considered as MSI-H if alterations occur in two or more repeats [150]. MSI is not common in NSCLC; according to several studies, MSI frequency is <1% [152,153].

Tumor mutational burden (TMB): TMB is defined as the total number of non-synonymous mutations present in a tumor. TMB could be used as a predictive biomarker for nivolumab (PD-1 targeted antibody) and ipilimumab (CTLA-4 targeted antibody) treatment [154]. NSCLC patients with higher TMB (TMB-H, ≥10 mutations per megabase) treated with a combination of nivolumab and ipilimumab showed a significantly longer progression-free survival (PFS), compared to patients receiving chemotherapy as a first-line treatment. Furthermore, TMB and PD-L1 expression were shown to be independent biomarkers [155].

Interferon-gamma (IFNγ): interferon-gamma is a cytokine with diverse roles in the innate and adaptive immune system. IFNγ plays a role in antiviral activity, antimicrobial activity, and antitumor activity [156]. It was reported in the literature that patients treated with anti-PD-L1 antibodies (such as durvalumab [157] or nivolumab [158]), with a higher expression of IFNγ, had longer progression-free and overall survival, compared to patients with a lower IFNγ expression.

Tumor infiltration lymphocytes (TILs): tumor infiltration lymphocytes (TILs) are immune cells that are present in tumors. Since some of them have a role in tumor progression and some in tumor regression, they are an important target in the evaluation for anti-cancer therapy [159]. Fumet et al. observed that, in NSCLS patients treated with nivolumab, a high expression of CD8+ TILs was significantly associated with a better response rate (RR) and progression-free survival (PFS) [159]. In patients treated with EGFR TKIs, a CD8+/CD4+ ratio could be a predictive response to immunotherapy. A lower ratio is indicative of a lower response rate, compared to a higher ratio [160].

T-cell immunoglobulin and mucin-domain containing-3 (TIM-3): T-cell immunoglobulin and mucin-domain containing-3 is a member of the TIM family of immune-regulatory proteins. TIM-3 is expressed by many immune cells, and it is being studied as a therapeutic target that likely modulates immune response [161]. Limagne et al. showed that a high level of TIM-3 expression in peripheral lymphoid cells after the initiation of nivolumab treatment is an important factor that negatively affects the response to anti-PD-1 therapy. Progressive patients had greater TIM-3 expression than stable and responding patients [162].

Table 4. Summary of ongoing clinical trials for novel immunotherapy biomarkers.

Gene	Drug	Eligible Patients	Trial Name
dMMR & MSI-H	SL-279252 (PD1-Fc-OX40L)	MSI high and mismatch repair deficient NSCLC patients, excluding subjects with known EGFR sensitizing (activating) mutation or an ALK fusion	NCT03894618 Phase 1
	L-NMMA + Pembrolizumab	MSI high and mismatch repair deficient NSCLC patients	NCT03236935 Phase 1
TMB	L-NMMA + Pembrolizumab	Unresectable or metastatic tumor, TMB ≥ 10 mut/Mb	NCT03236935 Phase 1
	Atezolizumab + Bevacizumab	Stage IIIB or IV non-squamous NSCLC with TMB ≥ 10 mut/Mb	NCT03836066 (TELMA) Phase 2
TIM-3	TSR-022	NSCLC patients that have received no more than 2 prior lines of therapy, which must include a platinum-based chemotherapy and an anti-PD-(L)1 antibody	NCT02817633 (AMBER) Phase 1

Table 4. *Cont.*

Gene	Drug	Eligible Patients	Trial Name
TILs	LN-145	NSCLC patients that have received a single line of systemic therapy that included checkpoint inhibitor and chemotherapy with documented radiographic disease progression on or following this single line of systemic therapy	NCT04614103 Phase 2
	LN-145 + Pembrolizumab	Locally advanced or metastatic NSCLC with $\leq$ 3 prior lines of systemic therapy, excluding checkpoint inhibitors or $\leq$ 4 prior lines if 2 or more of the lines are TKI therapy	NCT03645928 Phase 2
	LN-145	Stage III or Stage IV NSCLC, who have previously received 1–3 lines of prior systemic therapy	NCT03645928 Phase 2
	LN-145 + Ipilimumab and Nivolumab	Stage III or Stage IV NSCLC who have previously received 1 line of approved checkpoint inhibitor monotherapy as the only prior line of systemic therapy	NCT03645928 Phase 2

4. Prognostic Biomarkers

4.1. Prognostic Biomarkers Used in NSCLC Clinical Management

Prognostic biomarkers indicate the likelihood of a patient's clinical outcome, most commonly defined as overall survival, progression-free survival (PFS), or disease-free survival rate [32]. Identifying prognostic markers in lung tumor patients is important, because it allows the recognition of patient subpopulations that might anticipate different outcomes or might benefit from different types of therapies [163]. Unlike predictive markers that interact with the treatment to influence the outcome, it is not expected that the treatment effects will be different when patients groups are distinguished by prognostic markers alone [164]. The most reliable prognostic markers are reported on patients samples involved in large studies or placebo-controlled trials, because patient characteristics in cohorts are better defined and uniform [163]. Moreover, to enlarge patient cohorts and increase statistical power, scientists also use cost-effective ways to find potential prognostic markers with a meta-analysis of comparable trials or studies.

Prognostic markers can be genes, mRNA, proteins, or miRNAs. The most studied is protein expression, usually evaluated with immunohistochemistry. Advancements in technology, such as mass spectrometry, also influence a growing number of studies focusing on proteomic signature [165]. Similarly, tumor profiling, using microarrays or next-generation sequencing, generates new potential prognostic signatures based on the mRNA [166–169], methylation [170,171], or miRNA [172] status. There is no doubt that molecular tumor profiling is a very promising and productive research area that has arisen in the last decade, with numerous emerging biomarkers reported to date. However, despite the enormous amount of data available on molecular biomarkers, results are often not reproducible, partially due to the heterogeneity of study designs, techniques used, and interpretation of the data. Therefore, many molecular prognostic markers, to date, have not managed to pave their way in routine clinical use. In addition to molecular biomarkers, there are routinely used biomarkers for prognosis assessments that are well established in clinical settings, such as TNM stage, patient age, gender, and performance status. TNM stage, an internationally accepted classification system, uses tumor size (Tis-T4), nodal involvement (N0-N3), and the presence of distant metastasis (M0-M1c) to characterize the extent of the disease. Stage groups are defined based on different combinations of T, N, and M components [173]. LC stages correlate well with survival—for example, 90% of lung cancer patients diagnosed at the early stage, when tumor has not spread to surrounding lymph nodes, are predicted to reach the 5 year survival estimation, while only 12% of patients diagnosed at the advanced stage are expected to survive that long [174]. Regarding

metastasis, a single metastatic spot is not as detrimental as multiple metastatic sites [164]. Sometimes TNM staging is combined with the molecular testing of the tumor to guide prognostic assessment and treatment. Performance status (PS) is the assessment of patients' functionality level and their ability of self-care. Oncologists assess performance status with different tools, including the ECOG (Eastern Cooperative Oncology Group) performance status or KPS (Karnofsky performance status). The KPS scoring relies on a scale that ranges from 0 to 100%, in which 100% indicates no evidence of disease or symptoms and 0% indicates death. The ECOG scoring system, also called the World Health Organization (WHO) performance status, assesses performance status using a 5-point scoring system. A PS score 0 indicates normal activity and ability to function without restraints, while a score 5 indicates death [175]. The ECOG PS is often used as eligibility criteria for clinical trials, such as chemotherapy or immune therapy trials, for which the required PS is often 1 or 0.

4.2. Novel Prognostic Molecular Biomarkers

In this Section, we summarize the available literature for reported novel prognostic biomarkers (Table 5). Although some proposed novel prognostic biomarkers are still controversial, due to inconsistencies among reported studies, we believe that they show good potential and they might become relevant with time as the number of validation studies increases.

TP53: TP53 gene encodes the tumor-suppressor protein p53, an important player in cell cycle regulation, senescence, autophagy, apoptosis, and DNA repair in response to damaging agents [176]. Mutations in p53 lead to a loss of p53 tumor-suppressor functions, resulting in excessive cell proliferation and cancer promotion [177]. In NSCLCs, it seems that mutations of p53 are more frequent in SQCs compared to ADCs (77% vs. 47%, respectively) [178]. To date, several studies reported that the p53 mutational status in NSCLCs is associated with poorer survival and increased resistance to cancer therapy, compared to $TP53^{WT}$ [178–180]. However, some studies did not confirm p53 as a prognostic factor in NSCLCs [181].

Vascular endothelial growth factor (VEGF): tumor cells supply nutrients to grow and disseminate via existing blood vessels or angiogenesis. The vascular endothelial growth factor (VEGF) affects microvascular permeability, stimulates the growth of endothelial cells, and is pre-eminent in the formation of a new blood vessel in angiogenesis. VEGF overexpression is associated with tumor recurrence and metastasis, and is common in many cancer types, including lung cancer [182]. Several studies report that overexpression of the VEGF indicates poor prognosis in NSCLCs [183–185].

Class III β-tubulin (TUBB3): TUBB3 is an isotype of beta-tubulin that is normally found in various tissues [186–188], where polymers of tubulin form microtubules. Several studies indicate that high expression of TUBB3 is an indicator of poor prognosis in NSCLCs [189] and correlate an abundant TUBB3 expression with a reduced response to anti-tubulin-based chemotherapy, such as taxane or vinorelbine [190–192].

Ki-67: Ki-67 is encoded by the MKI67 gene. Since it is expressed in actively dividing cells throughout the cell cycle, reaching its expression peaks at the M phase, Ki-67 serves as a good proliferation marker [193]. The high expression of Ki-67 has been correlated with poor prognosis in several cancer types, including NSCLCs [194–196].

Excision repair cross complementing group 1 (ERCC1): ERCC1 protein plays an important role in the nucleotide excision repair pathway (NER) that is important for the maintenance of genomic stability. Studies indicate that a low expression of ERCC1 is an indicator of poor survival and that expression is generally higher in SQC histology [197,198].

Transforming growth factor-beta (TGF-β): TGF-β belongs to the cytokine family and is involved in cell proliferation, differentiation, and extracellular matrix production [199]. Although there is very little information on its prognostic potential, a few studies reported that a high TGF-β1 protein expression indicates poor prognosis [200,201]. Further investigations are needed to confirm these findings, but current studies on this issue are currently lacking.

Lymphocyte-activation gene 3 (LAG-3): lymphocyte-activation gene 3 is expressed on Tregs, and is involved in mediating their function [202]. It has been shown that NSCLC patients whose TILs were LAG-3$^-$ have longer recurrence-free survival (RFS) and OS versus NSCLC patients whose TILs were LAG-3$^+$. Moreover, a high expression of LAG-3 is correlated with a higher expression of PD-1 on TILs. When taking both LAG-3 and PD-L1 expression into account, patients whose tumor cells are PD-L1$^-$ and LAG-3$^-$ TILs have longer RFS than patients who are PD-L1$^+$ or LAG-3$^+$ or both positive [203]. However, Hald et al. have shown that the expression of LAG-3 on TILs in primary NSCLC tumors and metastatic lymph nodes is associated with improved survival [204], so further validation studies on its use as potential prognostic biomarker are needed.

KIAA1522: even though the KIAA1522's function is still unknown, in vitro experiments have shown that it is involved in the oncogenic KRAS signaling in lung cancer cells. In NSCLC patients, a lower OS has been linked with a high expression of KIAA1522, compared to those with a low expression of the protein, regardless of the stage and histological type (SQC and ADC). Furthermore, patients with a lower KIAA1522 expression that were treated with platinum-based chemotherapy have longer OS, in comparison to those with a lower KIAA1522 expression treated platinum-based chemotherapy [205].

Neutrophil-to-lymphocyte ratio (NLR) and platelet-to-lymphocyte ratio (PLR): inflammation plays an important role in both the development and propagation of lung cancer. Pretreatment neutrophil-to-lymphocyte ratio (NLR), as well as platelet-to-lymphocyte ratio (PLR), are signs of systemic inflammatory response, and they are closely related to the prognosis of various cancers [206]. Recently, there have been many reports of NLR and/or PLR as prognostic markers for various treatments. Studies show that, in patients with metastatic NSCLC, treatment with nivolumab elevated pretreatment levels of NLR and PLR, which are associated with a worse OS and a lower response rate (RR) [207,208].

Table 5. Summary of novel prognostic biomarkers.

Prognostic Biomarker	Alteration	Outcome
TP53	p53 mutations	Poorer survival, increased resistance to therapy
VEGF	High expression	Poor prognosis, tumor recurrence, metastasis
TUBB3	High expression	Poor prognosis
Ki-67	High expression	Poor prognosis
ERCC1	Low expression	Poor prognosis
TGF-β	High expression	Poor prognosis
LAG-3	Low expression High expression	Longer RFS and OS [203] Better survival [204]
KIAA1522	High expression High expression + platinum-based chemoterapy	Lower OS Longer OS
NLR & PLR	High NLR and PLR + Nivolumab	Worse OS, lower RR

OR—overall survival; RFS—recurrence-free survival; RR—response rate.

5. Summary and Conclusions

Lung cancer is a complex disease, and its successful treatment depends on well-defined patient characteristics, histologic type of tumor, assessed biomarkers, and good and prompt communication between pathologists and oncologists. Over the last decade, significant progress in developing therapy with complementary predictive biomarkers for NSCLCs has been made. While diagnostic biomarkers are well established in clinical routine, the number of predictive biomarkers (and their associated therapeutical options) will increase in the near future due to the numerous research efforts to identify new potential biomarkers and the new trials that are incorporating these findings. However, how will those rapid

changes affect routine clinical practice remains to be seen. Even though current biomarkers notably improved NSCLC treatment, there is still a need for novel predictors and targeted therapies that could help to achieve better outcomes and cost-effectiveness in treating patients with NSCLCs, especially those with SQC subtype.

Author Contributions: Conceptualization, J.K. and L.B.; writing—original draft preparation, M.Š. and A.V.; writing—review and editing, A.F., M.Š., J.B., F.D., M.J., and M.S.; visualization, M.Š., A.V., and J.B; supervision, J.K.; funding acquisition, J.K. All authors have read and agreed to the published version of the manuscript.

Funding: This work was supported by the Croatian Science Foundation, project number IP-2016-06-1441, LungInflaCare (JK).

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: Not applicable.

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

References

1. Ferlay, J.; Colombet, M.; Soerjomataram, I.; Parkin, D.M.; Piñeros, M.; Znaor, A.; Bray, F. Cancer statistics for the year 2020: An overview. *Int. J. Cancer* **2021**, *149*, 778–789. [CrossRef]
2. Society, A.C. Cancer Facts & Figures 2020. Available online: https://www.cancer.org/content/dam/cancer-org/research/cancer-facts-and-statistics/annual-cancer-facts-and-figures/2020/cancer-facts-and-figures-2020.pdf (accessed on 7 May 2020).
3. Osmani, L.; Askin, F.; Gabrielson, E.; Li, Q.K. Current WHO Guidelines and the Critical Role of Immunohistochemical Markers in the Subclassification of Non-Small Cell Lung Carcinoma (NSCLC). Moving from Targeted Therapy to Immunotherapy. *Semin. Cancer Biol.* **2018**, *52*, 103–109. [CrossRef] [PubMed]
4. Howlader, N.; Noone, A.M.; Krapcho, M.; Garshell, J.; Miller, D.; Altekruse, S.F.; Kosary, C.L.; Yu, M.; Ruhl, J.; Tatalovich, Z.; et al. *SEER Cancer Statistics Review, 1975–2016*; National Cancer Institute: Bethesda, MD, USA, 2019.
5. Ahmadzada, T.; Kao, S.; Reid, G.; Boyer, M.; Mahar, A.; Cooper, W. An Update on Predictive Biomarkers for Treatment Selection in Non-Small Cell Lung Cancer. *J. Clin. Med.* **2018**, *7*, 153. [CrossRef] [PubMed]
6. Román, M.; Baraibar, I.; López, I.; Nadal, E.; Rolfo, C.; Vicent, S.; Gil-Bazo, I. KRAS oncogene in non-small cell lung cancer: Clinical perspectives on the treatment of an old target. *Mol. Cancer* **2018**, *17*, 33. [CrossRef]
7. Cheng, T.Y.D.; Cramb, S.M.; Baade, P.D.; Youlden, D.R.; Nwogu, C.; Reid, M.E. The international epidemiology of lung cancer: Latest trends, disparities, and tumor characteristics. *J. Thorac. Oncol.* **2016**, *11*, 1653–1671. [CrossRef]
8. Allemani, C.; Matsuda, T.; Di Carlo, V.; Harewood, R.; Matz, M.; Nikšić, M.; Bonaventure, A.; Valkov, M.; Johnson, C.J.; Estève, J.; et al. Global surveillance of trends in cancer survival 2000–14 (CONCORD-3): Analysis of individual records for 37,513,025 patients diagnosed with one of 18 cancers from 322 population-based registries in 71 countries. *Lancet* **2018**, *391*, 1023–1075. [CrossRef]
9. Johnson, D.H.; Fehrenbacher, L.; Novotny, W.F.; Herbst, R.S.; Nemunaitis, J.J.; Jablons, D.M.; Langer, C.J.; DeVore, R.F.; Gaudreault, J.; Damico, L.A.; et al. Randomized phase II trial comparing bevacizumab plus carboplatin and paclitaxel with carboplatin and paclitaxel alone in previously untreated locally advanced or metastatic non-small-cell lung cancer. *J. Clin. Oncol.* **2004**, *22*, 2184–2191. [CrossRef] [PubMed]
10. Al-Saleh Mbbs, K.; Quinton, C.; Ellis, P.M. Medical Oncology Role of pemetrexed in advanced non-small-cell lung cancer: Meta-analysis of randomized controlled trials, with histology subgroup analysis. *Curr. Oncol.* **2012**, *19*, 9–15. [CrossRef] [PubMed]
11. Travis, W.D.; Brambilla, E.; Nicholson, A.G.; Yatabe, Y.; Austin, J.H.M.; Beth Beasley, M.; Chirieac, L.R.; Dacic, S.; Duhig, E.; Flieder, D.B.; et al. The 2015 world health organization classification of lung tumors. *J. Thorac. Oncol.* **2015**, *10*, 1243–1260. [CrossRef]
12. Travis, W.D.; Brambilla, E.; Noguchi, M.; Nicholson, A.G.; Geisinger, K.R.; Yatabe, Y.; Beer, D.G.; Powell, C.A.; Riely, G.J.; Van Schil, P.E.; et al. International association for the study of lung cancer/American Thoracic Society/European Respiratory Society international multidisciplinary classification of lung adenocarcinoma. *J. Thorac. Oncol.* **2011**, *6*, 244–285. [CrossRef]
13. WHO. *WHO Classification of Tumours Editorial Board. Thoracic Tumours*; International Agency for Research on Cancer: Lyon, France, 2021.
14. Latimer, K.; Mott, T. Lung Cancer: Diagnosis, Treatment Principles, and Screening. *Am. Fam. Physician* **2015**, *91*, 250–256. [PubMed]
15. Planchard, D.; Popat, S.; Kerr, K.; Novello, S.; Smit, E.F.; Faivre-Finn, C.; Mok, T.S.; Reck, M.; Van Schil, P.E.; Hellmann, M.D. ESMO Clinical Practice Guidelines for diagnosis, treatment and follow-up. *Ann. Oncol.* **2018**, *29* (Suppl. 4), iv192–iv237. [CrossRef]
16. Cagle, P.T.; Allen, T.C.; Bernicker, E.H.; Ge, Y.; Haque, A.; Barrios, R. Impact of recent developments in lung cancer on the practice of pathology. *Arch. Pathol. Lab. Med.* **2016**, *140*, 322–325. [CrossRef] [PubMed]

17. Bernardi, F.D.C.; Bernardi, M.D.C.; Takagaki, T.; Siqueira, S.A.C.; Dolhnikoff, M. Lung cancer biopsy: Can diagnosis be changed after immunohistochemistry when the H&E-Based morphology corresponds to a specific tumor subtype? *Clinics* **2018**, *73*, e361. [PubMed]
18. Lindeman, N.I.; Cagle, P.T.; Beasley, M.B.; Chitale, D.A.; Dacic, S.; Giaccone, G.; Jenkins, R.B.; Kwiatkowski, D.J.; Saldivar, J.S.; Squire, J.; et al. Molecular testing guideline for selection of lung cancer patients for EGFR and ALK tyrosine kinase inhibitors: Guideline from the College of American Pathologists, International Association for the Study of Lung Cancer, and Association for Molecular Pathology. *J. Thorac. Oncol.* **2013**, *8*, 823–859. [CrossRef]
19. Guibert, N.; Pradines, A.; Mazieres, J.; Favre, G. Current and future applications of liquid biopsy in nonsmall cell lung cancer from early to advanced stages. *Eur. Respir. Rev.* **2020**, *29*, 190052. [CrossRef] [PubMed]
20. Castro-Giner, F.; Gkountela, S.; Donato, C.; Alborelli, I.; Quagliata, L.; Ng, C.; Piscuoglio, S.; Aceto, N. Cancer Diagnosis Using a Liquid Biopsy: Challenges and Expectations. *Diagnostics* **2018**, *8*, 31. [CrossRef]
21. Duma, N.; Santana-Davila, R.; Molina, J.R. Non–Small Cell Lung Cancer: Epidemiology, Screening, Diagnosis, and Treatment. *Mayo Clin. Proc.* **2019**, *94*, 1623–1640. [CrossRef]
22. Shepherd, F.A.; Rodrigues Pereira, J.; Ciuleanu, T.; Tan, E.H.; Hirsh, V.; Thongprasert, S.; Campos, D.; Maoleekoonpiroj, S.; Smylie, M.; Martins, R.; et al. Erlotinib in Previously Treated Non–Small-Cell Lung Cancer. *N. Engl. J. Med.* **2005**, *353*, 123–132. [CrossRef]
23. Thatcher, N.; Chang, A.; Parikh, P.; Pereira, J.R.; Ciuleanu, T.; Von Pawel, J.; Thongprasert, S.; Tan, E.H.; Pemberton, K.; Archer, V.; et al. Gefitinib plus best supportive care in previously treated patients with refractory advanced non-small-cell lung cancer: Results from a randomised, placebo-controlled, multicentre study (Iressa Survival Evaluation in Lung Cancer). *Lancet* **2005**, *366*, 1527–1537. [CrossRef]
24. Shaw, A.T.; Kim, D.-W.; Nakagawa, K.; Seto, T.; Crinó, L.; Ahn, M.-J.; De Pas, T.; Besse, B.; Solomon, B.J.; Blackhall, F.; et al. Crizotinib versus Chemotherapy in Advanced ALK-Positive Lung Cancer. *N. Engl. J. Med.* **2013**, *368*, 2385–2394. [CrossRef] [PubMed]
25. Peters, S.; Gettinger, S.; Johnson, M.L.; Jänne, P.A.; Garassino, M.C.; Christoph, D.; Toh, C.K.; Rizvi, N.A.; Chaft, J.E.; Costa, E.C.; et al. Phase II trial of atezolizumab as first-line or subsequent therapy for patients with programmed death-ligand 1-selected advanced non-small-cell lung cancer (BIRCH). *J. Clin. Oncol.* **2017**, *35*, 2781–2789. [CrossRef]
26. Reck, M.; Rodriguez-Abreu, D.; Robinson, A.G.; Hui, R.; Csöszi, T.; Fülöp, A.; Gottfried, M.; Peled, N.; Tafreshi, A.; Cuffe, S.; et al. Pembrolizumab versus Chemotherapy for PD-L1-Positive Non-Small-Cell Lung Cancer. *N. Engl. J. Med.* **2016**, *375*, 1823–1833. [CrossRef]
27. Kohno, T.; Nakaoku, T.; Tsuta, K.; Tsuchihara, K.; Matsumoto, S.; Yoh, K.; Goto, K. Beyond ALK-RET, ROS1 and other oncogene fusions in lung cancer. *Transl. Lung Cancer Res.* **2015**, *4*, 156–164. [CrossRef]
28. Hanna, N.; Johnson, D.; Temin, S.; Baker, S.; Brahmer, J.; Ellis, P.M.; Giaccone, G.; Hesketh, P.J.; Jaiyesimi, I.; Leighl, N.B.; et al. Systemic therapy for stage IV non–small-cell lung cancer: American Society of clinical oncology clinical practice guideline update. *J. Clin. Oncol.* **2017**, *35*, 3484–3515. [CrossRef] [PubMed]
29. Paz-Ares, L.; Luft, A.; Vicente, D.; Tafreshi, A.; Gümüş, M.; Mazières, J.; Hermes, B.; Çay Şenler, F.; Csőszi, T.; Fülöp, A.; et al. Pembrolizumab plus Chemotherapy for Squamous Non–Small-Cell Lung Cancer. *N. Engl. J. Med.* **2018**, *379*, 2040–2051. [CrossRef] [PubMed]
30. 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*, 018-0360. [CrossRef]
31. Davies, J.C.; Wainwright, C.E.; Canny, G.J.; Chilvers, M.A.; Howenstine, M.S.; Munck, A.; Mainz, J.G.; Rodriguez, S.; Li, H.; Yen, K.; et al. Efficacy and safety of ivacaftor in patients aged 6 to 11 years with cystic fibrosis with a G551D mutation. *Am. J. Respir. Crit. Care Med.* **2013**, *187*, 1219–1225. [CrossRef]
32. Califf, R.M. Biomarker definitions and their applications. *Exp. Biol. Med.* **2018**, *243*, 213–221. [CrossRef]
33. Rastel, D.; Ramaioli, A.; Cornillie, F.; Thirion, B. CYFRA 21-1, a sensitive and specific new tumour marker for squamous cell lung cancer. Report of the first European multicentre evaluation. *Eur. J. Cancer* **1994**, *30*, 601–606. [CrossRef]
34. Okamura, K.; Takayama, K.; Izumi, M.; Harada, T.; Furuyama, K.; Nakanishi, Y. Diagnostic value of CEA and CYFRA 21-1 tumor markers in primary lung cancer. *Lung Cancer* **2013**, *80*, 45–49. [CrossRef] [PubMed]
35. Chen, F.; Wang, X.-Y.; Han, X.-H.; Wang, H.; Qi, J. Diagnostic value of Cyfra21-1, SCC and CEA for differentiation of early-stage NSCLC from benign lung disease. *Int. J. Clin. Exp. Med.* **2015**, *8*, 11295–11300. [PubMed]
36. Doseeva, V.; Colpitts, T.; Gao, G.; Woodcock, J.; Knezevic, V. Performance of a multiplexed dual analyte immunoassay for the early detection of non-small cell lung cancer. *J. Transl. Med.* **2015**, *13*, 55. [CrossRef] [PubMed]
37. Fang, R.; Zhu, Y.; Khadka, V.S.; Zhang, F.; Jiang, B.; Deng, Y. The Evaluation of Serum Biomarkers for Non-small Cell Lung Cancer (NSCLC) Diagnosis. *Front. Physiol.* **2018**, *9*, 1710. [CrossRef] [PubMed]
38. Patz, E.F.; Campa, M.J.; Gottlin, E.B.; Kusmartseva, I.; Guan, X.R.; Herndon, J.E. Panel of serum biomarkers for the diagnosis of lung cancer. *J. Clin. Oncol.* **2007**, *25*, 5578–5583. [CrossRef] [PubMed]
39. Jiang, Z.F.; Wang, M.; Xu, J.L. Thymidine kinase 1 combined with CEA, CYFRA21-1 and NSE improved its diagnostic value for lung cancer. *Life Sci.* **2018**, *194*, 1–6. [CrossRef] [PubMed]
40. Zang, R.; Li, Y.; Jin, R.; Wang, X.; Lei, Y.; Che, Y.; Lu, Z.; Mao, S.; Huang, J.; Liu, C.; et al. Enhancement of diagnostic performance in lung cancers by combining CEA and CA125 with autoantibodies detection. *Oncoimmunology* **2019**, *8*, e1625689. [CrossRef]

41. Yang, B.; Li, X.; Ren, T.; Yin, Y. Autoantibodies as diagnostic biomarkers for lung cancer: A systematic review. *Cell Death Discov.* **2019**, *5*, 126. [CrossRef] [PubMed]
42. Chapman, C.J.; Murray, A.; McElveen, J.E.; Sahin, U.; Luxemburger, U.; Türeci, Ö.; Wiewrodt, R.; Barnes, A.C.; Robertson, J.F. Autoantibodies in lung cancer: Possibilities for early detection and subsequent cure. *Thorax* **2008**, *63*, 228–233. [CrossRef] [PubMed]
43. Wu, K.L.; Tsai, Y.M.; Lien, C.T.; Kuo, P.L.; Hung, J.Y. The roles of microRNA in lung cancer. *Int. J. Mol. Sci.* **2019**, *20*, 1611. [CrossRef] [PubMed]
44. Weber, J.A.; Baxter, D.H.; Zhang, S.; Huang, D.Y.; Huang, K.H.; Lee, M.J.; Galas, D.J.; Wang, K. The MicroRNA Spectrum in 12 Body Fluids. *Clin. Chem.* **2010**, *56*, 1733–1741. [CrossRef]
45. Pan, J.; Zhou, C.; Zhao, X.; He, J.; Tian, H.; Shen, W.; Han, Y.; Chen, J.; Fang, S.; Meng, X.; et al. A two-miRNA signature (miR-33a-5p and miR-128-3p) in whole blood as potential biomarker for early diagnosis of lung cancer. *Sci. Rep.* **2018**, *8*, 16699. [CrossRef] [PubMed]
46. Aharonov, R.; Lebanony, D.; Benjamin, H.; Gilad, S.; Ezagouri, M.; Dov, A.; Ashkenazi, K.; Gefen, N.; Izraeli, S.; Rechavi, G.; et al. Diagnostic assay based on hsa-miR-205 expression distinguishes squamous from nonsquamous non-small-cell lung carcinoma. *J. Clin. Oncol.* **2009**, *27*, 2030–2037. [CrossRef]
47. Bishop, J.A.; Benjamin, H.; Cholakh, H.; Chajut, A.; Clark, D.P.; Westra, W.H. Accurate classification of non-small cell lung carcinoma using a novel microRNA-based approach. *Clin. Cancer Res.* **2010**, *16*, 610–619. [CrossRef] [PubMed]
48. Patnaik, S.; Mallick, R.; Kannisto, E.; Sharma, R.; Bshara, W.; Yendamuri, S.; Dhillon, S.S. MIR-205 and MIR-375 MicroRNA assays to distinguish squamous cell carcinoma from adenocarcinoma in lung cancer biopsies. *J. Thorac. Oncol.* **2015**, *10*, 446–453. [CrossRef] [PubMed]
49. Zhang, Y.K.; Zhu, W.Y.; He, J.Y.; Chen, D.D.; Huang, Y.Y.; Le, H.B.; Liu, X.G. MiRNAs expression profiling to distinguish lung squamous-cell carcinoma from adenocarcinoma subtypes. *J. Cancer Res. Clin. Oncol.* **2012**, *138*, 1641–1650. [CrossRef] [PubMed]
50. Gilad, S.; Lithwick-Yanai, G.; Barshack, I.; Benjamin, S.; Krivitsky, I.; Edmonston, T.B.; Bibbo, M.; Thurm, C.; Horowitz, L.; Huang, Y.; et al. Classification of the four main types of lung cancer using a microRNA-based diagnostic assay. *J. Mol. Diagn.* **2012**, *14*, 510–517. [CrossRef] [PubMed]
51. Gao, X.; Wang, Y.; Zhao, H.; Wei, F.; Zhang, X.; Su, Y.; Wang, C.; Li, H.; Ren, X. Plasma miR-324-3p and miR-1285 as diagnostic and prognostic biomarkers for early stage lung squamous cell carcinoma. *Oncotarget* **2016**, *7*, 59664–59675. [CrossRef] [PubMed]
52. Wang, C.; Ding, M.; Xia, M.; Chen, S.; Van Le, A.; Soto-Gil, R.; Shen, Y.; Wang, N.; Wang, J.; Gu, W.; et al. A Five-miRNA Panel Identified from a Multicentric Case-control Study Serves as a Novel Diagnostic Tool for Ethnically Diverse Non-small-cell Lung Cancer Patients. *EBioMedicine* **2015**, *2*, 1377–1385. [CrossRef] [PubMed]
53. Sozzi, G.; Boeri, M.; Rossi, M.; Verri, C.; Suatoni, P.; Bravi, F.; Roz, L.; Conte, D.; Grassi, M.; Sverzellati, N.; et al. Clinical Utility of a Plasma-Based miRNA Signature Classifier within Computed Tomography Lung Cancer Screening: A Correlative MILD Trial Study. *J. Clin. Oncol.* **2014**, *32*, 768–773. [CrossRef]
54. Montani, F.; Jacopo Marzi, M.; Dezi, F.; Dama, E.; Mary Carletti, R.; Bonizzi, G.; Bertolotti, R.; Bellomi, M.; Rampinelli, C.; Maisonneuve, P.; et al. miR-Test: A Blood Test for Lung Cancer Early Detection. *J. Natl. Cancer Inst.* **2015**, *107*, 63. [CrossRef] [PubMed]
55. Standfield, L.; Weston, A.R.; Barraclough, H.; Van Kooten, M.; Pavlakis, N. Histology as a treatment effect modifier in advanced non-small cell lung cancer: A systematic review of the evidence. *Respirology* **2011**, *16*, 1210–1220. [CrossRef] [PubMed]
56. Pennell, N.A.; Arcila, M.E.; Gandara, D.R.; West, H. Biomarker Testing for Patients with Advanced Non–Small Cell Lung Cancer: Real-World Issues and Tough Choices. *Am. Soc. Clin. Oncol. Educ. Book* **2019**, 531–542. [CrossRef] [PubMed]
57. Garrido, P.; Conde, E.; de Castro, J.; Gómez-Román, J.J.; Felip, E.; Pijuan, L.; Isla, D.; Sanz, J.; Paz-Ares, L.; López-Ríos, F. Updated guidelines for predictive biomarker testing in advanced non-small-cell lung cancer: A National Consensus of the Spanish Society of Pathology and the Spanish Society of Medical Oncology. *Clin. Transl. Oncol.* **2020**, *22*, 989–1003. [CrossRef] [PubMed]
58. Lindeman, N.I.; Cagle, P.T.; Aisner, D.L.; Arcila, M.E.; Beasley, M.B.; Bernicker, E.H.; Colasacco, C.; Dacic, S.; Hirsch, F.R.; Kerr, K.; et al. Updated molecular testing guideline for the selection of lung cancer patients for treatment with targeted tyrosine kinase inhibitors guideline from the college of American pathologists, the international association for the study of lung cancer, and the association for molecular pathology. *Arch. Pathol. Lab. Med.* **2018**, *142*, 321–346. [CrossRef] [PubMed]
59. Wu, Y.L.; Planchard, D.; Lu, S.; Sun, H.; Yamamoto, N.; Kim, D.W.; Tan, D.S.W.; Yang, J.C.H.; Azrif, M.; Mitsudomi, T.; et al. Pan-asian adapted clinical practice guidelines for the management of patients with metastatic non-small-cell lung cancer: A csco-esmo initiative endorsed by jsmo, ksmo, mos, sso and tos. *Ann. Oncol.* **2019**, *30*, 171–210. [CrossRef]
60. Popper, H.H.; Gruber-Mösenbacher, U.; Pall, G.; Müllauer, L.; Hochmair, M.; Krenbek, D.; Brcic, L.; Schmitz, K.; Lamprecht, B.; Eckmayr, J.; et al. The 2020 update of the recommendations of the Austrian working group on lung pathology and oncology for the diagnostic workup of non-small cell lung cancer with focus on predictive biomarkers. *Memo Mag. Eur. Med. Oncol.* **2020**, *13*, 11–26. [CrossRef]
61. Leukam, M.J.; Villaflor, V.M. Advances in molecular and immunologic targeted therapies for squamous cell carcinoma of the lung. *Transl. Cancer Res.* **2015**, *4*, 403–414. [CrossRef]
62. Cooper, W.A.; O'toole, S.; Boyer, M.; Horvath, L.; Mahar, A. What's new in non-small cell lung cancer forpathologists: The importance of accurate subtyping, EGFR mutations and ALK rearrangements. *Pathology* **2011**, *43*, 103–115. [CrossRef] [PubMed]

63. Lu, S. Development of treatment options for Chinese patients with advanced squamous cell lung cancer: Focus on afatinib. *Onco. Targets Ther.* **2019**, *12*, 1521–1538. [CrossRef]
64. Yang, Z.; Hackshaw, A.; Feng, Q.; Fu, X.; Zhang, Y.; Mao, C.; Tang, J. Comparison of gefitinib, erlotinib and afatinib in non-small cell lung cancer: A meta-analysis. *Int. J. Cancer* **2017**, *140*, 2805–2819. [CrossRef] [PubMed]
65. Daaboul, N.; Nicholas, G.; Laurie, S.A. Algorithm for the treatment of advanced or metastatic squamous non-small-cell lung cancer: An evidence-based overview. *Curr. Oncol.* **2018**, *25*, S77–S85. [CrossRef] [PubMed]
66. Wecker, H.; Waller, C.F. Afatinib. In *Recent Results in Cancer Research*; Springer: New York, NY, USA, 2018; pp. 199–215.
67. Zheng, D.; Hu, M.; Bai, Y.; Zhu, X.; Lu, X.; Wu, C.; Wang, J.; Liu, L.; Wang, Z.; Ni, J.; et al. EGFR G796D mutation mediates resistance to osimertinib. *Oncotarget* **2017**, *8*, 49671–49679. [CrossRef]
68. Wu, W.; Haderk, F.; Bivona, T.G. Non-canonical thinking for targeting ALK-fusion onco-proteins in lung cancer. *Cancers* **2017**, *9*, 164. [CrossRef] [PubMed]
69. Gadgeel, S. Approach to Anaplastic Lymphoma Kinase (ALK) Gene Rearranged Non–Small Cell Lung Cancer (NSCLC). In *Pulmonary Adenocarcinoma: Approaches to Treatment*; Elsevier: Amsterdam, The Netherlands, 2019; pp. 133–142.
70. Du, X.; Shao, Y.; Qin, H.-F.; Tai, Y.-H.; Gao, H.-J. ALK-rearrangement in non-small-cell lung cancer (NSCLC). *Thorac. Cancer* **2018**, *9*, 423–430. [CrossRef]
71. Rothschild, S.I.; Gautschi, O. Crizotinib in the treatment of non-small-cell lung cancer. *Clin. Lung Cancer* **2013**, *14*, 473–480. [CrossRef] [PubMed]
72. Forde, P.M.; Rudin, C.M. Crizotinib in the treatment of non-small-cell lung cancer. *Expert Opin Pharmacother* **2012**, *13*, 1195–1201. [CrossRef] [PubMed]
73. Morris, T.A.; Khoo, C.; Solomon, B.J. Targeting ROS1 Rearrangements in Non-small Cell Lung Cancer: Crizotinib and Newer Generation Tyrosine Kinase Inhibitors. *Drugs* **2019**, *79*, 1277–1286. [CrossRef]
74. Bergethon, K.; Shaw, A.T.; Ou, S.H.I.; Katayama, R.; Lovly, C.M.; McDonald, N.T.; Massion, P.P.; Siwak-Tapp, C.; Gonzalez, A.; Fang, R.; et al. ROS1 rearrangements define a unique molecular class of lung cancers. *J. Clin. Oncol.* **2012**, *30*, 863–870. [CrossRef]
75. Zhao, W.; Choi, Y.L.; Song, J.Y.; Zhu, Y.; Xu, Q.; Zhang, F.; Jiang, L.; Cheng, J.; Zheng, G.; Mao, M. ALK, ROS1 and RET rearrangements in lung squamous cell carcinoma are very rare. *Lung Cancer* **2016**, *94*, 22–27. [CrossRef]
76. Rotow, J.; Bivona, T.G. Understanding and targeting resistance mechanisms in NSCLC. *Nat. Rev. Cancer* **2017**, *17*, 637–658. [CrossRef]
77. Shaw, A.T.; Kim, T.M.; Crinò, L.; Gridelli, C.; Kiura, K.; Liu, G.; Novello, S.; Bearz, A.; Gautschi, O.; Mok, T.; et al. Ceritinib versus chemotherapy in patients with ALK-rearranged non-small-cell lung cancer previously given chemotherapy and crizotinib (ASCEND-5): A randomised, controlled, open-label, phase 3 trial. *Lancet Oncol.* **2017**, *18*, 874–886. [CrossRef]
78. Camidge, D.R.; Kim, H.R.; Ahn, M.-J.; Yang, J.C.-H.; Han, J.-Y.; Lee, J.-S.; Hochmair, M.J.; Li, J.Y.-C.; Chang, G.-C.; Lee, K.H.; et al. Brigatinib versus Crizotinib in ALK -Positive Non–Small-Cell Lung Cancer. *N. Engl. J. Med.* **2018**, *379*, 2027–2039. [CrossRef] [PubMed]
79. Peters, S.; Camidge, D.R.; Shaw, A.T.; Gadgeel, S.; Ahn, J.S.; Kim, D.-W.; Ou, S.-H.I.; Pérol, M.; Dziadziuszko, R.; Rosell, R.; et al. Alectinib versus Crizotinib in Untreated ALK -Positive Non–Small-Cell Lung Cancer. *N. Engl. J. Med.* **2017**, *377*, 829–838. [CrossRef] [PubMed]
80. Lim, F.; Ponce, S.; Patel, S.; Van Herpen, C.; Kurkjian, C.; Lou, Y.; Liu, Y.; Ramsingh, G.; Pal, S.; Neal, J. P1.01-113 Phase 1b Trial of Cabozantinib or Cabozantinib Plus Atezolizumab in Patients with Advanced Non-Small Cell Lung Cancer (NSCLC). *J. Thorac. Oncol.* **2019**, *14*, S405–S406. [CrossRef]
81. Guo, R.; Preeshagul, I.; Schoenfeld, A.; Mccarthy, C.; Makhnin, A.; Plodkowski, A.; Ginsberg, M.; Davare, M.; Delasos, L.; Somwar, R.; et al. P1.14-50 A Phase 2 Trial of Cabozantinib in ROS1-Rearranged Lung Adenocarcinoma. *J. Thorac. Oncol.* **2019**, *14*, S574–S575. [CrossRef]
82. Shaw, A.T.; Solomon, B.J.; Chiari, R.; Riely, G.J.; Besse, B.; Soo, R.A.; Kao, S.; Lin, C.C.; Bauer, T.M.; Clancy, J.S.; et al. Lorlatinib in advanced ROS1-positive non-small-cell lung cancer: A multicentre, open-label, single-arm, phase 1–2 trial. *Lancet Oncol.* **2019**, *20*, 1691–1701. [CrossRef]
83. Lin, Q.; Zhang, H.; Ding, H.; Qian, J.; Lizaso, A.; Lin, J.; Han-Zhang, H.; Xiang, J.; Li, Y.; Zhu, H. The association between BRAF mutation class and clinical features in BRAF-mutant Chinese non-small cell lung cancer patients. *J. Transl. Med.* **2019**, *17*, 298. [CrossRef] [PubMed]
84. Brustugun, O.T.; Khattak, A.M.; Trømborg, A.K.; Beigi, M.; Beiske, K.; Lund-Iversen, M.; Helland, Å. BRAF-mutations in non-small cell lung cancer. *Lung Cancer* **2014**, *84*, 36–38. [CrossRef]
85. Tissot, C.; Couraud, S.; Tanguy, R.; Bringuier, P.P.; Girard, N.; Souquet, P.J. Clinical characteristics and outcome of patients with lung cancer harboring BRAF mutations. *Lung Cancer* **2016**, *91*, 23–28. [CrossRef] [PubMed]
86. Bustamante Alvarez, J.G.; Otterson, G.A. Agents to treat BRAF-mutant lung cancer. *Drugs Context* **2019**, *8*, 212566. [CrossRef] [PubMed]
87. Moosavi, F.; Giovannetti, E.; Saso, L.; Firuzi, O. HGF/MET pathway aberrations as diagnostic, prognostic, and predictive biomarkers in human cancers. *Crit. Rev. Clin. Lab. Sci.* **2019**, *56*, 533–566. [CrossRef] [PubMed]
88. Paik, P.K.; Drilon, A.; Fan, P.D.; Yu, H.; Rekhtman, N.; Ginsberg, M.S.; Borsu, L.; Schultz, N.; Berger, M.F.; Rudin, C.M.; et al. Response to MET inhibitors in patients with stage IV lung adenocarcinomas harboring MET mutations causing exon 14 skipping. *Cancer Discov.* **2015**, *5*, 842–849. [CrossRef] [PubMed]

89. Scagliotti, G.; Von Pawel, J.; Novello, S.; Ramlau, R.; Favaretto, A.; Barlesi, F.; Akerley, W.; Orlov, S.; Santoro, A.; Spigel, D.; et al. Phase III multinational, randomized, double-blind, placebo-controlled study of tivantinib (ARQ 197) plus erlotinib versus erlotinib alone in previously treated patients with locally advanced or metastatic nonsquamous non-small-cell lung cancer. *J. Clin. Oncol.* **2015**, *33*, 2667–2674. [CrossRef] [PubMed]
90. 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]
91. Wolf, J.; Seto, T.; Han, J.-Y.; Reguart, N.; Garon, E.B.; Groen, H.J.M.; Tan, D.S.-W.; Hida, T.; De Jonge, M.J.; Orlov, S.V.; et al. Capmatinib (INC280) in METΔex14 -mutated advanced non-small cell lung cancer (NSCLC): Efficacy data from the phase II GEOMETRY mono-1 study. *J. Clin. Oncol.* **2019**, *37*, 9004. [CrossRef]
92. Cai, W.; Su, C.; Li, X.; Fan, L.; Zheng, L.; Fei, K.; Zhou, C. KIF5B-RET fusions in Chinese patients with non-small cell lung cancer. *Cancer* **2013**, *119*, 1486–1494. [CrossRef]
93. Wang, Y.; Xu, Y.; Wang, X.; Sun, C.; Guo, Y.; Shao, G.; Yang, Z.; Qiu, S.; Ma, K. RET fusion in advanced non-small-cell lung cancer and response to cabozantinib: A case report. *Medicine* **2019**, *98*, e14120. [CrossRef]
94. Gautschi, O.; Milia, J.; Filleron, T.; Wolf, J.; Carbone, D.P.; Owen, D.; Camidge, R.; Narayanan, V.; Doebele, R.C.; Besse, B.; et al. Targeting RET in patients with RET-rearranged lung cancers: Results from the global, multicenter RET registry. *J. Clin. Oncol.* **2017**, *35*, 1403–1410. [CrossRef]
95. Drilon, A.; Rekhtman, N.; Arcila, M.; Wang, L.; Ni, A.; Albano, M.; Van Voorthuysen, M.; Somwar, R.; Smith, R.S.; Montecalvo, J.; et al. Cabozantinib in patients with advanced RET-rearranged non-small-cell lung cancer: An open-label, single-centre, phase 2, single-arm trial. *Lancet Oncol.* **2016**, *17*, 1653–1660. [CrossRef]
96. Lee, S.H.; Lee, J.K.; Ahn, M.J.; Kim, D.W.; Sun, J.M.; Keam, B.; Kim, T.M.; Heo, D.S.; Ahn, J.S.; Choi, Y.L.; et al. Vandetanib in pretreated patients with advanced non-small cell lung cancer-harboring RET rearrangement: A phase II clinical trial. *Ann. Oncol.* **2017**, *28*, 292–297. [CrossRef] [PubMed]
97. Nakagawara, A. Trk receptor tyrosine kinases: A bridge between cancer and neural development. *Cancer Lett.* **2001**, *169*, 107–114. [CrossRef]
98. Vaishnavi, A.; Capelletti, M.; Le, T.A.; Kako, S.; Butaney, M.; Ercan, D.; Mahale, S.; Davies, K.D.; Aisner, D.L.; Pilling, A.B.; et al. Oncogenic and drug sensitive NTRK1 rearrangements in lung cancer. *Nat. Med.* **2013**, *19*, 1469–1472. [CrossRef] [PubMed]
99. Stransky, N.; Cerami, E.; Schalm, S.; Kim, J.L.; Lengauer, C. The landscape of kinase fusions in cancer. *Nat. Commun.* **2014**, *5*, 4846. [CrossRef] [PubMed]
100. Gullick, W.J. The epidermal growth factor system of ligands and receptors in cancer. *Eur. J. Cancer* **2009**, *45*, 205–210. [CrossRef]
101. Willem, M. Proteolytic processing of Neuregulin-1. *Brain Res. Bull.* **2016**, *126*, 178–182. [CrossRef]
102. Jonna, S.; Feldman, R.A.; Swensen, J.; Gatalica, Z.; Korn, W.M.; Borghaei, H.; Ma, P.C.; Nieva, J.J.; Spira, A.I.; Vanderwalde, A.M.; et al. Detection of NRG1 gene fusions in solid tumors. *Clin. Cancer Res.* **2019**, *25*, 4966–4972. [CrossRef]
103. Gay, N.D.; Wang, Y.; Beadling, C.; Warrick, A.; Neff, T.; Corless, C.L.; Tolba, K. Durable Response to Afatinib in Lung Adenocarcinoma Harboring NRG1 Gene Fusions. *J. Thorac. Oncol.* **2017**, *12*, e107–e110. [CrossRef]
104. Cheema, P.K.; Doherty, M.; Tsao, M.S. A Case of Invasive Mucinous Pulmonary Adenocarcinoma with a CD74-NRG1 Fusion Protein Targeted with Afatinib. *J. Thorac. Oncol.* **2017**, *12*, e200–e202. [CrossRef]
105. Liu, S.V.; Duruisseaux, M.; Tolba, K.; Branden, E.; Goto, Y.; Weinberg, B.A.; Renouf, D.J.; Doebele, R.C.; Heining, C.; Schlenk, R.F.; et al. Targeting NRG1-fusions in multiple tumour types: Afatinib as a novel potential treatment option. *Ann. Oncol.* **2019**, *30*, v791–v792. [CrossRef]
106. Bendell, J.C.; Lim, K.-H.; Burkard, M.E.; Lin, J.J.; Chae, Y.K.; Socinski, M.A.; Khan, G.; Reckamp, K.L.; Leland, S.; Plessinger, D.; et al. Abstract PO-003: CRESTONE—Clinical study of response to seribantumab in tumors with neuregulin-1 (NRG1) Fusions—A phase 2 study of the anti-HER3 mAb for advanced or metastatic solid tumors (NCT04383210). In *Proceedings of the AACR Virtual Special Conference on Pancreatic CancerCancer Research*; American Association for Cancer Research (AACR): Philadelphia, PA, USA, 2020; Volume 80, p. PO-003-PO-003.
107. Hanahan, D.; Weinberg, R.A. Hallmarks of cancer: The next generation. *Cell* **2011**, *144*, 646–674. [CrossRef] [PubMed]
108. Grigg, C.; Rizvi, N.A. PD-L1 biomarker testing for non-small cell lung cancer: Truth or fiction? *J. Immunother. Cancer* **2016**, *4*, 48. [CrossRef]
109. Johnson, R.M.G.; Dong, H. Functional expression of programmed death-ligand 1 (B7-H1) by immune cells and tumor cells. *Front. Immunol.* **2017**, *8*, 961. [CrossRef]
110. Dong, H.; Strome, S.E.; Salomao, D.R.; Tamura, H.; Hirano, F.; Flies, D.B.; Roche, P.C.; Lu, J.; Zhu, G.; Tamada, K.; et al. Tumor-associated B7-H1 promotes T-cell apoptosis: A potential mechanism of immune evasion. *Nat. Med.* **2002**, *8*, 793–800. [CrossRef]
111. Pawelczyk, K.; Piotrowska, A.; Ciesielska, U.; Jablonska, K.; Gletzel-Plucinska, N.; Grzegrzolka, J.; Podhorska-Okolow, M.; Dziegiel, P.; Nowinska, K. Role of PD-L1 Expression in Non-Small Cell Lung Cancer and Their Prognostic Significance according to Clinicopathological Factors and Diagnostic Markers. *Int. J. Mol. Sci.* **2019**, *20*, 824. [CrossRef] [PubMed]
112. Clarke, J.M.; George, D.J.; Lisi, S.; Salama, A.K.S. Immune Checkpoint Blockade: The New Frontier in Cancer Treatment. *Target. Oncol.* **2018**, *13*, 1–20. [CrossRef]

113. Tsoukalas, N.; Kiakou, M.; Tsapakidis, K.; Tolia, M.; Aravantinou-Fatorou, E.; Baxevanos, P.; Kyrgias, G.; Theocharis, S. PD-1 and PD-L1 as immunotherapy targets and biomarkers in non-small cell lung cancer. *J. BUON* **2019**, *24*, 883–888. [PubMed]
114. Yang, H.; Liang, S.Q.; Schmid, R.A.; Peng, R.W. New horizons in KRAS-mutant lung cancer: Dawn after darkness. *Front. Oncol.* **2019**, *9*, 953. [CrossRef] [PubMed]
115. 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]
116. Dai, S.; Zhou, Z.; Chen, Z.; Xu, G.; Chen, Y. Fibroblast Growth Factor Receptors (FGFRs): Structures and Small Molecule Inhibitors. *Cells* **2019**, *8*, 614. [CrossRef]
117. Chae, Y.K.; Ranganath, K.; Hammerman, P.S.; Vaklavas, C.; Mohindra, N.; Kalyan, A.; Matsangou, M.; Costa, R.; Carneiro, B.; Villaflor, V.M.; et al. Inhibition of the fibroblast growth factor receptor (FGFR) pathway: The current landscape and barriers to clinical application. *Oncotarget* **2017**, *8*, 16052–16074. [CrossRef]
118. Helsten, T.; Elkin, S.; Arthur, E.; Tomson, B.N.; Carter, J.; Kurzrock, R. The FGFR landscape in cancer: Analysis of 4853 tumors by next-generation sequencing. *Clin. Cancer Res.* **2016**, *22*, 259–267. [CrossRef] [PubMed]
119. Miao, J.L.; Liu, R.J.; Zhou, J.H.; Meng, S.H. Fibroblast growth factor receptor 1 gene amplification in nonsmall cell lung cancer. *Chin. Med. J.* **2016**, *129*, 2868–2872. [CrossRef] [PubMed]
120. Thomson, S.; Petti, F.; Sujka-Kwok, I.; Epstein, D.; Haley, J.D. Kinase switching in mesenchymal-like non-small cell lung cancer lines contributes to EGFR inhibitor resistance through pathway redundancy. *Clin. Exp. Metastasis* **2008**, *25*, 843–854. [CrossRef]
121. Lim, S.H.; Sun, J.M.; Choi, Y.L.; Kim, H.R.; Ahn, S.; Lee, J.Y.; Lee, S.H.; Ahn, J.S.; Park, K.; Kim, J.H.; et al. Efficacy and safety of dovitinib in pretreated patients with advanced squamous non-small cell lung cancer with FGFR1 amplification: A single-arm, phase 2 study. *Cancer* **2016**, *122*, 3027–3031. [CrossRef]
122. Nishio, M.; Horai, T.; Horiike, A.; Nokihara, H.; Yamamoto, N.; Takahashi, T.; Murakami, H.; Yamamoto, N.; Koizumi, F.; Nishio, K.; et al. Phase 1 study of lenvatinib combined with carboplatin and paclitaxel in patients with non-small-cell lung cancer. *Br. J. Cancer* **2013**, *109*, 538–544. [CrossRef] [PubMed]
123. Altorki, N.; Lane, M.E.; Bauer, T.; Lee, P.C.; Guarino, M.J.; Pass, H.; Felip, E.; Peylan-Ramu, N.; Gurpide, A.; Grannis, F.W.; et al. Phase II proof-of-concept study of pazopanib monotherapy in treatment-naive patients with stage I/II resectable non-small-cell lung cancer. *J. Clin. Oncol.* **2010**, *28*, 3131–3137. [CrossRef]
124. Reck, M.; Kaiser, R.; Mellemgaard, A.; Douillard, J.Y.; Orlov, S.; Krzakowski, M.; von Pawel, J.; Gottfried, M.; Bondarenko, I.; Liao, M.; et al. Docetaxel plus nintedanib versus docetaxel plus placebo in patients with previously treated non-small-cell lung cancer (LUME-Lung 1): A phase 3, double-blind, randomised controlled trial. *Lancet Oncol.* **2014**, *15*, 143–155. [CrossRef]
125. Jones, R.L.; Ratain, M.J.; O'dwyer, P.J.; Siu, L.L.; Jassem, J.; Medioni, J.; Dejonge, M.; Rudin, C.; Sawyer, M.; Khayat, D.; et al. Phase II randomised discontinuation trial of brivanib in patients with advanced solid tumours. *Eur. J. Cancer* **2019**, *120*, 132–139. [CrossRef] [PubMed]
126. Ng, T.L.; Yu, H.; Smith, D.; Boyle, T.A.; York, E.R.; Leedy, S.; Gao, D.; Heasley, L.; Hirsch, F.R.; Camidge, D.R. Preselection of lung cancer cases using FGFR1 mRNA and gene copy number for treatment with ponatinib. *J. Clin. Oncol.* **2018**, *36*, 12095. [CrossRef]
127. Spigel, D.R.; Cereda, R.; Litten, J.B.; Allen, A.R.; Giaccone, G.; Socinski, M.A.; Camidge, D.R.; Besse, B. A single arm, open-label, phase II study to assess the efficacy of lucitanib in patients with FGFR1 -amplified squamous NSCLC (sqNSCLC). *J. Clin. Oncol.* **2014**, *32*, TPS8119. [CrossRef]
128. Hu, X.; Wu, L.W.; Zhang, Z.Y.; Chen, M.L.; Li, Y.L.; Zhang, C. The anti-tumor effect of regorafenib in lung squamous cell carcinoma in vitro. *Biochem. Biophys. Res. Commun.* **2018**, *503*, 1123–1129. [CrossRef] [PubMed]
129. Fumarola, C.; Bozza, N.; Castelli, R.; Ferlenghi, F.; Marseglia, G.; Lodola, A.; Bonelli, M.; La Monica, S.; Cretella, D.; Alfieri, R.; et al. Expanding the Arsenal of FGFR Inhibitors: A Novel Chloroacetamide Derivative as a New Irreversible Agent with Anti-proliferative Activity Against FGFR1-Amplified Lung Cancer Cell Lines. *Front. Oncol.* **2019**, *9*, 179. [CrossRef] [PubMed]
130. Carafoli, F.; Mayer, M.C.; Shiraishi, K.; Pecheva, M.A.; Chan, L.Y.; Nan, R.; Leitinger, B.; Hohenester, E. Structure of the discoidin domain receptor 1 extracellular region bound to an inhibitory Fab fragment reveals features important for signaling. *Structure* **2012**, *20*, 688–697. [CrossRef]
131. Rammal, H.; Saby, C.; Magnien, K.; Van-Gulick, L.; Garnotel, R.; Buache, E.; El Btaouri, H.; Jeannesson, P.; Morjani, H. Discoidin domain receptors: Potential actors and targets in cancer. *Front. Pharmacol.* **2016**, *7*, 55. [CrossRef] [PubMed]
132. Hammerman, P.S.; Sos, M.L.; Ramos, A.H.; Xu, C.; Dutt, A.; Zhou, W.; Brace, L.E.; Woods, B.A.; Lin, W.; Zhang, J.; et al. Mutations in the DDR2 kinase gene identify a novel therapeutic target in squamous cell lung cancer. *Cancer Discov.* **2011**, *1*, 78–89. [CrossRef] [PubMed]
133. Fathi, Z.; Mousavi, S.A.J.; Roudi, R.; Ghazi, F. Distribution of *KRAS*, *DDR2*, and *TP53* gene mutations in lung cancer: An analysis of Iranian patients. *PLoS ONE* **2018**, *13*, e0200633. [CrossRef]
134. Kobayashi-Watanabe, N.; Sato, A.; Watanabe, T.; Abe, T.; Nakashima, C.; Sueoka, E.; Kimura, S.; Sueoka-Aragane, N. Functional analysis of Discoidin domain receptor 2 mutation and expression in squamous cell lung cancer. *Lung Cancer* **2017**, *110*, 35–41. [CrossRef]
135. Peters, S.; Zimmermann, S. Targeted therapy in NSCLC driven by HER2 insertions. *Transl. Lung Cancer Res.* **2014**, *3*, 84–88.
136. Hsu, J.L.; Hung, M.C. The role of HER2, EGFR, and other receptor tyrosine kinases in breast cancer. *Cancer Metastasis Rev.* **2016**, *35*, 575–588. [CrossRef]

137. Furrer, D.; Paquet, C.; Jacob, S.; Diorio, C. The Human Epidermal Growth Factor Receptor 2 (HER2) as a Prognostic and Predictive Biomarker: Molecular Insights into HER2 Activation and Diagnostic Implications. In *Cancer Prognosis*; IntechOpen: London, UK, 2018.
138. Nakamura, H.; Kawasaki, N.; Taguchi, M.; Kabasawa, K. Association of HER-2 overexpression with prognosis in nonsmall cell lung carcinoma: A metaanalysis. *Cancer* **2005**, *103*, 1865–1873. [CrossRef] [PubMed]
139. Hirsch, F.R.; Varella-Garcia, M.; Franklin, W.A.; Veve, R.; Chen, L.; Helfrich, B.; Zeng, C.; Baron, A.; Bunn, P.A. Evaluation of HER-2/neu gene amplification and protein expression in non-small cell lung carcinomas. *Br. J. Cancer* **2002**, *86*, 1449–1456. [CrossRef] [PubMed]
140. Kim, E.K.; Kim, K.A.; Lee, C.Y.; Shim, H.S. The frequency and clinical impact of HER2 alterations in lung adenocarcinoma. *PLoS ONE* **2017**, *12*, e0171280. [CrossRef]
141. Mazières, J.; Peters, S.; Lepage, B.; Cortot, A.B.; Barlesi, F.; Beau-Faller, M.; Besse, B.; Blons, H.; Mansuet-Lupo, A.; Urban, T.; et al. Lung cancer that harbors an HER2 Mutation: Epidemiologic characteristics and therapeutic perspectives. *J. Clin. Oncol.* **2013**, *31*, 1997–2003. [CrossRef] [PubMed]
142. Reck, M.; Spira, A.; Besse, B.; Wolf, J.; Skoulidis, F.; Borghaei, H.; Goto, K.; Park, K.; Griesinger, F.; Font, E.F.; et al. MO01.32 CodeBreaK 200: A Phase 3 Multicenter Study of Sotorasib, a KRAS(G12C) Inhibitor, versus Docetaxel in Patients with Previously Treated Advanced Non-Small Cell Lung Cancer (NSCLC) Harboring KRAS p.G12C Mutation. *J. Thorac. Oncol.* **2021**, *16*, S29. [CrossRef]
143. Mok, T.S.K.; Lawler, W.E.; Shum, M.K.; Dakhil, S.R.; Spira, A.I.; Barlesi, F.; Reck, M.; Garassino, M.C.; Spigel, D.R.; Alvarez, D.; et al. KRYSTAL-12: A randomized phase 3 study of adagrasib (MRTX849) versus docetaxel in patients (pts) with previously treated non-small-cell lung cancer (NSCLC) with KRASG12C mutation. *J. Clin. Oncol.* **2021**, *39*, TPS9129. [CrossRef]
144. Jänne, P.A.; Van Den Heuvel, M.M.; Barlesi, F.; Cobo, M.; Mazieres, J.; Crinò, L.; Orlov, S.; Blackhall, F.; Wolf, J.; Garrido, P.; et al. Selumetinib Plus Docetaxel Compared with Docetaxel Alone and Progression-Free Survival in Patients With KRAS-Mutant Advanced Non-Small Cell Lung Cancer: The SELECT-1 Randomized Clinical Trial. *JAMA* **2017**, *317*, 1844–1853. [CrossRef]
145. Goldman, J.W.; Mazieres, J.; Barlesi, F.; Dragnev, K.H.; Koczywas, M.; Göskel, T.; Cortot, A.B.; Girard, N.; Wesseler, C.; Bischoff, H.; et al. A Randomized Phase III Study of Abemaciclib Versus Erlotinib in Patients with Stage IV Non-small Cell Lung Cancer with a Detectable KRAS Mutation Who Failed Prior Platinum-Based Therapy: JUNIPER. *Front. Oncol.* **2020**, *10*, 578756. [CrossRef] [PubMed]
146. Dingemans, A.-M.C.; Smit, E.F.; De Langen, J.; van Tinteren, H. Chemotherapy in KRAS-mutated chemotherapy naive non-small cell lung cancer patients: A phase III comparing cisplatin-pemetrexed with carboplatin-paclitaxel-bevacizumab: NVALT 22 (NCT02743923). *J. Clin. Oncol.* **2019**, *37*, TPS9127. [CrossRef]
147. Smit, E.F.; Peters, S.; Dziadziuszko, R.; Dafni, U.; Wolf, J.; Wasąg, B.; Biernat, W.; Finn, S.; Kammler, R.; Tsourti, Z.; et al. A single-arm phase II trial of afatinib in pretreated patients with advanced NSCLC harboring a HER2 mutation: The ETOP NICHE trial. *J. Clin. Oncol.* **2017**, *35*, 9070. [CrossRef]
148. Gandhi, L.; Besse, B.; Mazieres, J.; Waqar, S.; Cortot, A.; Barlesi, F.; Quoix, E.; Otterson, G.; Ettinger, D.; Horn, L.; et al. MA04.02 Neratinib ± Temsirolimus in HER2-Mutant Lung Cancers: An International, Randomized Phase II Study. *J. Thorac. Oncol.* **2017**, *12*, S358–S359. [CrossRef]
149. Li, B.T.; Smit, E.F.; Goto, Y.; Nakagawa, K.; Udagawa, H.; Mazières, J.; Nagasaka, M.; Bazhenova, L.; Saltos, A.N.; Felip, E.; et al. Trastuzumab Deruxtecan in HER2-Mutant Non–Small-Cell Lung Cancer. *N. Engl. J. Med.* **2021**. [CrossRef] [PubMed]
150. Zhao, P.; Li, L.; Jiang, X.; Li, Q. Mismatch repair deficiency/microsatellite instability-high as a predictor for anti-PD-1/PD-L1 immunotherapy efficacy. *J. Hematol. Oncol.* **2019**, *12*, 54. [CrossRef] [PubMed]
151. Boyiadzis, M.M.; Kirkwood, J.M.; Marshall, J.L.; Pritchard, C.C.; Azad, N.S.; Gulley, J.L. Significance and implications of FDA approval of pembrolizumab for biomarker-defined disease. *J. Immunother. Cancer* **2018**, *6*, 35. [CrossRef]
152. De Marchi, P.; Berardinelli, G.N.; Cavagna, R.; De Paula, F.; Da Silva, E.A.; Miziara, J.; Leal, L.; Reis, R. EP1.04-11 Frequency of Microsatellite Instability (MSI) in Brazilian TKI Non-Treatable Non-Small Cell Lung Cancer (NSCLC) Patients. *J. Thorac. Oncol.* **2019**, *14*, S973. [CrossRef]
153. Takamochi, K.; Takahashi, F.; Suehara, Y.; Sato, E.; Kohsaka, S.; Hayashi, T.; Kitano, S.; Uneno, T.; Kojima, S.; Takeuchi, K.; et al. DNA mismatch repair deficiency in surgically resected lung adenocarcinoma: Microsatellite instability analysis using the Promega panel. *Lung Cancer* **2017**, *110*, 26–31. [CrossRef]
154. Alexander, M.; Galeas, J.; Cheng, H. Tumor mutation burden in lung cancer: A new predictive biomarker for immunotherapy or too soon to tell? *J. Thorac. Dis.* **2018**, *10*, S3994–S3998. [CrossRef] [PubMed]
155. Hellmann, M.D.; Ciuleanu, T.E.; Pluzanski, A.; Lee, J.S.; Otterson, G.A.; Audigier-Valette, C.; Minenza, E.; Linardou, H.; Burgers, S.; Salman, P.; et al. Nivolumab plus ipilimumab in lung cancer with a high tumor mutational burden. *N. Engl. J. Med.* **2018**, *378*, 2093–2104. [CrossRef] [PubMed]
156. Miller, C.H.; Maher, S.G.; Young, H.A. Clinical Use of Interferon-gamma. *Ann. N. Y. Acad. Sci.* **2009**, *1182*, 69–79. [CrossRef]
157. Karachaliou, N.; Gonzalez-Cao, M.; Crespo, G.; Drozdowskyj, A.; Aldeguer, E.; Gimenez-Capitan, A.; Teixido, C.; Molina-Vila, M.A.; Viteri, S.; Gil, M.D.L.L.; et al. Interferon gamma, an important marker of response to immune checkpoint blockade in non-small cell lung cancer and melanoma patients. *Ther. Adv. Med. Oncol.* **2018**, 10. [CrossRef]
158. DeNardo, D.G.; Andreu, P.; Coussens, L.M. Interactions between lymphocytes and myeloid cells regulate pro-versus anti-tumor immunity. *Cancer Metastasis Rev.* **2010**, *29*, 309–316. [CrossRef]

159. Fumet, J.D.; Richard, C.; Ledys, F.; Klopfenstein, Q.; Joubert, P.; Routy, B.; Truntzer, C.; Gagné, A.; Hamel, M.A.; Guimaraes, C.F.; et al. Prognostic and predictive role of CD8 and PD-L1 determination in lung tumor tissue of patients under anti-PD-1 therapy. *Br. J. Cancer* **2018**, *119*, 950–960. [CrossRef] [PubMed]
160. Uryvaev, A.; Passhak, M.; Hershkovits, D.; Sabo, E.; Bar-Sela, G. The role of tumor-infiltrating lymphocytes (TILs) as a predictive biomarker of response to anti-PD1 therapy in patients with metastatic non-small cell lung cancer or metastatic melanoma. *Med. Oncol.* **2018**, *35*, 25. [CrossRef]
161. Wolf, Y.; Anderson, A.C.; Kuchroo, V.K. TIM3 comes of age as an inhibitory receptor. *Nat. Rev. Immunol.* **2020**, *20*, 173–185. [CrossRef] [PubMed]
162. Limagne, E.; Richard, C.; Thibaudin, M.; Fumet, J.D.; Truntzer, C.; Lagrange, A.; Favier, L.; Coudert, B.; Ghiringhelli, F. Tim-3/galectin-9 pathway and mMDSC control primary and secondary resistances to PD-1 blockade in lung cancer patients. *Oncoimmunology* **2019**, *8*, e1564505. [CrossRef] [PubMed]
163. Zhu, C.Q.; Tsao, M.S. Prognostic markers in lung cancer: Is it ready for prime time? *Transl. Lung Cancer Res.* **2014**, *3*, 149–158. [CrossRef] [PubMed]
164. Paesmans, M. Prognostic and predictive factors for lung cancer. *Breathe* **2012**, *9*, 113–122. [CrossRef]
165. A 25-Signal Proteomic Signature and Outcome for Patients with Resected Non-Small-Cell Lung Cancer—PubMed. Available online: https://pubmed.ncbi.nlm.nih.gov/17551146/ (accessed on 1 July 2020).
166. Der, S.D.; Sykes, J.; Pintilie, M.; Zhu, C.Q.; Strumpf, D.; Liu, N.; Jurisica, I.; Shepherd, F.A.; Tsao, M.S. Validation of a histology-independent prognostic gene signature for early-stage, non-small-cell lung cancer including stage IA patients. *J. Thorac. Oncol.* **2014**, *9*, 59–64. [CrossRef]
167. Roepman, P.; Jassem, J.; Smit, E.F.; Muley, T.; Niklinski, J.; Van Develde, T.; Witteveen, A.T.; Rzyman, W.; Floore, A.; Burgers, S.; et al. An immune response enriched 72-gene prognostic profile for early-stage non-small-cell lung cancer. *Clin. Cancer Res.* **2009**, *15*, 284–290. [CrossRef]
168. Shedden, K.; Taylor, J.M.G.; Enkemann, S.A.; Tsao, M.S.; Yeatman, T.J.; Gerald, W.L.; Eschrich, S.; Jurisica, I.; Giordano, T.J.; Misek, D.E.; et al. Gene expression-based survival prediction in lung adenocarcinoma: A multi-site, blinded validation study. *Nat. Med.* **2008**, *14*, 822–827. [CrossRef]
169. Dvornikov, D.; Schneider, M.A.; Ohse, S.; Szczygieł, M.; Titkova, I.; Rosenblatt, M.; Muley, T.; Warth, A.; Herth, F.J.; Dienemann, H.; et al. Expression ratio of the TGFβ-inducible gene *MYO10* is prognostic for overall survival of squamous cell lung cancer patients and predicts chemotherapy response. *Sci. Rep.* **2018**, *8*, 9517. [CrossRef] [PubMed]
170. Methylation-Driven Genes PMPCAP1, SOWAHC and ZNF4 as Potential Prognostic Biomarkers in Lung Squamous Cell Carcinoma. Available online: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7002985/pdf/mmr-21-03-1285.pdf (accessed on 20 March 2020).
171. Gao, C.; Zhuang, J.; Zhou, C.; Ma, K.; Zhao, M.; Liu, C.; Liu, L.; Li, H.; Feng, F.; Sun, C. Prognostic value of aberrantly expressed methylation gene profiles in lung squamous cell carcinoma: A study based on The Cancer Genome Atlas. *J. Cell. Physiol.* **2019**, *234*, 6519–6528. [CrossRef] [PubMed]
172. Chen, B.; Gao, T.; Yuan, W.; Zhao, W.; Wang, T.-H.; Wu, J. Prognostic Value of Survival of MicroRNAs Signatures in Non-small Cell Lung Cancer. *J. Cancer* **2019**, *10*, 5793–5804. [CrossRef] [PubMed]
173. Detterbeck, F.C.; Boffa, D.J.; Kim, A.W.; Tanoue, L.T. The Eighth Edition Lung Cancer Stage Classification. *Chest* **2017**, *151*, 193–203. [CrossRef] [PubMed]
174. Goldstraw, P.; Chansky, K.; Crowley, J.; Rami-Porta, R.; Asamura, H.; Eberhardt, W.E.E.; Nicholson, A.G.; Groome, P.; Mitchell, A.; Bolejack, V. The IASLC Lung Cancer Staging Project: Proposals for Revision of the TNM Stage Groupings in the Forthcoming (Eighth) Edition of the TNM Classification for Lung Cancer on behalf of the International Association for the Study of Lung Cancer Staging and Prognostic Factors Committee, Advisory Boards, and Participating Institutions. *JTHO* **2015**, *11*, 39–51. [CrossRef]
175. Malalasekera, A.; Tan, C.S.Y.; Phan, V.; Yip, P.Y.; Vardy, J.; Clarke, S.; Kao, S. Eastern Cooperative Oncology Group score: Agreement between non-small-cell lung cancer patients and their oncologists and clinical implications. *Cancer Treat. Commun.* **2016**, *5*, 17–21. [CrossRef]
176. Mogi, A.; Kuwano, H. TP53 Mutations in Nonsmall Cell Lung Cancer. *J. Biomed. Biotechnol.* **2011**, *2011*, 583929. [CrossRef] [PubMed]
177. Huszno, J.; Grzybowska, E. TP53 mutations and SNPs as prognostic and predictive factors in patients with breast cancer (Review). *Oncol. Lett.* **2018**, *16*, 34–40. [CrossRef]
178. Xu, F.; Lin, H.; He, P.; He, L.; Chen, J.; Lin, L.; Chen, Y. A *TP53*-associated gene signature for prediction of prognosis and therapeutic responses in lung squamous cell carcinoma. *Oncoimmunology* **2020**, *9*, 1731943. [CrossRef]
179. Qin, K.; Hou, H.; Liang, Y.; Zhang, X. Prognostic value of TP53 concurrent mutations for EGFR- TKIs and ALK-TKIs based targeted therapy in advanced non-small cell lung cancer: A meta-analysis. *BMC Cancer* **2020**, *20*, 328. [CrossRef]
180. Gu, J.; Zhou, Y.; Huang, L.; Ou, W.; Wu, J.; Li, S.; Xu, J.; Feng, J.; Liu, B. TP53 mutation is associated with a poor clinical outcome for non-small cell lung cancer: Evidence from a meta-analysis. *Mol. Clin. Oncol.* **2016**, *5*, 705–713. [CrossRef]
181. Ma, X.; Le Teuff, G.; Lacas, B.; Tsao, M.S.; Graziano, S.; Pignon, J.P.; Douillard, J.Y.; Le Chevalier, T.; Seymour, L.; Filipits, M.; et al. Prognostic and predictive effect of TP53 mutations in patients with non-small cell lung cancer from adjuvant cisplatin-based therapy randomized trials: A LACE-bio pooled analysis. *J. Thorac. Oncol.* **2016**, *11*, 850–861. [CrossRef]

182. Han, H.; Silverman, J.F.; Santucci, T.S.; Macherey, R.S.; d'Amato, T.A.; Tung, M.Y.; Weyant, R.J.; Landreneau, R.J. Vascular Endothelial Growth Factor Expression in Stage I Non-Small Cell Lung Cancer Correlates with Neoangiogenesis and a Poor Prognosis. *Ann. Surg. Oncol.* **2001**, *8*, 72–79. [CrossRef] [PubMed]
183. Zhan, P.; Wang, J.; Lv, X.J.; Wang, Q.; Qiu, L.X.; Lin, X.Q.; Yu, L.K.; Song, Y. Prognostic value of vascular endothelial growth factor expression in patients with lung cancer: A systematic review with meta-analysis. *J. Thorac. Oncol.* **2009**, *4*, 1094–1103. [CrossRef] [PubMed]
184. Lin, Q.; Guo, L.; Lin, G.; Chen, Z.; Chen, T.; Lin, J.; Zhang, B.; Gu, X. Clinical and prognostic significance of OPN and VEGF expression in patients with non-small-cell lung cancer. *Cancer Epidemiol.* **2015**, *39*, 539–544. [CrossRef] [PubMed]
185. Seto, T.; Higashiyama, M.; Funai, H.; Imamura, F.; Uematsu, K.; Seki, N.; Eguchi, K.; Yamanaka, T.; Ichinose, Y. Prognostic value of expression of vascular endothelial growth factor and its flt-1 and KDR receptors in stage I non-small-cell lung cancer. *Lung Cancer* **2006**, *53*, 91–96. [CrossRef]
186. Jouhilahti, E.M.; Peltonen, S.; Peltonen, J. Class III β-tubulin is a component of the mitotic spindle in multiple cell types. *J. Histochem. Cytochem.* **2008**, *56*, 1113–1119. [CrossRef] [PubMed]
187. Sève, P.; Lai, R.; Ding, K.; Winton, T.; Butts, C.; Mackey, J.; Dumontet, C.; Dabbagh, L.; Aviel-Ronen, S.; Seymour, L.; et al. Class III β-tubulin expression and benefit from adjuvant cisplatin/vinorelbine chemotherapy in operable non-small cell lung cancer: Analysis of NCIC JBR.10. *Clin. Cancer Res.* **2007**, *13*, 994–999. [CrossRef]
188. Katsetos, C.D.; Herman, M.M.; Mörk, S.J. Class III β-tubulin in human development and cancer. *Cell Motil. Cytoskelet.* **2003**, *55*, 77–96. [CrossRef]
189. Reiman, T.; Lai, R.; Veillard, A.S.; Paris, E.; Soria, J.C.; Rosell, R.; Taron, M.; Graziano, S.; Kratzke, R.; Seymour, L.; et al. Cross-validation study of class III beta-tubulin as a predictive marker for benefit from adjuvant chemotherapy in resected non-small-cell lung cancer: Analysis of four randomized trials. *Ann. Oncol. Off. J. Eur. Soc. Med. Oncol.* **2012**, *23*, 86–93. [CrossRef]
190. Rosell, R.; Scagliotti, G.; Danenberg, K.D.; Lord, R.V.N.; Bepler, G.; Novello, S.; Cooc, J.; Crinò, L.; Sánchez, J.J.; Taron, M.; et al. Transcripts in pretreatment biopsies from a three-arm randomized trial in metastatic non-small-cell lung cancer. *Oncogene* **2003**, *22*, 3548–3553. [CrossRef] [PubMed]
191. Sève, P.; Mackey, J.; Isaac, S.; Trédan, O.; Souquet, P.J.; Pérol, M.; Lai, R.; Voloch, A.; Dumontet, C. Class III β-tubulin expression in tumor cells predicts response and outcome in patients with non-small cell lung cancer receiving paclitaxel. *Mol. Cancer Ther.* **2005**, *4*, 2001–2007. [CrossRef] [PubMed]
192. Sève, P.; Isaac, S.; Trédan, O.; Souquet, P.J.; Pachéco, Y.; Pérol, M.; Lafanéchère, L.; Penet, A.; Peiller, E.L.; Dumontet, C. Expression of class III β-tubulin is predictive of patient outcome in patients with non-small cell lung cancer receiving vinorelbine-based chemotherapy. *Clin. Cancer Res.* **2005**, *11*, 5481–5486. [CrossRef] [PubMed]
193. Endl, E.; Gerdes, J. Posttranslational Modifications of the KI-67 Protein Coincide with Two Major Checkpoints During Mitosis. *J. Cell. Physiol.* **2000**, *182*, 371–380. [CrossRef]
194. Wen, S.; Zhou, W.; Li, C.M.; Hu, J.; Hu, X.M.; Chen, P.; Shao, G.L.; Guo, W.H. Ki-67 as a prognostic marker in early-stage non-small cell lung cancer in Asian patients: A meta-analysis of published studies involving 32 studies. *BMC Cancer* **2015**, *15*, 520. [CrossRef]
195. Martin, B.; Paesmans, M.; Mascaux, C.; Berghmans, T.; Lothaire, P.; Meert, A.P.; Lafitte, J.J.; Sculier, J.P. Ki-67 expression and patients survival in lung cancer: Systematic review of the literature with meta-analysis. *Br. J. Cancer* **2004**, *91*, 2018–2025. [CrossRef]
196. Wei, D.M.; Chen, W.J.; Meng, R.M.; Zhao, N.; Zhang, X.Y.; Liao, D.Y.; Chen, G. Augmented expression of Ki-67 is correlated with clinicopathological characteristics and prognosis for lung cancer patients: An up-dated systematic review and meta-analysis with 108 studies and 14,732 patients. *Respir. Res.* **2018**, *19*, 150. [CrossRef]
197. Choi, C.M.; Yang, S.C.; Jo, H.J.; Song, S.Y.; Jeon, Y.J.; Jang, T.W.; Kim, D.J.; Jang, S.H.; Yang, S.H.; Kim, Y.D.; et al. Proteins involved in DNA damage response pathways and survival of stage I non-small-cell lung cancer patients. *Ann. Oncol.* **2012**, *23*, 2088–2093. [CrossRef]
198. Tiseo, M.; Bordi, P.; Bortesi, B.; Boni, L.; Boni, C.; Baldini, E.; Grossi, F.; Recchia, F.; Zanelli, F.; Fontanini, G.; et al. ERCC1/BRCA1 expression and gene polymorphisms as prognostic and predictive factors in advanced NSCLC treated with or without cisplatin. *Br. J. Cancer* **2013**, *108*, 1695–1703. [CrossRef]
199. Luwor, R.B.; Kaye, A.H.; Zhu, H.J. Transforming growth factor-beta (TGF-β) and brain tumours. *J. Clin. Neurosci.* **2008**, *15*, 845–855. [CrossRef]
200. Huang, A.L.; Liu, S.G.; Qi, W.J.; Zhao, Y.F.; Li, Y.M.; Lei, B.; Sheng, W.J.; Shen, H. TGF-β1 protein expression in non-small cell lung cancers is correlated with prognosis. *Asian Pacific J. Cancer Prev.* **2014**, *15*, 8143–8147. [CrossRef]
201. Li, J.; Shen, C.; Wang, X.; Lai, Y.; Zhou, K.; Li, P.; Liu, L.; Che, G. Prognostic value of TGF-β in lung cancer: Systematic review and meta-analysis. *BMC Cancer* **2019**, *19*, 691. [CrossRef] [PubMed]
202. Andrews, L.P.; Marciscano, A.E.; Drake, C.G.; Vignali, D.A.A. LAG3 (CD223) as a cancer immunotherapy target. *Immunol. Rev.* **2017**, *276*, 80–96. [CrossRef] [PubMed]
203. He, Y.; Yu, H.; Rozeboom, L.; Rivard, C.J.; Ellison, K.; Dziadziuszko, R.; Suda, K.; Ren, S.; Wu, C.; Hou, L.; et al. LAG-3 Protein Expression in Non–Small Cell Lung Cancer and Its Relationship with PD-1/PD-L1 and Tumor-Infiltrating Lymphocytes. *J. Thorac. Oncol.* **2017**, *12*, 814–823. [CrossRef] [PubMed]

204. Hald, S.M.; Rakaee, M.; Martinez, I.; Richardsen, E.; Al-Saad, S.; Paulsen, E.E.; Blix, E.S.; Kilvaer, T.; Andersen, S.; Busund, L.T.; et al. LAG-3 in Non–Small-cell Lung Cancer: Expression in Primary Tumors and Metastatic Lymph Nodes Is Associated with Improved Survival. *Clin. Lung Cancer* **2018**, *19*, 249–259.e2. [CrossRef] [PubMed]
205. Liu, Y.Z.; Yang, H.; Cao, J.; Jiang, Y.Y.; Hao, J.J.; Xu, X.; Cai, Y.; Wang, M.R. KIAA1522 is a novel prognostic biomarker in patients with non-small cell lung cancer. *Sci. Rep.* **2016**, *6*, 24786. [CrossRef] [PubMed]
206. Guthrie, G.J.K.; Charles, K.A.; Roxburgh, C.S.D.; Horgan, P.G.; McMillan, D.C.; Clarke, S.J. The systemic inflammation-based neutrophil-lymphocyte ratio: Experience in patients with cancer. *Crit. Rev. Oncol. Hematol.* **2013**, *88*, 218–230. [CrossRef]
207. Diem, S.; Schmid, S.; Krapf, M.; Flatz, L.; Born, D.; Jochum, W.; Templeton, A.J.; Früh, M. Neutrophil-to-Lymphocyte ratio (NLR) and Platelet-to-Lymphocyte ratio (PLR) as prognostic markers in patients with non-small cell lung cancer (NSCLC) treated with nivolumab. *Lung Cancer* **2017**, *111*, 176–181. [CrossRef] [PubMed]
208. Bagley, S.J.; Kothari, S.; Aggarwal, C.; Bauml, J.M.; Alley, E.W.; Evans, T.L.; Kosteva, J.A.; Ciunci, C.A.; Gabriel, P.E.; Thompson, J.C.; et al. Pretreatment neutrophil-to-lymphocyte ratio as a marker of outcomes in nivolumab-treated patients with advanced non-small-cell lung cancer. *Lung Cancer* **2017**, *106*, 1–7. [CrossRef]