



**HAL**  
open science

# Evaluation of Combined Chemotherapy and Genomic-Driven Targeted Therapy in Patient-Derived Xenografts Identifies New Therapeutic Approaches in Squamous Non-Small-Cell Lung Cancer Patients

Didier Decaudin, Fariba Némati, Vesnie Etienne, Harry Ahnine, Quentin Peretti, Laura Sourd, Rania El-Botty, Lea Huguet, Elodie Montaudon, Sarah Lagha, et al.

► **To cite this version:**

Didier Decaudin, Fariba Némati, Vesnie Etienne, Harry Ahnine, Quentin Peretti, et al.. Evaluation of Combined Chemotherapy and Genomic-Driven Targeted Therapy in Patient-Derived Xenografts Identifies New Therapeutic Approaches in Squamous Non-Small-Cell Lung Cancer Patients. *Cancers*, 2024, 16 (16), 10.3390/cancers16162785 . hal-04724615

**HAL Id: hal-04724615**

**<https://hal.science/hal-04724615v1>**

Submitted on 12 Nov 2024

**HAL** is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.







L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.



Distributed under a Creative Commons Attribution 4.0 International License

## Article

# Evaluation of Combined Chemotherapy and Genomic-Driven Targeted Therapy in Patient-Derived Xenografts Identifies New Therapeutic Approaches in Squamous Non-Small-Cell Lung Cancer Patients

Didier Decaudin <sup>1,2,\*</sup>, Fariba Némati <sup>1</sup> , Julien Masliah Planchon <sup>3</sup> , Agathe Seguin-Givelet <sup>4,5</sup>, Marine Lefevre <sup>6</sup> , Vesnie Etienne <sup>1</sup>, Harry Ahnine <sup>1</sup>, Quentin Peretti <sup>1</sup>, Laura Sourd <sup>1</sup>, Rania El-Botty <sup>1</sup>, Lea Huguet <sup>1</sup>, Sarah Lagha <sup>2</sup> , Nadia Hegarat <sup>2</sup> , Sergio Roman-Roman <sup>7</sup>, Ivan Bièche <sup>3</sup>, Nicolas Girard <sup>2,8</sup> and Elodie Montaudon <sup>1,\*</sup> 

- <sup>1</sup> Laboratory of Preclinical Investigation, Department of Translational Research, Institut Curie, PSL University Paris, 75005 Paris, France; fariba.nemati@curie.fr (F.N.); vesnie.etienne@curie.fr (V.E.); laura.sourd@curie.fr (L.S.); rania.el-botty@curie.fr (R.E.-B.); lea.huguet@curie.fr (L.H.)
  - <sup>2</sup> Department of Medical Oncology, Institut Curie, 75005 Paris, France; sarah.lagha@curie.fr (S.L.); nadia.hegarat@curie.fr (N.H.); nicolas.girard2@curie.fr (N.G.)
  - <sup>3</sup> Department of Genetic, Institut Curie, 75005 Paris, France; julien.masliahplanchon@curie.fr (J.M.P.); ivan.bieche@curie.fr (I.B.)
  - <sup>4</sup> Department of Thoracic Surgery, Curie-Montsouris Thorax Institute, Institut Mutualiste Montsouris, 75014 Paris, France; agathe.seguin-givelet@imm.fr
  - <sup>5</sup> Faculty of Medicine SMBH, Paris 13 University, Sorbonne Paris Cité, 75013 Bobigny, France
  - <sup>6</sup> Department of Pathology, Institut Mutualiste Montsouris, 75014 Paris, France; marine.lefevre@imm.fr
  - <sup>7</sup> Department of Translational Research, Institut Curie, PSL University Paris, 75006 Paris, France; sergio.roman-roman@curie.fr
  - <sup>8</sup> Paris Saclay University, University of Versailles Saint-Quentin-en-Yvelines (UVSQ), 91405 Versailles, France
- \* Correspondence: didier.decaudin@curie.fr (D.D.); elodie.montaudon@curie.fr (E.M.); Tel.: +33-1-56-24-62-46 (D.D.)



**Citation:** Decaudin, D.; Némati, F.; Masliah Planchon, J.; Seguin-Givelet, A.; Lefevre, M.; Etienne, V.; Ahnine, H.; Peretti, Q.; Sourd, L.; El-Botty, R.; et al. Evaluation of Combined Chemotherapy and Genomic-Driven Targeted Therapy in Patient-Derived Xenografts Identifies New Therapeutic Approaches in Squamous Non-Small-Cell Lung Cancer Patients. *Cancers* **2024**, *16*, 2785. <https://doi.org/10.3390/cancers16162785>

Academic Editor: Akiteru Goto

Received: 9 July 2024

Revised: 30 July 2024

Accepted: 5 August 2024

Published: 7 August 2024



**Copyright:** © 2024 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (<https://creativecommons.org/licenses/by/4.0/>).

**Simple Summary:** Non-small-cell lung cancer is characterized by high morbidity and mortality. Currently, the precision medicine approach in the adenocarcinoma subtype of non-small-cell lung cancer aims to identify genomic alterations that can be targeted in individual patients and offer them appropriate treatment. Several biomarker-guided therapies have been approved, targeting genes frequently altered in adenocarcinoma, such as *EGFR*, *BRAF*, *MET*, *ALK*, *ROS1*, *RET* and *NTRK*. To overcome the emergence of resistance and increase the efficacy of these targeted therapies, the combination of chemotherapy and targeted therapy has been studied and validated in adenocarcinoma patients with *EGFR* mutations. This study proposes to examine whether this type of combined approach, which is difficult to study in clinical trials, could be more widely used by targeting other genetic alterations in the MAPK/PI3K pathways or against alterations in the *CDNK2A* gene in adenocarcinomas but also in squamous-cell carcinomas.

**Abstract:** The combination of chemotherapy and targeted therapy has been validated in non-small-cell lung cancer (NSCLC) patients with *EGFR* mutations. We therefore investigated whether this type of combined approach could be more widely used by targeting other genetic alterations present in NSCLC. PDXs were generated from patients with NSCLC adenocarcinomas (ADCs) and squamous-cell carcinomas (SCCs). Targeted NGS analyses identified various molecular abnormalities in the MAPK and PI3K pathways and in the cell cycle process in our PDX panel. The antitumor efficacy of targeted therapies alone or in combination with chemotherapy was then tested in vivo. We observed that trametinib, BKM120, AZD2014 and palbociclib increased the efficacy of each chemotherapy in SCC PDXs, in contrast to a non-insignificant or slight improvement in ADCs. Furthermore, we observed high efficacy of trametinib in *KRAS*-, *HRAS*- and *NRAS*-mutated tumors (ADCs and SCCs), suggesting that the MEK inhibitor may be useful in a wider population of NSCLC patients, not just those with *KRAS*-mutated ADCs. Our results suggest that the detection of pathogenic variants by

NGS should be performed in all NSCLCs, and particularly in SCCs, to offer patients a more effective combination of chemotherapy and targeted therapy.

**Keywords:** squamous NSCLC; PDXs; NGS-defined targeted therapy; chemotherapy; combination

## 1. Introduction

Non-small-cell lung cancer (NSCLC) is the leading cause of cancer-related mortality worldwide [1]. NSCLC is divided into three major histological subtypes, from the most common to the least common: adenocarcinoma (ADC), squamous-cell carcinoma (SCC), and large-cell carcinoma. Most cases are diagnosed at an advanced or metastatic stage. Chemotherapy has been the mainstay of treatment for NSCLC patients for around three decades. Standard chemotherapy regimens include platinum-based therapy combined with an antimetabolic agent (cisplatin plus pemetrexed) for ADCs or a fusiform poison (carboplatin plus paclitaxel) for SCCs. Over the past decade, the sequencing of the human genome has enabled the emergence of new biological therapies for NSCLC, targeting specific molecular alterations known as driver mutations. In the clinic, patient treatment decisions are based on observed oncogenic molecular alterations [2], as these enable the administration of targeted agents that are more effective than traditional chemotherapy regimens [3,4]. This is particularly true for non-squamous NSCLC, where most of these alterations are observed. However, SCCs also display various oncogenic alterations that could guide these precision medicine approaches [5].

Recently, combination of EGFR tyrosine kinase inhibitors (TKI) with platinum-based chemotherapy demonstrated a significant survival benefit when compared to targeted agents alone in ADCs [6,7]. These data suggest such combinations may change the clonal evolution of NSCLC tumors, through the eradication of TKI-resistant clones leading to acquired resistance and disease progression.

A key question in the clinic is whether such combination approaches are relevant in other oncogenic alterations both in non-squamous and squamous NSCLCs. It is now well demonstrated that patient-derived xenografts (PDXs) constitute the best models for preclinical studies addressing these questions [8]. Indeed, the stability of the genomic and gene expression profiles of PDXs in mice at first transplantation and during the *in vivo* maintenance of the model is a key relevant characteristic of these models [9]. Furthermore, it has recently been shown that PDXs can be highly predictive models for therapeutic response in cancer patients [10].

We therefore investigated whether the combination of standard chemotherapy and targeted therapy in NSCLC PDXs, with both treatments defined according to histopathological subtypes and tumor molecular abnormalities, could improve *in vivo* antitumor responses.

## 2. Materials and Methods

### 2.1. Patients and Tumor Samples

The PDX bank was established from engraftment of fresh human tumors coming from surgically resected patients with confirmed NSCLC. The project was hosted under the institutional tissue collection umbrella protocol of Institut Mutualiste Montsouris dedicated to thoracic malignancies (EUdract 2017-A03081-52 approved by the Ethics Committee CPP SUD-EST I on 15 December 2017). Consent was obtained for all patients. The selection of patient was based on several criteria: confirmed diagnosis of NSCLC; all histological subtypes (ADC, large-cell neuroendocrine carcinoma, SCC); and chemo- and immune-naïve operable patients at the Institut Mutualiste Montsouris (Thoracic Département, Paris, France) (TNM staging 8th edition) (except for the patient at the origin of LCIM28 who received preoperative immunotherapy). For each patient, the following clinical data were collected: age, sex, smoking habits, tumor histology, and stage of disease. In total, 31 tumors

from confirmed NSCLC diagnoses were implanted into the fat pads of Swiss nude mice, as previously described [11].

## 2.2. Animals and Animal Facilities

Mice were purchased from Charles River Laboratories and maintained under specific pathogen-free conditions. The care and animal housing were in accordance with institutional guidelines and with the recommendations of the French Ethics Committee (authorization APAFiS No. 25870-2020060410487032-v1 given by the national authority). The housing facility was kept at 22 °C ( $\pm 2$  °C) with a relative humidity of 30–70%. The light/dark cycle was 12 h light/12 h dark.

## 2.3. Histopathological Analyses

Formalin-fixed, paraffin-embedded sections were de-paraffinized in xylene and rehydrated in a gradient of ethanol prior to processing. Hematoxylin, eosin, and saffron staining were carried. Histological examination was realized on each PDX and compared with the histologic features of the corresponding patient's tumor.

## 2.4. Somatic Genetic and Genomic Alteration Analyses

PDX genomes were analyzed by targeted next-generation sequencing (NGS). A custom NGS panel called DRAGON (Detection of Relevant Alterations in Genes involved in Oncogenetics by NGS) and marketed by Agilent under the name of SureSelect CD Curie CGP has been recently developed in the genetics department, specifically for the molecular analysis of tumors for single nucleotide variant, copy-number variation (CNV), microsatellite instability, and tumor mutational burden status. It is composed of 571 genes of interest in oncology from diagnosis, prognosis, and theranostics, including potential therapeutic targets (Supplemental Table S1). All studied genetic abnormalities and modalities of analyses are described in Passeri et al. [12].

## 2.5. In Vivo Efficacy Studies

Firstly, the sensitivity of PDXs to standard chemotherapy combinations was evaluated in 15 models. Based on histological characteristics, mice received pemetrexed (100 mg/kg, 1x/w, IP) plus cisplatin (4 mg/kg (1x/3w, IP) for ADC, carboplatin (66 mg/kg, 1x/3w, IP) plus paclitaxel (20 mg/kg, 1x/3w, IP) for SCC, etoposide (10 mg/kg, 3x/3w, IP) plus cisplatin (4 mg/kg, 1x/3w, IP) for large-cell neuroendocrine carcinoma, and gemcitabine (100 mg/kg, 1x/w, IP) plus cisplatin (1 mg/kg, 1x/w, IP) for sarcomatoid carcinoma.

Secondly, thirteen NSCLC PDXs (out of 16 established PDX models) in which an NGS study (panel "DRAGON") was performed were selected for in vivo experiments, in order to assess the efficacy of targeted therapies alone and in combination with chemotherapies. Afatinib (EGFR inhibitor), trametinib (MEK inhibitor), buparlisib (BKM120) (PI3K inhibitor), vistusertib (AZD2014) (mTORC1/2 inhibitor), palbociclib (CDK4/6 inhibitor), birabresib (BET bromodomain inhibitor), and tazemetostat (EZH2 inhibitor) were purchased from MedChemExpress. Afatinib, trametinib, BKM120, AZD2014, palbociclib, birabresib, and tazemetostat were administrated orally 5 days per week at a daily dosage of 15 mg/kg, 0.4 mg/kg, 15 mg/kg, 10 mg/kg, 50 mg/kg, 30 mg/kg (twice-a-day), and 150 mg/kg, respectively.

In in vivo experiments, the procedure of tumor graft, as well as tumor growth assessment, evaluation of the efficacy of tested therapies, and time sacrifice were performed, as previously reported [13]. The quality of the antitumor response was determined as follows: A decrease in tumor volume of at least 50% was classified as huge or complete response, a volume change between  $-50\%$  and  $+35\%$  was considered as stable disease, and an increase in tumor volume of at least 35% was identified as partial response (TGI: percentage of tumor growth inhibition  $> 50\%$ ) or progressive disease (TGI  $< 50\%$ ) (Supplemental Figure S1) [14]. To assess treatment response in relation to individual mouse variability, we determined the overall response rate (ORR) for each treated mouse. For each PDX model, the ORR

was calculated for each treated mouse as follows:  $ORR = [(RTV_t/mRTV_c)]^{-1}$ , where  $RTV_t$  is the relative tumor volume of the treated mouse and  $mRTV_c$  is the median relative tumor volume of the corresponding control group at the end of treatment.

Finally, to study the impact of treatments on the time to tumor growth delay, we also calculated the probability of progression (doubling time and time to reach  $RTV = 4$ ), as previously described [13]. Statistical analysis of tumor growth was performed using the unpaired Kruskal–Wallis test with Dunn’s multiple comparison test or the unpaired Mann–Whitney test for comparisons between two groups. Statistical analysis of the progression curves was performed using the log-rank test.

### 3. Results

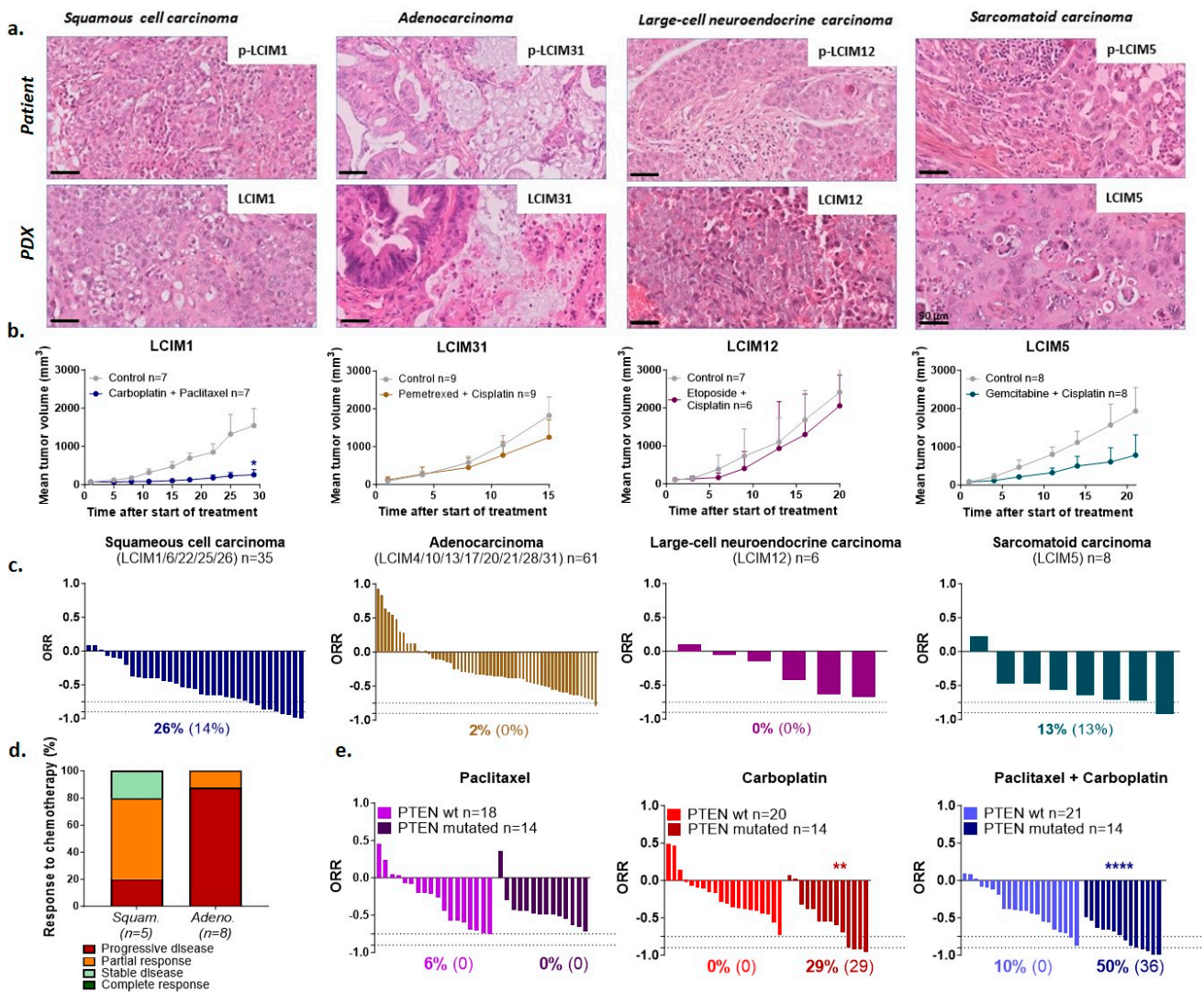
#### 3.1. Development of a Representative Panel of NSCLC PDXs and Identification of Targeted Genomic Alterations

A panel of 16 NSCLC PDXs, representative of the different histological subtypes of the disease, was obtained from the implantation of 31 primary tumor samples in nude mice (uptake rate: 51.6% and average tumor uptake time = 133 days). This panel includes eight ADCs (50%), five squamous NSCLCs (31%), two large cell carcinomas (13%), and one sarcomatoid sub-type (6%) (Supplemental Figure S2a). No significant correlation between in vivo tumor uptake and the clinical and molecular characteristics of NSCLC patients (cumulative survival, age, gender, smoking habits, TNM stage, and histology) was observed (Supplemental Table S2). Interestingly, a significant correlation between in vivo tumor uptake and PDL1 expression in patients’ tumor cells ( $p = 0.02$ ) was observed (Supplemental Figure S2c).

All the NSCLC PDXs were molecularly characterized at the genomic level by a targeted NGS analysis of 571 genes (the most frequently mutated genes in cancer) (Supplemental Table S1). Significantly mutated genes and CNV in putative cancer driver genes observed in our PDXs are shown in Supplemental Table S3. Of the 16 PDX models, 50% of the panel displayed pathogenic genomic alterations affecting the MAPK pathway (mutations of *KRAS* (25%), *NRAS*, *HRAS*, *RASA1*, *MAP2K4*, and *NF1*), 38% affecting the PI3K pathway (mutation or amplification of *PIK3CA*, mutations of *PIK3R1*, *PTEN* and *STK11*), and 38% of the panel presented mutations or homologous deletion of *CDKN2A/2B*. Only one PDX (ADC LCIM21) displayed an *EGFR* mutation (L858R exon21), representing 6% of the panel, as compared to 10% of *EGFR* mutations found in NSCLC patients.

#### 3.2. Low Sensitivity of Our PDX Panel to Standard Chemotherapies

Histological analyses between the primary patients’ tumors and their corresponding PDXs showed that xenografts resembled the primary tumors from which they derived (Figure 1a). The identification of histological subtypes enabled us to validate the models and select the chemotherapy combination to be tested in each PDX model. The choice of chemotherapy combinations was determined by clinical practice: carboplatine + paclitaxel for the five SCCs, pemetrexed + cisplatin for the eight ADCs, etoposide + cisplatin for the large-cell neuroendocrine carcinoma model, and gemcitabine + cisplatin for the sarcomatoid carcinoma. Overall, our models are not very sensitive to chemotherapy, whatever the histological subtype (Figure 1b,c) (individual data per model in Supplemental Figure S3). Only one among five SCCs responded to carboplatin + paclitaxel with tumor stabilization (Figure 1d). Interestingly, the two most chemosensitive SCC PDX models (LCIM1 and LCIM26) (Supplemental Figure S3) harbored an inactivating mutation of *PTEN*, in contrast to wild-type *PTEN* tumors (LCIM6, LCIM22 and LCIM25) (Figure 1e). Moreover, when we compared each mono-chemotherapy to the combination, we have observed that *PTEN*-mutated tumors are more sensitive to carboplatin (Figure 1e), a finding not reported so far in the clinic.

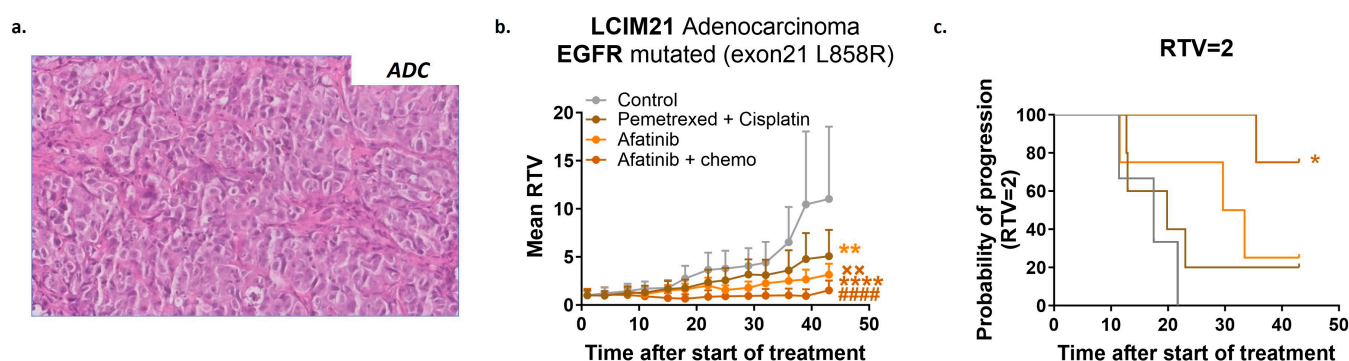


**Figure 1.** In vivo sensitivity of PDX NSCLC panel to standard chemotherapies. (a) Histological comparison with hematoxylin, eosin, and saffron staining between primary patients’ tumors (p-LCIM) and their corresponding xenografts (LCIM) (magnification  $\times 400$ ). (b) In vivo efficacy of carboplatin (66 mg/kg, 1x/3w, ip) plus paclitaxel (20 mg/kg, 1x/3w, ip) in squamous cell carcinomas, pemetrexed (100 mg/kg, 1x/w, ip) plus cisplatin (4 mg/kg, 1x/3w, ip) in adenocarcinomas, etoposide (10 mg/kg, 3x/3w, ip) plus cisplatin (4 mg/kg, 1x/3w, ip) in large-cell neuroendocrine carcinoma, and gemcitabine (100 mg/kg, 1x/w, ip) plus cisplatin (1 mg/kg, 1x/w, ip) in sarcomatoid carcinoma (RTV  $\pm$  SEM). (c) Overall response rate (ORR): a percentage in bold corresponds to an ORR lower than  $-0.75$  and a percentage in brackets corresponds to an ORR lower than  $-0.9$ . (d) Fraction of squamous carcinoma PDX panel and adenocarcinoma PDX panel responding to chemotherapy with progressive disease (TGI  $< 50\%$  + % of change  $> 35\%$ ), partial response (TGI  $> 50\%$  + % of change  $> 35\%$ ), stable disease (TGI  $> 50\%$  +  $35\% >$  % of change  $> -50\%$ ), or complete response (TGI  $> 50\%$  + % of change  $< -50\%$ ). (e) ORR to paclitaxel  $\pm$  carboplatin in squamous carcinoma NSCLC PDX with or without *PTEN* mutation; a percentage in bold corresponds to an ORR lower than  $-0.75$  and a percentage in brackets corresponds to an ORR lower than  $-0.9$ ; statistical analysis of the efficacy of the treatment was performed by Wilcoxon test: \* *PTEN*-mutated vs. *PTEN*-wt tumors; \*\*  $p < 0.01$  and \*\*\*\*  $p < 0.0001$ .

### 3.3. Additive Antitumor Effect of Combining Chemotherapy with an EGFR TKI in an ADC PDX with EGFR L858R Mutation

To investigate the value of combining a targeted therapy with chemotherapy in NSCLC, we first tested the antitumor effect of a clinically approved EGFR TKI, afatinib, in combina-

tion with pemetrexed + cisplatin in a PDX model of *EGFR* L858R-mutated ADC (histological analysis in Figure 2a). Looking at mean relative tumor volume (RTV), we observed in the LCIM21 model a high and significantly better efficacy of the combination of afatinib with chemotherapies (TGI = 86%) compared to afatinib (TGI = 71%) or chemotherapies (TGI = 54%) alone (Figure 2b) (unpaired *t*-test). In addition, by determining the probability of progression (two-fold increase in RTV), we observed a significant efficacy of the combination of EGFR TKI with chemotherapies compared with the control group ( $p = 0.0386$ ), which was not found in therapies administered alone (Figure 2c) (Mantel-Cox test). In conclusion, this first experiment demonstrated that the combination of targeted therapy and chemotherapy can improve antitumor response in a PDX model of *EGFR* L858R-mutated ADC, as reported into clinical trials [6,15].

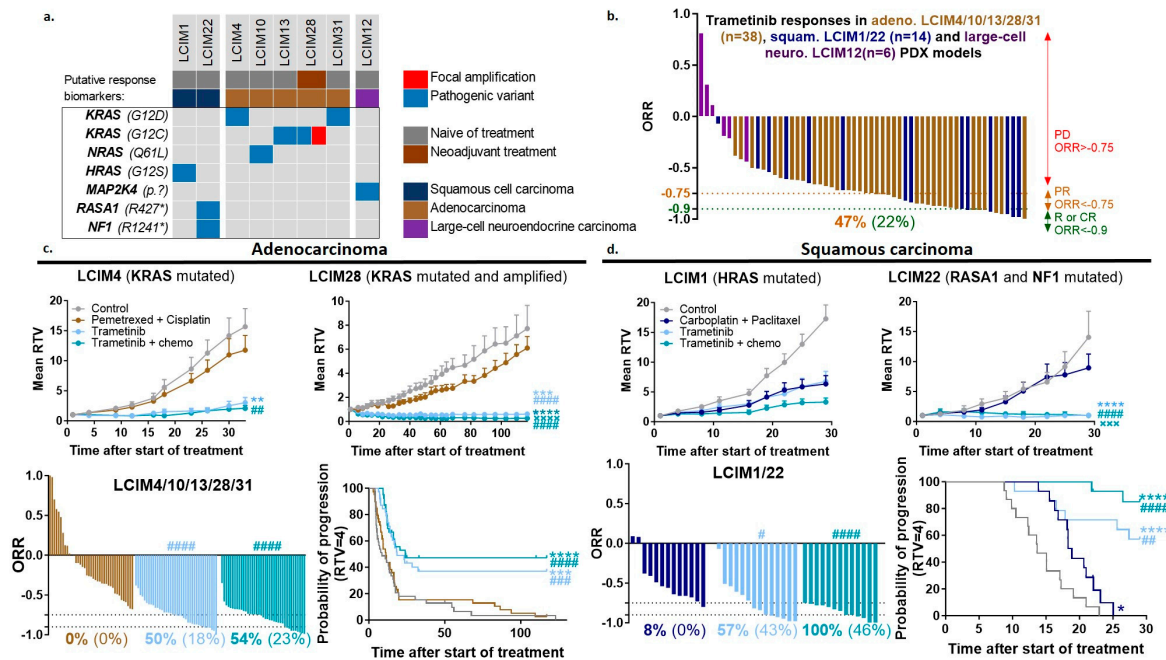


**Figure 2.** In vivo efficacy study of EGFR inhibitor  $\pm$  standard chemotherapy in EGFR-mutated adenocarcinoma NSCLC PDX. (a) HES histological staining of PDX LCIM21 adenocarcinoma (magnification  $\times 200$ ). (b) In vivo targeting of LCIM21 PDX by afatinib (15 mg/kg, 5x/w, per os) ( $n = 4$ ) in monotherapy (light orange line) and in combination (dark orange line) with pemetrexed (100 mg/kg, 1x/w, ip) plus cisplatin (4 mg/kg, 1x/3w, ip) ( $n = 4$ ) (brown line); statistical analysis of the efficacy of the treatment was performed by unpaired *t*-test (Mann–Whitney test). \* vs. control group, # vs. chemotherapy, x vs. afatinib. (c). Probability of progression after each tested treatment; the time to reach relative tumor volume RTV = 2 for each treated mouse has been calculated.

### 3.4. In Vivo Efficacy of Combining MEK Inhibitor with Chemotherapy in NSCLC PDX Models Showing Alterations in the MAPK Pathway

Trametinib, an MEK inhibitor, was tested alone and in combination with chemotherapy in eight NSCLC PDXs with MAPK pathway alterations. The targeting of the MAPK pathway in this study was not limited to *KRAS* mutations but extended to other genomic alterations found in our PDX models (*NRAS*, *HRAS*, *RASA1*, *MAP2K4*, *NF1*) (histological and genomic characteristics in Figure 3a). Independent of the type of mutations, treatment with trametinib alone induced a significant antitumor response, with an ORR  $< -0.75$  in 47% of treated mice and an ORR  $< -0.9$  in 22% of treated mice (Figure 3b). The strongest responses to trametinib were observed in LCIM22 SCC and LCIM28 ADC PDXs. Both models show a double genomic alteration in the MAPK pathway, *RASA1* and *NF1* mutations for LCIM22, and mutation and amplification of *KRAS* for LCIM28 (individual data per model in Supplemental Figure S4). When we examined ORR and probability of progression (RTV = 4), the addition of chemotherapy to trametinib appears to improve the quality of antitumor responses in SCC (57% of mice treated with trametinib have ORR  $< -0.75$  vs. 100% of mice treated with the combination) but not in ADC (Figure 3c,d).

These results suggest that clinical trials of MAPK pathway inhibitors should not be limited to *KRAS* gene alterations but should be extended to all mutations resulting in the activation of the pathway. Finally, we have shown the value of using trametinib in combination with chemotherapy in SCCs with activating alterations of the MAPK pathway, which needs to be validated on a larger scale in patients.



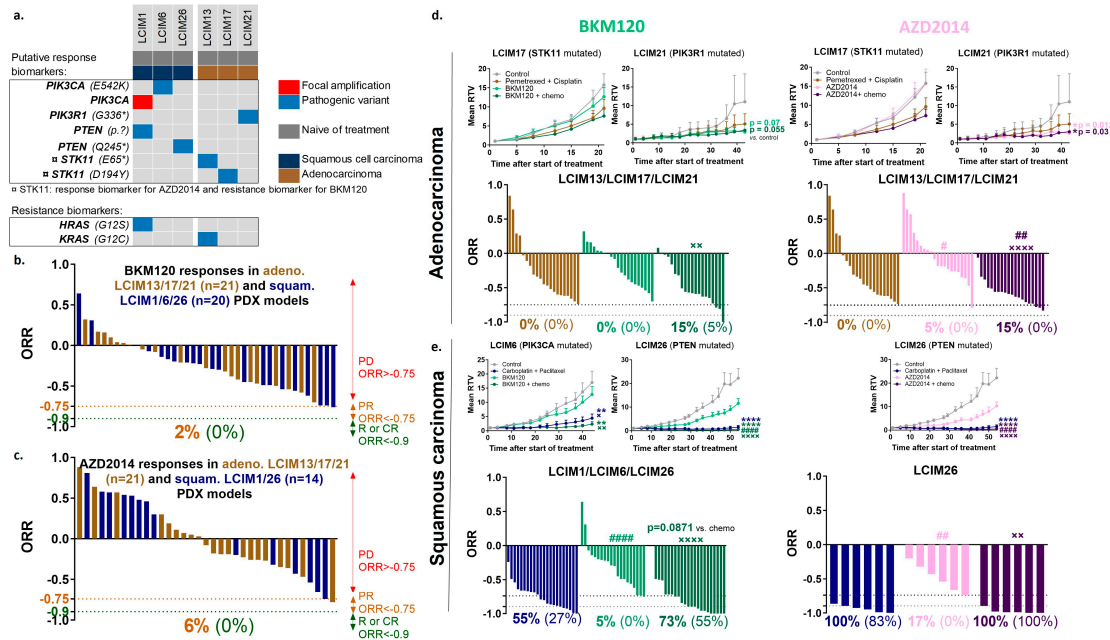
**Figure 3.** In vivo efficacy study of KRAS inhibitor ± standard chemotherapy in NSCLC PDX models showing alterations in the MAPK pathway. (a) Histological and genomic characteristics of PDX models with MAPK pathway alterations. (b) Overall response rate (ORR) to trametinib monotherapy (0.4 mg/kg, 5x/w, per os) in 8 NSCLC PDXs; a percentage in orange corresponds to an ORR lower than  $-0.75$  and a percentage in green corresponds to an ORR lower than  $-0.9$ . In vivo targeting of adenocarcinoma NSCLC PDXs by trametinib (light blue line) in combination (turquoise blue line) with pemetrexed (100 mg/kg, 1x/w, ip) plus cisplatin (4 mg/kg, 1x/3w, ip) (brown line) (c) and of squamous carcinoma PDXs by trametinib (light blue line) in combination (turquoise blue line) with carboplatin (66 mg/kg, 1x/3w, ip) plus paclitaxel (20 mg/kg, 1x/3w, ip) (dark blue line) (d). Mean RTV  $\pm$  SEM ( $n = 6$  to  $8$ ); statistical analysis of the efficacy of the treatment was performed by unpaired *t*-test (Mann–Whitney test). \* vs. control group, # vs. chemotherapy,  $\times$  vs. trametinib. ORR: a percentage in bold corresponds to an ORR lower than  $-0.75$  and a percentage in brackets correspond to an ORR lower than  $-0.9$ ; statistical analysis of ORR was performed by unpaired *t*-test (Mann–Whitney test); # vs. chemotherapy. Probability of progression after each tested treatment; the time to reach relative tumor volume RTV = 4 for each treated mouse has been calculated; statistical analysis of the efficacy of the treatment was performed by Mantel–Cox test. \* vs. control group, # vs. chemotherapy.

### 3.5. In Vivo Efficacy of Combining PI3K or mTOR Inhibitors with Chemotherapy in NSCLC PDX Models Showing Alterations in the PI3K Pathway

The antitumor activity of BKM120, a PI3K inhibitor, and AZD2014, an mTORC1/C2 inhibitor, was tested in our 6 NSCLC PDXs with various genetic alterations that activate the PI3K pathway (mutations of PIK3CA, PIK3R1, PTEN, STK11) (Figure 4a). Treatment with BKM120 and AZD2014 as monotherapies had no antitumor effect in our PI3K-altered PDX panel (all mutations and all histological subtypes), with ORR  $< -0.75$  in only 2% and 6% of treated mice, respectively (Figure 4b,c). The two PDXs with a mutation in the MAPK pathway (HRAS for LCIM1 and KRAS for LCIM13), which is a marker of resistance to PI3K inhibitors, had the lowest median ORR in response to PI3K and mTOR inhibitors alone in SCC and ADC subgroups, respectively (individual data per model in Supplemental Figure S5). In ADC models, the combination of chemotherapy with BKM120 or AZD2014 does not appear to improve the antitumor efficacy of PI3K and mTOR inhibitors (ORR  $< -0.75$  in 0% and 5% of mice treated with BKM120 or AZD2014, respectively, versus 15% and 15% of mice treated with the combination) (Figure 4d). In contrast, in our SCC models, combining chemotherapy with BKM120 or AZD2014 non-significantly improved



the quality of antitumor responses (BKM120: ORR < −90 in 55% of mice treated with the combination compared to 27% of mice treated with chemotherapy alone; AZD2014: ORR < −90 in 100% of mice treated with the combination compared to 83% of mice treated with chemotherapy alone) (Figure 4e).



**Figure 4.** In vivo efficacy study of PI3K or mTOR inhibitors ± standard chemotherapy in NSCLC PDX models showing alterations in the PI3K pathway. (a) Histological and genomic characteristics of PDX models with PI3K pathway alterations. Overall response rate (ORR) to BKM120 monotherapy (15 mg/kg, 5x/w, per os) in 6 NSCLC PDXs (b) and to AZD2014 monotherapy (10 mg/kg, 5x/w, per os) in 5 NSCLC PDXs (c) ( $n = 6$  to  $8$ ); a percentage in orange corresponds to an ORR lower than  $-0.75$  and a percentage in green corresponds to an ORR lower than  $-0.9$ . In vivo targeting of adenocarcinoma NSCLC PDXs by BKM120 (green line) and AZD2014 (pink line) in combination with pemetrexed (100 mg/kg, 1x/w, ip) plus cisplatin (4 mg/kg, 1x/3w, ip) (d) and of squamous carcinoma PDXs in combination with carboplatin (66 mg/kg, 1x/3w, ip) plus paclitaxel (20 mg/kg, 1x/3w, ip) (dark blue line) (e). Mean RTV ( $n = 6$  to  $8$ ); statistical analysis of the efficacy of the treatment was performed by unpaired  $t$ -test (Mann–Whitney test). \* vs. control group. ORR: a percentage in bold corresponds to an ORR lower than  $-0.75$  and a percentage in brackets corresponds to an ORR lower than  $-0.9$ ; statistical analysis of ORR was performed by unpaired  $t$ -test (Mann–Whitney test); # vs. chemotherapy, × vs. BKM120 or AZD2014.

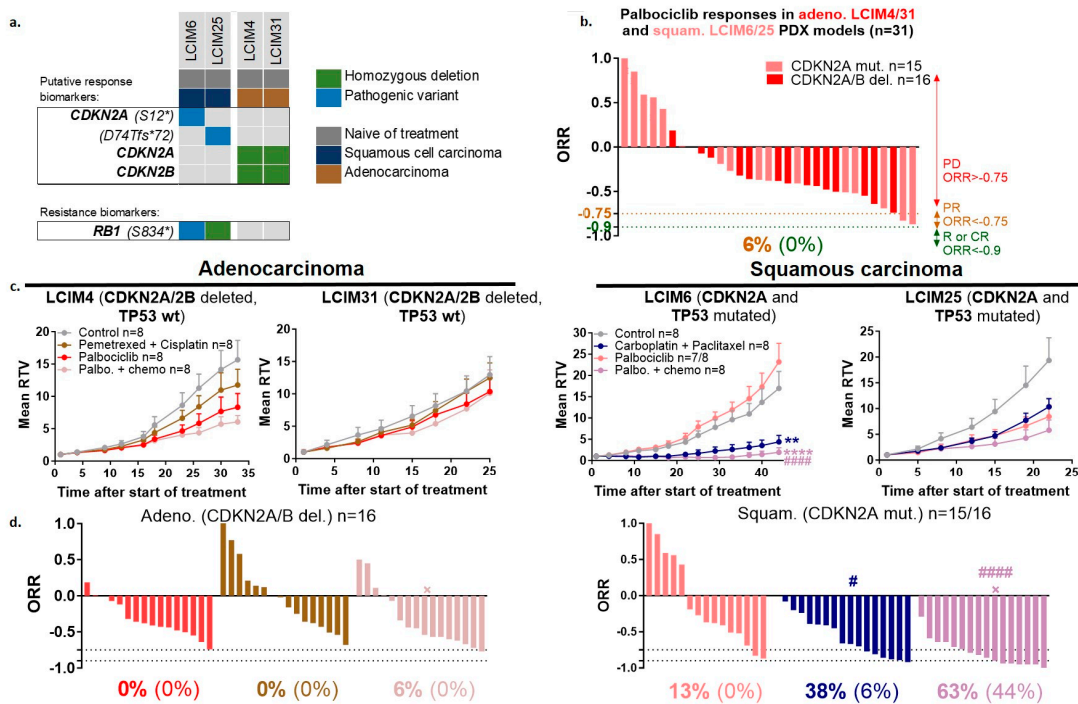
Similar to the results of targeting the MAPK pathway, these results demonstrate the value of combining chemotherapy with PI3K-targeted therapy in SCC. Beyond histological subtypes, it should be noted that the mutations present in our SCC models (mutations of *PTEN* and *PIK3CA* mutations) present a higher level of evidence for the use of PIK3CA pathway inhibitors than our ADC models (*PIK3R1* and *STK11* mutations, *STK11* being also a potential marker of resistance to BKM120).

Given the poor response to BKM120 and AZD2014 monotherapy in our models with PI3K pathway alterations, dual targeting of the PI3K pathway by concomitant treatment of mice with BKM120 and AZD2014 was tested (Supplemental Figure S6). Although slightly significant differences were observed between the monotherapy and dual therapy groups in the ADC and SCC models, we consider that the combination of the two inhibitors did not have a very potent additive antitumor effect (only 5% and 20% of mice treated with the combination had an ORR <  $-0.75$  in ADCs and SCC, respectively). In contrast, an additional experiment has shown that dual targeting of the PI3K and MAPK pathways with BKM120 plus trametinib induced a better antitumor effect than dual targeting of

the PI3K pathway with BKM120 plus AZD2014 in the *NF1*-mutated LCIM22 PDX model (Supplemental Figure S7).

### 3.6. In Vivo Efficacy Assessment of Targeted Therapy in NSCLC PDX Models Showing *CDKN2A*, *MYC*, or Epigenetic Alterations

To determine whether a *CDKN2A* mutation or homologous deletion (found in 38% of our NSCLC PDX panel) can be used as a biomarker of response to a CDK4/6 inhibitor in NSCLC, treatment with palbociclib alone or in combination with chemotherapies was carried out in two ADCs with *CDKN2A* homologous deletions and two SCCs with *CDKN2A* mutations (Figure 5a). Treatment with palbociclib alone did not induce an antitumor effect in the PDX tested, with an ORR < -0.75 only in 6% of palbociclib-treated mice (Figure 5b) (individual data per model in Supplemental Figure S8). When considering ORR, the addition of chemotherapy to palbociclib significantly improved the quality of antitumor responses in squamous-cell carcinomas (13% of palbociclib-treated mice had ORR < -0.75 versus 63% of combination-treated mice), but not in ADCs, despite the presence of RB1 alterations (a potential biomarker of palbociclib resistance) in squamous-cell carcinomas (Figure 5c,d). Finally, additional experiments have shown that treatment of PDXs displaying *MYC* (focal amplification) and epigenetic (mutations of *ARID1A* and *SMARCA4*) alterations using a BET bromodomain inhibitor and an *EZH2* inhibitor, respectively, did not induce antitumor effects in our NSCLC PDX models (Supplemental Figure S9).



**Figure 5.** In vivo efficacy study of CDK4/6 inhibitor ± standard chemotherapy in NSCLC PDX models showing *CDKN2A* alterations. (a) Histological and genomic characteristics of PDX models with *CDKN2A* alterations. (b) Overall response rate (ORR) to palbociclib monotherapy (50 mg/kg, 5x/w, per os) in 4 NSCLC PDXs; a percentage in orange corresponds to an ORR lower than -0.75 and a percentage in green corresponds to an ORR lower than -0.9. (c) In vivo targeting of 4 NSCLC PDXs by palbociclib in combination with chemotherapy (for adenocarcinomas: pemetrexed (100 mg/kg, 1x/w, ip) plus cisplatin (4 mg/kg, 1x/3w, ip), for squamous carcinomas: carboplatin (66 mg/kg, 1x/3w, ip) plus paclitaxel (20 mg/kg, 1x/3w, ip)); Mean RTV ± SEM (n = 8); statistical analysis of the efficacy of the treatment was performed by unpaired *t*-test (Mann–Whitney test). \* vs. control group, # vs. palbociclib. (d) ORR; a percentage in bold corresponds to an ORR lower than -0.75 and a percentage in brackets corresponds to an ORR lower than -0.9; statistical analysis of ORR was performed by unpaired *t*-test (Mann–Whitney test); # vs. palbociclib, × vs. chemotherapy.

#### 4. Discussion

In this article, we have evaluated whether combined chemotherapy and targeted therapy in NSCLC PDXs, both defined according to the histopathological subtypes and molecular abnormalities of the tumors, may be more efficient than chemotherapy or targeted therapy alone. In contrast to ADCs in which no significant additive effect was observed, our results show that, in squamous NSCLC, carboplatin + paclitaxel chemotherapy combined with targeted therapies directed against MAPK and PI3K pathways, or CDK4/6, were significantly more efficient than each treatment tested alone. We therefore consider that such an observation might be considered in the management of squamous NSCLC patients.

We have used for our work PDX models that are recognized to highly recapitulate the heterogeneity of human tumors and are strongly predictive of treatment responses in cancer patients [10,16]. In the NSCLC PDX panel that we developed, we observed a similar distribution of cancer subtypes, as well as similar and poor responses to standard chemotherapies in comparison to that observed in the clinics. We aim to highlight four peculiar points concerning our NSCLC PDX panel: First, in contrast to what has been reported in other cancer type PDXs [11,17,18], we did not observe that, in our series, NSCLC patients whose tumors were able to grow in mice had a shorter overall survival than patients whose tumors were not able to grow in mice (Supplemental Figure S2b); however, this may be due to the limited number of established models. Second, molecular analyses of our PDX panel were not limited to standard alterations such as *EGFR* or *KRAS* mutations, but to 571 genes (studied in the NGS panel called “DRAGON”) of interest in relation to a theranostic approach. Third, we observed that patients whose tumors expressed high levels of PDL1 significantly correspond to patients whose tumors did not grow in mice (Supplemental Figure S2c); this observation is at odds with a recent study in pancreatic cancer which reported that PDL1 expression correlated with tumor uptake in PDX models [19]. Fourth, we observed that *PTEN*-mutated tumors, exclusively observed in squamous PDXs, were more sensitive to carboplatin than wild-type *PTEN* tumors (Figure 1e). This observation contradicts previous studies showing that *PTEN* loss correlates with shorter recurrence-free survival in breast cancer and worse overall survival in head and neck squamous-cell carcinoma patients treated with chemotherapy [20,21]. It might therefore be of interest to evaluate the possible relationship between *PTEN* status in squamous NSCLC and response to carboplatin-based chemotherapy.

The combination of chemotherapy with a targeted therapy (gefitinib or osimertinib) in NSCLC patients has been proved to be superior to chemotherapy alone in the context of *EGFR* mutations [6,15,22]. A series of molecular targeted agents have been tested in combination to standard chemotherapy in NSCLC patients, as shown in Table 1. The results of clinical non-randomized studies have been reported. Few clinical randomized studies have been reported with selumetinib (an MEK inhibitor) [23], OGX-427 (an HSP 90 inhibitor) [24], Enzastaurine (a PKC and PI3K pathways inhibitor) [25], tirapazamine (which targets hypoxic cells) [26], and different antiangiogenic compounds [27–30]. Among all these studies, mainly performed in non-squamous NSCLC, only one showed a significant difference between chemotherapy alone (cisplatin + gemcitabine) and chemotherapy + bevacizumab in favor of the combination [29]. Otherwise, no significant improvement in terms of ORR, progression-free survival, and overall survival have been observed, and in particularly after treatment with the MEK inhibitor selumetinib in metastatic pretreated *KRAS*-mutated NSCLC [23]. This point underlines the fact that the evaluation of efficient combinations in *EGFR*-wild-type NSCLC remains a real and current therapeutic challenge.

**Table 1.** Randomized and non-randomized clinical trials evaluating chemotherapy plus targeted therapies in NSCLC patients.

Chemotherapies	Molecular Targeted Agents	Rando-Mized Studies	Sub-Type of Cancer	N of Patients	Results	Ref.
Docetaxel	Selumetinib (MEKi)	Yes	Metastatic pretreated KRAS-mutant NSCLC	44	mPFS 5.3 months (combi) versus 2.1 months (CT alone)	[23]
Carboplatin + pemetrexed	OGX-427 (HSP 27 mRNA)	Yes	Untreated metastatic non-squamous NSCLC	155	ORR, mPFS, and mOS: NS	[24]
Cisplatin + pemetrexed	Enzastaurin (PKC & AKT pathways)	Yes	Chemonaive metastatic NSCLC	22	Early interruption due to combination toxicity	[25]
Cisplatin + gemcitabine	Sorafenib (antiangiogenic agent)	Yes	Metastatic NSCLC	30	ORR, mPFS, and mOS: NS	[27]
Cisplatin + gemcitabine	Sorafenib (antiangiogenic agent)	Yes	Untreated metastatic non-squamous NSCLC	772	PFS: NS	[28]
Cisplatin + gemcitabine	Bevacizumab (antiangiogenic agent)	Yes	Untreated metastatic non-squamous NSCLC	1043	Combi > CT alone ( $p = 0.03$ )	[29]
Pemetrexed	Sunitinib (antiangiogenic agent)	Yes	Second-line metastatic NSCLC	130	High toxicity of combination impacted PFS and OS	[30]
Carboplatin + paclitaxel	Tirapazamine (hypoxic cells)	Yes	Metastatic NSCLC	367	Early interruption due to combination toxicity	[26]
Docetaxel	Celecoxib (COX-2i)	No	Recurrent NSCLC	56	ORR 11%	[31]
Pemetrexed + docetaxel	Apatinib (antiangiogenic TKI)	No	Refractory metastatic NSCLC	20	ORR 30% (PR)	[32]
Docetaxel	Apatinib (antiangiogenic TKI)	No	Metastatic non-squamous NSCLC	14	ORR 33%	[33]
Pemetrexed	Luminespib (HSP90i)	No	Metastatic non-squamous NSCLC	13	ORR 14%	[34]
Carboplatin + pemetrexed	Metformine	No	Treatment-naive metastatic non-squamous NSCLC	/	mPFS 4.5 months	[35]

In this view, our study appears of high interest to define, according to specific molecular abnormalities of the tumors (using an NGS panel), which combinations of chemotherapy plus targeted therapy are susceptible to improving the therapeutic management of both ADC and squamous NSCLCs. In contrast to current clinical practices, such an approach also

opens the possibility to evaluate the impact of the NGS-profile study of squamous NSCLC. Hence, the observation that the MEK inhibitor trametinib was also able to induce tumor regressions in *HRAS*-, *NRAS*-, and *RASA1*-mutated squamous NSCLC PDXs, in parallel to *KRAS*-mutated ADC PDXs (Figure 3), supports the enlargement of the number of NSCLC patients who could benefit from treatments including an MEK inhibitor and, consequently, appears to be of high clinical interest. The PDX model of SCC (LCIM22) that responded best to the MEK inhibitor had a double genomic alteration in the MAPK pathway, namely a *RASA1* mutation and an *NF1* mutation. These results are in accordance with a recent study showing that *RASA1* truncating mutations have a strong tendency to co-occur with *NF1* truncating mutations, and that alterations in these two negative regulators of RAS signaling, when concomitant, show profound sensitivity to MEK inhibition in vitro [36]. Moreover, the PDX model of ADC (LCIM28) that highly responded to the MEK inhibitor in our study exhibited a dual alteration of the MAPK pathway with a *KRAS* mutation plus a *KRAS* amplification. Trametinib has been shown to have clinical activity in a variety of malignancies, including *KRAS*-mutated NSCLC [37,38]. However, because of the presence of compensatory signaling pathways, targeting of *MEK* alone may not achieve a complete antitumor effect [39]. Our results show that a double alteration in the MAPK pathway seems to give a therapeutic advantage to ADC and SCC patients receiving MEK inhibitors. This observation underlines, as already described [40,41] the importance of determining the presence of a *KRAS* mutation in a tumor, as well as accurately assessing *KRAS* expression levels that may impact potentially therapeutic *KRAS*-targeted therapies.

In a similar view, it is noteworthy to note that various mutations affecting the PI3K pathway, such as *PIK3CA*, *PIK3R1*, *PTEN*, and *STK11* mutations, may define possible targeted therapies that could be combined with standard chemotherapy, even if the therapeutic weight of each mutation remains to be refined. Finally, our results do not validate *CDKN2A* alterations (mutations or deletions) as predictive biomarkers of response to palbociclib alone in NSCLC. However, once again, combining the CDK4/6 inhibitor with chemotherapy improved the response to chemotherapy only in squamous PDXs. The current clinical rule of evaluating mutational/copy-number status only in ADC NSCLC needs to be re-examined in the light of our results showing that the combination of chemotherapy plus targeted therapy was significantly more efficient than monotherapies in squamous PDXs. Although we lack mechanisms to explain the efficacy of targeted therapies combined with chemotherapy in CSC, and based on our significant preclinical data, we consider that a clinical trial can be launched to assess the impact of adding NGS-targeted therapy to chemotherapy. However, adverse reactions or toxicity may also be assessed in those cancer patients. Based on what we have observed in a relevant panel of NSCLC PDXs, our data support conducting further clinical trials to hopefully improve the prognosis of NSCLC patients.

## 5. Conclusions

In conclusion, this study has showed that, in contrast to adenocarcinomas, in squamous non-small-cell lung cancer, chemotherapy combined with targeted therapies directed at the PI3K or MAPK pathways, or against alterations in *CDKN2A* gene, was more effective than each treatment tested alone. Based on what we observed in a relevant panel of NSCLC PDXs, we suggest that the NSCLC clinical paradigm could be modified in the direction of systematically opening up NGS evaluation to squamous NSCLC, to define relevant combinations of therapies likely to improve the prognosis of patients with squamous NSCLC. Furthermore, our results suggest that the use of TKIs in combination with chemotherapy should not be limited to targeting *KRAS* or *PIK3CA* mutations, but should be extended to targeting *HRAS*, *NRAS*, *RASA1*, and *NF1* alterations for *KRAS* inhibitors, and *PTEN* and *PIK3R1* alterations for *PI3KCA* inhibitors.

**Supplementary Materials:** The following supporting information can be downloaded at: <https://www.mdpi.com/article/10.3390/cancers16162785/s1>. Table S1: Targeted NGS panel gene list. Table S2: Clinical and molecular characteristics of all NSCLC patients (n = 31; univariate analysis) and in vivo tumor take rate (%). Table S3: Molecular characteristics of NSCLC PDX panel. Figure S1: Classification of anti-tumor responses. Figure S2: Development of a NSCLC PDX panel. Figure S3: Overall response rate (ORR) per model to standard chemotherapies. Figure S4: Overall response rate (ORR) per model to KRAS inhibitor ± standard chemotherapy in NSCLC PDX models showing alterations in the MAPK pathway. Figure S5: Overall response rate (ORR) per model to PI3K or mTOR inhibitors ± standard chemotherapy in NSCLC PDX models showing alterations in the PI3K pathway. Figure S6: In vivo efficacy of dual targeting of the PI3K pathway in NSCLC PDX models. Figure S7: In vivo efficacy of dual targeting of the PI3K and MAPK pathways or dual targeting of the PI3K pathway in NF1-mutated NSCLC PDX model. Figure S8: Overall response rate (ORR) per model to CDK4/6 inhibitor ± standard chemotherapy in NSCLC PDX models showing CDKN2A alterations. Figure S9: In vivo efficacy study of MYC targeting and epigenetics targeting in NSCLC PDX models with genomic alterations.

**Author Contributions:** Conceptualization, D.D., I.B., N.G. and E.M.; Data curation, I.B. and E.M.; Formal analysis, D.D., J.M.P., R.E.-B. and E.M.; Funding acquisition, D.D.; Investigation, D.D., I.B. and E.M.; Methodology, F.N., V.E., H.A., Q.P., L.S., R.E.-B., L.H. and E.M.; Resources, A.S.-G., M.L., S.L., N.H. and N.G.; Supervision, D.D.; Validation, J.M.P., I.B. and N.G.; Writing—original draft, D.D., N.G. and E.M.; Writing—review & editing, J.M.P., A.S.-G., M.L., S.R.-R. and I.B. All authors have read and agreed to the published version of the manuscript.

**Funding:** This research received no external funding.

**Institutional Review Board Statement:** The animal study protocol was approved by the Ethics Committee of the Institut Curie CEEA-IC #118 (Authorization APAFiS No. 25870-2020060410487032-v1 given by National Authority).

**Informed Consent Statement:** Informed consent was obtained from all subjects involved in the study. Their referring oncologist informed all patients included in our study that their biological samples could be used for research purposes and patients signed an informed consent of non-opposition. In case of patient refusal, expressed either orally or written, residual tumor samples were excluded from our study.

**Data Availability Statement:** All data are already shared in the paper.

**Acknowledgments:** We wish to thank Ahmed Dahmani, Ludivine Morisset, Damien Treguez, Laura Adelon, Grégoire Huré and Marion Heckmann from the laboratory of preclinical investigation, who participated in the establishment of NSCLC PDXs. We also thank the Animal Platform of the Institut Curie. Finally, we thank Simon Lefranc from the CRB (“Centre de Recherche Biologique”) of the Institut Mutualiste Montsouris.

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

## References

1. Chen, X.; Mo, S.; Yi, B. The Spatiotemporal Dynamics of Lung Cancer: 30-Year Trends of Epidemiology across 204 Countries and Territories. *BMC Public Health* **2022**, *22*, 987. [[CrossRef](#)] [[PubMed](#)]
2. Hendriks, L.E.; Kerr, K.M.; Menis, J.; Mok, T.S.; Nestle, U.; Passaro, A.; Peters, S.; Planchard, D.; Smit, E.F.; Solomon, B.J.; et al. Electronic address: [Clinicalguidelines@esmo.org](mailto:Clinicalguidelines@esmo.org). Oncogene-Addicted Metastatic Non-Small-Cell Lung Cancer: ESMO Clinical Practice Guideline for Diagnosis, Treatment and Follow-Up. *Ann. Oncol.* **2023**, *34*, 339–357. [[CrossRef](#)] [[PubMed](#)]
3. Fukuoka, M.; Wu, Y.-L.; Thongprasert, S.; Sunpaweravong, P.; Leong, S.-S.; Sriuranpong, V.; Chao, T.-Y.; Nakagawa, K.; Chu, D.-T.; Saijo, N.; et al. Biomarker Analyses and Final Overall Survival Results from a Phase III, Randomized, Open-Label, First-Line Study of Gefitinib versus Carboplatin/Paclitaxel in Clinically Selected Patients with Advanced Non-Small-Cell Lung Cancer in Asia (IPASS). *J. Clin. Oncol.* **2011**, *29*, 2866–2874. [[CrossRef](#)]
4. Solomon, B.J.; Mok, T.; Kim, D.-W.; Wu, Y.-L.; Nakagawa, K.; Mekhail, T.; Felip, E.; Cappuzzo, F.; Paolini, J.; Usari, T.; et al. First-Line Crizotinib versus Chemotherapy in ALK-Positive Lung Cancer. *N. Engl. J. Med.* **2014**, *371*, 2167–2177. [[CrossRef](#)] [[PubMed](#)]
5. Cancer Genome Atlas Research Network. Comprehensive Genomic Characterization of Squamous Cell Lung Cancers. *Nature* **2012**, *489*, 519–525. [[CrossRef](#)] [[PubMed](#)]

6. Noronha, V.; Patil, V.M.; Joshi, A.; Menon, N.; Chougule, A.; Mahajan, A.; Janu, A.; Purandare, N.; Kumar, R.; More, S.; et al. Gefitinib Versus Gefitinib Plus Pemetrexed and Carboplatin Chemotherapy in EGFR-Mutated Lung Cancer. *J. Clin. Oncol.* **2020**, *38*, 124–136. [[CrossRef](#)] [[PubMed](#)]
7. Planchard, D.; Jänne, P.A.; Cheng, Y.; Yang, J.C.-H.; Yanagitani, N.; Kim, S.-W.; Sugawara, S.; Yu, Y.; Fan, Y.; Geater, S.L.; et al. Osimertinib with or without Chemotherapy in EGFR-Mutated Advanced NSCLC. *N. Engl. J. Med.* **2023**, *389*, 1935–1948. [[CrossRef](#)] [[PubMed](#)]
8. Miller, I.S.; Khan, S.; Shiels, L.P.; Das, S.; O' Farrell, A.C.; Connor, K.; Lafferty, A.; Moran, B.; Isella, C.; Loadman, P.; et al. Implementing Subtype-Specific Pre-Clinical Models of Breast Cancer to Study Pre-Treatment Aspirin Effects. *Cancer Med.* **2022**, *11*, 3820–3836. [[CrossRef](#)] [[PubMed](#)]
9. Reyat, F.; Guyader, C.; Decraene, C.; Lucchesi, C.; Auger, N.; Assayag, F.; De Plater, L.; Gentien, D.; Poupon, M.-F.; Cottu, P.; et al. Molecular Profiling of Patient-Derived Breast Cancer Xenografts. *Breast Cancer Res.* **2012**, *14*, R11. [[CrossRef](#)] [[PubMed](#)]
10. Izumchenko, E.; Paz, K.; Ciznadija, D.; Sloma, I.; Katz, A.; Vasquez-Dunddel, D.; Ben-Zvi, I.; Stebbing, J.; McGuire, W.; Harris, W.; et al. Patient-Derived Xenografts Effectively Capture Responses to Oncology Therapy in a Heterogeneous Cohort of Patients with Solid Tumors. *Ann. Oncol.* **2017**, *28*, 2595–2605. [[CrossRef](#)] [[PubMed](#)]
11. Némati, F.; Sastre-Garau, X.; Laurent, C.; Couturier, J.; Mariani, P.; Desjardins, L.; Piperno-Neumann, S.; Lantz, O.; Asselain, B.; Plancher, C.; et al. Establishment and Characterization of a Panel of Human Uveal Melanoma Xenografts Derived from Primary and/or Metastatic Tumors. *Clin. Cancer Res.* **2010**, *16*, 2352–2362. [[CrossRef](#)] [[PubMed](#)]
12. Passeri, T.; Dahmani, A.; Masliah-Planchon, J.; El Botty, R.; Courtois, L.; Vacher, S.; Marangoni, E.; Némati, F.; Roman-Roman, S.; Adle-Biassette, H.; et al. In Vivo Efficacy Assessment of the CDK4/6 Inhibitor Palbociclib and the PLK1 Inhibitor Volasertib in Human Chordoma Xenografts. *Front. Oncol.* **2022**, *12*, 960720. [[CrossRef](#)] [[PubMed](#)]
13. Montaudon, E.; El Botty, R.; Vacher, S.; Déas, O.; Naguez, A.; Chateau-Joubert, S.; Treguer, D.; de Plater, L.; Zemoura, L.; Némati, F.; et al. High In Vitro and In Vivo Synergistic Activity between mTORC1 and PLK1 Inhibition in Adenocarcinoma NSCLC. *Oncotarget* **2021**, *12*, 859–872. [[CrossRef](#)] [[PubMed](#)]
14. Marangoni, E.; Laurent, C.; Coussy, F.; El-Botty, R.; Château-Joubert, S.; Servely, J.-L.; de Plater, L.; Assayag, F.; Dahmani, A.; Montaudon, E.; et al. Capecitabine Efficacy Is Correlated with TYMP and RB1 Expression in PDX Established from Triple-Negative Breast Cancers. *Clin. Cancer Res.* **2018**, *24*, 2605–2615. [[CrossRef](#)] [[PubMed](#)]
15. Hosomi, Y.; Morita, S.; Sugawara, S.; Kato, T.; Fukuhara, T.; Gemma, A.; Takahashi, K.; Fujita, Y.; Harada, T.; Minato, K.; et al. Gefitinib Alone Versus Gefitinib Plus Chemotherapy for Non-Small-Cell Lung Cancer with Mutated Epidermal Growth Factor Receptor: NEJ009 Study. *J. Clin. Oncol.* **2020**, *38*, 115–123. [[CrossRef](#)] [[PubMed](#)]
16. Byrne, A.T.; Alférez, D.G.; Amant, F.; Annibaldi, D.; Arribas, J.; Biankin, A.V.; Bruna, A.; Budinská, E.; Caldas, C.; Chang, D.K.; et al. Interrogating Open Issues in Cancer Precision Medicine with Patient-Derived Xenografts. *Nat. Rev. Cancer* **2017**, *17*, 254–268. [[CrossRef](#)] [[PubMed](#)]
17. Passeri, T.; Dahmani, A.; Masliah-Planchon, J.; Naguez, A.; Michou, M.; El Botty, R.; Vacher, S.; Bouarich, R.; Nicolas, A.; Polivka, M.; et al. Dramatic In Vivo Efficacy of the EZH2-Inhibitor Tazemetostat in PBRM1-Mutated Human Chordoma Xenograft. *Cancers* **2022**, *14*, 1486. [[CrossRef](#)] [[PubMed](#)]
18. Savage, P.; Pacis, A.; Kuasne, H.; Liu, L.; Lai, D.; Wan, A.; Dankner, M.; Martinez, C.; Muñoz-Ramos, V.; Pilon, V.; et al. Chemogenomic Profiling of Breast Cancer Patient-Derived Xenografts Reveals Targetable Vulnerabilities for Difficult-to-Treat Tumors. *Commun. Biol.* **2020**, *3*, 310. [[CrossRef](#)] [[PubMed](#)]
19. Nilsson, L.M.; Vilhav, C.; Karlsson, J.W.; Fagman, J.B.; Giglio, D.; Engström, C.E.; Naredi, P.; Nilsson, J.A. Genetics and Therapeutic Responses to Tumor-Infiltrating Lymphocyte Therapy of Pancreatic Cancer Patient-Derived Xenograft Models. *Gastro Hep Adv.* **2022**, *1*, 1037–1048. [[CrossRef](#)]
20. Hayama, S.; Nakamura, R.; Ishige, T.; Sangai, T.; Sakakibara, M.; Fujimoto, H.; Ishigami, E.; Masuda, T.; Nakagawa, A.; Teranaka, R.; et al. The Impact of PIK3CA Mutations and PTEN Expression on the Effect of Neoadjuvant Therapy for Postmenopausal Luminal Breast Cancer Patients. *BMC Cancer* **2023**, *23*, 384. [[CrossRef](#)] [[PubMed](#)]
21. da Costa, A.A.B.A.; D'Almeida Costa, F.; Ribeiro, A.R.; Guimarães, A.P.; Chinen, L.T.; Lopes, C.A.P.; de Lima, V.C.C. Low PTEN Expression Is Associated with Worse Overall Survival in Head and Neck Squamous Cell Carcinoma Patients Treated with Chemotherapy and Cetuximab. *Int. J. Clin. Oncol.* **2015**, *20*, 282–289. [[CrossRef](#)]
22. Tsuboi, M.; Herbst, R.S.; John, T.; Kato, T.; Majem, M.; Grohé, C.; Wang, J.; Goldman, J.W.; Lu, S.; Su, W.-C.; et al. Overall Survival with Osimertinib in Resected EGFR-Mutated NSCLC. *N. Engl. J. Med.* **2023**, *389*, 137–147. [[CrossRef](#)] [[PubMed](#)]
23. Jänne, P.A.; Shaw, A.T.; Pereira, J.R.; Jeannin, G.; Vansteenkiste, J.; Barrios, C.; Franke, F.A.; Grinsted, L.; Zazulina, V.; Smith, P.; et al. Selumetinib plus Docetaxel for KRAS-Mutant Advanced Non-Small-Cell Lung Cancer: A Randomised, Multicentre, Placebo-Controlled, Phase 2 Study. *Lancet Oncol.* **2013**, *14*, 38–47. [[CrossRef](#)] [[PubMed](#)]
24. Spigel, D.R.; Shipley, D.L.; Waterhouse, D.M.; Jones, S.F.; Ward, P.J.; Shih, K.C.; Hemphill, B.; McCleod, M.; Whorf, R.C.; Page, R.D.; et al. A Randomized, Double-Blinded, Phase II Trial of Carboplatin and Pemetrexed with or without Apatorsen (OGX-427) in Patients with Previously Untreated Stage IV Non-Squamous-Non-Small-Cell Lung Cancer: The SPRUCE Trial. *Oncologist* **2019**, *24*, e1409–e1416. [[CrossRef](#)] [[PubMed](#)]
25. Vansteenkiste, J.; Ramlau, R.; von Pawel, J.; San Antonio, B.; Eschbach, C.; Szczesna, A.; Kennedy, L.; Visseren-Grul, C.; Chouaki, N.; Reck, M. A Phase II Randomized Study of Cisplatin-Pemetrexed plus Either Enzastaurin or Placebo in Chemonaive Patients with Advanced Non-Small Cell Lung Cancer. *Oncology* **2012**, *82*, 25–29. [[CrossRef](#)] [[PubMed](#)]

26. Williamson, S.K.; Crowley, J.J.; Lara, P.N.; McCoy, J.; Lau, D.H.M.; Tucker, R.W.; Mills, G.M.; Gandara, D.R.; Southwest Oncology Group Trial S0003. Phase III Trial of Paclitaxel plus Carboplatin with or without Tirapazamine in Advanced Non-Small-Cell Lung Cancer: Southwest Oncology Group Trial S0003. *J. Clin. Oncol.* **2005**, *23*, 9097–9104. [[CrossRef](#)]
27. Wang, Y.; Wang, L.; Liu, Y.; Yu, S.; Zhang, X.; Shi, Y.; Sun, Y. Randomize trial of cisplatin plus gemcitabine with either sorafenib or placebo as first-line therapy for non-small cell lung cancer. *Zhongguo Fei Ai Za Zhi* **2011**, *14*, 239–244. [[CrossRef](#)] [[PubMed](#)]
28. Paz-Ares, L.G.; Biesma, B.; Heigener, D.; von Pawel, J.; Eisen, T.; Bennouna, J.; Zhang, L.; Liao, M.; Sun, Y.; Gans, S.; et al. Phase III, Randomized, Double-Blind, Placebo-Controlled Trial of Gemcitabine/Cisplatin Alone or with Sorafenib for the First-Line Treatment of Advanced, Nonsquamous Non-Small-Cell Lung Cancer. *J. Clin. Oncol.* **2012**, *30*, 3084–3092. [[CrossRef](#)]
29. Reck, M.; von Pawel, J.; Zatloukal, P.; Ramlau, R.; Gorbounova, V.; Hirsh, V.; Leighl, N.; Mezger, J.; Archer, V.; Moore, N.; et al. Phase III Trial of Cisplatin plus Gemcitabine with Either Placebo or Bevacizumab as First-Line Therapy for Nonsquamous Non-Small-Cell Lung Cancer: AVAIL. *J. Clin. Oncol.* **2009**, *27*, 1227–1234. [[CrossRef](#)]
30. Heist, R.S.; Wang, X.; Hodgson, L.; Otterson, G.A.; Stinchcombe, T.E.; Gandhi, L.; Villalona-Calero, M.A.; Watson, P.; Vokes, E.E.; Socinski, M.A. A Randomized Phase II Study to Assess the Efficacy of Pemetrexed or Sunitinib or Pemetrexed Plus Sunitinib in the Second-Line Treatment of Advanced Non-Small-Cell Lung Cancer. *J. Thorac. Oncol.* **2014**, *9*, 214–221. [[CrossRef](#)]
31. Csiki, I.; Morrow, J.D.; Sandler, A.; Shyr, Y.; Oates, J.; Williams, M.K.; Dang, T.; Carbone, D.P.; Johnson, D.H. Targeting Cyclooxygenase-2 in Recurrent Non-Small Cell Lung Cancer: A Phase II Trial of Celecoxib and Docetaxel. *Clin. Cancer Res.* **2005**, *11*, 6634–6640. [[CrossRef](#)] [[PubMed](#)]
32. Tang, J.; Li, X.Y.; Liang, J.B.; Wu, D.; Peng, L.; Li, X. Apatinib Plus Chemotherapy Shows Clinical Activity in Advanced NSCLC: A Retrospective Study. *Oncol. Res.* **2019**, *27*, 635–641. [[CrossRef](#)] [[PubMed](#)]
33. Jiang, Q.; Zhang, N.-L.; Ma, D.-Y.; Tan, B.-X.; Hu, X.; Fang, X.-D. Efficacy and Safety of Apatinib plus Docetaxel as the Second or above Line Treatment in Advanced Nonsquamous NSCLC. *Medicine* **2019**, *98*, e16065. [[CrossRef](#)] [[PubMed](#)]
34. Noor, Z.S.; Goldman, J.W.; Lawler, W.E.; Telivala, B.; Braiteh, F.; DiCarlo, B.A.; Kennedy, K.; Adams, B.; Wang, X.; Jones, B.; et al. Luminespib plus Pemetrexed in Patients with Non-Squamous Non-Small Cell Lung Cancer. *Lung Cancer* **2019**, *135*, 104–109. [[CrossRef](#)] [[PubMed](#)]
35. Verma, S.; Chitikela, S.; Singh, V.; Khurana, S.; Pushpam, D.; Jain, D.; Kumar, S.; Gupta, Y.; Malik, P.S. A Phase II Study of Metformin plus Pemetrexed and Carboplatin in Patients with Non-Squamous Non-Small Cell Lung Cancer (METALUNG). *Med. Oncol.* **2023**, *40*, 192. [[CrossRef](#)]
36. Hayashi, T.; Desmeules, P.; Smith, R.S.; Drilon, A.; Somwar, R.; Ladanyi, M. RASA1 and NF1 Are Preferentially Co-Mutated and Define A Distinct Genetic Subset of Smoking-Associated Non-Small Cell Lung Carcinomas Sensitive to MEK Inhibition. *Clin. Cancer Res.* **2018**, *24*, 1436–1447. [[CrossRef](#)] [[PubMed](#)]
37. Blumenschein, G.R.; Smit, E.F.; Planchard, D.; Kim, D.-W.; Cadranet, J.; De Pas, T.; Dunphy, F.; Udud, K.; Ahn, M.-J.; Hanna, N.H.; et al. A Randomized Phase II Study of the MEK1/MEK2 Inhibitor Trametinib (GSK1120212) Compared with Docetaxel in KRAS-Mutant Advanced Non-Small-Cell Lung Cancer (NSCLC). *Ann. Oncol.* **2015**, *26*, 894–901. [[CrossRef](#)] [[PubMed](#)]
38. Infante, J.R.; Fecher, L.A.; Falchook, G.S.; Nallapareddy, S.; Gordon, M.S.; Becerra, C.; DeMarini, D.J.; Cox, D.S.; Xu, Y.; Morris, S.R.; et al. Safety, Pharmacokinetic, Pharmacodynamic, and Efficacy Data for the Oral MEK Inhibitor Trametinib: A Phase 1 Dose-Escalation Trial. *Lancet Oncol.* **2012**, *13*, 773–781. [[CrossRef](#)] [[PubMed](#)]
39. Gioeli, D.; Wunderlich, W.; Sebolt-Leopold, J.; Bekiranov, S.; Wulfkuhle, J.D.; Petricoin, E.F.; Conaway, M.; Weber, M.J. Compensatory Pathways Induced by MEK Inhibition Are Effective Drug Targets for Combination Therapy against Castration-Resistant Prostate Cancer. *Mol. Cancer Ther.* **2011**, *10*, 1581–1590. [[CrossRef](#)] [[PubMed](#)]
40. Chan-Seng-Yue, M.; Kim, J.C.; Wilson, G.W.; Ng, K.; Figueroa, E.F.; O’Kane, G.M.; Connor, A.A.; Denroche, R.E.; Grant, R.C.; McLeod, J.; et al. Transcription Phenotypes of Pancreatic Cancer Are Driven by Genomic Events during Tumor Evolution. *Nat. Genet.* **2020**, *52*, 231–240. [[CrossRef](#)] [[PubMed](#)]
41. Kerr, E.; Gaude, E.; Turrell, F.; Frezza, C.; Martins, C.P. Mutant Kras Copy Number Defines Metabolic Reprogramming and Therapeutic Susceptibilities. *Nature* **2016**, *531*, 110–113. [[CrossRef](#)]

**Disclaimer/Publisher’s Note:** The statements, opinions and data contained in all publications are solely those of the individual author(s) and contributor(s) and not of MDPI and/or the editor(s). MDPI and/or the editor(s) disclaim responsibility for any injury to people or property resulting from any ideas, methods, instructions or products referred to in the content.