Identification of Immuno-Targeted Combination Therapies Using Explanatory Subgroup Discovery for Cancer Patients with EGFR Wild-Type Gene

Olha Kholod ¹, William Basket ¹, Danlu Liu ², Jonathan Mitchem ¹,³,⁴, Jussuf Kaifi ¹,³, Laura Dooley ⁵ and Chi-Ren Shyu ¹,²,*,†

¹ MU Institute for Data Science and Informatics, University of Missouri, Columbia, MO 65212, USA
² Department of Electrical Engineering & Computer Science, University of Missouri, Columbia, MO 65212, USA
³ Department of Surgery, School of Medicine, University of Missouri, Columbia, MO 65212, USA
⁴ Harry S. Truman Memorial Veterans’ Hospital, Columbia, MO 65201, USA
⁵ Department of Otolaryngology, School of Medicine, University of Missouri, Columbia, MO 65212, USA
* Correspondence: shyuc@missouri.edu

Simple Summary: Cancer immunotherapy is a form of cancer treatment that uses a person’s own immune system to prevent, control, and eliminate cancer. However, immunotherapy alone may not be effective, especially in patients with limited treatment options. Immuno-targeted combination therapies have a potential to create synergetic effects with improved health outcomes. Therefore, there is a growing interest in searching for therapeutic combinations that could extend the benefits of immunotherapy. In this study, we designed a computational method that facilitated the identification of effective combination therapies for cancer patients with few treatment options. We determined several specific drug targets that substantially increased the odds of stable disease versus progressive disease for head and neck cancer, lung cancer, and melanoma. The identified treatment combinations were targets in several clinical trials. Moreover, our approach has the potential to improve the selection of patients for immuno-targeted combination therapies and lead to an overall improvement in health outcomes for cancer patients with limited treatment options.

Abstract: (1) Background: Phenotypic and genotypic heterogeneity are characteristic features of cancer patients. To tackle patients’ heterogeneity, immune checkpoint inhibitors (ICIs) represent some the most promising therapeutic approaches. However, approximately 50% of cancer patients that are eligible for treatment with ICIs do not respond well, especially patients with no targetable mutations. Over the years, multiple patient stratification techniques have been developed to identify homogenous patient subgroups, although matching a patient subgroup to a treatment option that can improve patients’ health outcomes remains a challenging task. (2) Methods: We extended our Subgroup Discovery algorithm to identify patient subpopulations that could potentially benefit from immuno-targeted combination therapies in four cancer types: head and neck squamous carcinoma (HNSC), lung adenocarcinoma (LUAD), lung squamous carcinoma (LUSC), and skin cutaneous melanoma (SKCM). We employed the proportional odds model to identify significant drug targets and the corresponding compounds that increased the likelihood of having stable disease versus progressive disease in cancer patients with the EGFR wild-type (WT) gene. (3) Results: Our pipeline identified six significant drug targets and thirteen specific compounds for cancer patients with the EGFR WT gene. Three out of six drug targets—FCGR2B, IGF1R, and KIT—substantially increased the odds of having stable disease versus progressive disease. Progression-free survival (PFS) of more than 6 months was a common feature among the investigated subgroups. (4) Conclusions: Our approach could help to better select responders for immuno-targeted combination therapies and improve health outcomes for cancer patients with no targetable mutations.

Keywords: immuno-targeted combination therapies; subgroup discovery; cancer
1. Introduction

Immunotherapy represents one of the most promising therapeutic approaches in cancer [1,2]. Immune checkpoint inhibitors (ICIs) can lead to long-term remission and improved survival in patients with locally advanced/metastatic cancer [3,4]. However, almost 50% of cancer patients that are eligible for treatment with ICIs do not respond [5,6]. This is particularly true in patients without targetable mutations [7]. Conventional cancer treatments, such as radiation therapy [8], cytotoxic chemotherapy [9,10], and targeted therapy [11,12], have immunomodulatory effects along with direct tumor-cell-killing activities. Their clinical utility in combination with ICIs potentially creates synergistic effects with improved and durable clinical response [13,14]. Therefore, there is a growing interest in searching for predictive biomarkers of therapeutic response and identifying therapeutic targets that could extend the benefits of ICIs.

Recent studies have shown that various factors contribute to the response to ICIs [15]. The tumor mutational burden (TMB) has emerged as one of the most crucial factors to determine the efficacy of ICIs [16,17]. The TMB quantitatively assesses the number of mutations per megabase (muts/Mb). High TMB values are an indication of better survival in cancer patients receiving immunotherapy [18]. However, the effectiveness of immunotherapy has been demonstrated in some patients with a low TMB, while unfavorable outcomes have been observed in a significant number of patients with a high TMB [19,20]. It is also unclear how patients that harbor specific genetic alterations would respond to immunotherapy. For example, anti-PD-1 monotherapy has been shown to be unable to improve survival outcomes for non-small cell lung cancer (NSCLC) patients with EGFR mutations, even in patients with high PD-L1 expression [21]. Meanwhile, the ATLANTIC trial has disputed this observation and has highlighted the benefits of ICIs for EGFR-mutated tumors [22]. Therefore, the question of how to effectively utilize thousands of cell-intrinsic and -extrinsic characteristics to identify patient subpopulations that would benefit from combinatorial treatments with ICIs remains unanswered.

Over the past decades, Subgroup Discovery (SD) methods have been used to find homogeneous subpopulations of patients that share common genetic profiles and may respond similarly to therapeutic regimens [23,24]. Existing SD approaches can be classified into two major categories: statistical methods and data-mining methods. Statistical methods include regression analysis [25], clustering techniques [26], and latent class analysis (LCA) [27]. For example, LCA is a finite mixture model that aims to uncover unobserved groups within a population. However, this technique does not automatically determine the number of latent classes and produces solutions that heavily rely upon expert knowledge, which significantly limits the capability to discover novel subgroups in large heterogenous cancer datasets. Data-mining methods for SD comprise two major categories based on the search strategy for potential candidates—heuristic approaches and exhaustive approaches [28]. For example, CN2-SD [29] is a heuristic algorithm that searches for the statistically “most interesting” subgroups that are as large as possible and have the most unusual distributional characteristics with respect to the property of interest. However, CN2-SD suffers from the standard scaling problem that appears in the evaluation of large datasets, including heterogenous cancer datasets.

In this work, we extended our Subgroup Discovery algorithm [30] to predict immuno-targeted combination therapies for EGFR WT patient subpopulations. We focused on EGFR WT subgroups because EGFR-TKIs are widely used for the first-line treatment of patients with EGFR-sensitizing mutations, leading to longer progression-free survival (PFS) [31,32]. However, beyond the first line, especially for EGFR WT tumors, the role of EGFR-TKIs is elusive. We employed a proportional odds model to identify significant drug targets and the corresponding compounds that increased the likelihood of stable disease versus progressive disease in cancer patients that had the EGFR WT gene. This approach could help to better select responders for immuno-targeted combination therapies and improve health outcomes for cancer patients with no targetable mutations.
2. Materials and Methods

Our informatic framework consists of two modules: Subgroup Discovery and Immuno-Targeted Combination Therapies Discovery. The Subgroup Discovery module identifies homogenous patient subgroups based on both phenotypic and genotypic parameters and explains the differences among these subgroups using gene expression patterns. The Immuno-Targeted Combination Therapies Discovery module predicts potential drug targets and the corresponding compounds for uses in combination therapies with immunotherapy for cancer patients with no targetable mutations. The details of the modules are described below.

2.1. Data Mapping

The TCGA dataset from Pan Cancer Atlas [33] consists of 1952 cancer patients. We focused on four different cancer types: (1) head and neck squamous carcinoma (HNSC), \( n = 515 \); (2) lung adenocarcinoma (LUAD), \( n = 510 \); (3) lung squamous carcinoma (LUSC), \( n = 484 \); and (4) skin cutaneous melanoma (SKCM), \( n = 443 \). We chose these cancer types because in several clinical trials involving patients with advanced lung cancer, skin cutaneous melanoma, and other solid tumors, they demonstrated significant response rates to nivolumab and pembrolizumab, where both are monoclonal IgG4 antibodies against PD-1 [34].

The input data consisted of phenotypic and genotypic variables for a disease population. The phenotypic variables divided disease population into subgroups, while the genotypic variables described the main characteristics (patterns) of these subgroups. Eleven phenotypic variables were chosen for this study, including demographic data (e.g., Age, Gender), clinical–pathologic data (e.g., Tumor Type, Tumor Mutational Burden (TMB)), treatment history (e.g., Administration of Radiotherapy), and behavioral data (e.g., Smoking Status). As a part of the human-in-the-loop process, a physician panel specialized in the care of cancer patients selected the phenotypic variables to be included in the analyses. Many of these phenotypic variables were categorical. For example, Smoking Status was categorized as follows: (1) Never Smoker, (2) Current Smoker, and (3) Former Smoker. We ensured all continuous variables in the dataset were categorized based on the clinical literature or experience. To deal with missing data, we excluded patients with too many missing values in both phenotypic and genotypic variables and used “NA” as a new category to represent missing values. However, “NA” variables were never used to form subgroups.

The genotypic data in this study were z-score-transformed values for 730 immune-related genes (including PD-1 and PD-L1 genes) [35] and 40 housekeeping genes between normal and tumor tissues. The categorization of the genotypic variables was based on z-score-transformed values, where downregulated genes corresponded to the range \((-\infty, -2)\); not significantly expressed genes corresponded to the range \((-2, 2)\); and upregulated genes corresponded to the range \((2, \infty)\). Therefore, each gene represented a genotypic variable.

2.2. Subgroup Discovery

2.2.1. Patient Stratification

The main goal of the Subgroup Discovery module is to identify homogeneous patient subpopulations. A set of common phenotypic and genotypic parameters specifies each unique subgroup. For example, Females with a TMB defined as high (more than 10 muts/Mb) with SKCM could be considered as a subgroup. The unique subgroup is reevaluated each time a phenotypic variable is included. The statistical significance of each subgroup is defined using the genotypic patterns that distinguish this subgroup from the rest of the outer population.

The Subgroup Discovery module includes three levels: Path Expansion, Floating Subgroup Selection, and Inclusion/Exclusion criteria. This method differs from the decision tree algorithm in a way that allows the same patient to be a member of multiple unique subgroups. For example, LUSC patients could be members of the (LUSC, Former Smoker)
subgroup and the (LUSC, Stage IIIA) subgroup. The key objective of the subgroup stratification process is to determine a large number of existing subgroups based on phenotypic parameters, where most patients in that subgroup shared unique genotypic patterns. This method is not greedy, because the algorithm tracks the top potential subgroups based on local optimal selection [36].

By iterating over various paths in the search space, the Floating Subgroup Selection traverses multiple paths. A phenotypic variable with the highest contrast score against the outer population (e.g., Tumor Type = LUAD in Figure 1) forms a base subgroup via the Path Expansion process. In the first inclusion step, it adds a new variable, e.g., EGFR = WT, to the base subgroup (Tumor Type = LUAD AND EGFR = WT). To eliminate less significant inclusion steps, the exclusion function is used after each inclusion step. For example, when in the third inclusion step, the subgroup is (Tumor Type = LUAD AND EGFR = WT AND Smoker = Former AND Gender = Female), the exclusion function eliminates the less significant move (Smoker = Former) from the existing subgroup if the newly created subgroup (Tumor Type = LUAD AND EGFR = WT AND Gender = Female) has a higher contrast score. When the algorithm reaches the subgroup with the highest contrast score, the exploratory search is terminated.

![Subgroup Discovery module](image)

**Figure 1.** Subgroup Discovery module. The floating and path expansion processes initiate multiple, distinct starting points at various computational nodes. Based on the contrast score, the node is added or eliminated at each point. Each point portrays a potential subgroup. By applying contrast pattern mining, each candidate subgroup is scored against the outer population. \( P \) refers to phenotypic feature.

### 2.2.2. Subgroup Contrast

The algorithm identifies the genotypic patterns that frequently occur within each candidate subgroup but are rare in the rest of the population. To evaluate the frequency for a specific genotypic pattern in the homogenous subgroup, the support metric [37]
(Equation (1)) is utilized. The growth rate metric \[38\] (Equation (2)) is employed to evaluate the contrast of the pattern in the selected subgroup.

\[
\text{Support}(p, D) = \frac{|\langle D, p \rangle|}{|D|}
\] (1)

where \( p \) is a pattern, \(|\langle D, p \rangle|\) is the number of patients with specific genotypic pattern, and \(|D|\) is the total number of patients in the collection.

\[
\text{Growth}(cp, S_{G1}, S_{G2}) = \frac{\text{Max}|s_1, s_2|}{\text{Min}|s_1, s_2|}
\] (2)

where \( cp \) is the contrast pattern, \( S_{G1} \) is the focus subgroup, \( S_{G2} \) is the outer population, \( s_1 \) is the support of \( cp \) in the focus subgroup, and \( s_2 \) is the support of \( cp \) in the outer population.

We employ a \( J \)-value \[39\] (Equation (3)) to calculate the contrast for all patterns in each subgroup.

\[
J\text{-value} = \frac{T \times J_{\text{org}} + M \times J_{\text{avr}}}{T + M}
\] (3)

where \( T \) is a parameter related to the population size preference that depends on the type of the disease under study and whether it is a rare disease or not, based on the concept of the Bayesian average \[40\].

There are six major parameters for the Subgroup Discovery module: \text{min\_support\_proportion}, \text{max\_depth}, \text{max\_breadth}, \text{min\_improvement\_significance}, \text{max\_checks} and \text{max\_pop\_complexity}. The \text{min\_support\_proportion} parameter describes the minimum proportion of the subgroup rows that a pattern must be present in and was set to 0.05. The \text{max\_depth} parameter represents the maximum pattern length and was adjusted to five. The \text{max\_breadth} stands for the maximum number of candidates at each search level and was modified to 50. The \text{min\_improvement\_significance} reflects the \( p \)-value needed to add any new element to a pattern and was set to 0.05. The \text{max\_checks} parameter describes a hard cap on the max number of patterns checked and was adjusted to 1000. Finally, the \text{max\_pop\_complexity} describes the maximum number of phenotypic variables that can define a subgroup and was modified to three.

### 2.3. Immuno-Targeted Combination Therapies Discovery

Our goal was to find significant drug targets and their corresponding compounds that could be used in immuno-targeted combination therapies for cancer patients with no targetable mutations. Specifically, we were interested in identifying significant drug targets that increased the likelihood of stable disease versus progressive disease in four cancer types: HNSC, LUAD, LUSC, and SKCM. For this purpose, we used the proportional odds model \[41\] on Prat, A., et al.’s dataset \[42\]. The dataset consists of 65 cancer patients, and 15 phenotypic and 770 genotypic variables. The outcome variable was categorical and had four levels: partial response, complete response, progressive disease, and stable disease. The continuous covariates were represented as genes with corresponding normalized gene expression values. We used the \text{MASS R package} \[43\] to fit the proportional odds model with the logit link function. We computed \( p \)-values via two-tailed \( z \)-tests to identify significant predictors of response to immunotherapy.

### 3. Results

#### 3.1. Identification of Candidate Subgroups for Immuno-Targeted Combination Therapies

The overview of our informatic pipeline is presented in Figure 2. The Subgroup Discovery module generated 9887 subgroups. To reduce the search space, we focused on subgroups that had Tumor Type (HNSC, LUAD, LUSC, or SKCM) as one of the phenotypic variables. The filtering procedure resulted in 1207 subgroups. To further focus on more specific subgroups, we selected those that had at least three phenotypic variables. In total, 1129 subgroups were used in our computational experiment.
3. Results

3.1. Identification of Candidate Subgroups for Immuno-Targeted Combination Therapies

The overview of our informatic pipeline is presented in Figure 2. The Subgroup Discovery module generated 9887 subgroups. To reduce the search space, we focused on subgroups that had Tumor Type (HNSC, LUAD, LUSC, or SKCM) as one of the phenotypic variables. The filtering procedure resulted in 1207 subgroups. To further focus on more specific subgroups, we selected those that had at least three phenotypic variables. In total, 1129 subgroups were used in our computational experiment.

Figure 2. Informatic pipeline. ① We ran the Subgroup Discovery algorithm on the combined TCGA dataset. The algorithm output 9887 subgroups. ② We retained EGFR WT subgroups that covered at least 20% of the initial dataset. ③ For these subgroups, we determined common DE genes ($n = 380$). ④ We then mapped these 380 genes to the list of 155 drug targets. Thus, we identified 25 targets that could be used in immuno-targeted combination therapies. ⑤ We determined significant drug targets that increased the likelihood of SD versus PD using the proportional odds model. ⑥ We matched significant genes to drugs that could be used in immuno-targeted combination therapies using the Drug Gene Interaction Database (DGIdb).

First, we investigated subgroup coverage in the whole dataset of 1948 cancer patients. The subgroup coverage ranged from 1.23% to 25.97%. Therefore, we decided to target subgroups or unions of subgroups that covered at least 20% of the whole dataset, which resulted in eleven subgroup unions. Secondly, we narrowed down our search to four subgroup unions that had both Tumor Type (HNSC, LUAD, LUSC, or SKCM) and the EGFR WT gene. We hypothesized that cancer patients from these four subgroup unions were unlikely to produce a favorable outcome upon treatment with EGFR inhibitors due to the lack of targetable mutations in this gene. However, these patients may have other mutations that can be sensitive to FDA-approved targeted treatments. Therefore, it is important to identify these additional drug targets to improve patients’ outcomes. In addition, immunotherapy alone may not produce durable responses for these patients. In the ongoing phase III KEYNOTE-042 trial of patients with treatment-naïve, advanced, EGFR/ALK WT NSCLC and at least 1% tumor PD-L1 expression, there was no statistically significant PFS benefit among patients receiving Pembrolizumab compared with those receiving chemotherapy, except for those with the highest level of PD-L1 expression [44]. However, EGFR WT cancer
patients may benefit from compounds that are used for immuno-targeted combination therapies, as was previously showed in EGFR WT NSCLC patients [45]. Thirdly, we identified common differentially expressed (DE) genes for these four subgroup unions. We started with identifying unique genotypic patterns for each subgroup union and then determined unique elements from these genotypic patterns. The intersection of unique genes from four subgroup unions produced 380 DE genes. The summary for each subgroup union is presented in Table 1.

Table 1. Subgroup summary for selected subgroup unions.

| Subgroup Union | # Patients | % of Patients with Cancer Type | % of Patients in Whole Dataset | # Subgroups of Size Three | # Unique Genotypic Patterns | # Unique DE Genes |
|----------------|------------|-------------------------------|-----------------------------|--------------------------|---------------------------|-----------------|
| HNSC           | 500        | 97.08                         | 25.66                       | 16                       | 8448                      | 693             |
| LUSC           | 466        | 96.68                         | 23.92                       | 16                       | 10,005                    | 652             |
| LUAD           | 444        | 87.06                         | 22.79                       | 20                       | 10,216                    | 656             |
| SKCM           | 407        | 92.29                         | 20.89                       | 15                       | 7428                      | 497             |

There were also some unique features for each subgroup union. Our analysis revealed that HNSC EGFR WT subgroups consisted of patients with a low TMB. The role of the TMB in predicting the outcome of immunotherapy for advanced HNSCC remains unclear. For example, one study showed that immunotherapy was more effective in metastatic HNSCC patients with a high TMB, and the median OS of these patients was 2.5 times as long as that of patients with a low TMB (25 vs. 10 months) [46]. However, data from over 10,000 patient tumors included in The Cancer Genome Atlas failed to support the application of a high TMB as a biomarker for treatment with ICIs in all solid cancer types [47]. The LUAD EGFR WT subgroups had a substantial number of patients with the BRAF WT gene. The clinical impact of BRAF mutational subtypes on lung adenocarcinoma remains to be defined. For example, the data from two large German lung cancer centers showed that patients with BRAF-mutated NSCLC had an inferior prognosis, which was not determined by the BRAF mutation functional class. However, in contrast to NSCLC with other driver mutations, BRAF-mutated NSCLC exhibited high susceptibility to immune checkpoint inhibitors [48]. The LUSC union consisted of patients who were former smokers. For example, LUSC patients with a history of smoking and a high TMB had longer PFS after treatment with chemoinmunotherapy or anti-angiogenesis therapy [49]. There were no distinctive phenotypic features for the SKCM union. These identified unique features should be considered when designing combinatorial treatment regimens for EGFR WT cancer patients.

3.2. Drug Target Prediction for EGFR Wild-Type Subgroups

We examined the Drug Gene Interaction Database (DGIdb) [50] to map therapeutic compounds that could be used in immuno-targeted combination therapies. This search produced 155 potential drug targets and 36 targeted treatments. We then mapped the 380 common DE genes from the subgroup unions to the list of 155 drug targets. In this analysis, we identified 25 targets and 16 compounds that may be used in combination with ICIs.

The Immuno-Targeted Combination Therapies Discovery module identified six significant drug targets: CDH5, FCGR2B, IGF1R, ITK, JAK2, and KIT (Table S1). The estimates in the output were given in units of ordered logits or ordered log odds. For example, for the FCGR2B gene, the likelihood of stable disease versus partial response, complete response, and progressive disease increased by 9.70 on the log odds scale. We also calculated the odds ratios and confidence intervals (Table S2). These were obtained either by profiling the likelihood function or using the standard errors and assuming a normal distribution (Table 2). For example, for every unit increase in the expression of the KIT gene, the odds of having stable disease was multiplied by 3.57 times, with all other variables remaining constant.
Table 2. Odds ratios and confidence intervals for significant genes.

| Drug  | OR           | 2.5%          | 97.5%          | p-Value        |
|-------|--------------|---------------|---------------|---------------|
| CDH5  | $2.875372 \times 10^{-2}$ | $8.965756 \times 10^{-4}$ | $9.221493 \times 10^{-1}$ | 0.0448808290  |
| FCGR2B| $1.644836 \times 10^4$     | $8.151472 \times 10^0$     | $3.319015 \times 10^6$     | 0.0003368373  |
| IGF1R | $2.238631 \times 10^3$     | $1.110336 \times 10^0$     | $4.513470 \times 10^6$     | 0.0469308638  |
| ITK   | $1.274047 \times 10^{-1}$ | $2.807962 \times 10^{-2}$ | $5.780693 \times 10^{-1}$ | 0.0075795133  |
| JAK2  | $1.047600 \times 10^{-5}$ | $1.525653 \times 10^{-10}$ | $7.193414 \times 10^{-1}$ | 0.0435977859  |
| KIT   | $3.571475 \times 10^0$     | $1.543342 \times 10^0$     | $8.264815 \times 10^1$     | 0.0029426309  |

Therefore, these six drug targets were predicted to benefit EGFR WT patients in immuno-targeted combination therapies (Table 3).

Table 3. Significant genes and potential compounds that can be used in immuno-targeted combination therapies.

| Drug Target | Compound                                | Cancer Type                        |
|-------------|-----------------------------------------|------------------------------------|
| CDH5        | Ruxolitinib, Lenalidomide               | Lung Squamous Carcinoma, Skin Cutaneous Melanoma |
| FCGR2B      | Bevacizumab, Cetuximab, Trastuzumab     | Lung Adenocarcinoma, Head and Neck Squamous Carcinoma |
| IGF1R       | Gefitinib                               | Lung Adenocarcinoma                |
| ITK         | Pazopanib, Ibrutinib                    | Skin Cutaneous Melanoma            |
| JAK2        | Bortezomib                              | Lung Adenocarcinoma                |
| KIT         | Axitinib, Cabozantinib, Pazopanib, Sunitinib | Head and Neck Squamous Carcinoma    |

To address the question about the potential drug interaction effects and toxicity of identified combinations, we employed the Drug Interaction Checker from the Drug Bank database [51]. Based on our search, the administration of PD-1 or PD-L1 inhibitors in combination with FCGR2B-targeting therapies could increase the risk of adverse effects. Specifically, these therapeutic combinations carry a risk of immunogenicity, which can produce a wide array of adverse effects, the most serious of which include anaphylaxis and serum sickness-type reactions [52]. A few studies suggested that the use of multiple immunoglobulin agents is relatively safe and may be more effective than monotherapy under certain conditions [53,54].

From clinicaltrials.gov, we were able to identify clinical trials for predicted immuno-targeted combination treatments (Table 4).
Table 4. Clinical trials for predicted immuno-targeted combinations.

| Trial ID                  | Treatment Combination         | Condition                                | Results/Conclusions                                                                 | Reference |
|--------------------------|------------------------------|------------------------------------------|-------------------------------------------------------------------------------------|-----------|
| MC1534, NCT03012230      | Pembrolizumab and Ruxolitinib | Stage IV triple negative breast cancer   | Estimated primary completion date: 1 April 2023.                                     | [55]      |
| BTCRC-HEM15-027, NCT03681561 | Nivolumab and Ruxolitinib | Relapsed or refractory classical Hodgkin lymphoma | Therapy combining Ruxolitinib with Nivolumab was well tolerated and yielded encouragingly high remission rates and durable responses in patients who had all failed with previous check-point inhibitors (CPIs). | [56]      |
| NCI-2020-08331, NCT04609046 | Nivolumab and Lenalidomide | Primary CNS lymphoma                     | Estimated primary completion date: 31 May 2024.                                     | [57]      |
| MK-3475-021/KEYNOTE-021, NCT02039674 | Pembrolizumab and Gefitinib | Non-small cell lung cancer               | First-line Pembrolizumab plus Pemetrexed-Carboplatin continued to show improved response and survival versus chemotherapy alone in advanced NSCLC, with durable clinical benefit in patients who completed 2 years of therapy. No new safety signals were observed with longer follow-up. | [58]      |
| MC1577, NCT03021460      | Pembrolizumab and Ibrutinib   | Stage III-IV melanoma                    | Estimated primary completion date: 1 February 2023.                                  | [59]      |
| OSU-18015, NCT03529925   | Nivolumab and Ibrutinib      | Metastatic solid tumors                  | Estimated primary completion date: 31 December 2021.                                 | [60]      |
| 020-008, NCT04265872     | Pembrolizumab and Bortezomib | Metastatic triple negative breast cancer  | Estimated Primary completion date: 1 October 2023.                                  | [61]      |
| PANDORA 001, NCT04995016 | Pembrolizumab and Axitinib   | Locally advanced non-metastatic clear cell renal cell carcinoma | Estimated primary completion date: 20 August 2022.                                   | [62]      |
| Winship4234-17, NCT03468218 | Pembrolizumab and Cabozantinib | Head and neck squamous cell cancer       | This phase II trial of Pembrolizumab + Cabozantinib met its primary endpoint of overall response rate (ORR). The regimen was well-tolerated, with very encouraging clinical activity in relapsed metastatic HNSCC, and warranted further exploration of this disease. | [63]      |
| CheckMate 016, NCT01472081 | Nivolumab, Pazopanib, and Sunitinib | Metastatic renal cell carcinoma       | The addition of standard doses of Sunitinib or Pazopanib to nivolumab resulted in a high incidence of high-grade toxicities limiting the future development of either combination regimen. | [64]      |
| 16-2300.cc, NCT03149822  | Pembrolizumab and Cabozantinib | Metastatic renal cell carcinoma         | This study of the combination of Pembrolizumab and Cabozantinib met the primary endpoint of ORR. Benefit was seen in first- and subsequent-line therapies. The safety profile was manageable. | [65]      |
Some of the clinical trials from Table 4 are still ongoing; therefore, they do not have information about primary and secondary outcomes. However, we were able to retrieve such information from other clinical trials, such as NCT02039674 and NCT01472081. In the NCT02039674 trial [58], Cohort F received Pembrolizumab (Pembro; 2 mg/kg) via IV infusion on Day 1 of each 3-week cycle plus Gefitinib (G; 250 mg) via oral tablets once a day every day of each 3-week cycle. Overall, seven participants were treated with this regiment, but none of these patients were able to complete the treatment due to death (n = 1), excluded medication (n = 4), or withdrawal from the study (n = 2).

In the NCT01472081 study [64], Arm S was treated with a combination of Nivolumab and Sunitinib, and Arm p was treated with a combination of Nivolumab and Pazopanib. Arm S received two different doses of Nivolumab: 2 mg/kg (SUN + NIV2; n = 7) and 5 mg/kg (SUN + NIV5; n = 26). Arm P received only 2 mg/kg dose of Nivolumab (PAZ + NIV2; n = 20). All-causality severe adverse effects (SAEs) of any grade were observed in 42% of the SUN + NIV2 cohort, 61% of the SUN + NIV5 cohort, and 65% of the PAZ + NIV2 cohort. Drug-related SAEs of any grade were observed in 28% of the SUN + NIV2 cohort, 46% of the SUN + NIV5 cohort, and 10% of the PAZ + NIV2 cohort. All-cause adverse effects (AEs) that led to the discontinuation of treatment were observed in 42% of the SUN + NIV2 cohort, 38% of the SUN + NIV5 cohort, and 25% of the PAZ + NIV2 cohort. These results suggested that a higher concentration of Nivolumab in combination with Sunitinib (SUN + NIV5 cohort) led to a higher chance of SAEs. By analyzing secondary responses, the SUN + NIV5 cohort achieved a lower rate of partial response to treatment, at 42%, in comparison with 71% in the SUN + NIV2 cohort. Therefore, lower concentrations of Nivolumab in combination with Sunitinib led not only to less adverse effects but also to better patient outcomes. Ideally, more trial settings are needed to study synergistic effect of two or more drugs. Unfortunately, we could not assess the synergistic effects of these treatments from the NCT01472081 study because there were no cohorts that solely underwent Nivolumab or Sunitinib treatment.

4. Discussion

One of the aims of this study was to find phenotypic commonalities among EGFR WT cancer patient subgroup unions that might be helpful in selecting responders for immunotherapeutic combination therapies. Unlike existing “black-box” models, our approach interpreted a combination of phenotypic characteristics by assessing the frequency of significant genomic patterns in the investigated subgroups. For this purpose, we examined the third phenotypic feature for each subgroup that was a part of the unions and covered at least 15% of the dataset. For all four unions—HNSC, LUAD, LUSC, and SKCM—progression-free survival (PFS) of more than 6 months was a common feature. Interestingly, in Impower150, clinical trial patients with EGFR WT metastatic lung adenocarcinoma had longer PFS upon combinatorial treatment with Atezolizumab (immunotherapy that targets PD-L1), Bevacizumab (targeted therapy against VEGF-A), and Paclitaxel and Carboplatin (both are chemotherapies) [66]. Finally, in the KEYNOTE-048 clinical trial, Pembrolizumab (immunotherapy that targets PD-L1) with chemotherapy improved overall survival versus Cetuximab (EGFR targeted therapy) with chemotherapy in patients with head and neck squamous cell carcinoma [67]. This demonstrated that combinatorial treatments with anti-PD-1 or anti-PD-L1 inhibitors with targeted therapy substantially prolonged the PFS of cancer patients with the EGFR WT gene.

The analysis of the dataset of Prat, A., et al. revealed that three out of six significant drug targets—FCGR2B, IGF1R, and KIT—substantially increased the odds of having stable disease versus progressive disease in EGFR WT cancer patients. The importance of this finding is further supported by the fact there is an ongoing clinical trial of BI-1206, a monoclonal antibody to FCGR2B, in combination with Rituximab (chemotherapy that targets CD20) in patients with indolent B-cell non-Hodgkin lymphoma that has relapsed or is refractory to Rituximab [68]. From another study, LUAD patients with high plasma levels of IGF-1 or high IGF-1R expression in tumors were associated with resistance to
anti-PD-1–programmed death-ligand 1 immunotherapy, which supported the need for the clinical evaluation of IGF-1 modulators in combination with a PD-1 blockade [69]. Finally, there is an ongoing clinical trial of Ipilimumab (immunotherapy that targets CTLA-4) and Imatinib Mesylate (KIT inhibitor) for treating patients with solid tumors that have spread to other places in the body or cannot be removed using surgery [70]. Therefore, these genes could be useful therapeutic targets for immuno-targeted combination therapies.

Based on our knowledge, there is no computational pipeline that can evaluate the synergetic effect from immuno-targeted combination therapies. Our evaluations were based on primary and secondary outcomes from existing clinical trials. The limitation of these trials was that they lacked the data on monotherapy effects. For example, the NCT01472081 trial evaluated the combinations of Nivolumab + Sunitinib and Nivolumab + Pazopanib. However, there was no information on treatment with Nivolumab, Sunitinib, or Pazopanib alone. Therefore, better clinical trial design is required to objectively evaluate the synergetic effect of immuno-targeted combination therapies.

5. Conclusions

Phenotypic and genotypic heterogeneity are characteristic features of cancer patients that limit therapeutic response. To tackle patient heterogeneity, immuno-targeted combination therapies represent a highly promising approach for patients with no targetable mutations [71]. However, matching patient subgroups to treatment options that improve their outcome remains a challenging task. In this work, we augmented our Subgroup Discovery algorithm to identify patient subpopulations that may benefit from immuno-targeted combination therapy. Specifically, we identified drug targets that increased the likelihood of stable versus progressive disease in cancer patients with HNSC, LUAD, LUSC, and SKCM. Our novel informatic pipeline identified six significant drug targets and thirteen specific compounds for EGFR WT cancer patients. Three out of six drug targets—FCGR2B, IGF1R, and KIT—were previously shown to substantially increase the odds of having a stable disease in other studies. We also showed that PFS of more than 6 months was a characteristic feature among the investigated EGFR WT subgroups. Moreover, the literature demonstrated that immuno-targeted combination therapies with anti-PD-1 or anti-PD-L1 inhibitors substantially prolonged PFS in EGFR WT cancer patients. Further validation of our findings in wet-lab experiments would be a significant step toward improving healthcare for cancer patients without targetable mutations.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/cancers14194759/s1, Table S1: Summary for the proportional odds model; Table S2: Odds ratios and confidence intervals for the proportional odds model.

Author Contributions: Conceptualization, O.K. and C.-R.S.; methodology, O.K. and D.L.; software, W.B.; validation, O.K. and C.-R.S.; formal analysis, O.K., W.B. and C.-R.S.; data curation, O.K.; writing—original draft preparation, O.K. and C.-R.S.; writing—review and editing, C.-R.S., J.M., J.K. and L.D.; visualization, O.K.; supervision, C.-R.S., J.M. and J.K.; project administration, C.-R.S.; funding acquisition, C.-R.S. All authors have read and agreed to the published version of the manuscript.

Funding: This research study was funded by University of Missouri Institute for Data Science and informatics Data-Driven and Artificial Intelligence Initiatives. O.K. and C.R.S. were funded by Shumaker Endowment for Bioinformatics. J.B.M. received funding from the Department of Veteran’s Affairs (K2BX004346-01A1). The content is solely the responsibility of the authors and does not necessarily represent the official views of the Department of Veterans’ Affairs. The funding bodies had no role in the study design; in the collection, analysis, interpretation of data; or in the writing of the manuscript.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.
Data Availability Statement: The TCGA dataset is available online at https://www.cbioportal.org (accessed on 9 February 2022). Prat, A., et al.’s dataset is available online at https://aacrjournals.org/cancers/articles/77/13/3540/619915/Immune-Related-Gene-Expression-ProFiling-After-PD (accessed on 9 February 2022).

Acknowledgments: We appreciate the critical input of members from Interdisciplinary Data Analytics and Search (IDAS) Laboratory at University of Missouri, Columbia.

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

References

1. Reck, M.; Rodríguez-Abreu, D.; Robinson, A.G.; Hui, R.; Csáoszi, 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] [PubMed]

2. Taefehshokr, N.; Baradaran, B.; Baghbanzadeh, A.; Taefehshokr, S. Promising approaches in cancer immunotherapy. *ImmunoBiology* 2020, 225, 151875. [CrossRef] [PubMed]

3. Gettinger, S.N.; Horn, L.; Gandhi, L.; Spigel, D.R.; Antonia, S.J.; Rizvi, N.A.; Powderly, J.D.; Heist, R.S.; Carvajal, R.D.; Jackman, D.M.; et al. Overall Survival and Long-Term Safety of Nivolumab (Anti-Programmed Death 1 Antibody, BMS-936558, ONO-4538) in Patients With Previously Treated Advanced Non-Small-Cell Lung Cancer. *J. Clin. Oncol.* 2015, 33, 2004–2012. [CrossRef] [PubMed]

4. Herbst, R.S.; Baas, P.; Kim, D.-W.; Felip, E.; Pérez-Gracia, J.L.; Han, J.-Y.; Molina, J.; Kim, J.-H.; Arvis, C.D.; Ahn, M.-J.; et al. Pembrolizumab versus docetaxel for previously treated, PD-L1-positive, advanced non-small-cell lung cancer (KEYNOTE-010): A randomised controlled trial. *Lancet Lond. Engl.* 2016, 387, 1540–1550. [CrossRef]

5. Blons, H.; Garinet, S.; Laurent-Puig, P.; Oudart, J.-B. Molecular markers and prediction of response to immunotherapy in non-small cell lung cancer, an update. *J. Thorac. Dis.* 2019, 11, S25–S36. [CrossRef] [PubMed]

6. Haslam, A.; Prasad, V. Estimation of the Percentage of US Patients With Cancer Who Are Eligible for and Respond to Checkpoint Inhibitor Immunotherapy Drugs. *JAMA Netw. Open* 2019, 2, e192535. [CrossRef]

7. Créquít, P.; Chaimani, A.; Yavchitz, A.; Attiche, N.; Cadranel, J.; Trinquant, L.; Ravaud, P. Comparative efficacy and safety of second-line treatments for advanced non-small cell lung cancer with wild-type or unknown status for epidermal growth factor receptor: A systematic review and network meta-analysis. *BMC Med.* 2017, 15, 193. [CrossRef]

8. Formenti, S.C.; Demaria, S. Systemic effects of local radiotherapy. *Lancet Oncol.* 2009, 10, 718–726. [CrossRef]

9. Coffelt, S.B.; de Visser, K.E. Immune-mediated mechanisms influencing the efficacy of anticancer therapies. *Trends Immunol.* 2015, 36, 198–216. [CrossRef]

10. Juan, Y.; Kraus, S.G.; Quaney, M.J.; Daniels, M.A.; Mitchem, J.B.; Teixeiro, E. FOLFOX Chemotherapy Ameliorates CD8 T Lymphocyte Exhaustion and Enhances Checkpoint Blockade Efficacy in Colorectal Cancer. *Front. Oncol.* 2020, 10, 586. [CrossRef]

11. Wilmott, J.S.; Long, G.V.; Howle, J.R.; Haydu, L.E.; Sharma, R.N.; Thompson, J.F.; Keffer, R.F.; Hersey, P.; Scolyer, R.A. Selective BRAF inhibitors induce marked T-cell infiltration into human metastatic melanoma. *Clin. Cancer Res.* 2012, 18, 1386–1394. [CrossRef] [PubMed]

12. Ott, P.A.; Hodi, F.S.; Buchbinder, E.I. Inhibition of Immune Checkpoints and Vascular Endothelial Growth Factor as Combination Therapy for Metastatic Melanoma: An Overview of Rationale, Preclinical Evidence, and Initial Clinical Data. *Front. Oncol.* 2015, 5, 202. [CrossRef] [PubMed]

13. Yan, Y.; Kumar, A.B.; Finnes, H.; Markovic, S.N.; Park, S.; Dronca, R.S.; Dong, H. Combining Immune Checkpoint Inhibitors With Conventional Cancer Therapy. *Front. Immunol.* 2018, 9, 1739. [CrossRef] [PubMed]

14. Corke, L.; Sacher, A. New Strategies and Combinations to Improve Outcomes in Immunotherapy in Metastatic Non-Small-Cell Lung Cancer. *Curr. Oncol.* 2021, 29, 38–55. [CrossRef] [PubMed]

15. Sharma, P.; Hu-Lieskovan, S.; Wargo, J.A.; Ribas, A. Primary, Adaptive, and Acquired Resistance to Cancer Immunotherapy. *Cell 2017*, 168, 707–723. [CrossRef]

16. Lim, A.R.; Rathmell, W.K.; Rathmell, J.C. The tumor microenvironment as a metabolic barrier to effector T cells and immunotherapy. *Elife* 2020, 9, e55185. [CrossRef]

17. Lee, M.; Samstein, R.M.; Valero, C.; Chan, T.A.; Morris, L.G.T. Tumor mutational burden as a predictive biomarker for checkpoint inhibitor immunotherapy. *Hum. Vaccines Immunother.* 2020, 16, 112–115. [CrossRef]

18. Lawlor, R.T.; Mattiolo, P.; Mafficini, A.; Hong, S.-M.; Priedda, M.L.; Taormina, S.V.; Malleo, G.; Marchegiani, G.; Pea, A.; Salvia, R.; et al. Tumor Mutational Burden as a Potential Biomarker for Immunotherapy in Pancreatic Cancer: Systematic Review and Still-Ongoing Studies. *Cancers 2021*, 13, 3119. [CrossRef]

19. Sade-Feldman, M.; Jiao, Y.J.; Chen, J.H.; Molina, J.; Kim, J.-H.; Arvis, C.D.; Ahn, M.-J.; et al. Genomic correlates of response to immune checkpoint blockade in microsatellite-stable solid tumors. *Nat. Genet.* 2018, 50, 1271–1281. [CrossRef]
21. Dotus, Y.; Horiike, A.; Yoshizawa, T.; Sonoda, T.; Koyama, J.; Saiki, M.; Ariyasu, R.; Uchibori, K.; Nishikawa, S.; Kitazono, S.; et al. Programmed death-ligand 1 expression after progressive disease with EGFR-TKI and efficacy of anti-programmed death-1 antibody in non-small cell lung cancer (NSCLC) harboring EGFR mutation. *J. Clin. Oncol.* **2018**, *36*, e21232. [CrossRef]

22. Garassino, M.C.; Cho, B.-C.; Kim, J.-H.; Mazieres, J.; Vansteenkiste, J.; Lena, H.; Corral Jaime, J.; Gray, J.E.; Powderly, J.; Chouaid, C.; et al. Durvalumab as third-line or later treatment for advanced non-small-cell lung cancer (ATLANTIC): An open-label, single-arm, phase 2 study. *Lancet Oncol.* **2018**, *19*, 521–536. [CrossRef]

23. Al-Taie, Z.; Hannink, M.; Mitchem, J.; Papageorgiou, C.; Shyu, C.-R. Drug Repositioning and Subgroup Discovery for Precision Medicine Implementation in Triple Negative Breast Cancer. *Cancers* **2021**, *13*, 6278. [CrossRef] [PubMed]

24. Park, J.V.; Park, S.J.; Yoo, J.S. Finding characteristics of exceptional breast cancer subpopulations using subgroup mining and statistical test. *Expert Syst. Appl.* **2019**, *118*, 533–562. [CrossRef]

25. Sheets, L.; Petroski, G.F.; Zhuang, Y.; Phinney, M.A.; Ge, B.; Parker, J.C.; Shyu, C.-R. Combining Contrast Mining with Logistic Regression To Predict Healthcare Utilization in a Managed Care Population. *Appl. Clin. Inform.* **2017**, *8*, 430–446. [CrossRef]

26. Rodriguez, M.Z.; Comin, C.H.; Casanova, D.; Bruno, O.M.; Amanio, D.R.; Costa, L.F.; Rodrigues, F.A. Clustering algorithms: A comparative approach. *PLoS ONE* **2019**, *14*, e0210236. [CrossRef]

27. Linzer, D.A.; Lewis, J.B. polCA: An R Package for Polytomous Variable Latent Class Analysis. *Scientometrics* **2006**, *69*, 131–152. [CrossRef]

28. Novak, P.K.; Lavrač, N.; Webb, G.I. Supervised Descriptive Rule Discovery: A Unifying Survey of Contrast Set, Emerging Pattern and Subgroup Mining. *J. Mach. Learn. Res.* **2009**, *10*, 377–403.

29. Lavrač, N.; Kavšek, B.; Flach, P.; Todorovski, L. Subgroup Discovery with CN2-SD. *IEEE J. Biomed. Health Inform.* **2020**, *24*, 1456–1468. [CrossRef]

30. Liu, D.; Baskett, W.; Beversdorf, D.; Shyu, C.-R. Exploratory Data Mining for Subgroup Cohort Discoveries and Prioritization. *Appl. Clin. Inform.* **2014**, *3*, 553–562. [CrossRef]

31. Liu, J.; Lichtenberg, T.; Hoadley, K.A.; Poisson, L.M.; Lazar, A.J.; Cherniack, A.D.; Kothavi, A.J.; Benz, C.C.; Levine, D.A.; Lee, A.V.; et al. An Integrated TCGA Pan-Cancer Clinical Data Resource to Drive High-Quality Survival Outcome Analytics. *Cell 2018*, *173*, 400–416.e11. [CrossRef] [PubMed]

32. Sequist, L.V.; Yang, J.C.-H.; Yamamoto, N.; O’Byrne, K.; Hirsh, V.; Mok, T.; Geater, S.L.; Orlov, S.; Tsai, C.-M.; Boyer, M.; et al. Erlotinib versus standard chemotherapy as first-line treatment for European patients with advanced EGFR mutation-positive non-small-cell lung cancer (EURTAC): A multicentre, open-label, randomised phase 3 trial. *Lancet Oncol.* **2012**, *13*, 239–246. [CrossRef]

33. Cesano, A. nCounter PanCancer Immune Profiling Panel (NanoString Technologies, Inc., Seattle, WA). *J. Immunother. Cancer* **2015**, *3*, 42. [CrossRef]

34. Agrawal, R.; Srikant, R.; Road, H.; Jose, S. Fast Algorithms for Mining Association Rules. In *Proceedings of the 20th International Conference on Very Large Data Bases*, Santiago, Chile, 12–15 September 1994; pp. 487–499. [CrossRef]

35. Cesano, A. nCounter PanCancer Immune Profiling Panel (NanoString Technologies, Inc., Seattle, WA). *J. Immunother. Cancer* **2015**, *3*, 42. [CrossRef]

36. Dong, G.; Li, J. Efficient Mining of Emerging Patterns: Discovering Trends and Differences. In *Proceedings of the Fifth ACM Conference on Very Large Data Bases*, Santiago, Chile, 12–15 September 1994; pp. 487–499. [CrossRef]

37. Agrawal, R.; Srikant, R.; Road, H.; Jose, S. Fast Algorithms for Mining Association Rules. In *Proceedings of the 20th International Conference on Very Large Data Bases*, Santiago, Chile, 12–15 September 1994; pp. 487–499. [CrossRef]

38. Egghe, L. Theory and practise of the g-index. *Sciento-metrics* **2006**, *69*, 131–152. [CrossRef]

39. Liu, Z.; Zhao, Q.; Zhu, Z.-X.; Yuan, S.-Q.; Yu, K.; Zhang, Q.; Zhang, X.; Sheng, H.; Hu, H.-Q.; Cheng, H.; et al. Systematic Analysis of the aberrances and Functional Implications of Ferroptosis in Cancer. *Iscience* **2020**, *23*, 101302. [CrossRef]

40. Agresti, A. *Categorical Data Analysis*, 2nd ed.; Wiley-Interscience: New York, NY, USA, 2002; ISBN 978-0-470-36093-3.

41. Prat, A.; Navarro, A.; Paré, L.; Reguart, N.; Galván, P.; Pascual, T.; Martínez, A.; Nuñor, P.; Comerma, L.; Alos, L.; et al. Immune-Related Gene Expression Profiling After PD-1 Blockade in Non-Small Cell Lung Carcinoma, Head and Neck Squamous Cell Carcinoma, and Melanoma. *Cancer Res.* **2017**, *77*, 3540–3550. [CrossRef]

42. Dong, G.; Li, J. Efficient Mining of Emerging Patterns: Discovering Trends and Differences. In *Proceedings of the Fifth ACM SIGKDD International Conference on Knowledge Discovery and Data Mining*, San Diego, CA, USA, 15–18 August 1999; pp. 43–52. [CrossRef]

43. Lv, Z.; Zhao, Q.; Zhu, Z.-X.; Yuan, S.-Q.; Yu, K.; Zhang, Q.; Zhang, X.; Sheng, H.; Hu, H.-Q.; Cheng, H.; et al. Systematic Analysis of the aberrances and Functional Implications of Ferroptosis in Cancer. *Isceience* **2020**, *23*, 101302. [CrossRef]

44. Prat, A.; Navarro, A.; Paré, L.; Reguart, N.; Galván, P.; Pascual, T.; Martínez, A.; Nuñor, P.; Comerma, L.; Alos, L.; et al. Immune-Related Gene Expression Profiling After PD-1 Blockade in Non-Small Cell Lung Carcinoma, Head and Neck Squamous Cell Carcinoma, and Melanoma. *Cancer Res.* **2017**, *77*, 3540–3550. [CrossRef]

45. Venables, W.N.; Ripley, B.D. *Modern Applied Statistics with S*. In *Statistics and Computing*; Springer: New York, NY, USA, 2002; ISBN 978-1-4419-3008-8.
46. Peng, Y.-P.; Wang, R.; Liu, Q.-D.; Xu, X.-W.; Wei, W.; Huang, X.-T.; Peng, X.-M.; Liu, Z.-G. Combination of Tumor Mutational Burden and Specific Gene Mutations Stratifies Outcome to ImmunoTherapy Across Recurrent and Metastatic Head and Neck Squamous Cell Carcinoma. *Front. Genet.* 2021, 12, 756506. [CrossRef] [PubMed]

47. McGrail, D.J.; Pilié, P.G.; Rashid, N.U.; Voorwerk, L.; Slatter, M.; Kok, M.; Jonasch, E.; Khasraw, M.; Heimberger, A.B.; Lim, B.; et al. High tumor mutation burden fails to predict immune checkpoint blockade response across all cancer types. *Ann. Oncol.* 2021, 32, 661–672. [CrossRef] [PubMed]

48. Wiesweg, M.; Preis, C.; Roep, J.; Metzenmacher, M.; Eberhardt, W.; Stropiep, U.; Wedeken, K.; Reis, H.; Herold, T.; Darwiche, K.; et al. BRAF mutations and BRAF mutation functional class have no negative impact on the clinical outcome of advanced NSCLC and associate with immunotherapy. *Eur. J. Cancer* 2021, 149, 211–221. [CrossRef]

49. Sun, L.-Y.; Cen, W.-J.; Tang, W.-T.; Long, Y.-K.; Yang, X.-H.; Ji, X.-M.; Yang, J.-J.; Zhang, R.-J.; Wang, F.; Shao, J.-Y.; et al. Smoking status combined with tumor mutational burden as a prognosis predictor for combination immune checkpoint inhibitor therapy in non-small cell lung cancer. *Cancer Med.* 2021, 10, 6610–6617. [CrossRef]

50. Freshour, S.L.; Kiwala, S.; Cotto, K.C.; Coffman, A.C.; McMichael, J.F.; Song, J.J.; Griffith, M.; Griffith, O.L.; Wagner, A.H. Integration of the Drug-Gene Interaction Database (DGIdb 4.0) with open crowdsourcing efforts. *Nucleic Acids Res.* 2021, 49, D1144–D1151. [CrossRef]

51. Wishart, D.S.; Feunang, Y.D.; Guo, A.C.; Lo, E.J.; Marcus, A.; Grant, J.R.; Sajad, T.; Johnson, D.; Li, C.; Sayeeda, Z.; et al. DrugBank 5.0: A major update to the DrugBank database for 2018. *Nucleic Acids Res.* 2018, 46, D1074–D1082. [CrossRef]

52. Corominas, M.; Gastaminza, G.; Lobera, T. Hypersensitivity reactions to biological drugs. *J. Investig. Allergol. Clin. Immunol.* 2021, 31, 225–231. [CrossRef]

53. Saba, N.F.; Ekpenyong, A.; McCook-Veal, A.; Patel, M.; Schmitt, N.C.; Stokes, W.A.; Bates, J.E.; Rudra, S.; Abousaud, M.I.; Tianjin Medical University Second Hospital. [CrossRef]

54. Genovese, M.C.; Breedveld, F.C.; Emery, P.; Cohen, S.; Matteson, E.L.; Baptiste, Y.; Chai, A.; Burke, L.; Reiss, W.; et al. Safety of biological therapies following rituximab treatment in rheumatoid arthritis patients. *Ann. Rheum. Dis.* 2009, 68, 1894–1897. [CrossRef]

55. Strauss, S.J.; Morschhauser, F.; Rech, J.; Repp, R.; Solal-Celigny, P.; Zinzani, P.L.; Engert, A.; Coiffier, B.; Hoelzer, D.F.; Wegener, W.A.; et al. Multicenter phase II trial of immunotherapy with the humanized anti-CD22 antibody, epratuzumab, in combination with rituximab, in refractory or recurrent non-Hodgkin’s lymphoma. *J. Clin. Oncol.* 2006, 24, 3880–3886. [CrossRef]

56. Peng, Y.-P.; Wang, R.; Liu, Q.-D.; Xu, X.-W.; Wei, W.; Huang, X.-T.; Peng, X.-M.; Liu, Z.-G. Combination of Tumor Mutational Burden and Specific Gene Mutations Stratifies Outcome to ImmunoTherapy Across Recurrent and Metastatic Head and Neck Squamous Cell Carcinoma. *Front. Genet.* 2021, 12, 756506. [CrossRef] [PubMed]

57. Wishart, D.S.; Feunang, Y.D.; Guo, A.C.; Lo, E.J.; Marcus, A.; Grant, J.R.; Sajad, T.; Johnson, D.; Li, C.; Sayeeda, Z.; et al. DrugBank 5.0: A major update to the DrugBank database for 2018. *Nucleic Acids Res.* 2018, 46, D1074–D1082. [CrossRef]

58. Genovese, M.C.; Breedveld, F.C.; Emery, P.; Cohen, S.; Matteson, E.L.; Baptiste, Y.; Chai, A.; Burke, L.; Reiss, W.; et al. Safety of biological therapies following rituximab treatment in rheumatoid arthritis patients. *Ann. Rheum. Dis.* 2009, 68, 1894–1897. [CrossRef]

59. Mayo Clinic. *A Phase 1 Study of PD-1 Inhibition with Pembrolizumab Combined with JAK2 Inhibition in Triple Negative Breast Cancer*; ClinicalTrials.gov.: Bethesda, MD, USA, 2022. Available online: https://clinicaltrials.gov/ct2/show/NCT03012230 (accessed on 22 September 2022).

60. Wiesweg, M.; Preis, C.; Roep, J.; Metzenmacher, M.; Eberhardt, W.; Stropiep, U.; Wedeken, K.; Reis, H.; Herold, T.; Darwiche, K.; et al. High tumor mutation burden fails to predict immune checkpoint blockade response across all cancer types. *Ann. Oncol.* 2021, 32, 661–672. [CrossRef] [PubMed]

61. National Cancer Institute (NCI).

62. Mayo Clinic.

63. Saba, N.F.; Ekpenyong, A.; McCook-Veal, A.; Patel, M.; Schmitt, N.C.; Stokes, W.A.; Bates, J.E.; Rudra, S.; Abousaud, M.I.; Tianjin Medical University Second Hospital. [CrossRef]

64. Amin, A.; Plimack, E.R.; Ernstoff, M.S.; Lewis, L.D.; Bauer, T.M.; McDermott, D.F.; Carducci, M.; Kollmannsberger, C.; Rini, B.I.; Heng, D.Y.C.; et al. Safety and efficacy of nivolumab in combination with sunitinib or pazopanib in advanced or metastatic renal cell carcinoma: The CheckMate 016 study. *J. Immunother. Cancer* 2018, 6, 109. [CrossRef] [PubMed]
65. Kessler, E.R.; Hu, J.; Srivastava, G.; Kemme, D.J.; Iruku, P.; Rana, V.; Schuster, S.R.; Amirault, M.; Callihan, E.; Flaig, T.W.; et al. Phase I/II trial of pembrolizumab and cabozantinib in the treatment of metastatic renal cell carcinoma (mRCC). *J. Clin. Oncol.* 2021, 39, 4544. [CrossRef]

66. Socinski, M.A.; Nishio, M.; Jotte, R.M.; Cappuzzo, F.; Orlandi, F.; Stroyakovskiy, D.; Nogami, N.; Rodríguez-Abreu, D.; Moro-Sibilot, D.; Thomas, C.A.; et al. IMpower150 Final Overall Survival Analyses for Atezolizumab Plus Bevacizumab and Chemotherapy in First-Line Metastatic Nonsquamous NSCLC. *J. Thorac. Oncol.* 2021, 16, 1909–1924. [CrossRef]

67. Burtness, B.; Harrington, K.J.; Greil, R.; Soulières, D.; Tahara, M.; de Castro, G.; Psyrri, A.; Basté, N.; Neupane, P.; Bratland, Å.; et al. Pembrolizumab alone or with chemotherapy versus cetuximab with chemotherapy for recurrent or metastatic squamous cell carcinoma of the head and neck (KEYNOTE-048): A randomised, open-label, phase 3 study. *Lancet Lond. Engl.* 2019, 394, 1915–1928. [CrossRef]

68. BioInvent International AB. Phase 1/2a Clinical Trial of BI-1206, a Monoclonal Antibody to CD32b (FcγRIIB), in Combination with Rituximab in Subjects with Indolent B-Cell Non-Hodgkin Lymphoma That Has Relapsed or Is Refractory to Rituximab; ClinicalTrials.gov: Bethesda, MD, USA, 2022. Available online: https://clinicaltrials.gov/ct2/show/NCT03571568 (accessed on 22 September 2022).

69. Ajona, D.; Ortiz-Espinosa, S.; Lozano, T.; Exposito, F.; Calvo, A.; Valencia, K.; Redrado, M.; Remírez, A.; Lecanda, F.; Alignani, D.; et al. Short-term starvation reduces IGF-1 levels to sensitize lung tumors to PD-1 immune checkpoint blockade. *Nat. Cancer* 2020, 1, 75–85. [CrossRef]

70. Reilley, M.; Bailey, A.M.; Subbiah, V.; Janku, F.; Naing, A.; Falchook, G.S.; Karp, D.D.; Piha-Paul, S.A.; Tsimberidou, A.M.; Fu, S.; et al. Phase I clinical trial of combination imatinib and ipilimumab in patients with advanced malignancies. *J. Clin. Oncol.* 2016, 34, 3054. [CrossRef]

71. Murciano-Goroff, Y.R.; Warner, A.B.; Wolchok, J.D. The future of cancer immunotherapy: Microenvironment-targeting combinations. *Cell Res.* 2020, 30, 507–519. [CrossRef]