Biomarkers and Outcomes in Diverse Cancers: Meta-Analysis of Early Phase Immuno-Oncology Trials

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Abstract

Background

Many immuno-oncology (IO) trials are conducted without biomarker selection. We performed a meta-analysis of phase I/II clinical trials evaluating immune checkpoint inhibitors (ICIs) to determine the association between biomarkers and clinical outcomes, if any.

Methods

We searched PubMed for phase I/II clinical trials of drugs approved by the Food and Drug Administration (labeled, off-label, or combined with investigational ICIs or other treatment modalities) from 2018 to 2020. We compared the objective response rate (ORR), progression-free survival (PFS), and overall survival (OS) between biomarker-positive and biomarker-negative groups, using data from correlative studies.

Results

Overall, 174 clinical studies that included 19,178 patients were identified and 131 studies investigated > 30 correlative biomarkers, that included PD-L1 expression (≥ 1%, 111 studies), tumor mutational burden (20 studies), and microsatellite instability/mismatch repair deficiency (10 studies). Overall, 123, 46, and 30 cohorts (drugs, tumor types, or biomarkers) with 11,692, 3,065, and 2,256 patient outcomes for ORR, PFS, and OS, respectively, were analyzed in correlation with biomarkers. Meta-analyses demonstrated that ICIs in patients with biomarker-positive tumors were associated with higher ORR (odds ratio 2.15 [95% CI, 1.79–2.58], p < 0.0001); and longer PFS (hazard ratio [HR] 0.55 [95% CI, 0.45–0.67], p < 0.0001), and OS (HR 0.65 [95% CI, 0.53–0.80], p < 0.0001) compared with those with biomarker-negative tumors. Significance for ORR and PFS was retained in multivariate analysis (p < 0.001) (OS, not included owing to the small number of trials reporting OS).

Conclusion

Our data suggest that IO biomarkers should be used in patient selection for ICIs. Prospective studies are warranted.

Introduction

Immunotherapy, including immune checkpoint inhibitors (ICIs), has changed the therapeutic landscape of cancer, providing significant clinical benefit in selected patients. ICIs exert their action by enhancing immune responses. To date, eight ICIs have been approved by the US Food and Drug Administration
(FDA) for the treatment of patients with cancer: one targeting cytotoxic T-lymphocyte-associated protein 4 (CTLA-4) (ipilimumab), four targeting programmed death-1 (PD-1) (pembrolizumab, nivolumab, cemiplimab, and dostarlimab), and three targeting programmed death-ligand 1 (PD-L1) (atezolizumab, durvalumab, and avelumab). These agents, alone or in combination, have led to improved outcomes in various tumor types. The use of ICIs has been associated with substantially improved clinical outcomes in patients with specific biomarkers, such as high microsatellite instability (MSI-H)/mismatch repair deficiency (MMRd), high tumor mutational burden (TMB-H), or high PD-L1 expression. However, approximately 80% of patients do not respond to ICIs and some responders eventually develop resistance to treatment. Serious immune-related adverse events and accelerated rate of growth or hyper-progressive disease (hyper-progression) have also been reported in some patients treated with immunotherapy.

To date, FDA-approved biomarkers for patient selection for cancer immunotherapy include PD-L1 expression on the tumor and/or immune cells, MSI-H/MMRd, and TMB-H (≥ 10 mutations/megabase [mut/mb]). Importantly, MSI-H/MMRd was the first FDA-approved biomarker predictive of response to pembrolizumab across tumor types. Other biomarkers predicting response to immunotherapy are currently under evaluation. Additionally, several biomarkers—including but not limited to loss of beta-2 (β2)-microglobulin, JAK1/2 mutations, and alterations in EGFR and MDM2 genes—have been associated with poorer clinical outcomes after treatment with immunotherapy.

Currently, many immunotherapy trials are conducted without biomarker selection. Biomarker use has often been associated with improved outcomes for genomically-selected targeted therapies. In addition, the use of predictive biomarkers has been correlated with improved efficacy outcomes in clinical trials evaluating FDA-approved anticancer agents.

We hypothesized that the use of biomarkers would correlate with better outcomes in clinical trials with ICIs for patients with cancer. To address this issue, we performed a systematic review and meta-analysis of phase I/II clinical trials evaluating FDA-approved ICIs that were published from 2018 through 2020.

**Methods**

**Search Strategy and Study Selection**

We conducted a PubMed search for “Clinical trials, Phase 1,” “Clinical trials, Phase 2,” and publication dates from January 1, 2018, to December 31, 2020; using the following filters: “Cancer”; and “Humans”. The 3-year study period was chosen before starting data extraction. The immunotherapeutic agents approved by the FDA until March 2021, i.e., “pembrolizumab”, “nivolumab”, “atezolizumab”, “durvalumab”, “cemiplimab”, “avelumab”, and “ipilimumab” were used in the search toolbar. Clinical trials with their labeled indications, off-label use or their combinations with investigational ICIs or other
treatment modalities were included. Studies describing supportive care or locoregional treatments; cellular, viral, or vaccine therapy; studies in the adjuvant or neoadjuvant setting; and pediatric studies were excluded.

Data Extraction And Categorization

Data extraction was conducted independently by three investigators (EF, HHV, and PM). Categorization was validated and any discrepancies were resolved in frequent meetings in the presence of the moderators (AMT and RK). To be included, the studies had to describe a phase 1, phase 1/2, or phase 2 cancer immunotherapy trial and evaluate an ICI as monotherapy or combined with other ICIs, chemotherapy, targeted agents, and/or any other anticancer treatment. The ORR was defined as the sum of the complete response (CR) and partial response (PR) rates. The median progression-free survival (PFS) [or time to progression (TTP)] and overall survival (OS) were extracted, and 95% confidence intervals (CIs) were recorded.

Biomarkers

Biomarkers were defined as biologic variables identified in tumor tissue or blood that were evaluated as potential predictors of response to immunotherapy. They were considered prospective when they were predetermined and taken into consideration for patient selection. In the remaining studies, biomarkers were analyzed retrospectively in correlative studies post hoc. Biomarkers were considered regardless of whether they were known to be predictive at the time the original study was published.

Statistical Analyses

Statistical analyses were performed by PM (biostatistician). Our initial plan was to examine both prospective and correlative biomarkers; however, the number of studies with prospective biomarkers was small. Therefore, we instead compared ORR, PFS, and OS between biomarker-positive and biomarker-negative groups, using data from the correlative studies (Fig. 1).

For clinical outcomes reported according to correlative biomarker analyses, we performed meta-analyses of odds ratios (ORs) for ORR and hazard ratios (HRs) for PFS/TTP and OS. OR was determined as follows: first, we calculated the probability of overall response among biomarker-positive patients in each study, and we evaluated the odds as the ratio of that probability over its complement. The same calculations were performed for biomarker-negative patients. The OR was defined as the ratio of these two odds.

For each meta-analysis, we first tested the hypothesis of homogeneous effects across studies (common effects, fixed effects model). If there was significant evidence for heterogeneity, the null hypothesis for homogeneity was rejected and the random effects model was used. The level of (residual)
heterogeneity was estimated using the DerSimonian-Laird estimator. The random effects model considers both within-study variation and between-study variation, including meta-analyses with moderators. ORs were calculated as the ratio of the odds of overall response in biomarker-positive versus biomarker-negative subgroups. Analyses of ORR were based on log ORs. For the time-to-event outcomes PFS and OS, the analyses were based on the log ratio of median event times for biomarker-negative versus biomarker-positive groups. We calculated study-specific variances as the sum of inverse sample sizes, which was reliably available for all studies under consideration. Tests and coding of variables were performed as previously published.

Two-sided \( P < 0.05 \) was considered statistically significant; \( P < 0.0001 \) was considered highly significant. Statistical analyses were performed in the R statistical software environment, using the packages metaphor, meta, and mvmeta.

For all three endpoints [objective response rates (ORR), PFS, and OS] we considered meta-regressions, that is, meta-analyses with moderators. The moderators considered the agent, tumor type, monotherapy versus combination therapy, line of therapy, and whether the tumor type was known to be associated with a favorable response to immunotherapy. The immunotherapeutic agents were categorized as follows: pembrolizumab; nivolumab; atezolizumab; avelumab; durvalumab; cemiplimab; pembrolizumab or nivolumab; nivolumab combined with ipilimumab; and pembrolizumab combined with ipilimumab therapy. Tumor types were grouped as follows: melanoma, breast cancer, non-small cell lung cancer (NSCLC), diverse tumors and other tumors. Treatment was categorized as monotherapy, combination therapy, or combination/monotherapy (when a checkpoint inhibitor was investigated as monotherapy and/or in combination with other treatments in the same trial). Line of therapy was categorized as first-line, \( \geq 2 \) lines, or other (multiple lines of therapy or not specified). Tumor types were categorized as those for which there were FDA-approved immunotherapy agents for the indication versus others.

For studies reporting clinical outcomes for multiple treatments, ORR, PFS, and OS were listed separately for each treatment in the analysis. Similarly, for studies that included multiple tumor types and reported clinical outcomes for specific tumor types, these outcomes were listed for each individual tumor type. For studies that reported outcomes for multiple biomarkers, each biomarker was included separately in the analysis. Hence, we refer to all aforementioned separately listed outcomes summaries as cohorts.

In phase I/II studies, all data were used, regardless of the dose level. Results were summarized in forest plots. The studies were weighted using the inverse variance method, which employed a 2 x 2 table of biomarker (positive or negative) and response to treatment (yes or no). To detect possible publication bias and sample size effects, we used funnel plots and trim-and-fill plots.

Results

Search Results And Clinical Trial Characteristics
Overall, 183 clinical studies were identified. Nine trials were excluded because they did not meet the inclusion criteria (two did not include patients with advanced/metastatic cancer, two did not include human studies, two did not include treatment with an approved immune checkpoint inhibitor, one referred to the same population already included in a previous study, one was a phase III trial, and one was a pooled analysis of a phase III and a phase II study). Overall, 174 trials met the criteria to be included in the analysis (Fig. 2) (59 phase I, 94 phase II, and 21 phase I/II studies).

In total, 19,178 patients were included in the 174 studies. Ninety-one studies (52.3%) investigated single-agent checkpoint inhibitors, 69 (39.7%) combination treatments, and 14 (8%) checkpoint inhibitors alone and combined with other treatments. Pembrolizumab was used in most trials (72; 41.4%). The most common malignancy was melanoma (27 trials, 15.5%), followed by NSCLC (26 trials, 14.9%), gastric/gastroesophageal cancer (17 trials, 9.8%), and breast cancer (14 trials, 8.1%). Eighteen (10.3%) trials included patients with diverse tumor types and 72 (41.4%) the remaining tumor types. Details of patient characteristics and treatments are summarized in Table 1.
<table>
<thead>
<tr>
<th>Characteristics</th>
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</tr>
</thead>
<tbody>
<tr>
<td><strong>Age, years</strong></td>
<td>202 cohorts (in 174 trials)</td>
</tr>
<tr>
<td>Average of per-study medians (range)</td>
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<tr>
<td><strong>Number of patients</strong></td>
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<tr>
<td>Median (range)</td>
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<tr>
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<td>II</td>
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<td>I/II</td>
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<td><strong>Treatment</strong></td>
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<td>Combination therapy</td>
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<tr>
<td>Monotherapy and/or combination therapy</td>
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<td>With targeted agents</td>
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<tr>
<td>With other immunotherapeutic agents*</td>
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<tr>
<td>With radiation therapy</td>
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<td><strong>Immune checkpoint inhibitor†</strong></td>
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<tr>
<td>Anti-CTLA4</td>
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* Other immunotherapies included: second checkpoint inhibitor (N = 13), TVEC (talimogene laherparepvec) (N = 2), vaccine (N = 2), interferon (N = 2), interleukin (N = 2), BCG (N = 1), and toll-like receptor 9 agonist (N = 1), antibody against CCR4 (N = 1).

† Two studies included either pembrolizumab or nivolumab.

‡ The following tumor types were included in ≤ 5 studies: mesothelioma (n = 5), urothelial (n = 5), adrenocortical (n = 4), sarcoma (n = 4), pancreatic (n = 3), small cell lung (n = 3), thymic (n = 3), germ cell (n = 2), merkel cell (n = 2), squamous cell skin (n = 2), prostate (n = 1), thyroid (n = 1), and glioblastoma (n = 1).

§ Several studies had multiple cohorts that they were analyzed individually.
<table>
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<th>Characteristics</th>
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<td><strong>Age, years</strong></td>
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<td>Combination anti-PD-1/anti-CTLA4</td>
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* Other immunotherapies included: second checkpoint inhibitor (N = 13), TVEC (talimogene laherparepvec) (N = 2), vaccine (N = 2), interferon (N = 2), interleukin (N = 2), BCG (N = 1), and toll-like receptor 9 agonist (N = 1), antibody against CCR4 (N = 1).

† Two studies included either pembrolizumab or nivolumab.

‡ The following tumor types were included in ≤ 5 studies: mesothelioma (n = 5), urothelial (n = 5), adrenocortical (n = 4), sarcoma (n = 4), pancreatic (n = 3), small cell lung (n = 3), thymic (n = 3), germ cell (n = 2), merkel cell (n = 2), squamous cell skin (n = 2), prostate (n = 1), thyroid (n = 1), and glioblastoma (n = 1).

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<table>
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<th>Characteristics</th>
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<tr>
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<tr>
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<tr>
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<tr>
<td>No biomarker</td>
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* Other immunotherapies included: second checkpoint inhibitor (N = 13), TVEC (talimogene laherparepvec) (N = 2), vaccine (N = 2), interferon (N = 2), interleukin (N = 2), BCG (N = 1), and toll-like receptor 9 agonist (N = 1), antibody against CCR4 (N = 1).

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§ Several studies had multiple cohorts that they were analyzed individually.

In total, we identified 123 cohorts reporting ORR, 46 reporting PFS, and 30 reporting OS, (drugs, tumor types, or biomarkers) with 11692, 3065, and 2256 patient outcomes for correlative analyses, respectively (Additional Table 1).

**Correlative Biomarker Studies**
Overall, 131 studies investigated correlative predictive biomarkers. The most frequently assessed biomarkers were PD-L1 expression (n = 111), TMB (n = 20), and MSI/MMRd (n = 10). The correlative biomarkers are listed in Additional Table 1.

An initial test of homogeneity for ORR, PFS, and OS analyses demonstrated evidence against homogeneity (p < 0.0001), i.e., the effect of immunotherapy biomarkers varied across studies; therefore, we used the random effects model.

ORR, PFS, and OS were significantly higher in cohorts with a biomarker than in those without a biomarker

ORR was compared between biomarker-positive and biomarker-negative subgroups for 123 cohorts (82 studies, including some with multiple cohorts; Additional Table 2). Overall, 11,692 data points were captured, including 2,320 reporting objective responses. We found statistically significant differences in ORR by biomarker presence. A meta-analysis (123 cohorts) demonstrated an estimated odds ratio of 2.15 (95% CI, 1.79 to 2.58, p < 0.0001), indicating that the presence of biomarkers (compared with absence of biomarkers) correlated with higher response rates. The median ORRs in the biomarker-positive versus biomarker-negative cohorts were 30% versus 16%, respectively. The estimated across-study (123 cohorts) variance ($\tau^2$) was 0.33, based on whether the hypothesis of common effects was rejected with a p-value of p < 0.0001, i.e., the effect of biomarkers on clinical outcomes varied across studies. The results of the meta-analysis in a forest plot including study-specific weights (last column), the estimated cohort-specific OR for ORR (with 95% CI) and an estimate of the OR of the ORRs for all the cohorts (with 95% CI) are shown in Fig. 3.

Additional Fig. 1 shows results from the meta-regressions by therapeutic agent, tumor type, monotherapy versus combination therapy, line of therapy, and tumor types with FDA-approved immune checkpoint blockade therapy. No significant effects of these covariates on the ORs of the ORRs were found in univariate analyses. As a graphical diagnostic for possible publication bias and small study effects, a funnel and trim-and-fill plot demonstrated no evidence of publication bias or small sample size effects. (Fig. 4a).

Comparisons of PFS by biomarker status were reported for 46 cohorts (36 studies, including some studies with separate summaries for multiple cohorts). Similarly, a hypothesis of common effects (across studies) was rejected (p < 0.0001) and we proceeded with inference in a random effects model. A funnel and trim-and-fill plot as a graphical diagnostic for possible publication bias and small study effects demonstrated no evidence of publication bias and small study effects (Fig. 4b). The estimated HR of PFS for biomarker-positive versus biomarker-negative subpopulations was 0.55 (95% CI, 0.45 to 0.67, p < 0.0001). Estimated HRs for each study and corresponding 95% CIs are shown in Fig. 5. Meta-regressions were again carried out by agent, tumor type, monotherapy versus combination therapy, line of therapy, and tumor types that are associated with a favorable response to immunotherapy. Results are summarized in Additional Fig. 2. Except for tumor type, no significant subgroup differences were found.
Comparison of OS by biomarker status was reported for 30 cohorts (25 studies, including studies with separate summaries for multiple cohorts). Again, a hypothesis of common effects (across studies) was rejected at $p < 0.0001$, and we proceeded with inference in a random effects model. The estimated hazard ratio of OS for biomarker-positive versus biomarker-negative subpopulations was 0.65 (95% C.I. 0.53 to 0.80, $p < 0.0001$). The estimated hazard ratios for each study and corresponding 95% C.I. are shown in Fig. 6. Meta-regression analysis did not show any significant effect for any of the considered moderators.

Multivariate analysis

Multivariate meta-analysis with a bivariate clinical response of ORR and PFS was carried out. OS was not included in the multivariate analysis because of the small number of studies reporting OS (only 15 studies reported ORR, PFS and OS for the same study). Using 32 cohorts that reported both ORR and PFS, we estimated a correlation coefficient of $r = 0.34$ between ORR and PFS. We then used all 125 cohorts with ORR, and/or PFS outcomes for a multivariate analysis, treating missing clinical outcomes as missing data, and fixing the correlation at the estimated $r$. We found estimates for the overall OR (biomarker-positive vs. biomarker-negative) for ORR as 2.11 (95% Cl. 1.77–2.51, $p < 0.001$) and for the overall HR for PFS as 0.56 (95% C.I. 0.43 to 0.72, $p < 0.001$).

Discussion

This is the first extensive meta-analysis and systematic review of phase I/II clinical trials assessing the effect of biomarkers on outcome in patients with solid tumors treated with ICIs. In 174 clinical studies comprising 19,178 patients, we found that patients with biomarker-positive tumors had higher ORR, PFS, and OS compared to patients with biomarker-negative tumors. This finding was confirmed in multivariate analysis for ORR and PFS, but multivariate analysis was not performed for OS because the number of studies reporting OS was too small. Our results suggest that biomarkers should be taken into consideration in the design of clinical trials with ICIs.

Despite the fact that the use of biomarkers has often been associated with improved outcomes for targeted therapies selected on the basis of genomic alterations,\textsuperscript{17,24–27} the use of biomarkers to select patients for ICI treatment remains a challenge. Limitations to the implementation of FDA-approved biomarkers\textsuperscript{22,28–30,48} for the selection of immunotherapy include the suboptimal development of biomarkers associated with response and toxicity to ICI therapies. For instance, the assessment of PD-L1 expression has not been standardized across tumor types. In patients with melanoma treated with ICIs, similar efficacy was reported irrespectively of tumor PD-L1 expression levels.\textsuperscript{49} Other investigators reported no significant difference in response rates in patients with PD-L1-positive and PD-L1-negative squamous NSCLC treated with nivolumab.\textsuperscript{50–52} In one of those studies, differences were found in the specificity and sensitivity of PD-L1 immunohistochemistry (IHC) assays for prediction of response to ICIs and associated clinical outcomes.\textsuperscript{52} A meta-analysis of seven randomized trials involving 3,871 patients with NSCLC confirmed that treatment with PD-1/PD-L1 inhibitors improves OS compared with
chemotherapy, irrespectively of PD-L1 expression levels. However, the investigators also reported that patients whose tumors were strongly PD-L1 positive by IHC derived the greatest survival benefit, and patients with a Kirsten Rat Sarcoma Viral Oncogene Homologue (KRAS)-mutant or epidermal growth factor receptor (EGFR) wild-type tumor had a greater survival benefit from ICIs than did patients with KRAS wild-type or EGFR-mutant NSCLC. The KEYNOTE-010 study demonstrated favorable clinical response to pembrolizumab in patients with advanced NSCLC whose tumors had high expression of PD-L1 (≥ 50%). PD-L1 assessment by IHC may be an imprecise predictor of response to immune checkpoint blockade, owing to technical difficulties or the complexity of the immune environment. Additionally, a high false-negative rate at low PD-L1 expression levels may prevent eligible patients from receiving targeted therapies. Additionally, as the ICI field has rapidly evolved over the past decade, more patients treated with ICIs have experienced immune-related toxicity, hyper-progression, and transient clinical responses.

The strengths of our analysis include the evaluation of substantial numbers of patients in consecutive clinical trials that met the criteria for the analysis and the detailed cohort stratification taking into consideration the ICI agents, tumor types, monotherapy versus combination therapy, line of therapy, and tumor types that are known to be associated with response to immunotherapy based on FDA approval status. Additionally, trials comprising diverse tumor types were included in the analysis, thus increasing the generalizability of the results.

Like other meta-analyses, our study has several limitations. First, the analysis included heterogenous patient populations with diverse tumor types who were treated with various ICIs as monotherapy or in combination with other therapies as different lines of treatment. On the other hand, this heterogeneity may point to generalizability of the results. Second, patients treated in the dose-escalation (with potentially suboptimal doses) and expansion phases of the studies were included. Third, we analyzed trials that were published only over a 3-year period. Fourth, our study did not aim to demonstrate whether a specific biomarker is predictive of benefit from ICIs; such an analysis would be limited by small sample sizes for each biomarker. Finally, we included only correlative biomarkers in the study owing to the limited number of studies (19 of 174, 10.9%) that examined prospective biomarkers. Importantly, bias could have been introduced by the fact that positive correlative studies are more likely to be reported.

The use of a single biomarker to select patients may have several limitations and, therefore, it is important to develop and implement additional or composite predictive biomarkers to select patients who are most likely to benefit from treatment with specific ICIs, while preventing unnecessary toxicity. Machine learning and artificial intelligence approaches may also accelerate immune biomarker assessment and discovery.

In conclusion, our meta-analysis demonstrated that the use of immune-related biomarkers is important in order to pinpoint those patients who will benefit from ICIs. Prospective clinical trials that implement individual or composite biomarkers that incorporate genomic, transcriptomic, and immune profiles, the host pharmaco-genome, and other factors are warranted.
Abbreviations

β2: beta-2

CR: complete response

CI: confidence interval

CTLA-4: cytotoxic T-lymphocyte-associated protein 4

EGFR: epidermal growth factor receptor

FDA: Food and Drug Administration

MSI-H: high microsatellite instability

HR: hazard ratios

ICI: immune checkpoint inhibitors

IO: immuno-oncology

KRAS: Kirsten Rat Sarcoma Viral Oncogene Homologue

MMRd: mismatch repair deficiency

NSCLC: non-small cell lung cancer

OR: odds ratios

ORR: objective response rate

PD-1: programmed death-1

PD-L1: programmed death-ligand 1

PFS: progression-free survival

PR: partial response

OS: overall survival

TTP: time to progression

TMB-H: tumor mutational burden

Declarations
Competing interests

Dr. Elena Fountzilas has the following financial relationships to disclose: Travel grants: Merck, Pfizer, and K.A.M Oncology/Hematology; Speaker fees: Roche, Leo, Pfizer; Stock ownership: Deciphera Pharmaceuticals, Inc.

Dr. Henry Hiep Vo reports no relevant conflicts of interest.

Dr. Peter Mueller reports no relevant conflicts of interest.

Dr. Razelle Kurzrock has the following financial relationships to disclose: Research Funding (Institution): Incyte, Genentech, Merck Serono, Pfizer, Sequenom, Foundation Medicine, Konica Minolta, Grifols, Biologic Dynamics, Boehringer Ingelheim, Medimmune, and Guardant. Consulting role: X-Biotech, Loxo, Biologic Dynamics, Turning Point, TD2, Bicara, and Actuate Therapeutics. Speaker fees: Roche. Ownership interest: IDbyDNA and CureMatch, Inc. Board member: CureMatch and CureMetrix.

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Data Availability Statements

The data underlying this article are available in the article and in its online additional material.

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Availability of data and materials

The dataset supporting the conclusions of this article is available upon request.

Author contribution:

Conceptualization: AMT, RZ

Data curation: EF, HHV

Formal Analysis: PM

Funding acquisition: AMT, PM, RK
Investigation: AMT, EF, HHV, PM, RK

Methodology: AMT, EF, HHV, PM, RK

Software: PM

Validation: AMT, EF, HHV, PM, RK

Writing – original draft: AMT, EF, HHV, PM, RK

Writing – review & editing: AMT, EF, HHV, PM, RK

References


Figures
Figure 1

Study design

Assessing the impact of biomarker-based treatment strategies in immuno-oncology clinical trials of diverse cancers. (A) Representation of immunotherapy trials without biomarkers. (B) Representation of immunotherapy trials with biomarkers.
**Figure 2**

PRISMA diagram

PRISMA diagram displaying numbers of patients and trials from the data extraction (2018 through 2020).

*Other immunotherapies include: second checkpoint inhibitor (N=13), talimogene laherparepvec (TVEC, n=2), vaccine (n=2), interferon (n=2), interleukin (n=2), Bacillus Calmette-Guerin (BCG, n=1), and toll-like receptor 9 agonist (n=1), antibody against CCR4 (N=1).

†Two studies included either pembrolizumab or nivolumab
Figure 3

Forest plot of objective response rates (ORR) across cohorts reporting ORR

Forest plot of ORR across all 123 cohorts reporting ORR in correlative analyses for biomarker-positive versus biomarker-negative subgroups. The figure shows that biomarkers correlated with higher response rates (odds ratio for biomarker-positive versus biomarker-negative patients was 2.15 [95% CI, 1.79 to 2.58,
p<0.0001]). Note: In some cases, investigators reported separate summaries by biomarkers, drugs, or tumor types. Such reports were treated as separate cohorts and included as separate lines in the forest plot. Corresponding references are found in the Additional Material.

Figure 4

Funnel and trim-and-fill plot for the meta-analysis for objective response rate (ORR) and progression-free survival (PFS)

Funnel and trim-and-fill plot for the meta-analysis for ORR (panel a) and PFS (panel b). Each point plots reported treatment effect (odds ratio and hazard ratio, respectively) on the horizontal axis and corresponding standard deviation on the vertical axis. Lack of symmetry, especially sparsity on the left side of the cone, would be considered evidence for publication bias. The trim-and-fill method fills in potentially missing studies with non-significant or negative outcomes, shown as open circles in the trim-and-fill plot. This figure shows that there was no evidence of selection bias.
Figure 5

Forest plot of log HR across cohorts that reported progression-free survival (PFS)

Forest plot of log HR across all 44 cohorts that reported PFS in correlative analyses for biomarker-negative versus biomarker-positive subgroups. The figure shows that the presence of biomarkers was correlated with longer PFS (HR for biomarker-positive versus biomarker-negative subpopulations was 0.55 (95% CI, 0.45 to 0.67, p<0.0001)). Corresponding references are found in the Additional Material.

* In some cases, investigators reported separate summaries by biomarkers, drugs, or tumor types. Such reports are treated as separate cohorts and included as separate lines in the forest plot.
<table>
<thead>
<tr>
<th>Study</th>
<th>(A) log(HR)</th>
<th>(B) SE</th>
<th>Hazard Ratio</th>
<th>(C) HR (D) 95%-CI (E) Weight</th>
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<td>1.10</td>
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</table>

**Random effects model**

Heterogeneity: $I^2 = 75\%$, $\tau^2 = 0.1550$, $p < 0.01$

Test for overall effect: $z = -4.06$ ($p < 0.01$)

![Forest plot of log HR across cohorts that reported overall survival (OS)](image)

<table>
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<th>1</th>
<th>2</th>
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</table>

**Figure 6**

Forest plot of log HR across cohorts that reported overall survival (OS)

Forest plot of log HR across all 21 cohorts that reported OS in correlative analyses for biomarker-negative versus biomarker-positive subgroups. The figure shows that the presence of biomarkers was correlated with longer OS. Hazard ratio was 0.65 (95% C.I. 0.53 to 0.80, p<0.0001). Corresponding references are found in the Additional Material.

**Supplementary Files**

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