Statistical and Practical Considerations for Clinical Evaluation of Predictive Biomarkers

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Predictive biomarkers to guide therapy for cancer patients are a cornerstone of precision medicine. Discussed herein are considerations regarding the design and interpretation of such predictive biomarker studies. These considerations are important for both planning and interpreting prospective studies and for using specimens collected from completed randomized clinical trials. Specific issues addressed are differentiation between qualitative and quantitative predictive effects, challenges due to sample size requirements for predictive biomarker assessment, and consideration of additional factors relevant to clinical utility assessment, such as toxicity and cost of new therapies as well as costs and potential morbidities associated with routine use of biomarker-based tests.


Precision medicine aims to guide therapy informed by biological characterization of a patient’s disease. For patients with cancer, these characterizations are typically achieved by molecular analysis of tumor biomarkers or sometimes by examination of host characteristics, such as variations in germline DNA. Two classes of biomarkers in oncology are prognostic markers and predictive markers (1): prognostic markers inform about likely disease outcome independent of the treatment received, and predictive markers provide information about likely outcomes with application of specific interventions. Therefore, predictive markers can help select among two or more therapy options. Predictive markers are of particular importance for targeted therapies, which often are expected to benefit only patients whose disease is characterized by presence of a biomarker.

When there is strong evidence that a targeted agent benefits only patients whose tumors have a particular biomarker, then evaluation of the targeted therapy in a trial that enrolls only patients whose tumors are positive for that marker would be appropriate. This type of trial design is called an enrichment design (2), and the marker is classified as an enrichment or selection marker (3). If there is uncertainty about the predictive biomarker and reasonable possibility that the therapy could benefit a broad population of patients, one can evaluate a candidate predictive biomarker by studying a group of unselected patients treated with the targeted therapy and another group of patients treated with some alternative therapy. The biomarker is predictive if the relative efficacy of the two treatments is different for the biomarker-positive patients than for the biomarker-negative patients. (Note that just demonstrating that biomarker-positive patients have better clinical outcome than biomarker-negative patients on a cohort of patients uniformly treated with the targeted therapy is insufficient to demonstrate that the biomarker is predictive because it may solely be prognostic.) A familiar example of a tumor marker that is both prognostic and predictive is estrogen receptor status in breast cancer, for which a positive status is associated with a more favorable prognosis generally and with specific benefit from endocrine therapy. Although much of the discussion here is framed around evaluation of predictive biomarkers for targeted therapies in retrospective studies, the same general principles would apply for evaluation of a predictive biomarker for selection between two different standard (non-targeted) therapies and for designing prospective clinical trials to evaluate predictive biomarkers.

The best setting in which to evaluate a predictive biomarker for an experimental targeted therapy is a randomized clinical trial (RCT) of the targeted therapy vs a standard treatment, where the biomarker status is obtained on the patients but not used to direct treatment (4). (Other possible RCT designs to evaluate biomarkers are possible but not as efficient as this design (2).) Ideally, a biomarker would be assessed prospectively in an RCT of the targeted agent. However, biomarker development often lags behind therapeutic development. The reasons for this asynchrony may include an incomplete understanding of the mechanism of action of a drug, the uncertainty about what form of a marker is most relevant (eg, DNA mutation, mRNA expression, protein expression), and technical difficulties with marker assay development (5). These reasons contribute, in part, to why biomarker studies are frequently conducted retrospectively on archived specimen collections several years after a cancer therapy has been developed. With a careful prospective design, such a retrospective analysis can provide convincing evidence in support of a predictive biomarker (6).

We assume that the assay for the biomarker or biomarker signature (a collection of biomarkers that are combined through some mathematical model and linked to a biological or clinical outcome) yields a fully specified binary marker (or is continuous and has been dichotomized with a cutoff based on appropriate statistical procedures (7,8)) that has demonstrated acceptable
analytical performance and sufficient robustness against influences of preanalytical factors (eg, warm ischemia time or duration of time that the paraffin blocks are stored before analysis) (9). Preanalytic factors may be of special concern because of the desire to extrapolate from retrospective biomarker analyses to how the biomarker will work in the clinic when the specimens are processed contemporaneously (6).

Under the assumptions that the biomarker is biologically relevant and analytically validated, we first define and describe in the next section predictive biomarkers that display clinical qualitative and quantitative interactions with the treatments. In the following section, we discuss the sample sizes required to reliably assess these interactions and discuss how to appropriately interpret estimates of predictive effect. This is followed by a discussion of the clinical utility of a predictive biomarker, which goes beyond whether or not a statistically significant interaction is present. We end with a brief discussion.

**Qualitative and Quantitative Interactions**

A biomarker is predictive if the treatment effect (experimental $E$ compared with standard $S$) is different for the biomarker-positive patients compared with the biomarker-negative patients. This is known as a (statistical) interaction between the treatment effect and biomarker status. An interaction can be qualitative or quantitative (10). Qualitative interaction can help guide treatment choice: $E$ is better than $S$ for the biomarker-positive patients and $E$ is not better than $S$ for the biomarker-negative patients (for which it could be equally efficacious or worse). With a quantitative interaction, $E$ is better than $S$ for both biomarker groups, but the amount of the treatment benefit is different for the two biomarker groups (otherwise there would be no interaction of any kind).

Examples of qualitative interactions are given in Figure 1. Gefitinib is better than chemotherapy as first-line treatment in epidermal growth factor receptor (EGFR) mutation–positive non–small cell lung cancer (NSCLC) patients (hazard ratio [HR] $= 0.48$; 95% confidence interval [CI] = 0.36 to 0.64; $P < .001$ (Figure 1A)) but worse than chemotherapy in EGFR mutation–negative patients (HR $= 2.85$; 95% CI = 2.05 to 3.98; $P < .001$) (Figure 1B); the interaction is statistically significant with $P$ less than .001 for the progression-free survival endpoint (11). A second example of qualitative interaction is given by the RCT of cetuximab + chemotherapy vs chemotherapy alone for first-line treatment of EGFR immunohistochemistry (IHC)–positive NSCLC patients (12). Based on the observed EGFR and outcome data, a division of the patients into low and high EGFR expression demonstrated a qualitative interaction: the addition of cetuximab improved overall survival for the patients with tumors with high EGFR expression (HR $= 0.73$; 95% CI = 0.58 to 0.93; $P = .01$) (Figure 1C) but offered no benefit for patients with tumors with low EGFR expression (HR $= 0.99$; 95% CI = 0.84 to 1.16; $P = .88$) (Figure 1D); the interaction is statistically significant with $P$ equal to .04 (13). (Because the EGFR cutpoint was not pre-specified, these results would need to be validated on a new dataset.)

Examples of quantitative interactions are given in Figure 2. Pazopanib improves progression-free survival (PFS) relative to placebo in locally advanced or metastatic renal cell carcinoma patients with high interleukin 6 (IL-6) plasma concentrations (Figure 2A) and low IL-6 concentrations (Figure 2B), but more so for the high IL-6 subgroup (HR $= 0.31$; 95% CI = 0.31 to 0.58; $P < .0001$) than for the low IL-6 subgroup (HR $= 0.55$; 95% CI = 0.28 to 0.71; $P < .002$); the interaction is statistically significant ($P = .009$) (14). A second example of a quantitative interaction is given by the interaction between maintenance treatment of NSCLC with erlotinib and tumor EGFR mutation status: wild-type (Figure 2C) vs mutant (Figure 2D) (15). There is an interaction (statistically significant with $P < .001$), and the interaction is quantitative: erlotinib improves PFS for patients with wild-type EGFR tumors (HR $= 0.78$; 95% CI $= 0.63$ to 0.96; $P = .02$) and for patients with EGFR-mutant tumors (HR $= 0.10$; 95% CI $= 0.04$ to 0.25; $P < .0001$), but much more for the patients with EGFR-mutant tumors.

Qualitative interactions can provide a clear indication of treatment choice: patients who are biomarker-negative should get the standard treatment ($S$), and, when the experimental treatment ($E$) is sufficiently better than $S$ in the biomarker-positive subgroup, these patients should receive $E$. Whether a biomarker that has quantitative interaction with treatment would be useful in directing treatment is a more difficult question and is discussed below.

**Sample Sizes and Ability to Draw Conclusions**

In designing an RCT to assess an experimental treatment vs a control treatment, one typically designates a minimally clinically interesting treatment difference (for example, a target hazard ratio of 0.75) that one would not want the trial to miss detecting. One then chooses the sample size of the trial so that there will be a high probability of having a statistically significant result at the end of the trial (the power of the trial) if the true treatment difference is this target treatment difference or more extreme.

One approach for testing whether a biomarker is a predictive biomarker is to estimate the statistical interaction and to assess whether that interaction is statistically significant. Unfortunately, the required sample size to test whether an interaction is statistically significant can be much larger than the required sample size to assess a treatment difference. For example, with a biomarker with 50% positive prevalence, it requires approximately four times the sample size to detect a 0.75 interaction (defined, for example, by a hazard ratio of 0.75 in a biomarker-positive subgroup and a hazard ratio of 1.00 in a biomarker-negative subgroup) as it does to detect a treatment difference with a hazard ratio of 0.75 (16). If the biomarker positivity prevalence is not 50%, the required sample size will be even larger. For example, if 20% of the patients have positive biomarkers and 80% have negative biomarkers, then the required sample size to detect the 0.75 interaction would be more than six times the required sample size to detect the same size treatment difference. If the investigator is using data and tissue from a retrospective RCT, not all patients will have tumor tissue available and the assay will not be successful for all tissues, further reducing the effective sample size.

A potential solution to the sample size problem is to pool biomarker data from multiple treatment trials that are asking similar questions. This approach is consistent with published guidelines for conducting prospective–retrospective studies that include a requirement that similar results for a biomarker be observed in...
at least two comparable studies for a biomarker to reach level IB evidence for medical utility (6). For example, based on a meta-analysis of multiple trials of the anti-EGFR monoclonal antibodies cetuximab or panitumumab for metastatic colorectal cancer, it was shown that these agents provide no benefit for patients with KRAS-mutated tumors (17). However, caution in pooling is required because a biomarker may have a qualitative interaction with treatment in one setting but not in another “similar” setting. For example, EGFR mutation status has a qualitative interaction with the treatment choice of the EGFR tyrosine kinase inhibitor gefitinib vs chemotherapy in first-line treatment of NSCLC (Figure 1, A and B) but only a quantitative interaction with the choice of EGFR tyrosine kinase inhibitor erlotinib vs placebo as maintenance therapy for NSCLC (Figure 2, C and D). Another cautionary example is the situation when a predictive biomarker in one disease setting may not be predictive in another disease setting. For example, although KRAS mutation has been shown to be a negative predictive factor for benefit from anti-EGFR monoclonal antibodies in colorectal cancer (17), data to date have not supported its predictive value in NSCLC (18,19). Although because of limited sample sizes there will not be sufficient power to detect the minimally clinically interesting treatment difference in each biomarker subgroup, there may be power to detect a larger interaction effect that is still considered plausible. For example, suppose a targeted agent is tested in an unselected population with the overall treatment hazard ratio of 0.75. One expects the treatment only to work in the biomarker-positive patients that comprise one-half of the population and not to work in the one-half of the

**Figure 1.** Examples of qualitative interactions. Gefitinib vs carboplatin + paclitaxel for first-line treatment of non–small cell lung cancer patients with EGFR mutation–positive tumors (A) and EGFR mutation–negative tumors (B) [adapted from Figure 2 of Mok et al (11). Reprinted with permission. Copyright 2009 Massachusetts Medical Society.]. Cetuximab + chemo vs chemotherapy for first-line treatment of non–small cell lung cancer patients with high-expressing EGFR immunohistochemistry (IHC)–positive tumors (C) and low-expressing EGFR IHC–positive tumors (D) [adapted from Figure 4 of Pirker et al. (13). Reprinted with permission. Copyright 2012 Elsevier]. PFS = progression-free survival.
patients who are biomarker negative (ie, hazard ratio = 1.0 in the biomarker-negative subgroup). Then, the treatment hazard ratio in the biomarker-positive patients would have to be approximately 0.56 for the overall hazard ratio to be 0.75 (log 0.75 = [log 0.56 + log 1.00] / 2). This corresponds to an interaction of 0.56, and a required sample size that is the same as the treatment trial (not four times larger) to achieve the same power. However, note that the analysis in this situation will not have much power to detect an interaction corresponding to a clinically meaningful treatment effect in the biomarker-positive patients (HR = 0.75) with no effect in the biomarker-negative patients. Therefore, it is important to describe the sample size limitations in reports of retrospective biomarker analyses, which are best conveyed by confidence intervals for the treatment effects in the biomarker subgroups.

Example of Difficulties Arising From Limited Sample Sizes

We consider the RCT of temozolomide + radiotherapy vs radiotherapy alone for glioblastoma multiforme (20), for which the biomarker MGMT methylation status was studied on a subset of the patients for whom tumor specimens and assay results were available (n = 206 patients of the 573 randomized) (21). The biomarker-stratified results are displayed in Table 1 and show a statistically significant PFS benefit of the addition of temozolomide for both biomarker strata. There was a statistically significant survival benefit from temozolomide in the MGMT methylated subgroup (P = .007 based on two-sided log-rank test), but this benefit did not reach statistical significance in the MGMT unmethylated subgroup (P = .06 based on two-sided log-rank test). However, because patients with unmethylated MGMT did relatively poorly regardless of treatment and the temozolomide benefit is less for patients with unmethylated MGMT (ie, larger hazard ratios), Hegi et al. conclude, “Patients with glioblastoma containing a methylated MGMT promoter benefited from temozolomide, whereas those who did not have a methylated MGMT promoter did not have such a benefit.” Given the wide confidence interval for the hazard ratio in the unmethylated group, one cannot confidently reach a conclusion from these data alone (21) about the potential value of MGMT methylation status as a predictive marker for benefit from temozolomide in patients with glioblastoma. Further
clinical follow-up on these patients resulted in demonstration of a statistically significant overall survival benefit in the unmethylated MGMT group (HR = 0.6; 95% CI = 0.4 to 0.8; Table 2 in Stupp et al. (22)), with an apparent long-term survival benefit (Figure 3).

A rough calculation based on the information provided in Stupp et al. (22) suggests that the hazard ratio in the methylated MGMT group is approximately 0.6 (95% CI = 0.4 to 0.9). These calculations are consistent with Stupp et al. (22) who reported, “Survival was significantly longer in patients treated with temozolomide and radiotherapy than in patients treated with radiotherapy alone, both in patients with a methylated and unmethylated MGMT promoter.” Although MGMT methylation status is highly prognostic, currently available data do not provide sufficient evidence that MGMT methylation status is predictive. Recognizing that the nature of the treatment effects in the two MGMT subgroups are quite different (Figure 3), evaluation of this biomarker in other studies will be needed to provide further clarity to its potential as a predictive biomarker. This example highlights the challenges associated with establishing the predictive value of a biomarker using data from a previously completed RCT. Specifically, such studies often lack statistical power to reliably ascertain whether there is a treatment effect in the biomarker-negative subgroup. As such, statistical significance in one biomarker subgroup but not in the other biomarker subgroup is insufficient for establishing the predictive value of a biomarker. Such evaluations should be done in conjunction with a critical examination of the estimate and the confidence interval of the treatment difference in both biomarker subgroups, with the recognition that P values are influenced by available sample size and number of events.

**From Interaction to Clinical Utility**

The most important clinical question that needs to be answered is whether the biomarker can classify patients into biomarker subgroups for which different choices of treatment regimen may be indicated. Although the presence of a treatment-by-biomarker interaction provides some insights into whether differential degrees of treatment benefit exist in two biomarker-defined subgroups (ie, the biomarker is predictive), it does not by itself inform us which specific treatment is superior for each biomarker subgroup. In particular, the recommended treatment in the biomarker-negative subgroup may not be clear in the case of a quantitative interaction because there will be treatment benefit in that subgroup but it may be relatively modest compared with the benefit seen in the biomarker-positive group. In this case, it is useful to incorporate into the decision-making process other practical factors such as toxicities induced by the therapies, the cost of the therapies, and the morbidity/cost associated with routine use of a biomarker assay to identify the biomarker-negative patients. For example, in the study by Brugger et al. (15), erlotinib maintenance therapy improved PFS compared with placebo for NSCLC patients with both mutant EGFR tumors and wild-type EGFR tumors. However, although statistically significant, the degree of benefit was less in the EGFR wild-type subgroup. In this situation, it is useful to weigh the added benefit from erlotinib against its cost and toxicity profile, the availability of other treatments, and issues related to routine testing for EGFR mutation.

An additional consideration in using archived specimens from clinical trials is that the clinical utility of the biomarker is being explicitly examined only for the subset of patients in the RCT who have specimens available. For example, the biomarker may only be evaluable on larger tumors in a retrospective analysis where the specimens are being used for multiple research purposes. How these patients may be different from the patients for whom eventual clinical use of the biomarker is being contemplated should be considered when evaluating the clinical utility of the biomarker.

To address the treatment question, it is best to estimate the hazard ratio with its confidence interval for each of the biomarker subgroups. This information, along with presentation of the survival curves for each biomarker subgroup and the practical considerations mentioned above, can help decide the recommended treatment for each subgroup if the confidence intervals for the hazard ratios are sufficiently narrow. If the recommended treatment is the same for both biomarker subgroups (eg, treat with experimental therapy), then the biomarker is not clinically useful, regardless of its interaction with the treatment. For example, one would not
want to withhold pazopanib to renal carcinoma patients with low IL-6 values, based on Figure 2, A and B, and one might argue that temozolomide should be used for glioblastoma patients regardless of MGMT methylation status based on long-term survivors, as seen in Figure 3B (23,24). Whether erlotinib maintenance therapy for NSCLC is appropriate regardless of EGFR mutation status (Figure 2, C and D) is also a difficult issue (25).

When the confidence intervals for the hazard ratios are wide for one or both of the biomarker subgroups, then it may be impossible to make an informed treatment recommendation for each of the subgroups. In this case, it will be impossible to say whether the biomarker has any clinical utility. One solution to this problem is to obtain more information—for example, by pooling data from related trials as described previously or increasing follow-up time to observe more events and increase precision of the estimated treatment effects. Moreover, additional biological understanding of the biomarker and mechanism of action of the targeted agent may be obtained through further preclinical studies that increase confidence in the strength of the biomarker’s predictive effect.

Discussion

A key part of designing a study to evaluate a putative predictive biomarker using specimens from an RCT is to specify what one will potentially be able to conclude from the study with sample size planned for the new trial or available from the previously conducted trial. In particular, although one may be able to conclude that the experimental treatment works much better than the standard treatment in the biomarker-positive subgroup (if it truly does), one may not be able to conclude whether the treatments are equally efficacious in the biomarker-negative subgroup (even if they truly are). This limitation is due partially to the inherently larger sample size required for testing an interaction compared with testing a treatment effect but is particularly important for analyses based on previously conducted trials where one does not have control of the sample size and because of the retrospective nature of the study specimens may not be available or be successfully assayed from all patients. In some circumstances, it may be reasonable to pool data from multiple trials to overcome the limited sample size in individual studies. Successful pooling relies on the clinical settings to be similar across the trials and requires that the biomarkers be assayed on all specimens using a common assay or assays that are sufficiently comparable. In addition, when evaluating the clinical utility of a biomarker, one needs to consider the possibility that biomarker assays may improve over time, so a treatment effect seen in the biomarker-negative subgroup may be partially due to some biomarker-positive patients being incorrectly classified as biomarker-negative; this treatment effect may no longer be present if a more accurate assay is used in future clinical practice. An example of a biomarker assay that has evolved over time is the HER2 assay in breast cancer. The immunohistochemical assay for HER2 that was used to screen for entry into the pivotal clinical trials of trastuzumab in metastatic breast cancer was replaced with a US Food and Drug Administration–approved companion diagnostic immunohistochemical assay at the time of approval of trastuzumab. Subsequently other immunohistochemical assays and fluorescence in situ hybridization tests for HER2 were widely adopted in clinical practice. Discordance rates of 20% or more were observed between different testing methods in clinical trial and community settings, prompting calls for HER2 testing standards (26).

In this commentary, we have focused on studies designed to demonstrate therapeutic superiority. In some settings, establishing noninferiority of a new therapy compared with the standard of care may justify change in clinical practice (eg, when the new therapy is less toxic and/or less costly than the standard one). However, because demonstration of noninferiority typically requires a larger sample size than that of superiority, the retrospective designs described here are unlikely to provide the precision needed to establish noninferiority unless they are based on previously conducted RCTs designed to demonstrate noninferiority. For example, consider a retrospective biomarker study based on data from a randomized trial designed to demonstrate superiority of a new therapy over the standard treatment. If the biomarker study shows that survival with the new treatment is statistically significantly better than with the standard treatment for the biomarker-positive subgroup, while the survival curves for the new and standard treatment arms are virtually identical to each other in the biomarker-negative subgroup, one may be tempted to conclude that the new therapy is at...
least as good as the standard treatment across the entire population. However, because of the typically limited sample sizes in subgroup analyses performed retrospectively, estimates of treatment effects restricted to subgroups may lack precision. This limited precision may preclude one from ruling out a nontrivial survival detriment due to the new treatment in the biomarker-negative subgroup.

Routine collection and banking of specimens from clinical trials provides a rich resource for conducting retrospective analyses of promising biomarkers. However, specimen collection is expensive and time consuming, and these precious resources, once depleted, are usually nonrenewable. Similar to futility monitoring for a treatment effect in a prospective randomized clinical trial, one could perform biomarker assays in stages with the possibility to stop performing biomarker assays if it becomes clear early that the hypothesized biomarker effect will not be confirmed (27). In addition, patient specimens must be appropriately collected and stored and should be prioritized for well-designed studies that are most likely to delineate the clinical utility of promising biomarkers. For additional design considerations relevant to establishing clinical utility of biomarker assays or tests, readers are referred elsewhere (28).

References


Notes

The views expressed in this article are the personal opinions of the authors and do not necessarily reflect policy of the US National Cancer Institute.

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