Statistical Issues in Translational Cancer Research

Stephen L. George

Abstract The explosion of knowledge about the basic biological processes and the genetics of cancer has led to increasing optimism that this knowledge can be put to practical clinical use in the near future. Indeed, important examples of translational approaches can already be found in the areas of drug discovery and development, disease diagnosis and classification, selection of therapeutic regimens for individual patients, and designing clinical trials. These are important developments but, as with any new approach, there is a danger of unwarranted enthusiasm and premature clinical application of laboratory results based on insufficient evidence. To carry out the translation of knowledge into practice with maximal efficiency and effectiveness, it is essential to conduct studies with appropriate designs and analyses based on sound statistical principles. This article provides an overview of some of these principles applied to assay development, validation of predictive models, and the design of clinical trials for targeted therapies.

Background

In an effort to address the pervasive problem of inadequacies in the design, analysis, and reporting of tumor marker prognostic studies, a set of reporting recommendations, REMARK, has been developed and adopted by many prominent journals, including Clinical Cancer Research (1). Many of the recommendations are directly or indirectly related to statistical issues, and the articles in this edition of CCR Focus will address some of these principles at key points along the translation pathway including assay development (2, 3), validation of predictive models (4), and the design of clinical trials (5). This is a very broad topic, with an extensive literature (6, 7), and no article or series of articles can hope to cover all of the issues. However, the specific topics covered serve as examples of the types of issues that arise and that need to be addressed carefully if we hope to fulfill the promise of translational cancer medicine. More detailed discussion of these and other statistical topics in clinical trials and in translational research can be found in various specialized texts (8–13).

In this overview article, after some brief comments on assay development, attention is focused on two important topics: model validation and targeted designs. Some common errors to be avoided in attempting to validate models are listed and some general results on the efficiency of targeted designs are presented along with the advantages and disadvantages of these designs.

At the risk of oversimplification, the following terminology will be used throughout to focus the discussion and to emphasize the generality of the concepts.

Biomarker. The official NIH definition of a biomarker is “a characteristic that is objectively measured and evaluated as an indicator of normal biological processes, pathogenic processes, or pharmacologic responses to a therapeutic intervention” (14). For our purposes, a biomarker is the end result of a bioassay, a laboratory technique for processing biological material from humans, expressed quantitatively or categorically. Imaging results could also be included as a biomarker if we interpret “bioassay” broadly enough. The measurement outcome of a biomarker might be binary (e.g., positive or negative), categorical (e.g., high, medium, low), quantitative (e.g., the level of some serum protein), or something more complicated (e.g., a genomic signature or “metagene”).

Statistical model. A formal representation of the statistical relationship between measured variables. In our case, we are concerned primarily with the relationship between patient characteristics (e.g., clinical or demographic data; biomarkers; other data) and patient status (e.g., presence or absence of disease; disease stage) with a clinical outcome (e.g., response to treatment; toxicity; survival). It is sometimes useful to distinguish “prognostic” models, those models that simply attempt to relate patient characteristics to patient outcomes, from “predictive” models, those that attempt to select specific treatments for individual patients based on those characteristics, but both are examples of statistical models.

Validation process. The process of assessing the extent to which a statistical model is successful at doing what it purports to do. Often the validation process includes a second study or series of studies aimed at confirming an earlier study to reduce bias and increase precision (15, 16). Sometimes the process is
loosely referred to as validation of the biomarker, but this is not precisely correct. Any biomarker can be defined and used in an unlimited number of ways in a statistical model; it is the particular statistical model being studied that requires validation.

**Assay Development: Getting It Right from the Beginning**

Although the necessity of applying appropriate statistical methods in defining and assessing statistical models based on a biomarker is obvious, the derivation of the biomarker itself is often implicitly considered a nonstatistical process with no need for a formal statistical approach. This attitude can lead to serious mistakes. Assay development and validation is an important part of the lengthy biomarker development pathway ranging from biomarker discovery through assay optimization and standardization and eventually clinical application (17).

Two articles in this issue of *CCR Focus* address important issues in assay development and validation. Chau et al. (3) discuss validation of the analytic methods behind a biomarker (i.e., validation of the assay), emphasizing a “fit for purpose” approach (18) rather than an approach that might be more appropriate for routine clinical laboratory validation. Owzer et al. (2), using the example of microarray data, address the procedures used in reducing an unmanageably large set of molecular data to a more manageable, but still perhaps quite large, number of summary features to be used in further development, called the “preprocessing” step. The procedures in this step have a profound effect on the later high-level analyses. The microarray example is especially timely because of the recent efforts to use gene expression data to predict clinical outcome or to select treatment for patients in a wide variety of diseases (19–22). Some of the issues to be considered include background correction, normalization, summary measures, filtering, quality control, outlier detection, and adjustments for batch effects. Variations in how these issues are addressed can have large effects on the specific features available for subsequent analyses. Indeed, it is easy to imagine that variation in preprocessing procedures can have a more important influence on the eventual results than variation in how the summary measures are used in a statistical model.

**Validation of Predictive Models: Proving that a Model Works**

The only useful function of a statistician is to make predictions, and thus to provide a basis for action.

W.E. Deming

It is difficult to make predictions, especially about the future.

variously attributed to Niels Bohr, Storm Peterson, and others

The fundamental idea behind the concept of personalized medicine is that it is possible to identify patterns of demographic, clinical, genomic, and other types of biological data that can be used together to benefit individual patients. Ordinarily, the association between patient characteristics and outcome must be expressed through a statistical model. If the ultimate purpose of the modeling process is to select the optimal, or at least a very good, treatment for an individual patient, the model is called a predictive model. However, this is not an easy task. It is essential to carry out appropriate validation procedures to ensure that the model works well in practice. The purpose of validation is not to see if the model under study is “correct,” but to verify that it is useful, that it can be used as advertised, and that it is fit for purpose (18). In addition to the article by Taylor et al. (4) in this *Focus*, the principles and features of an adequate validation process have been extensively described in the literature (15, 16, 19, 23, 24), although these principles are often not followed well in practice.

Given here are some elementary blunders to be avoided in developing and attempting to validate a putative predictive model.

- Lack of a priori specification of the process used to derive the model in the first place. Without such specification, it is difficult to judge the appropriateness of the process.
- Small sample sizes. In settings with a lot of data on each subject (e.g., microarray data), the importance of having an adequate number of subjects is often misunderstood. A large number of variables per subject is not the same as a large number of subjects.
- Testing the model on the data used to derive the model. There are ways to do “internal” validation (see below), but this must be done with care. A simple application of the model to the data used in the development of the model will yield greatly overly optimistic estimates of the predictive accuracy of the model.
- Over-fitting, or including too many variables in the model (25–28). As a simple example, for any set of *n* data points \((x, y)\) with unique \(x’s\) an \(n - 1\) degree polynomial can be derived that fits the data exactly. This polynomial would be unlikely to be useful either for summarizing the data or for prediction and would be unlikely to be proposed for such purposes. However, similar, if less obvious, examples of over-fitting of models are common (25).
- Over-reliance on “statistical significance” of a new variable. Even if the addition of a variable is found to be statistically significant, this does not mean that the model including the variable will be any better at prediction than the model without the variable (20, 21).
- Failure to test the model on a truly external data set. Proper external validation is a requirement for any proposed predictive model.
- Failure to account for multiplicity. When a very large number of tests are conducted, some statistical adjustment must be made to preserve overall error rates.

As implied by this list, it is important to distinguish between internal validation carried out on the original data set and external validation carried out on independent data produced under different circumstances. Internal validation refers to various cross-validation procedures such as dividing the original data randomly into two sets: a “training” data set to develop the model and a “test” data set to assess the predictive accuracy. There are many well-known variations on this theme,
including resampling schemes designed to refine the model and to assess its operating characteristics. Internal validation is necessary but not sufficient. There is no substitute for a well-done external validation. Ideally, such external validation would be carried out not only on independent data, preferably collected prospectively rather than retrospectively, but by investigators independent of those who developed the model being validated.

**Design of Clinical Trials: Efficiency through the Use of Biomarkers**

I abhor averages. I like the individual case. A man may have six meals one day and none the next, making an average of three meals per day, but that is not a good way to live.

Louis D. Brandeis

The development of the randomized clinical trial is arguably the most important contribution to scientific medicine of the 20th century. Beginning in the late 1940s, clinical trials rapidly became the gold standard throughout the world for assessing the relative effects of therapies for diseases and medical conditions. By the end of the century and continuing into the 21st century, thousands of trials have been and are being conducted worldwide in virtually all disease areas. Regulatory agencies now require properly designed and executed randomized clinical trials as the primary source of information on the safety and efficacy of therapies proposed for marketing approval in specified indications.

The design of randomized clinical trials is based on sound scientific principles for the reduction of bias and for increasing the precision and for controlling error rates in the testing of prespecified statistical hypotheses. Powerful tools for controlling bias, including randomization of treatment assignment, blinded treatment assignments, blinded evaluation of outcome, and intent-to-treat analyses, have become so widely accepted that they now seem to be almost self-evident requirements, but it is important to remember that these are relatively new developments in scientific medicine. The hallmark of modern randomized clinical trials, in contrast to studies prior to the time of widespread application of such trials, is that sufficient numbers of similar patients, those meeting prespecified eligibility criteria, need to be treated alike following a written protocol covering all aspects of the treatment and the details of the trial. Assuming that the trial is well designed and executed, the number of patients so treated is the primary determinant of the precision of the results and the reliability of the conclusions from the trial. The concept of moving from individualized treatment regimens given idiosyncratically to each patient based primarily on the prior experience of the treating physician to treating large numbers of patients in a similar fashion is central to the success of randomized clinical trials.

Although the assumptions underlying the design of a randomized clinical trial do not require that the patients are homogeneous in all important respects, the primary conclusions are usually stated on a population basis. For example, the conclusion might be that the overall survival distribution of patients entered on study was significantly better for patients randomized to receive treatment A than for those randomized to receive treatment B. The difference is often characterized, sometimes misleadingly, by some simple summary of the overall distribution (e.g., the estimated median overall survival on treatment A was 18 months compared with 13 months on treatment B) although the commonly used test statistics (e.g., a log-rank test of the overall survival distributions) compare the overall distributions. Secondary exploratory analyses might be conducted to see if the treatment effect differs by certain patient characteristics, but the overall comparison is properly understood to represent the primary result of the trial.

Recently, efforts to develop therapies targeted to some specific biological target have raised questions about the proper way to evaluate such therapies in randomized clinical trials. The oft-stated ultimate goal of such targeted therapy is individualized therapy based on a patient’s demographic (e.g., age, race, gender, etc.) and biological characteristics (e.g., biological markers, genomic data, specific biological pathway targets, etc.). That is, the specific therapy given to each patient would be tailored to the particular patient being treated, designed to achieve the best possible outcome for that patient. Although this concept has strong intuitive appeal, the irony is that this brings us full circle back to the preclinical trials era of individualized therapy. A major difference, of course, is that in the new setting the individualization is presumably based on solid scientific evidence of how to tailor the therapy. In the old setting, the basis for the individualization was at best not clear. Despite the appeal of the concept, there are challenges in the design of clinical trials to examine the effectiveness of individualized, or at least more tailored, therapies.

It is intuitively obvious that if we have a reliably measured biomarker that can distinguish patients likely to benefit from a therapy from those unlikely to benefit, we should be able to design much more efficient clinical trials. Simon (5) helps quantify this intuition in an article in the current CCR Focus issue as well as in previously published work (29–32). Consider the case of targeted therapy, in which the therapy has been designed to affect a particular biological pathway, mutation, receptor, or some other aspect of the cancer being treated. This assumes that we have some type of validated assay to distinguish those with the target from those without the target. We expect that the therapy will work much better, if not exclusively, in patients with the target than in those without the target. Well-known examples are found in breast cancer (e.g., Tamoxifen for ER+ patients; Herceptin for patients who overexpress HER2), colorectal cancer (e.g., the antiangiogenic agent bevacizumab targeted to vascular endothelial growth factors)...

### Table 1. Treatment effects in patients with and without a target $R$

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<th>$R^-$</th>
<th>$R^+$</th>
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<td>$\mu + \epsilon$</td>
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<td><strong>Trt 1</strong></td>
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<td><strong>Trt effect</strong></td>
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factor family pathways; cetuximab targeted to epidermal growth factor receptor family pathways), and non–small-cell lung cancer (e.g., tyrosine kinase inhibitors such as gefitinib and erlotinib targeted to epidermal growth factor receptor mutations; bevacizumab). For simplicity and to focus attention on the key points, in the following section only a single targeted therapy is considered, although it is possible to consider the more complicated case of two or more such therapies (33).

Some Technical Notes on Targeted Therapies

A full appreciation of the efficiency of targeted designs and the advantages and disadvantages of these designs requires an understanding of technical details such as those presented in this section. For those wishing to skip the details, the salient results are summarized in the concluding section.

In designing randomized clinical trials involving targeted therapies, consider three possible options:

- **Traditional design**—randomize all patients without regard to target; assess overall treatment effect.
- **Targeted design**—randomize only patients with positive assays; assess treatment effect in these patients; no information will be available about the treatment effect in assay negative patients.
- **Hybrid design**—randomize all patients as in the traditional design, but also conduct the assay on all patients; assess the overall treatment effect as well as the effect in the targeted subgroup.

The relative efficiency of these designs can be measured in various ways:

- Relative numbers of patients required on trial.
- Relative duration of accrual or time to completion of the trial.
- Relative cost of the trial, including screening and assay costs.

Table 1 presents notation for the average outcome in two treatment groups, control (Trt 0) and targeted therapy (Trt 1), and two groups of patients, those with the target in question (R+); those without (R−): δ₀ is the treatment effect in R+ patients, δ₁ in R− patients; ε is the prognostic effect of the target itself (i.e., those patients in R⁺ have a different mean than those in R⁻ regardless of treatment); and the prevalence of R⁺ patients is 1 − χ.

In this setting, the factors affecting the relative efficiency include the relative treatment effect (θ = δ₀ / δ₁) in those patients in R⁺ compared with those in R⁻, the prevalence (1 − χ) of R⁺ patients, the accuracy of the assay (through ω, the positive predictive value of the assay), and the costs of the assay and treatment. Specifically, the relative number of patients required on the traditional design (n) relative to the number required on the targeted design (n₁) is

\[
n/n₁ \approx \left( \frac{1 - \omega}{\gamma \theta + (1 - \chi)} \right)^2
\]

That is, the relative efficiency of the targeted design in terms of the required number of patients is high if θ is small (i.e., relatively little treatment benefit in the R⁺ patients), if 1 − χ is small (i.e., few R⁺ patients in the population), and if the positive predictive value (ω) of the assay is high. For example, if θ = 0.25, 1 − χ = 0.20, and ω = 0.90, the required number of patients in a traditional design is more than five times higher than a targeted design. If the targeted therapy does not benefit R⁺ patients at all (i.e., θ = 0), the required number of patients for the traditional design is more than 20 times higher than a targeted design. These considerations help define situations in which targeted therapies may not work well in unscreened populations.

The picture is somewhat less favorable for a targeted design if we consider the time required for accrual of the requisite number of patients and the cost of screening. For example, the relative average time to accrue the requisite number of patients is \(n / n₁\) (1 − χ). If the prevalence of R⁺ is low, fewer patients are required in a targeted design, but it takes longer to accrue these patients and more patients have to be screened to identify those suitable for the trial.

These considerations suggest that if we can be reasonably certain that the benefit of the targeted therapy for the R⁺ patients will be low and the assay is known to be highly accurate, a targeted design will be preferable to a traditional design. However, if we are not certain of these items, various “hybrid” designs may be considered (34–36). In one such design, all patients are entered; the assay is conducted on all patients; and the trial is designed to test the null hypothesis that there is no treatment effect both overall and in the R⁺ subset. In statistical terminology, this means that we test the following null (\(H₀\)) and alternative (\(H₁\)) hypotheses:

\[
H₀ : δ = 0 \quad \text{and} \quad δ₁ = 0 \\
H₁ : δ > 0 \quad \text{or} \quad δ₁ > 0
\]

One approach, controlling the overall type I error rate \(z\), is to test \(δ = 0\) at level \(z₁\) and \(δ₁ = 0\) at level \(z₂\). The correlation between the test statistics (approximately \(\sqrt{1 - χ}\)) allows us to set \(z₁\) and \(z₂\) such that \(z₁ + z₂ > z\). We can design the trial to achieve a power of 1 - β for the subset test (i.e., determine \(n₁\)) and enter patients until the requisite number of R⁺ patients are enrolled. For example, if \(z = 0.05\), \(1 - β = 0.50\), the correlation is approximately 0.71, so we might choose \(z₁ = 0.04\) and \(z₂ = 0.04\) and still achieve an overall error rate \(z = 0.05\). The required total number of patients will be approximately \(2n₁\).

Conclusions

Appropriate statistical methods are required throughout the entire translational pathway, including areas where it is currently neglected or relegated to a minor role. In the validation of putative predictive models, the most important consideration is undoubtedly the need for proper external validation, but other issues such as over-reliance on statistical significance tests, over-fitting of models, small sample sizes, and lack of prespecification of the validation process continue to be problematic. In the design of studies of targeted therapy,
restriction to patients with positive assays for the target in question can lead to greatly increased efficiency in terms of numbers of patients if the treatment effect is primarily limited to the assay-positive patients; the prevalence of such patients is low; and the assay is accurate. The relative efficiency in terms of length of study and cost may be considerably less because of the need to screen all patients and the low prevalence. In addition, such targeted designs do not give information about the effect of therapy in those screened out and may restrict future development to a particular marker-defined subset. This could be an important consideration because the relevant markers will almost certainly change over time. If one is uncertain about whether the treatment effect will be much higher in the assay-positive patients, if the prevalence is relatively high, or if the assay does not have a high positive predictive value, some type of design in which all patients are included is required. In all of these areas, it is incumbent on translational researchers to be aware of the issues and to involve biostatisticians in developing novel approaches that can improve on current techniques.

Disclosure of Potential Conflicts of Interest
The author has no potential conflicts of interest to disclose.

References
33. Vach W, dePont CR. Making efficient use of patients in designing phase III trials investigating simultaneously a set of targeted therapies with different targets. [see comment]. Biometrical Journal 2006;48:897–907.