Chapter 2

Clinical Trial Design in the Age of Molecular Profiling

Alexander Spira and Kirsten H. Edmiston

Abstract

The accelerating science of molecular profiling has necessitated a rapid evolution in clinical trial design. Traditional clinical research begins with Phase I studies to characterize dose-limiting toxicities and defines maximally tolerated doses of drugs in small numbers of patients. Traditional Phase II studies test these drugs at the doses discovered during Phase I drug development in small numbers of patients evaluating efficacy and safety. Phase III studies test new therapies to demonstrate improved activity or improved tolerability compared with a standard of care regimen or a placebo. The rapid advances in the understanding of signal transduction, and the identification of new potential diagnostic and therapeutic targets, now require the design and implementation of molecular clinical trials that are very different than traditional Phase I, II, or III trials. The main differentiating factor is the use of a molecular end point to stratify a subset of patients to receive a specific treatment regimen. This chapter focuses on the issues surrounding (a) the definition of clinical end points and the assessment of tumor response; (b) clinical trial design models to define the targeted pathway; and (c) the need for appropriate biomarkers to monitor the response.

Key words: Biomarkers, Clinical trial, Design, End points, Imaging, Pharmacodynamics, Tumor

1. Introduction

1.1. The Definition of Clinical End Points and Assessment of Tumor Response

Establishing the efficacy of a cancer drug is obviously the most important goal of oncology drug development. The gold standard end point is overall survival (OS), i.e., the time from diagnosis to death. Progression-free survival (PFS) is a shorter end point related more directly to the quality of life. Traditionally, response rates (RRs) as determined by tumor volume changes from imaging studies, and physical exams, have been used by investigators and patients to measure drug efficacy (e.g., “What are the chances of Drug X will cause a meaningful shrinkage in my cancer?”). Due to a multitude of reasons, tumor RR is not the best end point, and is not generally accepted in obtaining regulatory approval. Instead, the
more stringent measurement of overall survival as the “defining” end point reflects what is viewed as the most significant clinical benefit. While overall survival is frequently utilized by the US Food and Drug Administration (FDA) as a major end point in the drug approval process, it presents significant problems in molecular target-based therapy (1). Phase II and III studies can often take one or more years to accrue adequately large numbers of patients. An additional number of years must pass before the appropriate numbers of patients live out the natural course of their disease. The prolonged time frame required to assess OS is the reason that this end point may not be useful for phase (Phase II) studies. Overall survival is also affected by the use of subsequent lines of therapies as well as improvements in supportive care that may obscure the effects of a particular therapeutic agent. Moreover, any prolonged time courses add to the cost of clinical trial development as one gets further along in drug development. With the increase in costs to bring novel agents to the market, pharmaceutical manufacturers have reduced incentive to evaluate novel risky therapies.

Determining the efficacy of a therapy by OS alone ignores reduction of symptoms and improvements in the quality of life. Based on all the drawbacks of using the traditional OS end point, clinicians are now utilizing alternative measurement end points that are reached earlier in the treatment course and may be more meaningful to the patient. The approval of gemcitabine in advanced pancreatic cancer was precisely based on symptomatic improvement as the survival benefit was negligible (as were the response rates). Newer clinical trials are incorporating quality-of-life (QOL) measurements, such as the “Lung Cancer Symptom Scale” which is a subjective QOL questionnaire filled out by patients and nurses that reports subjective symptoms, in addition to the standard measurements of response rate and overall survival.

Clinical trial end points, such as time to progression (TTP), which is the time from randomization to the time of progressive disease, may be used as a surrogate for OS. TTP and PFS have been correlated with OS in patients with rectal cancer (2). These end points offer several advantages over the traditional OS in clinical trials design. Both TTP and PFS permit smaller sample sizes and shorter study durations (e.g., months as compared to years). In addition, TTP and PFS do not require demonstration of tumor mass shrinkage. Thus, these end points are useful in trials designed to evaluate cytostatic agents that arrest growth but do not shrink the tumor. TTP and PFS may be measured in real time after a single line of therapy. Consequently, when these measures are used, the designation of a response is not confounded by subsequent events. The disadvantage of TTP and PFS, as compared to OS, in clinical trials is the requirement for costly, frequent, and careful imaging assessment for progression (3, 4).

In order to rapidly assess response, particularly in Phase I and II studies, surrogate measurement techniques have been adopted and
standardized to assess results and to compare results across trials. Since 2000, the Response Evaluation Criteria in Solid Tumors (RECIST) criteria have been used to assess response (5). These criteria use the premise that tumor shrinkage reflects a positive outcome of antineoplastic therapy. Measurements for RECIST are based on two-dimensional measurements derived from computed tomography (CT) scans or magnetic resonance imaging (MRI). The RECIST criteria require the radiologist to measure the aggregate of all target tumors in their longest dimensions. Complete resolution of all tumors for at least 4 weeks is a complete response (CR); a 30% shrinkage is a partial response (PR); a 20% increase is progressive disease (PD); and everything else is stable disease (SD) (Table 1). As an example, the drug sorafenib was recently approved for use in patients with advanced hepatocellular carcinoma (HCC) (6, 7). The use of sorafenib was found to prolong survival in patients with HCC by 3 months and was associated with a 31% increase in OS at 1 year. Nevertheless, the RECIST response rate was only 2% (6). The same drug has been studied in patients with advanced renal cell cancer (8). The response rate was only 10%; but this constituted a significant prolongation in survival that led to FDA approval of this agent in advanced renal cell cancer.

There are a number of reasons why RECIST-defined response rates may be problematic endpoints. First, tumor cytotoxicity may not result in rapid shrinkage, especially in tumors that induce large amounts of stroma (rather than cellular elements that may “die” with chemotherapy). Stroma-rich tumors include sarcomas, lung cancers, and pancreatic cancers. According to RECIST criteria, a tumor that shrinks 29% and a tumor that grows 19% are both considered stable disease while these two responses are clearly different (9). Furthermore, even with modern imaging, there is some scan variability, especially with lesions close to 10 mm, the minimal slice size used in modern CT imaging. Not all cancers are amenable to accurate imaging on scans. For example, a lung cancer can cause adjacent lung atelectasis (collapse) that is difficult to discern from adjacent tumor and hence make accurate calculations difficult. Lastly,

### Table 1
Summary of RECIST response criteria (9)

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<tr>
<th>Stage</th>
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<tr>
<td>Complete response (CR)</td>
<td>Complete resolution of the tumor for a least 4 weeks</td>
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<tr>
<td>Partial response (PR)</td>
<td>Greater than 30% decrease in tumor sustained for a least 4 weeks</td>
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<tr>
<td>Progressive disease (PD)</td>
<td>At least 20% increase in tumor size with no CR, PR, or SD documented before the increase of disease</td>
</tr>
<tr>
<td>Stable disease (SD)</td>
<td>Neither PR nor PD criteria are met</td>
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RECIST criteria cannot be used in tumors with primarily bone lesions since these lesions do not shrink, making it difficult in the assessment of diseases, such as prostate cancer, as well as in many hematologic malignancies that cannot be measured with tumor size.

Recent advances in positron emission tomography (PET), MRI, and dynamic contrast-enhanced MRI (DCE-MRI) have yielded functional methods for tumor staging and assessment of tumor response. PET scans use (18)F-fluorodeoxyglucose (FDG) which accumulates in metabolically active tumor cells and can be described as a “functional” assessment of malignancies. After treatment, decrease in FDG uptake and metabolism correlates with a reduction in tumor viability. To achieve pharmacodynamic end points of tumor response, measurements are made by combining size and tracer uptake activity (standardized uptake value, “SUV”). FDG-PET scans have been very effective in measuring therapeutic responses in gastrointestinal stromal tumors, head and neck, breast, lung, esophageal lymphomas, and high-grade sarcomas (3). Despite many studies demonstrating its usefulness, FDG-PET still has not been utilized in most clinical research due to cost and difficulties in reimbursement as well as lack of an “official” reference standard to date, akin to RECIST (10). Rather than adopting FDG-PET, the US National Cancer Institute uses traditional CT scans as a method of evaluation for assessing response.

MRI and DCE-MRI are increasingly being utilized within clinical trials particularly to measure pharmacodynamic end points for novel antiangiogenic and antivascular targeting agents (11). MRI can provide a functional assessment of tumor physiology through the pattern of progressive enhancement and the change in washout kinetics. In this manner, MRI can provide a functional impression of tumor response superior to tumor shrinkage (Fig. 1).

Fig. 1. Example of RECIST criteria for determining tumor size following treatment. Cystosarcoma phylloides of the breast metastatic to the left pelvis before (a) and after (b) chemotherapy. The size of the tumor increased by approximately 20% by RECIST criteria, yet was felt to be largely necrotic on imaging. At the time of surgery, the tumor was largely necrotic consistent with a good response to chemotherapy. Arrowheads indicate the mass. Photo courtesy of A. Spira, Virginia Oncology Services.
This has been well-demonstrated in high-grade soft-tissue sarcomas with the correct prediction of tumor response in 80% of evaluable patients after isolated limb perfusion (12). In addition, MRI is particularly useful for assessing the central nervous system and the bone marrow infiltration that are not well-seen by FDG-PET. MRI is also useful for assessing metastasis and disease progression.

Overall, the accurate assessments of clinical and imaging response rates are critical to establish the pharmacodynamic response of the potential therapy within the target organ. Many clinical trials were done in the time period (1980s and 1990s) prior to the adoption of RECIST criteria. Moreover, the resolution, sensitivity, and specificity of imaging technology have advanced tremendously in the last 10 years. In this light, the use of historical comparisons as reference points to older studies is fraught with potential for error. Additional work needs to be done to integrate these newer noninvasive imaging modalities within clinical trials to provide the “pharmacological audit trail” necessary for drug validation and approval (13).

In many respects, the concept of patient stratification in clinical trials has been around for many years. Many clinical trials have been designed to compare Drug A against Drug B, or placebo, for all patients with a particular stage of a general type of cancer. An example is carboplatin and paclitaxel that were judged to be active in lung cancer (14). The overall observed response was the net combination of the number of beneficial outcomes offset by the number of patients receiving no benefit/toxic outcomes. Traditional chemotherapeutic agents are typically classified as cytotoxic drugs, targeting many pathways of cellular replication and division. Over the past 10 years, there have been rapid advances in molecular profiling technologies (as described in the subsequent chapters) and greater understanding of the specific pathways and upstream regulating molecules responsible for the malignant process. In parallel, there has been an expansion of national and international research collaborations, an increase in data sharing among clinical groups, and an enlargement in the size of patient cohorts in major trials (15). Consequently, researchers are now able to accurately profile patient tumors and design tailored therapy clinical trial models with a high degree of sophistication.

Trials that subdivide patients into defined groups are designed to answer specific treatment questions based on clinical or histologic characteristics (16, 17). These trials often require large numbers of patients and strong research collaborations. The following example demonstrates the value of patient stratification by histology.
Lung cancer is frequently called “non-small-cell lung cancer (NSCLC)” due to the fact that years of drug development never demonstrated that the identification of a subtype (usually, squamous versus adenocarcinoma) had any bearing on the use of a particular chemotherapeutic choice, hence the broad definition of NSCLC that is usually treated with platinum-based therapy (in the USA, usually carboplatin and paclitaxel) (14).

Pemetrexed is an intravenous methotrexate analog that was FDA approved in 2004 for pleural mesothelioma and as second-line NSCLC therapy due to its tolerability and safety. In an attempt to bring pemetrexed into the first-line setting, gemcitabine/pemetrexed was compared with gemcitabine/cisplatin. Based upon previous leads, histology was identified prestudy to be part of a subgroup analysis (18). Of note, patients with squamous histology did worse and lived for a shorter time when treated with pemetrexed (9.4 months vs. 10.8 months), but patients with adenocarcinoma lived longer when treated with pemetrexed (12.6 months vs. 10.9 months). This data led to the subsequent FDA labeling change specifying that pemetrexed could only be used in nonsquamous (and mainly adenocarcinoma) histologies. To date, this is the only study using traditional chemotherapy (vs. targeted chemotherapy) that demonstrated an impact of histology on outcome.

Within the treatment of breast cancer, a number of different multigene marker sets have been developed and validated to predict clinical outcomes more accurately than traditional clinicopathologic features. These include the 21 gene set (19, 20) and a 70 gene set (21, 22). The 21 gene biomarker (oncotype DX®) has been established and well-validated in hormone receptor-positive, lymph node-negative patients. It is used to predict the low, intermediate, and high recurrence risks. The oncotype DX® score identifies patients who would benefit from hormonal therapy only or would benefit from chemotherapy and hormonal therapy in combination. To further evaluate this prognostic multigene biomarker in clinical decision making, the North American Breast Cancer Intergroup developed the TAILORx trial in 2006 (23). Using a two-way stratified design model, the study first stratifies the patients based on their oncotype DX® recurrence risk score (RRS). Patients with a low RRS (<18) get hormonal therapy alone. Patients with a high RRS (>31) receive chemotherapy and hormonal therapy. Those patients with an intermediate RRS (18–30) are randomized to receive either hormonal therapy only or a combination of chemotherapy and hormonal therapy. This design enabled the Intergroup to take advantage of what was already known about the biomarker in clinical practice to address an important question in a practical manner. This study also utilizes a noninferiority design for the intermediate group and has the statistical power to detect a 3% or greater difference between the randomized arms (24). This design also provides further, although indirect, validation of the biomarker.
To clinically validate the 70 gene set biomarker (originally identified by Van’t Veer and coworkers (22)), the EORTC and TRANSBIG used a classifier randomization design. The multigene biomarker score was compared to a common clinical pathological prognostic tool (Adjuvant! Online (25, 26)) to identify patients for adjuvant chemotherapy in node-negative breast cancer (Microarray In Node Negative Disease may Avoid Chemotherapy, MINDACT, trial) (27, 28). This study sought to confirm that patients with a “low-risk” molecular prognosis and “high-risk” clinical prognosis could be safely spared chemotherapy without affecting disease-free survival. In addition, this study compared anthracycline-based chemotherapy to a docetaxel–capecitabine regimen and evaluates the efficacy and safety of 7 years of single-agent Letrozole to sequential 2 years of tamoxifen followed by 5 years of Letrozole (see Note 1).

In the development of “−omic” (genomic, proteomic, and metabolomic)-targeted therapies, it is critical to test both the utility of predictive/target biomarker as well as the utility of particular therapeutic agent, whether they are inhibitor- or biomarker-directed antibodies (29). A predictive biomarker is one that predicts the differential efficacy of a particular therapy based on the biomarker status (15). Identifying those patients who benefit and those patients who experience toxicity without efficacy is essential given the potential morbidity and cost of these targeted therapies. Many molecular targeted drugs can reach $10,000 per month of treatment, hence making the selection of targeted therapy even more appropriate in the setting of rising health costs.

The earliest example of patient stratification was the demonstration that estrogen-receptor (ER)-positive breast cancers could be treated with hormonal manipulation by tamoxifen and subsequently aromatase inhibitors. The case study of HER-2/neu and the subsequent development of trastuzumab are instructive in development of clinical trials to validate biomarkers.

HER-2/neu belongs to a family of transmembrane receptor tyrosine kinases that influence cell growth, differentiation, and survival. It is expressed in many normal cells as well as breast cancer cells in particular. Amplification of the HER-2/neu gene, overexpression of the HER-2/neu protein, or both occurs in approximately 25–30% of patients with breast cancer. HER-2/neu-positive patients have a high rate of recurrence and a short disease-free interval after adjuvant conventional anthracycline-based chemotherapy (30–32).

The first antibody discovered against HER-2/neu was trastuzumab, which is a very active intravenous antibody that targets the HER2 extracellular domain (33). The only clinically available biomarker predictor of responsiveness to trastuzumab is HER-2/neu status (30–32). During the initial development and testing of trastuzumab, there were two methods to demonstrate HER-2/neu status – immunohistochemistry (IHC) and fluorescence in situ.
hybridization (FISH). Traditional pathology looks for activity by the amount of immunohistochemical staining a tumor specimen has for a particular agent. Typically, the pathologist applies the anti-HER2/NEU antibodies, developing agents, and then scores what they see under the microscope (typically, 0, 1+, 2+, or 3+ staining). The disadvantage of IHC scoring is the variability in the quality of the staining, subjectivity by the scoring pathologist, and tumor sampling error. With the advent of FISH testing for HER2 amplification, a much more accurate measurement of HER2 expression can be done without the aforementioned subjectivity (34). FISH measures the fluorescence of the tagged HER2 gene. The amount of fluorescence is proportional to the amplification (number of copies) of the gene. The reproducibility of IHC and FISH testing at the local labs was compared to centralized labs during the Breast Intergroup Trial N9831 on the role of trastuzumab in breast cancer (35). This trial reported strong concordance between central IHC and FISH testing (92%) but poor concordance (74%) between local and central testing for HER-2/neu, thus underscoring the need for standardized testing for biomarkers. To establish consistency in tumor marker prognostic studies across preclinical and clinical trials, the US National Cancer Institute and the European Organization for Research and Treatment came together in July 2000 to publish the REporting recommendations for tumour MARKer prognostic studies (REMARK) (Table 2) (36).

To demonstrate clinical utility, Phase I clinical trials showed that the antibody is safe and confined to the tumor (unpublished

### Table 2

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<tr>
<th>REporting recommendations for tumour MARKer prognostic studies (REMARK) (36)</th>
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<tr>
<td><strong>Introduction</strong></td>
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<tr>
<td>1. State the marker examined, study objectives, and any prespecified hypotheses</td>
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<tr>
<td><strong>Materials and Methods</strong></td>
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<td>Patients</td>
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<td>2. Describe the characteristics (e.g., disease stage or comorbidities) of the study patients, including their source and inclusion and exclusion criteria</td>
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<td>3. Describe treatments received and how chosen (e.g., randomized or rule based)</td>
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<tr>
<td><strong>Specimen characteristics</strong></td>
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<td>4. Describe the type of biological material used (including control samples), and methods of preservation and storage</td>
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<td><strong>Assay methods</strong></td>
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<tr>
<td>5. Specify the assay method used and provide (or reference) a detailed protocol, including specific reagents or kits used, quality control procedures, reproducibility assessments, quantitation methods, and scoring and reporting protocols. Specify whether and how assays were performed blinded to the study end point</td>
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Table 2 (continued)

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<th>Study design</th>
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<td>6. State the method of case selection, including whether prospective or retrospective and whether stratification or matching (e.g., by stage of disease or age) was employed. Specify the time period from which cases were taken, the end of the follow-up period, and the median follow-up time.</td>
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<tr>
<td>7. Precisely define all clinical end points examined.</td>
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<td>8. List all candidate variables initially examined or considered for inclusion in models.</td>
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<tr>
<td>9. Give rationale for sample size; if the study was designed to detect a specified effect size, give the target power and effect size.</td>
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<tr>
<th>Statistical analysis methods</th>
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<tr>
<td>10. Specify all statistical methods, including details of any variable selection procedures and other model-building issues, how model assumptions were verified, and how missing data were handled.</td>
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<tr>
<td>11. Clarify how marker values were handled in the analyses; if relevant, describe methods used for cut-point determination.</td>
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<th>Results</th>
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<td>Data</td>
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<td>12. Describe the flow of patients through the study, including the number of patients included in each stage of the analysis (a diagram may be helpful) and reasons for dropout. Specifically, both overall and for each subgroup extensively examined report the numbers of patients and the number of events.</td>
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<tr>
<td>13. Report distributions of basic demographic characteristics (at least age and sex), standard (disease specific) prognostic variables, and tumor marker, including numbers of missing values.</td>
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<th>Analysis and presentation</th>
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<td>14. Show the relation of the marker to standard prognostic variables.</td>
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<td>15. Present univariate analyses showing the relation between the marker and outcome, with the estimated effect (e.g., hazard ratio and survival probability). Preferably provide similar analyses for all other variables being analyzed. For the effect of a tumor marker on a time-to-event outcome, a Kaplan–Meier plot is recommended.</td>
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<tr>
<td>16. For key multivariable analyses, report estimated effects (e.g., hazard ratio) with confidence intervals for the marker and, at least for the final model, all other variables in the model.</td>
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<tr>
<td>17. Among reported results, provide estimated effects with confidence intervals from an analysis in which the marker and standard prognostic variables are included, regardless of their significance.</td>
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<td>18. If done, report results of further investigations, such as checking assumptions, sensitivity analyses, internal validation.</td>
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<th>Discussion</th>
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<td>19. Interpret the results in the context of the prespecified hypotheses and other relevant studies; include a discussion of limitations of the study.</td>
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<td>20. Discuss implications for future research and clinical value.</td>
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Subsequent Phase II trials demonstrated that many women with HER2-positive metastatic disease who had relapsed after chemotherapy had a response to trastuzumab; as suggested by the preclinical data, the efficacy of trastuzumab when given with chemotherapy was superior to its effectiveness when used alone (37). The central issues in the Phase I and II studies were to identify...
the optimal biologic dose and the best dosing schedule to optimize target binding and inhibition \((38)\). Slamon et al. reported the results of the Phase III trial in which women with cancers that overexpressed HER2 who had not previously received chemotherapy for metastatic disease were randomly assigned to receive chemotherapy alone or chemotherapy plus trastuzumab \((39)\). The primary end points of the study were the time to disease progression and the incidence of adverse effects. Secondary end points were the rates and the duration of responses, the time to treatment failure, and overall survival. The addition of trastuzumab to chemotherapy was associated with a longer time to disease progression (median, 7.4 vs. 4.6 months; \(P<0.001\), a higher rate of objective response (50% vs. 32%, \(P<0.001\)), a longer duration of response (median, 9.1 vs. 6.1 months; \(P<0.001\)), a lower rate of death at 1 year (22% vs. 33%, \(P=0.008\)), longer survival (median survival, 25.1 vs. 20.3 months; \(P=0.046\)), and a 20% reduction in the risk of death \((39)\). Further work is being conducted to refine the clinical utility for trastuzumab and additional biomarkers to identify the further subset of HER-2/neu-positive patients that respond to trastuzumab.

The next generation of therapies is designed to suppress specific cellular protein signal pathways (rather than a general cytotoxic approach), driving the cancer cell. Clinically, the most successful example of this approach involves the highly selective tyrosine kinase inhibitor, imatinib, in the treatment of chronic myelogenous leukemia and gastrointestinal stromal tumors. The new generation of agents is small molecules with selective tyrosine kinase inhibitory activity (e.g., erlotinib, imatinib, sunitinib, sorafenib) or antibodies to epidermal growth factor receptors (EGFRs), CD20, or vascular endothelial growth factor (cetuximab, bevacizumab, panitumumab, rituximab). The evolving role of \(K\)-ras mutations as a predictor of the lack of the effectiveness of EGFR inhibitors underscores the importance of understanding the comprehensive pathway in targeted therapies.

The EGFR is transmembrane receptor that is a family of molecules both required for normal cell development as well as for the proliferation and growth of malignant cells, particularly lung, pancreas, head and neck, and colorectal cancers \((40)\). Both small-molecule inhibitors of the receptor are currently available (erlotinib, gefitinib) as well as monoclonal antibodies (panitumimab and cetuximab) that inhibit this family of receptors.

At best, the efficacy of these drugs are modest, with an RR of 8% with cetuximab in colorectal cancer, a prolonged stable disease rate of 31% compared with 11% given the best supportive care alone, and an improvement in survival of 1.5 months; more
than 50% of patients did not go beyond a single disease assessment due to early progressive disease (41). Even though the EGFR signaling pathway is important in cancer cell proliferation, it is not the only step in this pathway and can easily be bypassed by alternative or downstream pathways. Subsequent to one of the pivotal studies that led to the approval of cetuximab, the role of K-ras was studied (42). K-ras is a small G-protein downstream of EGFR, and mutations in exon 2 can become “activating,” hence isolating K-ras activity from upstream EGFR signaling. Karpetis et al. found that patients with wild-type (wt) K-ras treated with cetuximab had nearly double the survival (4.8 vs. 9.5 mos) compared with BSC; yet those patients with mutant K-ras treated with survival had the identical median survival (4.5 vs. 4.6 months) (Fig. 2) (41, 42). Hence, by understanding pathway activation, one could identify the appropriate treatment for a patient. Similar results were also seen for the monoclonal antibody panitumimab in colorectal cancer (43). It is expected that there will be other downstream molecules that are likely to be just as important as K-ras, as well as other parallel pathways as well, independent of the EGFR pathway.

Small-molecule EGFR inhibitors have been available for several years. They are associated with modest response rates, on the order of 10%. While these drugs are modestly effective for the overall population, a small percentage (8–15%) of patients have a dramatic response that lasted far longer than the average (i.e., years vs. months). Soon after the responder subgroup was first identified, clinical and epidemiologic criteria were found that predicted a higher-than-normal likelihood of benefit. These criteria included the following set: Asian race, adenocarcinoma histology, female, non-smokers. Researchers went on to identify the molecular basis for the dramatic responses by studying changes in the EGFR molecule that would correlate with these responses. Two groups of researchers in Boston, MA, identified mutations in the ATP-binding pocket of EGFR that strongly correlated with tumor response to gefitinib (44, 45). A subsequent study looked at the use of gefitinib as front-line therapy in patients with EGFR-activating mutations, and found a striking response rate of 55% (46). In this study, interestingly, only two patients with EGFR-activating mutations (out of 34) demonstrated resistance (i.e., early progression) on gefitinib, and subsequent analysis demonstrated that one had a subsequent mutation in EGFR associated with gefitinib resistance (T790M) while the other had MET amplification, which is also associated with gefitinib resistance (46). This work further underscores the need to understand the targeted pathway as well as the impact of various receptor mutations on determining responsiveness to specific targeted therapies.
Fig. 2. Comparison of overall survival for patients post cetuximab treatment based on K-ras mutation status. Non-small-cell lung cancer patients harboring wild-type or mutated K-ras were treated with cetuximab. Panel (A) shows results for patients with mutated K-ras tumors, and panel (B) for patients with wild-type K-ras tumors. Cetuximab as compared with best supportive care alone was associated with improved overall survival among patients with wild-type K-ras tumors but not among those with mutated K-ras tumors. The difference in treatment effect according to mutation status was significant (test for interaction, $P=0.01$). Reprinted with permission from NEJM (2008). Copyright © 2008 Massachusetts Medical Society. All rights reserved.
3. Conclusions

We hope to leave the reader with several thoughts going forward. First, trial design must ideally be hypothesis driven, biomarker guided, and individualized based on the biology of tumors. As good as modern imaging is, the use of response rate and overall survival has many limitations and should be applied only in the context of the overall clinical picture of the patient’s disease course and QOL. The traditional Phase II response rate end point based on RECIST is being supplanted by OS end points and “TTP”, i.e., the waiting time before the tumor begins to grow again after initial growth arrest. The thought of “prolonged stable disease” emerged, based on the above understanding, as a viable end point and surrogate for survival.

In the future, it is imperative to design studies that match a therapy with a specific molecular correlate of response: e.g., companion diagnostic. Techniques, such as genomic microarrays, reverse-phase protein microarray analysis, DNA mutation studies, and even IHC, are very important in identifying drug target pathways. Targeted therapy, by definition, treats a pathologic signaling pathway that drives the cancer. The responsibility falls to the designers of the clinical trial to identify the nodes in the pathway affected by a particular drug and to use this information to predict what surrogates or molecular end points can be used to stratify patients. In the past, patients were stratified by histology alone. In the future, DNA mutations in a particular gene, RNA transcript profiles, or proteomic profiles, including the activation state or phosphorylation of a protein in the target signal pathway, will constitute molecular “theranostics” (47). Stratification of patients by molecular profiling increases the likelihood of response while sparing toxicity with no treatment benefit. Molecular stratification may allow drugs with significant activity to be detected in small populations, obviating the time delay to accrue and study large populations of patients. Ideally, the oncologist of the future will not treat patients based on their specific organ category of disease, i.e., adenocarcinoma of the colon. Instead, they will treat the molecular pathway defect itself, which may be independent of histology.

4. Notes

References


Molecular Profiling
Methods and Protocols
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