Methodology of clinical trials with new molecular-targeted agents: where do we stand?

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In recent years, we have witnessed growing interest in the methodology of clinical trials with molecular-targeted agents. In phase I studies, alternative end points to toxicity have been proposed to define the optimal biological dose: the identification of a ‘target effect’, the measurement of ‘surrogates’ for biological activity and the assessment of drug plasma levels. However, these end points are not routinely incorporated into the study design and have rarely formed the primary basis for dose selection. In phase II studies, response rate remains the preferred end point in the early evaluation of new drugs. However, this approach might lead to rejection of potentially useful drugs when significant tumor shrinkage cannot be demonstrated. Therefore, a number of alternative end points have been proposed for agents that are not expected to cause a major tumor regression: time to progression, progression-free survival, overall survival, early progression rate and growth modulation index. In phase III trials, where efficacy in terms of survival remains the most important goal of the research, the major issues are the adequate selection of patients and the optimal clinical setting of evaluation of drugs. In conclusion, many important questions regarding the methodology of clinical research with target-based agents remain open and need to be defined by research in the near future.

Key words: targeted therapy, clinical trials, methodology

Introduction

Results currently obtained with standard chemotherapy for advanced solid tumors are still unsatisfactory. In recent years, the increased knowledge of molecular mechanisms that regulate cancer cell proliferation and survival, and the availability of a great number of new biological agents with well known, specific molecular targets, has led to a growing interest in the methodology of clinical trials with these drugs [1–4]. However, there are some crucial points, regarding the methodology of clinical research with target-based agents that still need to be defined:

- Is toxicity an adequate end point of phase I studies with molecular-targeted agents? Is it important to evaluate a ‘target effect’? How and where should the ‘target effect’ be evaluated?
- Which is the best end point to evaluate treatment activity?
- How should patients for phase III studies be selected? Which patients are expected to benefit from a targeted agent?

In this review, we will discuss the most important issues in the design and conduction of clinical trials with molecular-targeted agents, with a special emphasis on methodological implications.

Phase I studies

Traditional phase I studies for chemotherapeutic agents are designed to find the maximum tolerated dose (MTD) and the dose-limiting toxicity (DLT) of the drugs. The assumptions underlying phase I designs are that for most cytotoxic agents there is a direct relationship between the dose of a drug, its antitumor effect and toxicity. Therefore, toxicity and activity increase with the increasing of the dose of the drug and there is a recommended dose that provides clinical activity with acceptable toxicity [5–7]. Thus, toxicity has been seen as a surrogate for potentially effective doses. With biological agents, acting on highly specific targets expressed in cancer cells, the MTD may not be reached if the drug has a much wider therapeutic ratio: therefore, an increase of the doses to toxic levels may be not necessary to achieve the maximum activity and it may be an irrelevant end point. There are alternative end points for these agents that can be usefully employed in phase I studies: the identification of a molecular drug effect (the ‘target effect’), the measurement of ‘surrogates’ for biological activity and the assessment of drug plasma levels. However, these end points are not routinely incorporated into the study design and have rarely been the primary basis for dose selection [8, 9]. The identification of the ‘target effect’ through pharmacodynamic assays is proof of principle and can be proof of activity of the drug. The main application of pharmacodynamic studies is to help in the selection of the minimum target inhibiting dose (MTID) and the optimal schedule of administration of a drug [10].
Dose escalation trial designs based on molecularly targeted end points have recently been proposed, with the aim of facilitating the testing of agents and achieving clinical benefit at much lower doses than would be obtained from a standard toxicity-driven design [11]. Failure to identify a dose that inhibits the target should probably be a main cause for interrupting the development of an agent. There are, however, several problems with the use of the target inhibition as a common end point of phase I studies [12, 13]. First is the difficulty in identifying the ‘pivotal’ target of a biological agent that plays a crucial role in the mechanism of action of the drug and in the metabolism of the cell. A target, although considered relevant for an agent on the basis of preclinical data, may not be responsible for the antitumor activity of the drug, due to other effects at the cellular level. A second issue is the lack of pharmacodynamic assays which have been adequately validated in preclinical models and are sufficiently sensitive, specific and precise [10].

Another issue is the frequent lack of adequate tissue sample in which to measure target inhibition, often requiring the use of ‘surrogate’ tissue, such as skin or peripheral leukocytes. The measurement of other surrogates for biological activity could be a reasonable end point for phase I trials. The demonstration of a biological optimal dose in leukocytes can contribute to designing the dose escalation of the drug. However, results obtained in surrogate tissues should be interpreted cautiously. For example, pharmacodynamic studies of anti-EGFR agents have shown that blockade of the EGFR in the skin is associated with a significant reduction in the activation of signaling pathways downstream the EGFR, induction of p27kip1, and reduction in cell proliferation. In contrast, blockade of EGFR in the tumor might not be associated with effects on downstream signaling and tumor cell proliferation [14].

These findings suggest that biochemical effects of target-based agents in the tumor might not be predicted by analysis of surrogate tissues. Another approach exploits new imaging techniques, such as MRI and PET scan, with the aim of measuring changes in vascular permeability, blood flow and glucose uptake [15, 16]. The assessment of the blood levels of drugs can be useful, supposing that blood levels correlate with target inhibition and biological activity in animal models. In conclusion, when designing phase I trials with a molecular-targeted agent, it is important to measure not only the toxicity and the MTD, but also the pharmacokinetic and the pharmacodynamic of the agent, with the aim of identifying the MTID. If MTD and MTID correspond, the selection of the optimal biological dose is easy. If there is a difference between MTD and MTID, then the selection of the dose recommended for further development of the drug will be based on the evaluation of toxicity, on the distance between the two doses and, especially, on the ‘confidence’ of the researchers on the ‘target effect’.

**phase II studies**

The traditional end point of a phase II study is to demonstrate the activity of a drug, evaluating the response rate with standard criteria (e.g. RECIST) [17], with the aim of identifying the most promising agents to test in randomized phase III trials [18]. Objective response is still the most useful end point for phase II studies with molecular-targeted agents. The response rate has been reported at 10%–15% for patients treated with these agents as monotherapy (trastuzumab, gefitinib, erlotinib, cetuximab, etc). However, in most cases, this approach is not adequate for the development of molecular-targeted agents, which are thought to be cytostatic rather than cytotoxic, and are only expected to inhibit tumor growth, without tumor shrinkage and evidence of objective response. Moreover, in some cases the evidence of disease progression is not necessarily a sign of inactivity of a biological agent, considering that a delay in tumor growth may be the expression of cytostatic activity. Therefore, if we consider response rate as the only end point for a phase II study with a molecular-targeted agent, there is the potential risk of underestimating or rejecting potentially active drugs.

For all these reasons, a major challenge in the last few years has been the research of alternative statistical designs or new end points that could adequately select new biological drugs for further development. Possible alternative end points for phase II trials with agents which are not expected to cause major tumor regression: time to progression (TTP), progression-free survival (PFS), overall survival (OS) and early progression rate. Median TTP has the advantage of being a well standardized end point, but it needs frequent and rigorous procedures of evaluation. Moreover, it is affected by the variability of tumor growth. PFS is more reliable than TTP, because it also includes death as an event. OS could be used in phase II trials when the prognosis of patients is poor or when a ‘sequence’ of treatments has to be studied. Early progression rate is the proportion of patients with early progression, generally evaluated within the first 6–8 weeks of treatment [2].

An agent is declared ‘uninteresting’ and rejected from further evaluation if the progression rate with this agent is above a ‘maximum’ progression rate that is established *a priori* and is different according to tumor type.

A more complex end point for phase II trials is the growth modulation index that compares, for each patient, the TTP observed with the cytostatic agent as second-line treatment with the TTP observed with the first-line therapy [19]. If the TTP is at least 33% longer during the treatment with the cytostatic agent, then the drug can be considered active and suitable for further investigation.

A possible alternative methodological approach is the randomized discontinuation design, in which all patients initially receive the investigational agent [20, 21]. After a fixed period (3–4 months), patients who respond to treatment continue to receive the drug, whereas those with disease progression are taken off study, while patients with stable disease are randomly assigned to continue the administration of the drug or to interrupt it (observation/placebo) for a fixed period. The randomized discontinuation design can be an efficient means for early development of molecular-targeted agents when a reliable assay for identifying sensitive patients is not available [22]. The objective of this design is to use an enrichment strategy to focus on the patients who are more likely to benefit from the drug [23]. With this approach it is possible to distinguish the stabilization of disease due to natural history from those due to the effect of the treatment.
Another methodological approach often chosen to test the activity of new drugs is the randomized phase II design [24]. However, caution should be used in the proper interpretation of these trials that are not formally comparative. The only aim of the standard arm is to calibrate the interpretation of the results obtained in the experimental arm, but of course promising activity of the latter should be confirmed in a subsequent, comparative phase III trial [25]. In conclusion, response rate can no longer be considered the only primary end point for phase II studies with molecular-targeted agents: alternative end points and new methodological design, although not yet actually validated, should be carefully considered in addition to or instead of response rate when selecting agents of interest for further evaluation.

**phase III studies**

Phase III trials with molecular-targeted agents have similar designs and the same end points in terms of efficacy (OS, quality of life, etc.) to those with cytotoxic agents. However, several key questions remain to be answered, in particular regarding the selection of patients and the optimal clinical setting for the evaluation of molecular targeted compounds. Histological diagnosis in oncology is the cornerstone for treatment planning and, until now, clinicians have considered that tumors with the same histology should be treated as a single disease. However, we have all learned that response to the same treatment can be different in spite of the same histology and that variations in response are due to differences in gene expression [26]. Therefore, molecular characterization of tumors may supplant or add to traditional histological diagnosis as eligibility criteria for phase III trials and might become an important stratification variable.

The identification of a molecular target that allows the identification of responding versus non-responding patients to a molecular-targeted agent could have important implications for the design of randomized trials evaluating the efficacy of the drug. In fact, the presence of unrecognized molecular heterogeneity, conferring different risks to patients, can result in a falsely negative study. Such a study could be underpowered and may fail to detect a truly effective new therapy, leading to the rejection of a potentially useful drug [27]. With molecular characterization, patient selection will be more target-oriented and no longer, or much less, disease-oriented.

It is possible in the near future that, rather than studies in breast, colon, lung cancer, etc., there will be studies in patients with tumors expressing a particular target, stratifying for disease. However, the absence of selection of patients based on molecular criteria should not preclude the possibility of studying *a posteriori* predictive factors of activity. From this point of view, the availability of tumor tissue samples of patients enrolled in clinical trials appears crucial and it should be considered mandatory for future phase III clinical trials with molecular-targeted agents.

A second key question concerns the optimal clinical setting of evaluation for these compounds, preferably constituted of patients with a small tumor burden, as suggested by preclinical studies. Patients with a large tumor burden, pretreated with several lines of chemotherapy and resistant to conventional therapies, may receive minimal benefit from therapy with target-based agents. For this reason, the best option to validate most of these compounds is probably the adjuvant setting.

**conclusions**

There are several key issues in the design and conduction of clinical trials with molecular-targeted agents (Table 1). Part of the difficulty in translating preclinical efficacy to the clinical setting is the lack of valid, predictive preclinical models; the dose and schedule tested may be sub-optimal and bulky disease may not be responsive. Several unanswered questions also remain regarding the optimal trial design in the early clinical development of molecular-targeted agents. The final efficacy of these agents should be validated in appropriately designed phase III trials, which must include tissue or circulating surrogate biomarkers of efficacy, biologically-driven criteria of patient selection, well-defined schedules of treatment and predictive markers of activity and toxicity. In phase III trials, another intriguing issue seems to be the choice of the proper modality of administration of these drugs in combination with cytotoxic agents, hormone therapy and radiotherapy. It is unlikely that

| Table 1. Differences between clinical trials with conventional chemotherapeutic agents and with molecular-targeted agents |
|---|---|---|
| Phase I | Chemotherapeutic agents | Molecular targeted agents |
| **Objectives** | Maximum tolerated dose (MTD) | Minimum target-inhibiting dose (MTID) |
| **Toxicity** | Toxicity | Toxicity |
| **Pharmacokinetics** | Pharmacokinetics | Pharmacodynamics |
| **Patients selection** | Various tumors | Various tumors (target oriented?) |
| **Dose** | Increasing up to MTD | Increasing up to MTID (not necessarily up to MTD) |
| **End point** | Toxicity | Effective inhibition of molecular target |

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<th>Chemotherapeutic agents</th>
<th>Molecular targeted agents</th>
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<td><strong>Patients selection</strong></td>
<td>Disease-oriented</td>
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<td><strong>Dose</strong></td>
<td>One dose-level before MTD</td>
<td>MTID? MTD?</td>
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<td><strong>End point</strong></td>
<td>Objective responses</td>
<td>Objective responses? Progression rate? Time to progression? Other?</td>
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<th>Chemotherapeutic agents</th>
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a single molecular-targeted agent, administered in the setting of advanced disease, may result in the cure of the tumor and the majority of biological approaches have not yet demonstrated the possibility of inducing complete responses.

In conclusion, a large number of molecular-targeted drugs are actually in clinical development. A tightened collaboration between laboratory and clinical researchers is required in order to avoid missing activity and to define relevant biological end points. This would lead to true ‘translational’ research, crucial for the correct development of these new antitumoral therapies.

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references