Sequential research-related biopsies in phase I trials: acceptance, feasibility and safety

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Background: Sequential tumour biopsies are of potential interest for the rational development of molecular targeted therapies.

Patients and methods: From June 2004 to July 2009, 186 patients participated in 14 phase I clinical trials in which sequential tumour biopsies (13 trials) and/or sequential normal skin biopsies (6 trials) were optional. All patients had to sign an independent informed consent for the biopsies.

Results: Tumour biopsies were proposed to 155 patients and 130 (84%) signed the consent while normal skin biopsies were proposed to 70 patients and 57 (81%) signed the consent. Tumour biopsies could not be carried out in 41 (31%) of the 130 consenting patients. Tumour biopsies were collected at baseline in 33 patients, at baseline and under treatment in 56 patients. Tumour biopsies were obtained using an 18-gauge needle, under ultrasound or computed tomography guidance. Only nine minor complications were recorded. Most tumour biopsy samples collected were intended for ancillary molecular studies including protein or gene expression analysis, comparative genomic hybridization array or DNA sequencing. According to the results available, 70% of the biopsy samples met the quality criteria of each study and were suitable for ancillary studies.

Conclusions: In our experience, the majority of the patients accepted skin biopsies as well as tumour biopsies. Sequential tumour and skin biopsies are feasible and safe during early-phase clinical trials, even when patients are exposed to anti-angiogenic agents. The real scientific value of such biopsies for dose selection in phase I trials has yet to be established.

Key words: biopsy, cancer, early clinical trials, phase I, research biopsies, sequential biopsies

Introduction

Considerable progress has been achieved in the management of cancer over the last decade with the advent of molecular targeted agents (MTA) [1]. Greater knowledge of cancer biology has led to the isolation of many new and promising molecular targets and compounds targeting these abnormalities.

Recently, tyrosine kinase inhibitors or monoclonal antibodies targeting epidermal growth factor receptor (EGFR), Human epidermal growth factor receptor 2, Vascular endothelial growth factor factor and Vascular endothelial growth factor receptor yielded a substantial clinical benefit in patients with advanced breast cancer (trastuzumab, lapatinib and bevacizumab), colon cancer (cetuximab, panitumimab and bevacizumab), head and neck cancer (cetuximab), non-small-cell lung cancer (bevacizumab, erlotinib and gefitinib) and metastatic renal cell carcinoma (bevacizumab, sunitinib and sorafenib). For some of these targeted drugs, translational research carried out on tumour samples during drug development led to the identification of major biomarkers such as EGFR and KRAS mutations, allowing a better selection of patients for a given treatment.

For these reasons, biomarkers reflecting drug activity have been widely proposed in the literature as candidate end points for newer agents, and clinical pharmacogenomics provide a powerful approach for investigating a myriad of putative markers during the early phase of drug development [2–4]. Moreover, drug development in oncology has one of the highest failure rates compared with that of drugs used to fight other diseases. The primary reason for such high failure rates is believed to be the lack of rigorous and predictive animal models, which properly reflect experiences in human beings [5, 6]. In this respect, identification of biomarkers during phase I clinical trials is appealing as they could serve, if prospectively validated, as surrogates for drug efficacy or toxicity. MTA and in particular kinase inhibitors are of two types: very specific clean drugs and multi-targeted drugs. Even though major targets are identified in the preclinical setting, reality in the clinic may be different.

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Predictive markers based on a single biopsy often fail to select the right patients while sequential biopsies may have added value by better apprehending the real pharmacodynamics of the drug and link it to the clinical response.

This study retrospectively evaluates the feasibility and safety of optional and mandatory sequential tumour and skin biopsies in patients with advanced cancer enrolled in early clinical trials at the Institut Gustave Roussy (Villejuif, France) between 2006 and 2009.

patients and methods

patients
Between June 2004 and July 2009, using a computerised database and systematic chart reviews, we identified the records of all patients enrolled in phase I trials who had undergone skin or tumour biopsies at the Institut Gustave Roussy (Early Clinical trials Unit, 'Service des Innovations Thérapeutiques Précoces', SITEP). We also identified the records of all patients who had undergone a tumour biopsy in the Interventional Radiology Department using the institutional computerised database (for cross-checking).

biopsies
Image-guided biopsies of the liver, abdominal masses, lung and deep-seated lymph nodes were carried out with serial passes using an 18-gauge cutting needle inserted through a 17-gauge needle guide, under ultrasound (US) or computed tomography (CT) guidance (Figure 3). The imaging modality that allowed the best tumour visualisation and easy access was chosen for image guidance. US was preferred for liver and soft tissue biopsy when possible, and CT was used for lung biopsies and when the tumour was inaccessible under US guidance. Most of these biopsies were optional with independent informed consent. Some patients had more than two biopsies, and some at relapse or later, at the time of progression during the same phase 1 trial. Sequential biopsies were mostly carried out at the recommended therapeutic dose.

quality of tumour biopsy samples
Tumour biopsy samples from six trials were analysed at the Institut Gustave Roussy, whereas the biopsy samples from the seven other trials were sent to the pharmaceutical sponsor for analysis. Sequential tumour biopsy samples of optimal quality were defined as a pair of sequential biopsy samples that were suitable for performing the analysis defined in each protocol. These analyses included Immuno histo chemistry, comparative genomic hybridization array, DNA sequencing or RNA-based gene expression analysis.

statistical analysis
All variables and patient characteristics were analysed with SPSS v.13 (Microsoft, Rdemond, DC).

results

patient characteristics
From June 2004 to July 2009, we participated in 13 phase I clinical trials in which patients were requested to give their consent to optional sequential tumour biopsies (13 trials) as well as skin biopsies (6 trials), so that we would be able to undertake correlative laboratory studies. A total of 186 patients with histologically confirmed malignant solid tumours were enrolled in those trials. Fifty-nine percent were male, median age was 59 years (range, 18–72 years), and all had satisfactory Eastern Cooperative Oncology Group performance status scores (range 0–1). The most common histological types were lung tumours (21%), colorectal adenocarcinoma (11%) and sarcomas (12%) (Table 1). Twenty-one patients received monoclonal antibodies while 166 received MTA with or without chemotherapy.

skin and tumour biopsies at baseline
Among the 186 patients who participated in these trials, a total of 156 accepted to undergo research-related biopsies: 130 accepted tumour biopsies and 57 normal skin biopsies. The acceptance rate were 81% and 84% for skin and tumour biopsies, respectively (Figures 1 and 2). Fifty-six patients did not sign the informed consent to undergo tumour biopsies, 31 patients were not offered the procedure by the investigator (15 of those patients were enrolled in an expansion cohort no longer requiring biopsies) and 25 directly refused to participate in biopsy-based ancillary studies. Nevertheless, a total of 145 biopsies were carried out in 89 (68%) of the 130 consenting patients. The reasons for failure to obtain tumour material in 41 patients who accepted a tumour biopsy were because the procedure was deemed too dangerous (5 patients) by the interventional radiology physicians or because of logistic problems (i.e. competitive blood sampling for pharmacokinetics, positron emission tomography scanning the same day; 18 patients), technical problems (i.e. CT scan breakdown; 8 patients) or unknown reasons (10 patients).

biopsies on therapy
Forty patients underwent two sequential skin biopsies, and 57 patients had two sequential tumour biopsies (Figure 4).

In the 33 patients with only the baseline tumour biopsy, the second biopsy was not carried out due to early progression in 17, minor complications at the time of the first biopsy in 4, the patient’s refusal in 5 and to unknown reasons in 7.

Table 1. Patient characteristics

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Number of patients</th>
</tr>
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<tbody>
<tr>
<td>Number of patients</td>
<td>186</td>
</tr>
<tr>
<td>Median age at diagnosis (years)</td>
<td>59 (22–72)</td>
</tr>
<tr>
<td>Gender</td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>111 (59%)</td>
</tr>
<tr>
<td>Female</td>
<td>75 (41%)</td>
</tr>
<tr>
<td>Performance status at inclusion</td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>71 (38%)</td>
</tr>
<tr>
<td>1</td>
<td>115 (62%)</td>
</tr>
<tr>
<td>Tumour types</td>
<td></td>
</tr>
<tr>
<td>Lung</td>
<td>40 (21%)</td>
</tr>
<tr>
<td>Colon</td>
<td>21 (11%)</td>
</tr>
<tr>
<td>Sarcoma</td>
<td>22 (12%)</td>
</tr>
<tr>
<td>Gynaecological</td>
<td>22 (12%)</td>
</tr>
<tr>
<td>Melanoma</td>
<td>18 (10%)</td>
</tr>
<tr>
<td>Head and Neck</td>
<td>12 (7%)</td>
</tr>
<tr>
<td>Gastrointestinal other than colon</td>
<td>12 (7%)</td>
</tr>
<tr>
<td>Mesothelioma</td>
<td>10 (5%)</td>
</tr>
<tr>
<td>Breast</td>
<td>6 (3%)</td>
</tr>
<tr>
<td>Urological</td>
<td>5 (2%)</td>
</tr>
<tr>
<td>Other</td>
<td>18 (10%)</td>
</tr>
<tr>
<td>Prior treatment</td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>176 (95%)</td>
</tr>
<tr>
<td>No</td>
<td>10 (5%)</td>
</tr>
</tbody>
</table>
The most frequent sites of these sequential tumour biopsies were: liver (38%), lung (19%) and lymph nodes (7%).

Image-guided biopsies of the liver, abdominal masses, lung and deep-seated lymph nodes were carried out with serial passes using an 18-gauge cutting needle inserted through a 17-gauge needle guide, under US or CT guidance.

safety
No complications were observed with skin biopsies. Nine minor complications were recorded during 145 tumour biopsies: one episode of subcapsular hepatic bleeding, five cases of grade I pneumothorax (that did not require any additional treatment and resolved spontaneously), two episodes of bleeding at the biopsy site (cervix and skin, relieved with simple local measures) and one episode of acute pain.

tumour biopsies and anti-angiogenic therapies
Thirty-two of the sequential biopsies were carried out in patients treated with an anti-angiogenic agent. Two of them experienced complications: minor subcapsular hepatic bleeding and grade I pneumothorax. The rate of complications was similar in patients biopsied under anti-angiogenics (2/32, 6%) compared with those who were not under anti-angiogenics (7/113, 6%).

quality of tumour biopsy samples
The information concerning the number of adequate quality sequential biopsy samples allowing the conduct of the predefined analysis was obtained from the Institut Gustave Roussy genomics platform for six trials analysed in our centre and from five of the pharmaceutical sponsors. Of the 57 paired tumour biopsy samples, 6 were lost due to packaging issues or during dispatch to the sponsor, leaving 46 pairs of biopsy samples analysed, because the results of 5 paired biopsy samples were not obtained from the sponsor. Seventy percent of all the biopsy samples met the quality criteria required for each ancillary molecular study (percentage of tumour cells, nucleic acid quantity and quality specifications ...).

In trials where more than five patients had undergone a biopsy, the rate of adequate quality samples was 80%. Gynaecological and liver tumour samples yielded the best quality. The quality of the biopsied material was poorer in the case of skin metastasis and lung samples, mostly due to an insufficient percentage of tumour cells in the sampled tissue.

discussion
In this study, we demonstrated that the vast majority of patients included in early trials gave their consent for sequential biopsies even if they were carried out exclusively for research.

Figure 1. Flow chart skin biopsies.

Figure 2. Flow chart tumour biopsies.
purposes with no individual benefit. This is in line with data reported by other phase I trialists. In the everolimus phase I trial, Tabernero et al. [7] reported an acceptance rate of 78% and 60% for serial skin biopsies and sequential tumour biopsies, respectively. In a gefitinib pharmacodynamics study in patients with a gastrointestinal tumour, Rojo et al. [8] obtained a 93% success rate for a tumour biopsy at baseline but a 50% success rate for biopsies on therapy.

In our cohort, only 16% of the patients actively refused tumour biopsies. Interestingly, the informed consent form regarding optional biopsies was not proposed to 15 of the patients treated in our phase I trials, despite the fact that this is an early clinical trials unit with a strong commitment to collecting such biopsy material for research. It is noteworthy that most of the ‘missed’ biopsy proposals were observed at the time of the expansion cohort in the context of a fast and competitive multicenter recruitment period. Even if we did not explore the reasons for these ‘missed proposals’ in this study, two hypotheses could be put forward. First, some investigators may have considered that time-consuming scheduling of biopsies causes delayed treatment in a competitive environment. Secondly, during the expansion cohort, some investigators who were already aware of the limited magnitude of drug activity might have been less eager to propose tumour biopsies. Certain strategies could increase the commitment of investigators to propose research-related biopsies, like providing a strong rationale and detailed description of the analysis to be carried out and reporting preliminary results, at least on quality assessment, during an ongoing study.

Furthermore, for multicenter trials, a predefined publication policy with a higher impact for patients included with optional biopsies (i.e. a patient with a paired biopsy would be equivalent to two patients with no biopsies) could also be discussed to increase commitment to obtaining such material.

In our series, only 4% of potential biopsies were considered technically dangerous in consenting patients. Moreover, >14% of the biopsies were not carried out because of a slot scheduling problem in the interventional radiology unit and other logistic problems. This observation underlines the need to define a preferential route between the early trials unit and the interventional radiology unit in order to guarantee high-quality tumour biopsy samples as well as the investigator’s commitment to proposing optional biopsies to patients who have given their informed consent. Furthermore, the capabilities of the interventional radiology unit should be designed to be able to perform a predefined number of research-related biopsies without interfering with ‘diagnostic’ or ‘therapeutic’ procedures.

Phase I trials are designed to evaluate the safety and toxicity of new therapeutic agents, to ascertain the pharmacokinetics of those agents and to determine the safest dose for subsequent testing. Several early clinical trials also aim to assess the biological activity of drugs, even in phase I or II, which underscores the importance of tumour biopsies and functional imaging [9,10]. Biomarkers have been proposed as potentially useful end points for phase I trials of new anticancer agents. The aims of these approaches are to identify the mechanisms of treatment effects (in vivo, at the tumour level), the main biological pathway/gene ideally targeted or associated with response to therapy or treatment toxicity, putative biomarkers associated with the biological effects of treatment and early putative surrogate markers able to provide information on treatment efficiency or toxicity. Biomarkers could be useful for correlating clinical data with target modulation. Sequential skin biopsies are very easy to perform before and after the administration of an investigational drug [7, 8, 11, 12]. In some cases, pre- and post-treatment normal skin biopsy samples could be used as a surrogate method for evaluating target modulation rather than serial tumour biopsies, with the advantage of being more accessible and with a lower risk of morbidity. Evidence from phase I trials of cetuximab targeting the EGFR suggests that normal skin biopsies can serve as surrogate tissue to demonstrate target modulation by the experimental agent [12]. Changes in gene or protein expression, gene mutations or protein phosphorylation between baseline and on-treatment skin tissue are ways of analysing the biological effect of new treatments on molecular pathways in cancer cells in vivo. However, skin biopsies do not provide information on whether the target has been modulated in malignant cells. The advent of molecularly targeted drugs entering clinical trials has stimulated the use of biomarkers to correlate clinical empirical data with the presence of the target in the tumour. Archived slides of primary tumours are sometimes analysed to characterise molecular abnormalities of cancer, whereas some techniques require fresh frozen material. However, cancer is a Darwinian evolutionary process during which its molecular characteristics are modified during the natural history of the disease, from the primary lesion to the metastatic disease, but also under the effects of drugs in the different lines of treatment. Thus, the molecular analysis of old archived material may not be the best reflection of the biology of advanced and metastatic disease. Paired sequential tumour biopsy samples at baseline and on treatment allow us to collect fresh frozen samples suitable for most analytical techniques and also to focus on drug effects based on a low number of samples without under powering the statistical analysis. Moreover, sequential biopsy samples can help investigators...
identify a genomic profile of response, particularly in the case of drugs without clear preclinical predictive factors for response, because the 'background noise' of the first biopsy sample will be eliminated. In fact, we recently demonstrated the potential value of gene expression analysis using sequential biopsy samples in a phase I trial of a new cyclin-dependent kinase inhibitor [11]. Despite the high heterogeneity of tumour biopsy specimens and the limited number of patients, we demonstrated that gene expression profiling of sequentially collected biopsy material is a powerful way to identify putative biomarkers in early clinical trials [10, 11].

In the context of phase I trials, the quality of biopsy material is a major issue for optimal biomarker analysis. Recommendations on the design of early trials with biomarker evaluation should be completed with guidelines on how to obtain 'optimal' quality biopsy material for these evaluations [13]. Currently, this point is dependent on the expertise of the interventional radiology team, on the use of new procedures such as biopsying under contrast-enhanced US in vascularised tumours [14] and on the relevance of standard operating procedures for archiving and shipping or transporting the biopsy sample to the final laboratory.

Figure 4. (A) Description of patients and achieved tumour biopsies. (B) Description of patients and achieved skin biopsies.
The rate of sufficient biopsy material for analysis is not usually reported by early clinical trial investigators. Our data are poorer than those published by Rojo et al. [8], who reported a 90% rate of sufficient quality material in sequential paired stomach and gastro-oesophageal junction biopsy samples for immunohistochemical analysis [8]. One of the explanations for a lower percentage of sufficient quality biopsy specimens in our experience could be that most of them were sampled at metastatic sites and the predefined analysis mostly involved genomic analysis on RNA which is more fragile. Even if these invasive procedures could be safe for our patients, in experienced hands, there is an intrinsic risk of harming them. Correlative biomarker studies in phase I trials are the first steps to understanding drug activity in vivo in human tumours. However, solid preclinical data are not always available to justify the conduct of a biomarker analysis nor is there always an appropriate trial design enabling one to answer the ‘biomarker’ question or a predefined timing for the sample analysis. For example, only half of the results of the biomarker analyses in our series were communicated to the investigators when this manuscript was written. During recent years, these issues have raised justified concern in the medical and patient communities about the ethical dimension of performing optional or mandatory biopsies in clinical trials. This concern led to the elaboration of guidelines for the design of biomarker studies that should be considered by phase I trialists when examining a new phase I trial proposal at their institutions [13, 15].

In summary, our experience shows that despite the high acceptance rates from patients (>80%), various logistics and technical obstacles still exist, resulting in a much lower number of analysable samples (~50% of the original sample set). This underscores the importance of identifying and dealing with major bottlenecks in the process in order to improve the success rate of research biopsy sampling.

conclusions

In our study, the majority of the patients accepted skin biopsies (81%) as well as tumour biopsies (84%) despite written information clearly indicating that the biopsy specimens would be exclusively used for research purposes. This result is in agreement with a recent report, which showed that most patients enrolled in clinical trials with sequential tumour biopsies easily tolerate biopsy procedures and readily give permission for their specimens to be tested for research purposes [16, 17]. Strong collaboration and commitment between the oncologist, interventional radiologist and researchers are crucial for a tumour biopsy programme in early trials. The scientific value and ethical dimension of such biopsies were not addressed in the present series and remain an area of intense debate in the oncology community [13, 15, 18].

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references