Requirements to Assess Feasibility of Phase 0 Trials during Major Abdominal Surgery: Variability of PARP Activity

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Abstract

Purpose: The aim of this study was to evaluate the feasibility of phase 0 trials in the setting of a routine surgical procedure. Logistic considerations, tissue sampling and tissue handling, and variability of a biomarker during surgery, in here PARP, were evaluated.

Experimental Design: Patients with highly suspicious or proven diagnosis of advanced ovarian cancer, planned for debulking surgery were asked to allow sequential tumor biopsies during surgery. Biopsies were frozen immediately and PARP activity was measured subsequently.

Results: Baseline biopsies were obtained from eight patients after a median time of 88 minutes (minimum of 50 to maximum of 123 minutes). Second and third biopsies were obtained after a median of 60 (32–96) and 101 (79–130) minutes, respectively. Mean tumor load was 44% (5%–100%), with a cellular viability of 98% (85%–100%). Median baseline PARP activity was 1035 pg/mL (range, 429–2663 pg/mL). The observed interpatient variability at baseline was large: SD was 0.59 after natural logarithm transformation.

Conclusions: Conducting phase 0 trials during surgery seems to be feasible in terms of logistic considerations. In preparation of a phase 0 trial during surgery, a feasibility study like this should be conducted to rule out major interactions of the surgical intervention with respect to the targeted biomarker.

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Introduction

Phase 0 trials have recently been introduced as new clinical research concept to improve the efficiency of drug development (1, 2). If the new agent fails to show biologic activity in a phase 0 trial, further clinical development, with an ineffective treatment of many patients in phase I to III trials and excessive costs is abandoned. It has been shown previously that subtherapeutic doses of a PARP-inhibitor reduced PARP-activity indicated through PAR expression in sequential tumor biopsies in patients with a broad variety of refractory advanced malignancies (1). These biopsies were obtained in local anesthetics percutaneously or by a dermal biopsy punch in preclinical (3) and in the first in-human phase 0 trial (1). One limitation of this approach is the need of tumor formations accessible to biopsies, which is not warranted in all tumor entities. Another problem is the application of this procedure in patients who were heavily pretreated with refractory disease. Tumors treated with several lines of chemotherapy show genetic differences in comparison with primary tumors (4, 5), develop resistances (6, 7), and thus are not representative for primary, often curable disease. One way might be to conduct phase 0 trials during primary surgery for different diseases, like colon, gastric, endometrial, and ovarian cancer.

This work was sought to evaluate prospectively the feasibility to conduct phase 0 trials during surgery for advanced ovarian cancer undergoing primary debulking surgery. Therefore, the focus was on logistic considerations during surgery and on variability of PARP as biomarker.

Materials and Methods

Patients counseled for debulking surgery for advanced ovarian cancer gave written informed consent to allow sequential tumor biopsies during surgery. This prospective feasibility study was appraised and approved by the Local Governmental Ethics committee of the Landesaerztekammer Hessen, Germany.

The goal of surgical intervention was complete debulking. Therefore, median longitudinal incision from the pubic bone to the xiphoid process was made and a thorough examination of the abdominal cavity was carried out. A
tumor formation which was thought to outwear in situ for at least 2 further hours was identified. A part of this tumor was cut out and immediately transferred to a nonsterile workplace. A biopsy needle (Temno 18-gauge; Nicolai GmbH) was passed into the tumor and the tissue was collected. The collected material was frozen immediately and this procedure was repeated at the same tumor localization after 1 and 2 hours. Frozen specimens were stored at −80°C until use. The amount of viable and necrotic tissue content was approximated in each sample.

The detailed steps of tumor extract preparation and PAR assay were described elsewhere before (3). The commercial available PARP in vivo pharmacodynamic assay was used for the analyses (Trevigen Inc., Gaithersburg, MD) and manufacturers’ instructions were followed accurately. In brief, the serially diluted PAR standards and diluted test samples (Jurkat Cell Lysate Standards) and sample buffer (background control) were assayed in triplicate. To reach the maximal binding equilibrium, the wells were covered with sealing film and incubate the strip wells overnight at 4°C. PAR polyclonal detecting antibody was diluted 1:250 fold in antibody diluent and incubated at 25°C one hour before use. The horseradish peroxidase (HRP) conjugate was diluted 1:250 in antibody diluent and incubated at 25°C one hour before use. Diluted goat anti-rabbit IgG-HRP conjugate (50 μL per well) was added, and wells were incubate at 25°C for 1 hour. Prewarmed Place PeroxyGlow A and B reagents were mixed with equal volumes and immediate chemiluminescent readings were conducted. The validation criteria of the assay were met, and double measurement was conducted between July, 2010 and November, 2010. One appendix carcinoma and 1 metastatic gastric cancer were diagnosed in the frozen section; thus both patients were excluded from further biopsies. One patient had recurrent ovarian cancer of endometrioid histology. This patient was included in the tumor biopsy analyses, but was excluded for PARP activity analyses.

One patient underwent interval and 6 patients underwent primary debulking surgery. Median age of the included patients was 62.5 years, ranging from 40 to 71 years.

Median time between begin of anesthesia and first tumor specimen sampling (n = 8) was 87.5 minutes (range, 50.0–123.0 minutes) and median time between specimen sampling out of the abdominal cavity (n = 24) and freezing was 1.25 minutes (range, 0.5–13.5 minutes). Second biopsies after baseline assessment were obtained after a median time of 60 minutes (range, 32.0–96.0 minutes) and third biopsies after 100.5 minutes (range, 79.0–130.0 minutes). Median weight of samples obtained with the 18G biopsy needle was 5.0 mg (range, 1.4–12.4 mg).

Out of all 24 examined samples, mean tumor load was 44.2% (ranging between 5% and 100%). Cellular viability was high and nonnecrotic tissue was observed in mean 97.8% (ranging between 85% and 100%) of all cases (Fig. 1).

Reliability of the results was high with SDs in the measurements ranging between 3.7 and 84.5. Median PARP activity measured by the concentration of the active metabolite PAR was 1033.8 pg/ml (range, 422.0–2730.5 pg/ml), 893.6 pg/ml (range, 284.9–2188.8 pg/ml) and 1241.8 pg/ml (range, 506.0–1702.2 pg/ml) of the biopsy at beginning of surgery, at 2nd biopsy and 3rd biopsy, respectively.

Translational Relevance

Phase 0 trials have been introduced to the armamentarium of clinical drug development most recently. Patients with heavily pretreated cancers are subjected to the drug in development and activity of an influenced biomarker is analyzed in tumors before and after treatment. This study shows that it is possible to conduct phase 0 trials during major abdominal surgery and to omit problems apparent during already conducted phase 0 trials. Tumor formations do not have to be accessible by biopsy and chemotherapy-naïve tumors better reflecting tumor biology than heavily pretreated tumors are used.

Results

Ten patients gave written consent to enter this study conducted between July, 2010 and November, 2010. One appendix carcinoma and 1 metastatic gastric cancer were diagnosed in the frozen section; thus both patients were excluded from further biopsies. One patient had recurrent ovarian cancer of endometrioid histology. This patient was included in the tumor biopsy analyses, but was excluded for PARP activity analyses.

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(Table 1). However, in repeated measures ANOVA no statistically significant differences between the 3 time points were observed \( (P = 0.657) \).

To use this concept for further interventional procedure, the cutoff values for 2nd and 3rd biopsy compared with 1st biopsy were evaluated, at which a significant change would have been observed. It is shown in Table 1 that the baseline SD across all patients was 0.59 after natural logarithm transformation and the corresponding cutoff value was \(-1.07\), corresponding to a 65\% reduction. In accordance to the fact that the patients in our study did not receive any treatment, no significant alterations of PARP activity in comparison with baseline assessment were observed in 6 out of 7 evaluated patients, neither in second nor in third biopsies (Fig. 2). With respect to the relevance criterion, 2 patients showed at least a 50\% reduction of baseline PARP activity: ID 2 in the second biopsy and ID 8 in the third biopsy, respectively.

Discussion

One way to validate the activity of new drugs in humans is to conduct phase 0 studies (1). A necessary prerequisite is a reliable biomarker, which indicates activity of an enzyme or the presence of an active metabolite (1, 2). One phase 1 trial in prostate cancer patients used a PD endpoint assayed in surgical specimens of patients who were treated with an antisense oligonucleotide some weeks before (9). However, this study is the first, to our knowledge, to investigate the variability of a biomarker in cancer during a major abdominal surgical procedure, with a specimen taken at the beginning and again in the course of surgical intervention. This prospective study was rather designed to highlight the feasibility to conduct phase 0 trials during major surgical intervention with PARP activity as biomarker than to carry out a phase 0 trial. Consequently, main criteria defining a phase 0 trial were not met with this study because no agent was administered, and thus no effect on target could be evaluated. Instead, the primary questions addressed by this study were whether it is possible to obtain sequential tumor biopsies during major surgery and whether reliable values of PARP activity could be obtained.

Although sequential samples should have gathered at 1 and 2 hours after the baseline sample, second biopsy was taken timely in 57.1\% of all cases and third biopsy in only 28.6\%. This is not inevitable in most cases, but as ongoing surgery requires concentration and intraoperative complications have to be solved immediately, tight schedules might not be maintained. We approximated the quantitative levels of cellular content of the samples at each time point by histopathologic examination. Although sampled tumor formations, identified in the abdominal cavity, were obvious knotty and looked malignant, the mean tumor load of all samples was under 50\%, but with high cellular viability. In the preclinical model, using human melanoma cells in mice, subcutaneous tumor cell suspensions were injected, and it was reported that cellular content of the subsequent tumor formations showed "good cellular content" with no further quantification (3). Kummar and colleagues have not reported the tumor load of the samples in their study (1), but it is most likely, that at least the nonskin-associated biopsies were contaminated with other tissues, too.

One of the critical steps in using PAR as biomarker might be the rapid degradation (10–12), thus straightforward freezing after obtaining the biopsy is very important. In this study, it was possible to remove the tumor, to obtain a biopsy and to freeze the sample in less than 1 minute in 62.5\% of all samples. Another most important aspect of PAR as biomarker in tumor tissue is the viability of tumor cells because necrotic cells have higher levels of PAR (13). This might be one of the reasons why the PARP activity in the ovarian cancer specimens we analyzed was essentially lower. The PAR content of the melanoma cell formations in the latter samples ranged between 4,102 and 7,066 pg/100 \( \mu \)g protein, whether the range in our cohort was 291.58 and 2,704.90 pg/100 \( \mu \)g protein. Other possible
The variability of PARP activity during major abdominal surgery is a critical aspect for planning phase 0 trials in cancer treatment. This variability can be influenced by factors such as the baseline PARP activity, the surgical procedure, and the anesthesiologic medication. The variability is assessed using statistical criteria combined with a biological effect, which helps to determine if a treatment-related PD response is significant.

### Table 1. Logarithmized PARP activity kinetics during surgery

<table>
<thead>
<tr>
<th>Patient ID</th>
<th>PARP activity at baseline* (SD)</th>
<th>PARP activity of 2nd biopsy* (SD)</th>
<th>PARP activity of 3rd biopsy* (SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>6.78 (0.022)</td>
<td>7.46 (0.155)</td>
<td>6.25 (0.021)</td>
</tr>
<tr>
<td>3</td>
<td>7.62 (0.024)</td>
<td>5.72 (0.012)</td>
<td>7.22 (0.004)</td>
</tr>
<tr>
<td>4</td>
<td>7.06 (0.006)</td>
<td>7.68 (0.006)</td>
<td>7.38 (0.045)</td>
</tr>
<tr>
<td>5</td>
<td>6.91 (0.015)</td>
<td>6.30 (0.034)</td>
<td>7.42 (0.052)</td>
</tr>
<tr>
<td>6</td>
<td>6.94 (0.012)</td>
<td>6.80 (0.012)</td>
<td>6.60 (0.048)</td>
</tr>
<tr>
<td>7</td>
<td>6.06 (0.012)</td>
<td>5.68 (0.014)</td>
<td>6.91 (0.031)</td>
</tr>
<tr>
<td>8</td>
<td>7.90 (0.032)</td>
<td>7.20 (0.016)</td>
<td>7.12 (0.021)</td>
</tr>
<tr>
<td>Median</td>
<td>6.94</td>
<td>6.80 (0.81)</td>
<td>7.13 (0.42)</td>
</tr>
<tr>
<td>SD all points</td>
<td>0.59</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Median activity of 3 measurements with each 2 measurement repetitions.

For phase 0 trials, it has been suggested to use 2 criteria indicating a significant PD response at individual patient level, a statistical criterion combined with a biological criterion. For individual patients, a treatment-related PD effect will be considered statistically significant at the 1-sided significance level of 10% if the change from baseline exceeds 1.8 times the baseline SD. A "minimum magnitude criterion" of 50% reduction of baseline PARP activity indicating a biological effect should be added (2). We would have found 1 patient with significant PARP activity reduction using those criteria (reaching the cutoff value of −1.07 and at least 50% reduction of PARP activity) although no active agent was administered, fitting to the defined error probability of 10%.

As previously described (1, 8), the interpatient baseline variance of the baseline PARP activity in our cohort was large in comparison with the expected intrapatient variance, but as we analyzed only 1 sample of each tumor this approach had to be used (2). In further investigations, several tumor samples at each time point should be analyzed to avoid unnecessary high variances and to obtain more exact cutoff values at which a significant activity change is present. The abundance of available tumor tissue during surgery is one further great advantage of the presented approach. Ethical concerns with respect to phase 0 trials have been described, whereas one important aspect was the limited access to tumor tissue via transectan biopsies, which is solved using this approach (14).

This experimental in-human study design (Fig. 3) in patients with ovarian cancer might be useful for at least 2 scenarios: first, urgently needed new drugs for the treatment of ovarian cancer affecting short half-life biomarkers might be tested in phase 0 trials. Moreover, this study design might be adapted to other entities which have to be treated surgically.

It remains speculative if other biomarkers would be stable during surgical procedures, like shown in here for PARP activity. Thus, it has to be shown previously that the primary PD endpoint is neither affected by surgical nor anesthetic interventions.

Second, it might be a useful system to identify patients with ovarian cancer who will be primary refractory or resistant to standard treatment with platinum containing drug regimens which is due to different mechanisms. The cytotoxic effect of platinum comes about building adducts between DNA strands, resulting in elevated frequencies of DNA strand breaks. These strand breaks will again be repaired in part by PARP. It has been shown previously that tumor cells being treated with cisplatin exhibit elevated PARP activity (15, 16). In the preclinical study conducted by Kinders and colleagues, topotecan application resulted in an increase of PARP activity, although this effect was not statistically significant (3). Patients with ovarian cancer undergoing debulking surgery might be treated with low doses of cisplatin during surgery. Consecutive high levels of PARP activity would indicate platinum-sensitive tumors, whereas nonelevated PARP activity might indicate primary nonplatinum-sensitive tumors.

An important aspect for planning phase 0 trials in during surgery is the meticulous selection of agents not affecting blood clotting, interacting with anesthetic medication, or antibiotics, which might lead to effect attenuation of these essential medications.

Limiting factors of this work were the low tumor load of analyzed samples, and a somewhat low PARP activity of the samples. Nevertheless, we were able to show that it is possible to obtain sequential tumor biopsies during major surgery and to get reliable values of a highly instable biomarker and to describe a threshold at which a significant increase and decrease of PARP activity could be assumed. Investigation of target variability would help to ascertain the feasibility of conducting a phase 0 trial in the setting of a routine surgical procedure, but it has to be shown.
previously that the primary PD endpoint, as we have shown in here for PARP activity, is neither affected by surgical nor anesthetic interventions.

Disclosure of Potential Conflicts of Interest
F. Heitz, P. Harter, and A. du Bois received an unrestricted grant for basic research projects from AstraZeneca.

Authors’ Contributions
Conception and design: F. Heitz, A. du Bois, P. Harter.
Acquisition of data: F. Heitz, A. du Bois, S. Scheil-Bertram, R. Hils, C. Kaub, P. Harter.

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