Featured Article

Vaccination of Patients with Advanced Ovarian Carcinoma with the Anti-Idiotype ACA125: Immunological Response and Survival (Phase Ib/II)

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Abstract

Purpose: A Phase I/IIb multicenter study was conducted to evaluate the safety and immunogenicity of the anti-idiotypic antibody vaccine ACA125 that functionally imitates the tumor antigen CA125 in 119 patients with advanced ovarian carcinoma. A preliminary report on the initial 42 patients demonstrated safety and immunogenicity.

Experimental Design: Using the complete intention-to-treat population (n = 119) who received a mean of 9.7 ACA125 applications, survival was analyzed with respect to immunological responses.

Results: In 81 patients (68.1%), a specific anti-anti-idiotype antibody (Ab3) response could be induced. Additionally, the development of CA125-specific antibodies (Ab1) and antibody-dependent cell-mediated cytotoxicity of CA125-positive tumor cells was observed in 50.4% and 26.9% of patients, respectively. The median survival of all patients was 19.4 months (range, 0.5–56.1 months). Ab3-positive patients showed a significantly longer survival (median, 23.4 months; P < 0.0001) as compared with Ab3-negative patients (median, 4.9 months). A positive Ab3 response remained associated with longer survival when controlling for other prognostic factors including FIGO (International Federation of Gynecologists and Obstetricians) stage, response to and type of first-line chemotherapy, number of previous treatments, or concomitant antitumor therapy. With regard to safety, repeated vaccination was well tolerated. No serious adverse events related to the application of ACA125 occurred.

Conclusions: Although the uncontrolled design of this study prevents definitive conclusions with respect to subgroups, the data support a relationship between Ab3 response and survival time. Thus, the need for further randomized, controlled clinical trials to establish efficacy of the vaccine ACA125 seems to be indicated.

Introduction

Ovarian cancer represents a chemotherapy-sensitive malignancy for which the majority of patients are in clinical remission after primary surgical debulking and adjuvant platinum-based therapy. However, 70–90% of patients relapse, and whereas many patients return to a partial or complete remission, the chance of further relapse is fairly certain (1). A variety of systemic and regional consolidation approaches with standard chemotherapy are being investigated. This has also led to the development of different immunotherapeutic treatment strategies within the last few years (2). One of those strategies is the vaccination with anti-idiotypic (Id) antibodies (Ab2), which is based on the immune network hypothesis of Nils Jerne (3). According to this theory, the variable antigen-binding regions of antibodies (Ab1) contain Id determinants that are immunogenic and induce the formation of Ab2. A subset of these antibodies is able to functionally mimic the three-dimensional structure of the original antigen. Thus, selective immunization with Ab2 could induce a specific immune response directed against the original antigen (4–7).

In patients with colorectal carcinoma (8–12), malignant melanoma (13–15), and advanced ovarian carcinoma (16–20) the induction of humoral and cellular immune responses using murine anti-Id antibodies could be demonstrated and was associated with an improved survival.

In the treatment of ovarian carcinoma, several studies have been performed with a monoclonal murine anti-Id called monoclonal antibody (mAb) ACA125. This antibody functionally imitates the CA125 antigen and induces humoral as well as cellular anti-CA125 immunity in animals (21, 22) and humans (16, 18, 23). A clinical Phase I study in patients with recurrent ovarian carcinoma showed that 67% of the patients developed specific anti-Id antibody (Ab3) directed against the vaccine. Side effects were limited to local pain (WHO NCIC Common Toxicity Criteria (NCIC) grade I) at the vaccination sites and abdominal pain (WHO NCIC grade II) in one single case (16).
The aim of the present Phase Ib/II study, which includes the Phase I patients reported by Wagner et al. (16), was to report safety with additional patient accrual and to evaluate a general estimate for efficacy of the entire cohort of patients with platinum-pretreated recurrences of ovarian carcinoma.

Patients and Methods

Study Design and Patients. The present study was designed as a multicenter (Department of Obstetrics and Gynecology of the Universities of Tuebingen, Bonn, Ulm, and Frankfurt a.M., Germany), open-label, Phase Ib/II trial with one treatment group. Patient accrual was started in 1994 and continued until 2002. Data were captured using standardized case report forms. Eligible patients (n = 119) met the following criteria: signed informed consent; age > 18 years; diagnosis of ovarian cancer, carcinoma of the fallopian tube, peritoneal carcinoma, or CA125-positive malignant tumor; and history of debulking surgery and previous platinum-based chemotherapy. Patients with other active malignancies, active uncontrolled infection, active autoimmune disease, immune deficiency, or known allergy to murine proteins were excluded from the study. ACA125 treatment was terminated in case of any serious adverse events due to the administration of ACA125 or withdrawal of consent by the patient.

Treatment Schedule. The vaccination scheme that was performed in the present study is based on pilot data (16, 18, 20). Treatment was started with four immunizations at 2-week intervals, followed by monthly applications of ACA125. Treatment duration was dependent on the clinical course, with treatment continued in the absence of disease progression, withdrawal of consent, or serious adverse events. Vaccinations were performed with 2 mg of alum-coupled anti-Id ACA125 injected into the gluteal muscle. The 2-mg dose was chosen because it demonstrated immunogenicity with few side effects in a previous Phase I study (16, 18, 20). Patients who discontinued ACA125 therapy due to progressive disease (as determined by increase of serological CA125 value and physical examination) were allowed to reenter the study after termination of additional antitumor treatment such as chemotherapy, surgery, or immune-augmenting therapy.

Monitoring of Clinical Responses. Survival time was calculated as the time between the day of the first application of ACA125 and the day of death. For patients who were still alive at the end of the observation period, the time between the first day of ACA125 application and the day of the last assessment was calculated. The clinical responses were documented by the investigator according to WHO criteria.

Determination of Human Antimouse Antibodies (HAMAs). Anti-allo- and anti-isotypic nonspecific HAMA titers were determined by a commercially available ELISA (Medac, Hamburg, Germany). HAMA response was termed positive if the concentration exceeded 100 ng/ml during ACA125 treatment.

Determination of Anti-Anti-Id (Ab3) Antibodies. Ab3 titers were assessed by ELISA as described previously (16, 20). Briefly, HAMAs that may interfere with the measurements were eliminated before Ab3 determination using mouse IgG agarose. Purified sera were allowed to bind to ACA125 F(ab')2-coated microtiter plates. Complete mAb ACA125 was added, followed by incubation with horseradish peroxidase-labeled goat antimouse IgG. After appropriate washings, color development was measured photometrically. Ab3 concentrations in patient sera are given in arbitrary units/ml corresponding to 1 ng/ml Ab1 (mAb OC125, anti-CA125). Ab3 responses were termed positive if Ab3 concentration exceeded 1000 arbitrary units/ml during ACA125 treatment.

Determination of CA125-Specific Antibodies (Ab1'). Binding of antibodies in patient sera to CA125-positive (OAW-42) and CA125-negative (SKOV-3) human ovarian carcinoma cell lines was measured by flow cytometry. Tumor cells (2 × 10⁵) were resuspended in PBS/0.01% NaN₃/5% FCS and incubated with anti-CA125 mouse mAb OC125 as positive control or pre- and postimmune sera (diluted 1:50) from patients immunized with anti-Id ACA125 (1 h at 4°C). After washing, FITC-conjugated rabbit antihamster IgG (DAKO, Hamburg, Germany) or FITC-conjugated rabbit antimouse IgG (DAKO), respectively, was added for 30 min at 4°C. A negative control included cells that were incubated with conjugated secondary antibodies alone. Flow cytometric analysis was performed on FACSCalibur (Becton Dickinson, Heidelberg, Germany). Binding was expressed as the percentage of positively stained cells after subtraction of the negative control, which was set on approximately 5% positive staining. Binding capacity was rated positive if the binding of postimmune sera to CA125-positive OAW-42 cells exceeded 15% after subtracting the negative control, and the difference of the binding capacity between pre- and postimmune sera was >5%.

Ab1’ immunocomplexes (i.e., antibodies bound to circulating CA125) were detected after dissociation of the complexes by acid/heat treatment of patients’ sera. Serum (100 µl) was added to 190 µl of 0.15 M glycine HCl (pH 2.0), mixed, and incubated for 5 min at 56°C. Processed sera were neutralized immediately with 10 µl of Tris (pH 10.7) and tested by ELISA as follows: microtiter plates were coated with CA125 antigen (1000 units/ml) and with culture supernatant from the CA125-negative cell line SKOV-3 as control. Plates were blocked and incubated with pre- and postimmune sera (diluted 1:10) of immunized patients. Bound Ab1’ was detected with the complete anti-Id mAb ACA125, followed by incubation with horseradish peroxidase-labeled goat antimouse IgG (Fc specific). Binding of Ab1’ was expressed as A₄05 nm value of 1:10 diluted serum after subtracting background values.

Antibody-Dependent Cell-Mediated Cytotoxicity (ADCC). To evaluate ADCC effects mediated by CA125-specific Ab3 antibodies, cryopreserved peripheral blood mononuclear cells isolated from heparinized blood of healthy donors by Ficoll density centrifugation were incubated with patient sera. The percentage of lysis of CA125-positive and CA125-negative cells was determined as follows. CA125-positive OAW-42 and CA125-negative SKOV-3 human ovarian cancer cells (1 × 10⁴ cells in RPMI 1640/10% FCS) were seeded in round-bottomed 96-well culture plates. Ten µl of heat-inactivated sera were added to the target cells for 1 h at 4°C. Subsequently, target cells were washed and resuspended in assay medium (RPMI 1640/1% BSA). Effector cells (cryopreserved peripheral blood mononuclear cells) were thawed and washed with assay medium. A total of 2.5 × 10⁵ peripheral blood mononuclear
cells/well were added to the target cells, resulting in an E:T cell ratio of 25:1, and incubated at 37°C and 5% CO2 for 18 h. Lysis of tumor cells was determined using the LDH Cytotoxicity Detection Kit (Roche Diagnostics, Mannheim, Germany). Appropriate controls were included: background of medium alone and peripheral blood mononuclear cell without target cells; spontaneous lactate dehydrogenase release of target cells, and maximal release of target by addition of 1% Triton X-100. Each measurement was carried out in triplicates. Pre- and postimmune samples were tested side-by-side in one experiment.

Specific release was defined as follows:

\[
\% \text{ release} = \frac{([\text{experimental release} - \text{effector background}]) - [\text{spontaneous release}]}{\text{maximum release} - \text{spontaneous release}} \times 100
\]

The percentage of sera-mediated cell lysis was expressed as follows:

\[
\% \text{ ADCC} = \% \text{ release of peripheral blood mononuclear cells with sera} - \% \text{ release of peripheral blood mononuclear cells without sera}
\]

ADCC was termed positive if specific lysis of CA125-positive OAW-42 cells exceeded 10% lysis after treatment with ACA125 and the difference of cell lysis between pre- and postimmune sera was >5%. Sera showing a positive ADCC reaction were retested in a second setting.

**Data Quality Assurance and Statistical Analysis.** Data collection was monitored by CellControl Biomedical Laboratories AG and statistical analyses were performed by GKM Gesellschaft für Therapieforschung mbH. For survival analyses, the Kaplan-Meier estimation procedure was used. Median and mean of the survival distribution function and the range of survival times were calculated. Statistical differences between subgroups were tested using the log-rank test (\( \alpha = 0.05 \)).

**Results**

**Duration of Exposure to ACA125 Vaccine and Adverse Events.** A total of 119 patients with advanced/recurrent epithelial ovarian carcinoma (average age, 56.9 years) received ACA125 vaccinations for a median of 4.9 months (range, 0.5–54.8 months). A total of 1156 applications were injected across all patients with a mean number of 9.7 ACA125 applications/patient (Table 1).

Forty-six adverse events were definitely or probably related to the application of ACA125. The majority of those events were defined as grade I (93.5%) according to the NCIC. The most frequent adverse events were local reactions at the injection sites (\( n = 25 \)), followed by cases of nausea (\( n = 5 \)), fever (\( n = 3 \)), vomiting (\( n = 3 \)), chills (\( n = 2 \)), and leukopenia (\( n = 2 \)). No severe adverse events due to ACA125 application were observed. In general, repeated vaccination with anti-Id ACA125 was well tolerated.

**Monitoring of Immune Responses Induced by Anti-Id ACA125.** A positive HAMA response (HAMA > 100 ng/ml) was detected in 93 of 119 patients (78.2%). Twenty-seven patients showed detectable HAMA titers before starting ACA125 treatment. Eighty-one patients (68.1%) developed specific Ab3 responses directed against the anti-Id vaccine including six patients with pre-existing low Ab3 titers (range, 55–3761 arbitrary units/ml) before treatment (Table 2).

To prove the specificity of the induced immune response, binding of patient sera to the tumor antigen CA125 was assessed by ELISA and flow cytometric analysis. CA125-specific antibody (Ab1’) binding to CA125-positive human ovarian carcinoma cells was detected in 47 patients (39.5%). Additionally,
the formation of Ab1’ immunocomplexes was seen in 42 patients by ELISA. A total of 60 patients (50.4%) developed either Ab3 or Ab1’ immunocomplexes. An in vitro assay for monitoring an ADCC by CA125-specific Ab1’ was performed in 108 patients. In 32 cases, sera of patients immunized with anti-Id ACA125 showed increased lysis of CA125-positive ovarian carcinoma cells compared with the corresponding pre-immune sera (Table 2).

**Survival Rate Stratified by Immune Response.** The entire population of patients (n = 119) who received a mean of 9.7 applications of the anti-Id vaccine ACA125 showed a median survival of 19.4 months (Table 3). Before study enrollment, patients had been treated with a variable number and type of chemotherapeutic agents. A total of 53.7% of patients obtained additional antitumor treatment during or after ACA125 application.

Based on the determination of specific Ab3 responses, 81 (68.1%) of the patients were considered as immunological responders to ACA125 immunotherapy. The efficacy of the vaccine ACA125 was further evaluated by subgroup analyses with respect to survival time and Ab3 response. The median survival time for patients with a positive Ab3 response was 23.4 months, in contrast to 4.9 months for patients without detectable Ab3 titers. The difference in survival between both subgroups was statistically significant (P < 0.0001, log-rank-test; Table 3). Comparison of Ab3 response rates and survival time across the four participating study centers, including center 1 with 54 patients, center 2/3 with 33 patients, and center 4 with 32 patients, showed no effects that could influence the results of the whole patient cohort. Similar Ab3 response rates (center 1, 68.5%; center 2/3, 66.7%; center 4, 68.8%) as well as median overall survival (center 1, 20.6 months; center 2/3, 15.3 months; center 4, 17.1 months) were detected in individual study centers.

Because this study includes Phase I patients (n = 42) initially reported by Wagner et al. (16), a partial analysis of these patients separated from the 77 new patients was performed. Taking into account that the 42 patients were reanalyzed, as some of them continued ACA125 treatment, the respective survival data differ from the previous report (16). However, no significant differences concerning Ab3 response rate (66.7% for 42 patients versus 68.8% for 77 new patients), median overall survival (17.1 months for 42 patients versus 19.4 months for 77 patients), and median survival of Ab3 responder (23.4 months for 42 patients versus 30.9 months for 77 patients; P = 0.96) were detected. Thus, both collective groups of patients contribute equally to the results of the entire cohort of 119 patients.

Besides Ab3 as a surrogate marker for ACA125-specific immunity, a potential relationship between additional immunological parameters, such as HAMA, Ab1’ (free Ab1’ and Ab1’ immunocomplexes), ADCC response, and survival time was analyzed in the present study. As shown in Fig. 1, patients with a positive HAMA, Ab1’, or ADCC response had a longer median survival as compared with the respective nonresponders. For all immune parameters analyzed, the differences in the survival time were statistically significant, although this was

### Table 2  Frequencies of pre-existing and vaccine-induced immune responses

<table>
<thead>
<tr>
<th>Immune parameter</th>
<th>Before ACA125 treatment</th>
<th></th>
<th></th>
<th></th>
<th>After ACA125 treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>% positive</td>
<td>Mean</td>
<td></td>
<td>% positive</td>
<td>Mean</td>
</tr>
<tr>
<td>HAMA*</td>
<td>9.2 (n = 11)</td>
<td>927 ± 743 ng/ml</td>
<td>78.2 (n = 93)</td>
<td>316272 ± 955554 ng/ml</td>
<td></td>
</tr>
<tr>
<td>Ab3</td>
<td>1.7 (n = 2)</td>
<td>2691 ± 1514 arb. U/ml</td>
<td>68.1 (n = 81)</td>
<td>722327 ± 3169217 arb. U/ml</td>
<td></td>
</tr>
<tr>
<td>Free Ab1’ binding to CA125+ cells</td>
<td>26.9 (n = 32)</td>
<td>28.0 ± 15.4</td>
<td>39.5 (n = 47)</td>
<td>35.4 ± 17.1</td>
<td></td>
</tr>
<tr>
<td>Ab1’ immunocomplexes</td>
<td>0 (n = 0)</td>
<td>0%</td>
<td>35.3 (n = 42)</td>
<td>0.25% ± 0.228 (OD405)</td>
<td></td>
</tr>
<tr>
<td>Free Ab1’ and/or Ab1’ complexes</td>
<td>26.9 (n = 32)</td>
<td>18.9 ± 7.2%</td>
<td>50.4 (n = 60)</td>
<td>23.1 ± 17.4%</td>
<td></td>
</tr>
<tr>
<td>ADCC against CA125+ cells</td>
<td>8.4 (n = 10)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Positive immune responses are defined as >100 ng/ml for HAMA, >1000 arb. U/ml for Ab3, >15% positive staining for Ab1’ binding to CA125+ cells, >OD405=0.05 for Ab1’ immunocomplexes at 1:30 serum dilution, >10% antibody-mediated lysis of CA125+ cells for ADCC.

* Ab3, anti-anti-idiotypic antibody.

### Table 3  Survival time of immunological responders (Ab3+) versus Ab3 non-responder patients depending on the FIGO stage

<table>
<thead>
<tr>
<th>FIGO stage</th>
<th>All FIGO stages</th>
<th>All FIGO stages*</th>
<th>FIGO III stages</th>
<th>FIGO III stages*</th>
</tr>
</thead>
<tbody>
<tr>
<td>ACA125-treated patients</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No.</td>
<td>119</td>
<td>100</td>
<td>74</td>
<td>62</td>
</tr>
<tr>
<td>Overall median survival (months)</td>
<td>19.4</td>
<td>20.6</td>
<td>20.1</td>
<td>20.9</td>
</tr>
<tr>
<td>Ab3 responder</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No.</td>
<td>81</td>
<td>81</td>
<td>49</td>
<td>49</td>
</tr>
<tr>
<td>Median survival (months)</td>
<td>23.4</td>
<td>23.4</td>
<td>24.2</td>
<td>24.2</td>
</tr>
<tr>
<td>Ab3 non-responder</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No.</td>
<td>38</td>
<td>19</td>
<td>25</td>
<td>13</td>
</tr>
<tr>
<td>Median survival (months)</td>
<td>4.9</td>
<td>8.9</td>
<td>4.9</td>
<td>7.9</td>
</tr>
</tbody>
</table>

* Ab3 non-responder with survival ≥2 months (≥4 vaccinations) excluded.

* Ab3, anti-anti-idiotypic antibody.

* Of survival time Ab3 responder versus non-responder.
less striking in case of ADCC response (Fig. 1). Simultaneous
detection of Ab3 and ADCC did not lead to further improve-
ment of survival compared to Ab3 responder without measur-
able ADCC induction ($P = 0.308$). Therefore, the influence of
ADCC induction in Ab3-positive patients on survival time re-
ains unclear.

**Subgroup Analysis with Respect to Ab3 Response and Survival Time.** Further subgroup analysis of Ab3 responders *versus* nonresponders included a variety of possible confounding
factors, which could have an impact on survival. No differ-
ences were seen between the two groups with respect to age,
FIGO (International Federation of Gynecologists and Obstetri-
cians) stage, duration of disease, best overall response to previ-
uous therapy, and concomitant antitumor therapy (Table 1). Apart
from this, Ab3 responder patients received, on average, more
ACA125 applications compared with the Ab3 nonresponder
group (mean, 12.3 ± 9.5 applications for Ab3-positive patients
*versus* 4.3 ± 2.1 applications for Ab3-negative patients). How-
ever, a mean number of 4.4 ± 3.1 applications (median, 4.0
applications) was sufficient to induce detectable Ab3 titers in
the Ab3 responder group, and therefore the Ab3 nonresponder
received, on average, enough vaccinations to develop a specific
immune response. Nevertheless, a consistent survival advantage
of Ab3 responder patients could also be demonstrated after exclusion
of all Ab3 nonresponder patients with survival of ≤2
months who received ≤4 ACA125 applications. The median
survival of the remaining Ab3 nonresponder ($n = 19$) was 8.9
months and was significantly different from the survival of the
Ab3 responder (median survival, 23.4 months; $P < 0.0008$; Table 3).

Because the entire patient cohort contains patients with
different prior treatments as well as patients who received
ACA125 as the last treatment option, further subgroup analyses
regarding FIGO stage, type of first-line chemotherapy, and
number of previous therapies were performed. As shown in
Table 3, the efficacy of ACA125 therapy in patients with FIGO
stage III tumors ($n = 74$) is equivalent to the whole cohort of
patients ($n = 119$), with consistent survival advantage of the
Ab3 responder subgroup ($P < 0.0001$). Considering the type of
first-line chemotherapy, a significant difference in survival time
between Ab3 responder and Ab3 nonresponder was maintained
regardless of whether platinum-based regimens were used as
first-line chemotherapy for patients with FIGO stage III disease
(Table 4).

The potential for improved immune competence of
Ab3 responders might be considered as one factor that could
influence the clinical results rather than the specific immunity
induced by the vaccine, FIGO III patients with one previous
chemotherapy *versus* patients with three or more prior treat-
ments were further analyzed. The latter cohort contains prog-
ostically poor patients who have the potential for a weakened
immune system when compared with patients after first pro-
gression. Again, the survival advantage of Ab3 responders is
maintained, despite different numbers of previous therapies
(Table 4).

Antitumor therapy administered concomitantly with
ACA125 vaccination led to a prolongation of survival in the
entire cohort of patients, although this prolongation was not
statistically significant (log-rank $P = 0.0901$). The benefit in
survival mediated by additional antitumor therapy seems to be
more pronounced in patients without immunological (Ab3) re-
sponses as compared with Ab3-positive patients (Table 5). Thus,
it seems that both an additional antitumor treatment and
the induction of specific immune responses by ACA125 vacci-
nation positively influence survival time.

Clinical responses to subsequent chemotherapies after
ACA125 vaccination included a complete response in 4 patients

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**Table 4** Survival time of immunological responders (Ab3+) versus Ab3 non-responder patients depending on the type of 1st line chemotherapy and the number of previous therapies

<table>
<thead>
<tr>
<th>FIGO III stages</th>
<th>platinum/taxol + 1st line therapy</th>
<th>platinum/taxol – 1st line therapy</th>
<th>1 previous chemotherapy</th>
<th>&gt;3 previous chemotherapies</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ab3 responder</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No.</td>
<td>18</td>
<td>31</td>
<td>13</td>
<td>18</td>
</tr>
<tr>
<td>Median survival (months)</td>
<td>29.7</td>
<td>24.2</td>
<td>26.1</td>
<td>11.2</td>
</tr>
<tr>
<td>Ab3 non-responder</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No.</td>
<td>9</td>
<td>16</td>
<td>10</td>
<td>7</td>
</tr>
<tr>
<td>Median survival (months)</td>
<td>14.4</td>
<td>4.9</td>
<td>10.6</td>
<td>4.9</td>
</tr>
<tr>
<td>Statistics</td>
<td>$p = 0.0269$</td>
<td>$p &lt; 0.0001$</td>
<td>$p = 0.0455$</td>
<td>$p = 0.0240$</td>
</tr>
</tbody>
</table>

*a* Ab3, anti-idiotype antibody.

*b* Of survival time Ab3 responder versus non-responder.
in 50.4% of all patients, and these Ab1 were able to mediate an ADCC reactivity against CA125-positive cells in 26.9% of patients. Therefore, it could be concluded that the determination of polyclonal Ab3 responses does not necessarily correlate with detectable anti-CA125 reactivity and possible cytotoxic effector mechanisms in some cases. However, it must be taken into consideration that induced anti-CA125 antibodies could have been bound to CA125-expressing tumor cells or to circulating CA125 antigen so that the amount of Ab1’ produced might be underestimated by measurement of free Ab1’ concentrations. Indeed, it could be shown that in 35% of patients, relevant amounts of circulating Ab1’ immunocomplexes were present after vaccination.

For all tested immune parameters, a significant survival advantage in immunological responders versus nonresponders could be demonstrated. It may be assumed that the humoral immune response as determined by HAMA, Ab1’, and surrogate marker Ab3 influences the survival of treated patients. By contrast, the survival advantage was less profound but still significant in ADCC-positive versus ADCC-negative patients. Thus, ADCC reactivity might be regarded as only one of several potent effector mechanisms induced by ACA125 treatment.

In Ab3 responder patients, the median survival time was 23.4 months, which is significantly higher than that in patients without induction of a specific Ab3 response (median survival, 4.9 months). This result is not biased by patient characteristics, including FIGO stage at the time of diagnosis and response to and type of first-line chemotherapy, or by concomitant antitumor therapy (e.g., chemotherapy, interleukin). Although there was a difference in both subgroups with respect to the total number of applications, a positive Ab3 response was detected after at least four vaccinations in the Ab3 responder group, which is comparable with the mean total application number in the nonresponder group. Therefore, it was considered that Ab3-negative patients received enough vaccinations to induce vaccine-specific immunity, but even the exclusion of all Ab3 nonresponders with survival time of ≤ 2 months (4 ≤ 4 vaccinations) from statistical analysis did not significantly affect the previous results. Thus, the difference in survival appears to be dependent on the induced immune response to ACA125, and the higher number of ACA125 applications in the Ab3-positive cohort may have resulted from beneficial effects of treatment. Additionally, a generally improved immune competence of the Ab3 responder does not seem to contribute to this survival advantage because Ab3 responders are equally distributed among patients with one or more than three previous chemotherapies. This is further supported by the observation that no significant difference regarding Ab3 response and survival rates exists among the four study centers. Apart from this, phenotypic analysis of lymphocytes has revealed a reduced B-cell count below normal range among both Ab3 responder and nonresponder patients (five patients were tested; data not shown), which implies that the immune system gives rise to specific Ab3 antibodies even in patients with a reduced B cell count.

A complete remission was seen in only four patients (3.4%). Thirty patients (25%) showed stabilization, tumor progression was observed in 62 patients (52%), and a clinical response assessment was missing for 23 patients. For all tested immune parameters, a significant survival advantage in immunological responders versus nonresponders could be demonstrated. It may be assumed that the humoral immune response as determined by HAMA, Ab1’, and surrogate marker Ab3 influences the survival of treated patients. By contrast, the survival advantage was less profound but still significant in ADCC-positive versus ADCC-negative patients. Thus, ADCC reactivity might be regarded as only one of several potent effector mechanisms induced by ACA125 treatment.

In Ab3 responder patients, the median survival time was 23.4 months, which is significantly higher than that in patients without induction of a specific Ab3 response (median survival, 4.9 months). This result is not biased by patient characteristics, including FIGO stage at the time of diagnosis and response to and type of first-line chemotherapy, or by concomitant antitumor therapy (e.g., chemotherapy, interleukin). Although there was a difference in both subgroups with respect to the total number of applications, a positive Ab3 response was detected after at least four vaccinations in the Ab3 responder group, which is comparable with the mean total application number in the nonresponder group. Therefore, it was considered that Ab3-negative patients received enough vaccinations to induce vaccine-specific immunity, but even the exclusion of all Ab3 nonresponders with survival time of ≤ 2 months (4 ≤ 4 vaccinations) from statistical analysis did not significantly affect the previous results. Thus, the difference in survival appears to be dependent on the induced immune response to ACA125, and the higher number of ACA125 applications in the Ab3-positive cohort may have resulted from beneficial effects of treatment. Additionally, a generally improved immune competence of the Ab3 responder does not seem to contribute to this survival advantage because Ab3 responders are equally distributed among patients with one or more than three previous chemotherapies. This is further supported by the observation that no significant difference regarding Ab3 response and survival rates exists among the four study centers. Apart from this, phenotypic analysis of lymphocytes has revealed a reduced B-cell count below normal range among both Ab3 responder and nonresponder patients (five patients were tested; data not shown), which implies that the immune system gives rise to specific Ab3 antibodies even in patients with a reduced B cell count.
although additional beneficial effects related to conventional palliative treatment could not be ruled out at this stage.

Whereas proof of efficacy will need to await the planned randomized controlled clinical trial with ACA125, it is possible to put the time to progression and overall survival data in the context of historical controls to give a general estimate of activity of the vaccine. In general, the outcomes from randomized trials of recurrent ovarian cancer in patients treated with chemotherapy depend on whether patients are platinum sensitive (relapse >6 months after prior platinum therapy) or platinum resistant (relapse ≤6 months after prior platinum therapy).

Eighty-one of 119 patients (68%) treated with ACA125 received platinum-based first-line chemotherapy in our study (Table 1) and can therefore be separated into platinum-sensitive (46%) and -resistant groups (54%). The median overall survival time was 20.3 months (versus 19.4 months in the entire cohort of ACA125-treated patients). Based on a literature review (25–29), the median survival time across all available randomized studies for recurrent ovarian cancer is between 9.9 and 15.8 months, with the median at 13.4 months. Included studies are reviewed in Table 6. The patients in the ACA125 treatment group and in the historical control group are comparable with respect to the proportion of platinum-resistant and platinum-sensitive patients (Table 6). Realizing the limitations of such a comparison, the median overall survival of patients receiving ACA125 exceeds that seen in all available randomized studies. The survival advantage of 6.9 months (51%) favoring ACA125 is more striking when one considers that the ACA125-treated patients have received multiple prior chemotherapy regimens (mean, 2.6 regimens), as compared with one prior regimen in the studies used as historical controls giving them a worse prognosis.

In conclusion, these data provide evidence that the vaccine ACA125 is safe and may be effective in ovarian cancer patients, a possibility that has to be evaluated in further randomized, controlled trials.

Acknowledgments

We thank Ute Hilcher and Beate Kootz for excellent technical assistance.

Table 6  Median survival and proportion of platinum resistant patients in historical control group

<table>
<thead>
<tr>
<th>Study</th>
<th>Drug</th>
<th>Platinum (%)</th>
<th>Median survival (months)</th>
</tr>
</thead>
<tbody>
<tr>
<td>European Canadian Randomized Trial of Paclitaxel in Relapsed Ovarian Cancer: high-dose vs. low-dose and long versus short infusion (25)</td>
<td>Taxol</td>
<td>na</td>
<td>na</td>
</tr>
<tr>
<td>Topotecan Versus Paclitaxel for the Treatment of Recurrent Epithelial Ovarian Cancer (26)</td>
<td>Topotecan</td>
<td>46%</td>
<td>54%</td>
</tr>
<tr>
<td>Recurrent Epithelial Ovarian Carcinoma: a Randomized Phase III Study of Pegylated Liposomal Doxorubicin Versus Topotecan (27)</td>
<td>Paclitaxel</td>
<td>48%</td>
<td>52%</td>
</tr>
<tr>
<td>Randomized Trial of Single Agent Paclitaxel Given Weekly Versus Every Three Weeks and with Peroral Versus Intravenous Steroid Premedication to Patients with Ovarian Carcinoma Previously Treated with Platinum (28)</td>
<td>Doxorubicin</td>
<td>46%</td>
<td>54%</td>
</tr>
<tr>
<td>Randomized trial of Oral versus Intravenous Topotecan in Patients with Relapsed Epithelial Ovarian Cancer (29)</td>
<td>Topotecan</td>
<td>47%</td>
<td>53%</td>
</tr>
<tr>
<td></td>
<td>Paclitaxel weekly</td>
<td>46%</td>
<td>54%</td>
</tr>
<tr>
<td></td>
<td>Paclitaxel three weekly</td>
<td>50%</td>
<td>50%</td>
</tr>
<tr>
<td></td>
<td>Topotecan oral</td>
<td>43%</td>
<td>57%</td>
</tr>
<tr>
<td></td>
<td>Topotecan i.v.</td>
<td>43%</td>
<td>57%</td>
</tr>
</tbody>
</table>

*na, not analyzed.

References

Vaccination of Patients with Advanced Ovarian Carcinoma with the Anti-Idiotype ACA125: Immunological Response and Survival (Phase Ib/II)

Silke Reinartz, Siegmund Köhler, Harald Schlebusch, et al.


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