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Delta tocotrienol in recurrent ovarian cancer. A phase II trial

Caroline Brenner Thomsen\textsuperscript{a,b,c}, Rikke Fredslund Andersen\textsuperscript{b}, Karina Dahl Steffensen\textsuperscript{a,c}, Parvin Adimi\textsuperscript{a}, Anders Jakobsen\textsuperscript{b,c,*}

\textsuperscript{a} Department of Oncology, Vejle Hospital, Beriderbakken 4, 7100, Vejle, Denmark
\textsuperscript{b} Department of Clinical Biochemistry, Vejle Hospital, Beriderbakken 4, 7100, Vejle, Denmark
\textsuperscript{c} Institute of Regional Health Research, University of Southern Denmark, Windsløwparken 9, 5000, Odense, Denmark

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\textbf{ABSTRACT}

Delta tocotrienol has anti-neoplastic activity as demonstrated in several in-vitro and in-vivo investigations. The effect relies on inhibition of different pathways. It also has antiangiogenic activity, and an additive effect to bevacizumab may be expected.

The present study was a phase II trial of bevacizumab combined with tocotrienol in chemotherapy refractory ovarian cancer. The study also included analysis of circulating tumor specific HOXA9 methylated DNA (HOXA9 meth-ctDNA) during treatment.

The study included 23 patients. The rate of disease stabilization was 70\% with very low toxicity. The median PFS was 6.9 months and the median OS 10.9 months, which is rather high compared to the current literature. A division of the patients according to level of HOXA9 meth-ctDNA already after the first cycle of chemotherapy resulted in two groups of patients with different prognoses. Patients with an increasing level of HOXA9 meth-ctDNA had a median PFS and OS of 1.4 and 4.3 months, respectively, compared to 7.8 and 12 months in the group with stable or decreasing levels.

The combination of bevacizumab and tocotrienol is potent in chemotherapy refractory ovarian cancer. The level of HOXA9 meth-ctDNA after one cycle of chemotherapy holds important prognostic information.

1. Introduction

Despite considerable progress in cancer management, recurrent ovarian cancer still represents a therapeutic challenge. Most ovarian cancer patients will recur within few years after the primary treatment, and although second and even third line chemotherapy is efficient, the majority of the patients eventually become chemotherapy resistant with no or only few treatment options, all without curative potential.

Bevacizumab has been established as an integrated part of both first\textsuperscript{[1,2]} and second line\textsuperscript{[3,4]} treatment of ovarian cancer. The combination with chemotherapy has improved the treatment results, but still, the majority of patients will die from their disease. The situation calls for new approaches to resistant disease with the perspective of improved quality of life and prolonged life span. The toxicity of such treatment should be very low or non-existing.

Tocotrienols are vitamin E analogues with several biological targets. A considerable literature\textsuperscript{[5–7]}, mainly on in-vitro experiments but also a few in-vivo studies, has indicated a clear effect on malignant cells with respect to proliferation and invasion. Several mechanisms and effects on different pathways have been suggested\textsuperscript{[8]} among which inhibition of two important transcription factors NF-κB and STAT3 rank high. The delta form is especially active in relation to malignancies. It suppresses the vascular endothelial growth factor (VEGF) and inhibits proliferation of endothelial cells resulting in reduced tube formation\textsuperscript{[9,10]}. The angiogenic activity may be an essential part of its anti-neoplastic effect. Therefore, an additive effect to bevacizumab could at least in theory be expected, which motivated the present study.

Aberrant methylation occurs in almost all malignant tumors and\textsuperscript{1} tumor specific methylated DNA (meth-ctDNA) can be measured in the plasma from patients with various tumors. Plasma meth-ctDNA has been approved by the FDA as a tool in colorectal cancer screening\textsuperscript{[11]}. In ovarian cancer focus has also been on early detection and screening, but recently, HOXA9 meth-ctDNA has been suggested as a prognostic marker\textsuperscript{[12]}. We have presented a study on BRCA 1/2 positive patients treated with the PARP inhibitor veliparib\textsuperscript{[13]}. The results indicate a considerable prognostic importance of HOXA9 meth-ctDNA and a further study\textsuperscript{[14]} holds promise as to early selection of responding patients.

\textsuperscript{*}Corresponding author at: Vejle Hospital, Department of Oncology, Beriderbakken 4, 7100, Vejle, Denmark.
\textsuperscript{E-mail address: anders.jakobsen@rsyd.dk} (A. Jakobsen).

\textsuperscript{1}Tumor specific methylated DNA; meth-ctDNA

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The present study aimed to investigate the combination of bevacizumab and tocotrienol in a phase II trial of multiresistant ovarian cancer. We also wanted to elucidate the possible clinical importance of HOXA9 meth-ctDNA in relation to treatment.

2. Patient eligibility

The inclusion criteria were histologically verified endothelial, primary fallopian or primary peritoneal cancer; prior treatment with at least two different cytostatic regimens including platinum; progression on previous treatment; measurable disease by RECIST 1.1 or GCIG CA125 criteria; age ≥ 18 years; performance status 0–2; adequate organ function as estimated by WBC ≥ 3.0×10^9/l or neutrophils (ANC) ≥ 1.5×10^9/l; platelet count ≥ 100×10^9/l; hemoglobin ≥ 6 mmol/l; serum bilirubin < 2.0×ULN; serum transaminase ≤ 2.5×ULN; serum creatinine ≤ 1.5×ULN; urine dipstick for protein negative; if positive, the protein contents of a subsequent 24-hour urine testing was to be < 1 g; written informed consent. The exclusion criteria were other malignant diseases within five years prior to enrolment except for basal cell or squamous cell carcinoma of the skin and other types of cancer with minimal risk of recurrence; other experimental therapy or participation in another clinical trial within 28 days prior to treatment initiation; underlying medical disease not adequately treated; uncontrolled hypertension; cerebral vascular attack within 6 months before start of treatment; clinically significant cardiovascular disease as estimated by NYHA ≥ 2.

The trial was approved by the Regional Committee on Health Research Ethics for Southern Denmark (S-20130145), the Danish Medicines Agency (2013111478), and registered with ClinicalTrials.gov (NCT02399592). All participating patients provided informed consent.

3. Treatment

The patients received bevacizumab (Avastin®) 10 mg/kg i.v. every three weeks. Tocotrienol capsules were given as a continuous treatment 300 mg orally three times daily. Tocotrienol was a special formulation produced by American River Nutrition, USA, based on a patented method with 90% delta tocotrienol and supplied for the trial by DuraPharma, Denmark, as Traptol® free of charge. No dose reduction was scheduled in case of toxicity. The treatment continued until progression, grade 3 toxicity, or patient wish to discontinue. In case of bevacizumab discontinuation due to toxicity > grade 3, the treatment with tocotrienol continued.

4. Efficacy

Response was evaluated by chest and abdominal CT scans at every three cycles in patients with measurable disease according to RECIST 1.1 [15] and by the GCIG CA125 criteria [16] in patients with non-measurable disease. Patients having received at least three treatment cycles were eligible for response evaluation unless progression had occurred. The primary endpoint was the rate of disease control (DC). Secondary endpoints were quality of life, safety, progression free survival (PFS), and overall survival (OS).

5. Quality of life assessment

Quality of life was evaluated by EORTC QLQ-C30 before start of treatment, at the first response evaluation, and again at progression.

6. Toxicity assessment

Before the start of each treatment cycle toxicity was recorded as adverse events according to the CTCAE criteria version 4.

7. Analysis of HOXA9 meth-ctDNA

Blood samples were collected at baseline and at every treatment cycle. Nine ml blood was collected in EDTA tubes and plasma was isolated by centrifugation (2000 g, 10 min) within four hours of sampling. Plasma was stored at −80 °C until analysis. After thawing, plasma was centrifuged again at 10,000 g for 10 min and exogenous control DNA (CPPI) was added [17]. DNA was extracted from up to 4 ml of plasma using the QiaSymphony DSP Circulating DNA kit (Qiagen, Hilden, Germany) on the QiaSymphony SP instrument (Qiagen, Hilden, Germany). DNA was eluted in 60 ul. 340 ul water was added and samples were analysed by qPCR for CPPI and B2M as described previously [17] using 3 ul of DNA per well. The remaining DNA was concentrated to 20 ul on Amicon Ultra – 0.5 centrifugal filter units (Millipore, Billerica, MA, USA) and bisulfite converted in 50 ul reactions using the EZ DNA methylation lightning kit (Zymo Research, Irvine, CA, USA) according to the manufacturer’s instructions. Universal human methylated control DNA (Zymo Research, Irvine, CA, USA), genomic DNA from lymphocytes and water were converted alongside the samples as positive and negative controls. Converted DNA was eluted in 15 ul and analysed by digital PCR on a BioRad QX100 system (BioRad, Hercules, CA, USA) in two 20 ul reactions containing 2X Supermix for probes, 5 ul of DNA and primer/probe mix for Albumin [18] and HOXA9.

Comparison of the baseline HOXA9 meth-ctDNA levels with the levels at cycle two allowed for a division of the patients into two groups. The group with a level increasing above the 95% confidence interval of baseline was designated Increasing Value patients and compared with the group having stable or decreasing values designated Stable Value patients.

8. Statistics

The study was an open, single arm, phase II trial based on Simon’s two-stage design [19]. The target level at six months of treatment was 75% with DC and the lower level of interest set at 50%. The risk of type 1 error was set at 5% and power at 80%. Given these restrictions the first step would include 14 patients with at least seven having DC. The second step would include 23 patients with another nine having DC.

PFS and OS was calculated from the day of enrolment by the Kaplan-Meier method and compared by the Log-rank test. The PFS and OS of the Increasing Value patients were compared with that of the Stable Value patients and included in the analysis at the date of cycle two. Statistical calculations were performed using NCSS version 10 (NCSS Statistical Software, Kaysville, UT, USA).

9. Results

The study included 23 patients from March 2015 to January 2018. Patient characteristics are given in Table 1. It appears that most patients were diagnosed with stage III disease, and serous histopathology was the dominating type. The median number of prior chemotherapy regimens was 4.0. All patients were platinum resistant and more than half of them had previously received bevacizumab with progression on treatment. It also appears that all patients but one were in good performance status (0–1). The median number of treatment cycles was six with 20% of the patients treated for more than 12 months.

9.1. Response

Table 2 shows the response rate according to the RECIST 1.1 and CA125 criteria, respectively. Three patients were not evaluable for response, two because of withdrawal of consent and one due to worsening of hypertension. It appears that 20 patients were evaluable by either RECIST or CA125. One patient showed response according to the RECIST criteria while at the same time having SD according to CA125.
9. Toxicity

Three patients discontinued treatment, one because of unspecific gastrointestinal toxicity, one due to rectal bleeding, and one patient with hypertension stopped because of increased blood pressure despite intensive antihypertensive treatment. These are all well-known side effects to bevacizumab. There was no gastroenterial perforation, thromboembolism or other serious toxicity, and no treatment related death.

9.3. Quality of life

The quality of life during treatment is shown in Fig. 1 according to the patient reported global health assessment. It appears that it was stable during treatment with a clear drop at progression.

9.4. Survival

PFS by intention to treat is shown in Fig. 2. The median PFS was 6.9 months with 20% of the patients being alive without progression for more than a year. There was no difference between patients previously treated with bevacizumab and bevacizumab naive patients. Overall survival is also shown in Fig. 2 with a median of 10.9 months and 25% of the patients being alive after 24 months. Again, there was no difference according to previous bevacizumab treatment.

The prognostic importance of HOXA9 meth-ctDNA is shown in Fig. 3A and B. The increasing value patients had a median PFS of 1.4 months compared to 7.8 months in stable value patients, $p = 0.01$. The median OS was 4.3 and 12.0 months, respectively, $p = 0.01$.

10. Discussion

The present paper suggests an additive effect of tocotrienol to bevacizumab in chemotherapy refractory ovarian cancer, although it did not meet its primary endpoint of 75% DC at six months. On the other hand, an overall DC of 70% is high. The same applies to the DC rate of 50% at six months in our study compared to phase II trials on single agent bevacizumab reporting only 25% DC for more than 12 weeks [20]. A similar rate of 31% DC after six months of treatment was reported in a more recent, however retrospective, analysis [21].

Many patients with advanced recurrent disease and a short life expectancy are in good performance status with a wish for further treatment. Their general condition, however, deteriorates rapidly and the current literature indicates a median PFS of 2-4 months and a median OS of 5-7 months. This is in agreement with our previous phase II trial with a combination of veliparib and topotecan in the same patient category [22]. It may be discussed if such treatment is of benefit to the

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**Table 1**

<table>
<thead>
<tr>
<th>Patient characteristics.</th>
<th>N = 23</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Age, median (range)</strong></td>
<td>70 (41-81)</td>
<td></td>
</tr>
<tr>
<td><strong>FIGO stage</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>I</td>
<td>1</td>
<td>4</td>
</tr>
<tr>
<td>II</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>III</td>
<td>16</td>
<td>70</td>
</tr>
<tr>
<td>IV</td>
<td>6</td>
<td>26</td>
</tr>
<tr>
<td><strong>Histology</strong></td>
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<tr>
<td>Serous</td>
<td>21</td>
<td>91</td>
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<tr>
<td>Endometrioid</td>
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</tr>
<tr>
<td>Other</td>
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<td>4</td>
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<tr>
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<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>3</td>
<td>13</td>
</tr>
<tr>
<td>2</td>
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<td>3</td>
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</tr>
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<td>4</td>
</tr>
<tr>
<td><strong>Number of prior regimens</strong></td>
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<td></td>
</tr>
<tr>
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<td>4</td>
<td>17</td>
</tr>
<tr>
<td>2</td>
<td>4</td>
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</tr>
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<td>0</td>
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<td>9</td>
</tr>
<tr>
<td>8</td>
<td>1</td>
<td>4</td>
</tr>
<tr>
<td><strong>Previous Avastin treatment</strong></td>
<td>14</td>
<td>61</td>
</tr>
<tr>
<td><strong>Performance status</strong></td>
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<tr>
<td>1</td>
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<td>43</td>
</tr>
<tr>
<td>≥ 2</td>
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<td>4</td>
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</tbody>
</table>

**Table 2**

<table>
<thead>
<tr>
<th>Response rate.</th>
<th>GCIG CA125</th>
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</thead>
<tbody>
<tr>
<td><strong>RECIST, N = 23</strong></td>
<td>PR</td>
</tr>
<tr>
<td><strong>GCIG CA125</strong></td>
<td>N=20</td>
</tr>
<tr>
<td>PR</td>
<td>0</td>
</tr>
<tr>
<td>SD</td>
<td>1</td>
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<tr>
<td>PD</td>
<td>0</td>
</tr>
<tr>
<td>NE</td>
<td>0</td>
</tr>
</tbody>
</table>
The present paper is the first to classify patients according to the effect of chemotherapy after one treatment cycle. The dynamics of meth-ctDNA provides important clinical information. The results suggest an increase after three weeks of treatment, compared to baseline, to imply rapid progression and death. Thus, it seems reasonable to conclude that analysis of HOXA9 meth-ctDNA allows for early stop of ineffective treatment. On the other hand, the group with stable or decreasing levels had a considerable benefit with a median PFS of 7.8 months and a median OS of 12.0 months. Thus, HOXA9 meth-ctDNA is an interesting marker, but there is not yet enough data to justify its use in the daily clinic.

The toxicity of the combined treatment was low. In agreement with the low level of side effects the quality of life remained unchanged during treatment and only deteriorated at disease progression.

The limitations of the present study should not be forgotten. First of all, it is a phase II, non-randomized trial with a small number of patients, which does not allow for a final conclusion. It should also be noted that it is a single center study.

11. Conclusion

The present study indicates that the combination of bevacizumab and delta tocotrienol is effective in multiresistant ovarian cancer. The analysis of HOXA9 meth-ctDNA allows for early discontinuation of ineffective treatment and thus represents an important step towards personalized medicine, which should be addressed in prospective clinical trials.

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Declarations of interest

None.

References


[8] None.

Fig. 3. (a) Progression free survival according to HOXA9 meth-ctDNA level after one cycle of chemotherapy. (b) Overall survival according to HOXA9 meth-ctDNA level after one cycle of chemotherapy.


The present study suggests that a prolonged lifespan can be reached without negative influence on quality of life. It could be claimed that the prolongation is based on bevacizumab alone, but the PFS was only 4.2 months and the OS 6.7 months in our previous trial on single agent bevacizumab in the same category of patients [25]. It has been suggested that bevacizumab has activity after progression on treatment, but the effect was only 1.4 months in a randomized trial [26] and not likely to explain the findings in the present study.

It seems obvious to relate the antineoplastic effect of delta tocotrienol seen here with its angiogenic effect, which has been clearly demonstrated both in vitro and in vivo. It is also clear that the angiogenic effect is linked with tocotrienol and not seen with tocopherol [27,28]. The drug used in our trial, Traptol®, is a special product with 90% delta tocotrienol, which may explain the clinical effect in our study. The dose given was high compared to conventional use of tocotrienol, but it was well tolerated over several months and in agreement with a phase I study in which the dose was increased to 3200 mg daily for six weeks [29].

The clinical experience with delta tocotrienol is sparse but eagerly awaited as underlined in a recent comprehensive review [30]. A study in early breast cancer did not find any effect of tocotrienol on cancer specific survival [31]. The present study is the first to use delta tocotrienol in ovarian cancer, which is a challenging disease, and new treatment approaches are of major interest. This is also the first study to combine bevacizumab and delta tocotrienol. Bevacizumab is used in a spectrum of different tumors and an additional effect by an atoxic drug holds high priority.


