First-line bevacizumab in combination with chemotherapy for HER2-negative metastatic breast cancer: pooled and subgroup analyses of data from 2447 patients


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Background: Bevacizumab has consistently demonstrated improved progression-free survival (PFS) and response rate when combined with first-line chemotherapy for HER2-negative metastatic breast cancer (mBC). However, the lack of a significant overall survival (OS) difference continues to attract debate, and identification of patients deriving greatest benefit from bevacizumab remains elusive.

Patients and methods: Individual patient data from three randomised phase III trials in the first-line HER2-negative mBC setting were analysed, focusing specifically on efficacy in poor-prognosis patients.

Results: The meta-analysis (n = 2447) demonstrated a PFS hazard ratio (HR) of 0.64 (95% confidence interval [CI] 0.57–0.71; median 9.2 months with bevacizumab versus 6.7 months with non-bevacizumab therapy) and response rate of 49% versus 32%, respectively. The OS HR was 0.97 (95% CI 0.86–1.08; median 26.7 versus 26.4 months, respectively. In patients with triple-negative mBC, the HRs for PFS and OS were 0.63 (95% CI 0.52–0.76) and 0.96 (95% CI 0.79–1.16), respectively. Median PFS was 8.1 months with bevacizumab versus 5.4 months with chemotherapy alone, median OS was 18.9 versus 17.5 months, respectively, and 1-year OS rates were 71% versus 65%.

Conclusions: Bevacizumab improves efficacy, including 1-year OS rates, both overall and in subgroups of poor-prognosis patients with limited treatment options.

Key words: anti-angiogenesis, bevacizumab, first-line therapy, metastatic breast cancer, triple-negative breast cancer, vascular endothelial growth factor

Introduction

Since 2011, the role of bevacizumab in HER2-negative metastatic breast cancer (mBC) has been examined by various regulatory authorities. The US Food and Drug Administration (FDA) and the European Committee for Medicinal Products for Human Use reviewed the same datasets but drew divergent conclusions. In the USA, the FDA revoked approval of bevacizumab in this setting, whereas the European Commission considered that the benefit:risk balance remains positive for bevacizumab in combination with paclitaxel. Furthermore, the European Commission extended the indication to include bevacizumab in combination with capecitabine, although the indication in combination with docetaxel was withdrawn. There was one notable point of agreement between the authorities: the urgent need to identify those patients deriving the most substantial benefit from bevacizumab.

Biomarker analyses of the AVADO (mBC), AVAGAST (gastric cancer) and AViTA (pancreatic cancer) trials suggest that pre-treatment plasma vascular endothelial growth factor (VEGF)-A concentration may have potential predictive as well as prognostic value [1–3]. The ongoing randomised phase III MERiDiAN trial (ClinicalTrials.gov identifier NCT01663727) in HER2-negative mBC is prospectively evaluating plasma VEGF-A as a potential biomarker for bevacizumab efficacy. Currently, however, plasma VEGF-A cannot be used to guide treatment decisions. In many health care systems, there are substantial barriers to prescribing bevacizumab to all patients presenting with HER2-negative mBC who are eligible for first-line bevacizumab combined with chemotherapy. The practical challenge is to deliver the available resources to those in whom it might make the biggest impact or who have limited treatment options.

The use of bevacizumab in patients with triple-negative (estrogen-, progesterone- and HER2-negative) breast cancer...
(TNBC) has attracted considerable interest, primarily because of the lack of targeted therapy for these patients and failure of drugs developed in this setting, but also because of the observed effect of bevacizumab in the subgroup of patients with TNBC treated in the E2100 trial. In these patients, median progression-free survival (PFS) was 10.6 months with bevacizumab plus paclitaxel versus 5.3 months with paclitaxel alone (hazard ratio [HR] 0.49; 95% confidence interval [CI] 0.34–0.70), the overall response rate was 43% versus 22%, respectively, and median overall survival (OS) was 20.5 versus 16.3 months (HR 0.89; 95% CI 0.66–1.19) [4, 5].

To increase the likelihood of detecting signals, particularly in subgroups of patients defined by disease and patient characteristics, we carried out a meta-analysis including data from 2447 patients.

methods
study design
A pooled analysis of individual patient data from three randomised phase III trials (E2100, AVADO and RIBBON-1) of first-line bevacizumab-containing therapy for HER2-negative mBC was undertaken, focusing especially on patient subgroups with a poor prognosis.

The designs of the three trials have been previously described [6–8]. In all three trials, patients who had received no prior chemotherapy for mBC were randomised to chemotherapy either alone or combined with bevacizumab. PFS was the primary end point. OS was a secondary end point. AVADO and RIBBON-1 were not powered for OS; E2100 had 80% power to detect an OS HR of 0.71. In all trials, bevacizumab was continued until disease progression, unacceptable toxicity or withdrawal of consent.

There were several differences between the trials. AVADO and RIBBON-1 were double-blinded placebo-controlled trials, whereas E2100 was an open-label study. The chemotherapy backbone was weekly paclitaxel in E2100, 3-weekly docetaxel in AVADO, capecitabine in one cohort of RIBBON-1 and either a taxane or an anthracycline-based combination in the second cohort of RIBBON-1. Chemotherapy was continued until disease progression, unacceptable toxicity or withdrawal of consent in E2100 and RIBBON-1, but in AVADO, docetaxel was given for a maximum of nine cycles and bevacizumab was continued as a single agent after stopping docetaxel. In AVADO and RIBBON-1, patients in either arm could receive bevacizumab with their second-line therapy after progression, whereas further bevacizumab was not permitted or planned in E2100. One-year OS rate was a pre-specified secondary end point in RIBBON-1 and AVADO but not in E2100.

statistical analyses
The three trials had similar procedures for collecting baseline data and identical definitions for the primary and secondary outcome measures. These features, together with the similar PFS HRs with overlapping 95% CIs, provided the rationale for pooling the trials. Treatment-by-study interactions were calculated to further assess the appropriateness of pooling data.

The docetaxel plus bevacizumab 7.5 mg/kg arm in AVADO was excluded from the pooled analysis as this bevacizumab dose is not approved in mBC. PFS data were censored for patients who received non-protocol anti-cancer therapy before disease progression. The primary analysis of PFS was based on independent review facility assessment for E2100 and on investigator assessment for AVADO and RIBBON-1. For PFS, the data cut-offs for this meta-analysis were 9 February 2005 for E2100, 31 October 2007 for AVADO and 31 July 2008 for RIBBON-1. For OS, the data cut-offs were 21 October 2006 for E2100, 30 April 2009 for AVADO and 23 February 2009 for RIBBON-1.

PFS and OS were estimated using the Kaplan–Meier method. A Cox model was used to provide a HR estimate with a single stratification factor, ‘study’ (E2100, AVADO, RIBBON-1 [capecitabine], RIBBON-1 [taxane/anthracycline]) for the pooled analyses. Exploratory analyses were conducted on a range of subgroups identified by patient, tumour or pre-treatment characteristics with a focus on patients with a median OS of <24 months with chemotherapy alone. Within the subgroups, unstratified HRs were summarised and Kaplan–Meier methodology was used to estimate the median PFS and OS in each arm. PFS and OS were compared between treatment arms using two-sided log-rank tests. One-year survival rates were compared using the normal approximation method (Greenwood estimate for standard error). The subgroup with metastatic TNBC was explored in more detail because of the clinical interest in bevacizumab for this subgroup as described above. All P-values were two-sided and the type I error rate was set at 0.05 with no multiplicity adjustment.

results
patient population
The analysis population included 2447 patients: 1439 receiving bevacizumab and 1008 receiving non-bevacizumab therapy. Of these, 363 and 258 patients, respectively, had TNBC. The imbalance between the bevacizumab and non-bevacizumab arms is due to the 2:1 randomisation in the RIBBON-1 trial. The E2100 trial was slightly over-represented in the TNBC subgroup: 37% of patients in the TNBC subgroup were from E2100, whereas patients treated in E2100 comprised only 30% of the pooled population.

Median follow-up for survival (both treatment arms pooled) was 35 months in E2100, 29 months in AVADO, 23 months in the capecitabine cohort of RIBBON-1 and 26 months in the anthracycline/taxane cohort of RIBBON-1.

Patient characteristics were generally balanced between the treatment arms, both in the overall population and in the subgroup of patients with TNBC (Supplementary Table S1, available at Annals of Oncology online). The TNBC subset was characterised by a shorter disease-free interval and greater likelihood of prior adjuvant taxane exposure compared with the overall analysis population. P-values for the treatment-by-study interaction effects for PFS in the overall patient population were not significant, indicating no differential treatment effect between trials.

efficacy
In the overall analysis population, the stratified HR for PFS was 0.64 (95% CI 0.57–0.71). Median PFS was 9.2 months in the bevacizumab arm and 6.7 months in the non-bevacizumab arm (Figure 1A). Overall response rates (in patients with measurable disease, n = 1893) were 49% versus 32%, respectively (P < 0.0001), consistent with the significant benefit from bevacizumab reported in the individual trials. There was, however, no difference in OS between the two treatment arms (Table 1, Figure 1B).

PFS and OS in subgroups identified according to baseline characteristics were generally similar to results in the overall population. The unstratified HR for PFS ranged from 0.54 to 0.71 for all subgroups with a sample size >200 patients (Figure 2A). The
Figure 1. Kaplan–Meier curves in the entire pooled population: (A) PFS; (B) OS. BEV, bevacizumab.

Table 1. Summary of efficacy (exploratory analyses) in selected ‘poor-prognosis’ subgroups

<table>
<thead>
<tr>
<th>Subgroup</th>
<th>PFS HR (95% CI)</th>
<th>Median PFS, months</th>
<th>OS HR (95% CI)</th>
<th>Median OS, months</th>
<th>1-year OS rate difference, % (95% CI)</th>
<th>1-year OS rate, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>All (n = 2447)</td>
<td>0.64 (0.58–0.71)</td>
<td>9.2</td>
<td>0.98 (0.87–1.09)</td>
<td>26.7</td>
<td>5.1 (1.8–8.4)</td>
<td>81.6</td>
</tr>
<tr>
<td>Triple-negative breast cancer (n = 621)</td>
<td>0.63 (0.52–0.76)</td>
<td>8.1</td>
<td>0.96 (0.79–1.16)</td>
<td>18.9</td>
<td>6.1 (1.5 to 13.6)</td>
<td>70.9</td>
</tr>
<tr>
<td>Visceral disease (n = 1707)</td>
<td>0.66 (0.59–0.75)</td>
<td>8.5</td>
<td>0.96 (0.85–1.09)</td>
<td>24.8</td>
<td>5.2 (1.1–9.4)</td>
<td>78.5</td>
</tr>
<tr>
<td>≥3 metastatic sites (n = 980)</td>
<td>0.64 (0.55–0.75)</td>
<td>8.3</td>
<td>0.93 (0.79–1.10)</td>
<td>22.1</td>
<td>8.2 (2.4–14.0)</td>
<td>76.9</td>
</tr>
<tr>
<td>Prior (neo)adjuvant chemotherapy (n = 1525)</td>
<td>0.60 (0.53–0.68)</td>
<td>9.2</td>
<td>0.87 (0.76–1.00)</td>
<td>26.4</td>
<td>7.3 (3.0–11.6)</td>
<td>81.9</td>
</tr>
<tr>
<td>Prior (neo)adjuvant taxane (n = 558)</td>
<td>0.54 (0.44–0.67)</td>
<td>9.2</td>
<td>0.72 (0.57–0.90)</td>
<td>26.7</td>
<td>10.6 (3.2–17.9)</td>
<td>81.9</td>
</tr>
</tbody>
</table>

*Estimated using unstratified Cox regression.

BEV, bevacizumab; CI, confidence interval; HR, hazard ratio; OS, overall survival; PFS, progression-free survival.
unstratified HR for OS in these groups was typically close to 1 (Figure 2B).

In subgroups of patients with a median OS of <24 months in the non-bevacizumab arm, the HR for PFS consistently favoured bevacizumab, ranging from 0.54 to 0.66. Median PFS was ≤6.5 months with chemotherapy alone in all of the subgroups, but exceeded 8 months in patients treated with bevacizumab. The HR for OS was generally below 1 but with the upper boundary of the 95% CI crossing 1, except for the subgroup of patients pre-treated with a taxane-containing regimen. In this population, the unstratified HR for OS was 0.72 (95% CI 0.57–0.90; median 26.7 months with bevacizumab versus 20.5 months with chemotherapy), although this finding should be interpreted with caution in this exploratory analysis of multiple subgroups. One-year OS rates consistently favoured the bevacizumab arm.

In the subgroup of patients with TNBC, the PFS HR (unstratified analysis) was 0.63 (95% CI 0.52–0.76; P < 0.0001) (Figure 3A). The objective response rate was significantly higher with bevacizumab-containing therapy than with chemotherapy.

**Figure 2.** Subgroup analysis of: (A) PFS (unstratified); (B) OS. BEV, bevacizumab; ER, estrogen receptor; HR, hazard ratio; OS, overall survival; PFS, progression-free survival; PgR, progesterone receptor.
alone (42% versus 23%, respectively; \( P < 0.0001 \)). In addition, primary resistant disease (best response of progressive disease) was significantly less common among patients receiving bevacizumab-containing therapy than in those treated with chemotherapy alone (11% versus 28%, respectively; \( P < 0.0001 \)). The HR for OS (unstratified analysis) was 0.96 (95% CI 0.79–1.16; \( P = 0.6732 \)) (Figure 3B). One-year survival rates were 71% with bevacizumab-containing therapy versus 65% with chemotherapy alone.

In additional exploratory analyses, an OS HR of 0.61 (95% CI 0.40–0.94) was observed in the subset of patients with hormone receptor-negative mBC who had received prior taxane therapy (median OS 25.6 months with bevacizumab versus 15.0 months with non-bevacizumab therapy).

**safety**

The most frequent grade \( \geq 3 \) adverse events with bevacizumab-containing therapy were neutropenia, sensory neuropathy and hypertension (Supplementary Table S2, available at *Annals of Oncology* online). The only adverse events with a >2% higher incidence in the bevacizumab compared with the non-bevacizumab groups were hypertension, proteinuria, febrile neutropenia and neutropenia. At the time of data cut-off, 51% of patients treated with bevacizumab and 56% of those receiving non-bevacizumab therapy in the safety population had died. Deaths in 2.1% and 1.8% of patients, respectively, were considered to be treatment-related. The safety profile in the TNBC subgroup was very similar to that observed in the overall population.

**Figure 3.** Triple-negative subgroup: (A) PFS; (B) OS. BEV, bevacizumab.
discussion

This pooled analysis of individual patient data from three randomised phase III trials demonstrated significantly improved PFS and response rate in patients receiving first-line bevacizumab combined with chemotherapy versus chemotherapy alone. However, there was no significant difference in OS. The impact of long post-progression survival and the potential confounding influence of subsequent lines of therapy and crossover to bevacizumab (which significantly improves PFS in the second-line mBC setting [9]) are discussed extensively in the literature [10–13]. In subgroups with relatively short OS and thus shorter post-progression survival, the likelihood of detecting an OS benefit may be expected to increase. In the exploratory subgroup analyses reported here, no OS benefit from bevacizumab was seen in the subgroups defined by a median OS of <24 months. However, 1-year OS rates consistently favoured the bevacizumab arm, suggesting that in those with the highest risk of rapid progression and short OS after starting chemotherapy for mBC, bevacizumab may have had a more marked effect. Nevertheless, a priori identification of these patients remains a clinical conundrum.

The TNBC subgroup represents, to our knowledge, the largest reported population of patients randomised to treatment for metastatic TNBC. Moreover, it is a ‘pure’ population, with all patients treated in the first-line setting rather than in mixed treatment lines. Among the 621 patients with TNBC, 232 were treated in E2100, suggesting that the effect in TNBC was influenced but not driven by E2100. The HR was 0.63 for PFS (median 8.1 months for bevacizumab versus 5.4 months for chemotherapy alone) and 0.96 for OS (median 18.9 versus 17.5 months, respectively). In the literature, median PFS in TNBC typically ranges from 2 to 6 months and median OS is around 12 months [14]. The improvement in 1-year OS rate with bevacizumab suggests that within the TNBC subgroup, patients at risk of rapid progression may benefit, although we are not able to identify these particularly poor-prognosis patients. The effect of bevacizumab in TNBC is supported by a subgroup analysis of the RIBBON-2 trial in the second-line HER2-negative mBC setting. A numerical trend towards improved OS was observed among 159 patients receiving bevacizumab in combination with chemotherapy versus chemotherapy alone (HR 0.85; 95% CI 0.58–1.26; median 17.8 months versus 13.5 months, respectively) [15]. Efforts to identify subtypes within the heterogeneous TNBC population are hampered by the lack of molecular characterisation of tumours in patients from these trials.

Several meta-analyses of bevacizumab have been published in recent years, most of which include trials across a range of treatment lines and tumour types. Many focus on safety, but five report efficacy analyses specifically in breast cancer [16–20]. Of these, three included mixed settings and in some cases, early OS results from two of the first-line trials. More recently, Rossari et al. [18] conducted a meta-analysis including only trials in the first-line mBC setting, similar to the present meta-analysis. The investigators followed Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidance [21], but for some trials, HRs were estimated or extrapolated from published graphs. An additional difference between Rossari et al.’s report and our meta-analysis is the handling of the bevacizumab 7.5 mg/kg arm in AVADO. We chose to include only cohorts treated with bevacizumab 15 mg/kg every 3 weeks to avoid a possible confounding effect of varied bevacizumab doses, whereas Rossari et al. included the comparison of bevacizumab 7.5 mg/kg versus placebo and the comparison of bevacizumab 15 mg/kg versus placebo, thus effectively counting the results in the placebo arm twice within the meta-analysis. A recent Cochrane review [19] included the three trials included in our analysis and that of Rossari et al., and additionally the TRIO 010 randomised phase II trial evaluating paclitaxel in combination with motesanib, bevacizumab or placebo as first-line therapy for mBC [22].

Use of individual patient data with longer follow-up represents an important methodological advantage of the present meta-analysis. Despite the differences between the various meta-analyses and the inclusion of an additional trial in the Cochrane review, for which we did not have access to individual patient data, the PFS HRs were quite similar, ranging from 0.64 in the present meta-analysis to 0.70 in Rossari et al.’s report. Furthermore, none of the three meta-analyses in the first-line setting demonstrated an OS difference. Of note, the present meta-analysis is the first to focus on subgroups of patients with a poor prognosis and to report 1-year OS rates within these subgroups. The apparent effect of bevacizumab on 1-year OS rates suggests that even within these clinically defined subgroups, the aggressiveness of disease and patients’ prognoses are quite heterogeneous and it is perhaps those at risk of very rapid progression who gain most from the addition of bevacizumab to chemotherapy. For example, in the subgroup of patients pretreated with taxanes, the HR for OS was 0.72 (95% CI 0.57–0.90; median 26.7 months with bevacizumab versus 20.5 months with chemotherapy alone). However, this hypothesis does not help in identifying such patients a priori. Whether there is any overlap between those patients exhibiting the most aggressive disease and those with high pre-treatment concentrations of plasma VEGF-A is not known.

A major limitation of this meta-analysis is the ad hoc nature of the subgroup analyses, defined using the clinical rationale of a lack of effective options rather than by a biological hypothesis as to why bevacizumab should be most effective in these specific subgroups. Thus the suggestion of an effect may simply reflect the greater number of events, especially for 1-year OS, increasing the chance of detecting a difference between treatments. Nevertheless, OS was not significantly different in the bevacizumab versus non-bevacizumab arms. Another weakness is the lack of adjustment for multiple testing. The number of subgroup analyses carried out increases the probability of a false-positive finding [23], and we acknowledge that firm conclusions about bevacizumab cannot be drawn from these post hoc analyses.

Safety results are consistent with the well-defined safety profile for bevacizumab in a range of tumours. Bevacizumab was associated with an increased incidence of hypertension and proteinuria but no increase in gastrointestinal perforations, consistent with findings specifically in breast cancer [18, 24]. Other meta-analyses in breast cancer have shown increased risks of grade ≥3 bleeding [17, 24], neurotoxicity [17, 18], left ventricular dysfunction/cardiac events [18, 24] and febrile
neutropenia [17]. In our individual patient data meta-analysis, the incidences of grade ≥3 bleeding and left ventricular dysfunction were slightly increased with bevacizumab, but remained below 2%. The increase in congestive heart failure reported by Choueiri et al. [25] was not identified in the Cochrane review, which also included data from the single-arm ATHENA study (N = 2264) in routine oncology practice [26].

A recent meta-analysis of bevacizumab trials in various tumour types suggested a higher treatment-related mortality rate in patients receiving bevacizumab- versus non-bevacizumab-containing regimens [27]. However, no such increase was seen among patients with breast cancer treated with bevacizumab, consistent with findings from the present meta-analysis and the Cochrane review of four bevacizumab mBC trials, which showed a lower odds ratio for treatment-related deaths with bevacizumab versus non-bevacizumab-containing therapy (odds ratio 0.60; 95% CI 0.36–0.99) [19].

In summary, this pooled analysis of individual patient data from three randomised phase III trials indicates that the magnitude of bevacizumab benefit is similar irrespective of baseline characteristics. However, in clinical practice, the absolute benefit should perhaps be considered in light of the patient’s prognosis and available treatment options. If resources are limited, bevacizumab treatment could be directed towards patients in whom it may have the greatest impact compared with chemotherapy alone. Moving forward, the highest research priority with bevacizumab in mBC is prospective evaluation of candidate biomarkers, such as plasma VEGF-A, so that treatment decisions can be guided by robust, prospectively generated data rather than by post hoc subgroup analyses.

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Overabundant FANCD2, alone and combined with NQO1, is a sensitive marker of adverse prognosis in breast cancer

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Background: Defective DNA repair is central to the progression and treatment of breast cancer. Immunohistochemically detected DNA repair markers may be good candidates for novel prognostic and predictive factors that could guide the selection of individualized treatment strategies.

Patients and methods: We have analyzed nuclear immunohistochemical staining of BRCA1, FANCD2, RAD51, XPF, and PAR in relation to clinicopathological and survival data among 1240 paraffin-embedded breast tumors, and additional gene expression microarray data from 76 tumors. The antioxidant enzyme NQO1 was analyzed as a potential modifier of prognostic DNA repair markers.

Results: RAD51 [hazard ratio (HR) 0.81, 95% confidence interval (CI) 0.70–0.94, P = 0.0050] and FANCD2 expression (HR 1.50, 95% CI 1.28–1.76, P = 1.50 \times 10^{-7}) were associated with breast cancer survival. High FANCD2 expression correlated with markers of adverse prognosis but remained independently prognostic in multivariate analysis (HR 1.27, 95% CI 1.08–1.49, P = 0.0043). The FANCD2-associated survival effect was most pronounced in hormone receptor positive, HER2-negative tumors, and in tumors with above-median NQO1 expression. In the NQO1-high subset, patients belonging to the highest quartile of FANCD2 immunohistochemical scores had a threefold increased risk of metastasis or death (HR 3.10, 95% CI 1.96–4.92). Global gene expression analysis indicated that FANCD protein overabundance is associated with the upregulation of proliferation-related genes and a downregulated nucleotide excision repair pathway.

Conclusion: FANCD2 immunohistochemistry is a sensitive, independent prognostic factor in breast cancer, particularly when standard markers indicate relatively favorable prognosis. Taken together, our results suggest that the prognostic effect is linked to proliferation, DNA damage, and oxidative stress; simultaneous detection of FANCD2 and NQO1 provides additional prognostic value.

Key words: DNA repair, breast cancer, prognosis, FANCD2, NQO1

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