RESEARCH ARTICLE

Risk of Serious Neutropenic Events in Cancer Patients Treated with Bevacizumab: A Meta-analysis

Fan Zhou[1&*], Jiang-Hua Shao[1,2&], Lin-Quan Wu[1,2], Xiang-Bao Yin[1], Xin Yu[1]

Abstract

Bevacizumab has been approved for use in combination with chemotherapy to treat many types of cancer but associated neutropenic events, including febrile neutropenia, have been reported. To estimate the incidence and relative risk of neutropenic events in cancer patients treated with bevacizumab combination therapy, we searched PubMed, EMBASE, and Web of Science literature databases, as well as abstracts presented at the American Society of Clinical Oncology conferences, to identify relevant studies published from January 1966 to December 2011. Studies that compared bevacizumab plus chemotherapy or biological therapy with chemotherapy or biological therapy alone, and that had adequate safety data profiles, were selected for analysis. Statistical analyses were conducted to calculate the summary incidence rates, relative risks (RRs), and 95% confidence intervals (CIs) using fixed- or random-effects models. A total of 22 clinical trials involving 15,056 patients were included in the analysis. The summary incidences of high-grade neutropenia (HGN) and high-grade febrile neutropenia (HGFN) in patients receiving bevacizumab was 27.3% (95% CI: 26.4%-28.3%) and 3.91% (95% CI: 3.51%-4.37%), respectively. The risks of HGN (RR=1.10; 95% CI: 1.02-1.19; *P*=0.02) and HGFN (RR=1.31; 95% CI: 1.08–1.59; *P*=0.005) were significantly increased in bevacizumab-treated patients, compared to those who did not receive bevacizumab. The RR of bevacizumab-associated HGN, but not HGFN, varied significantly with tumor types (*P*=0.005). The increased risk of bevacizumab-associated neutropenic events was dose-dependent, as the RR was greater at a dose of 5 mg/kg/week than at 2.5 mg/kg/week. Our findings suggest that bevacizumab addition to cancer therapy significantly increases the risk of serious neutropenic events, and this risk may be dose-dependent.

Keywords: Bevacizumab - neutropenia - febrile neutropenia - cancer - meta-analysis

Asian Pacific J Cancer Prev, **14 (4)**, 2453-2459

Introduction

Vascular endothelial growth factor (VEGF) plays an important role in tumor growth, invasion, and metastasis by promoting tumor angiogenesis (Folkman, 2002; Kerbel, 2008). The human VEGF family consists of five members: VEGF-A, VEGF-B, VEGF-C, VEGF-D, and placental growth factor (PIGF), of which VEGF-A is considered the most important angiogenic factor in cancer (Sakurai et al., 2011). Bevacizumab (manufactured by Genentech Inc. as Avastin), a humanized monoclonal antibody that can bind to VEGF-A, has been demonstrated to effectively inhibit VEGF receptor binding, thereby preventing tumor angiogenesis (Rini et al., 2008). As a result, bevacizumab has been approved for use in combination with chemotherapy to treat many types of advanced cancers, including colorectal cancer, non–small cell lung cancer (NSCLC), breast cancer, renal cell carcinoma, and glioblastoma multiforme (Hurwitz et al., 2005; Rini et al., 2008; Baar et al., 2009; Miles et al., 2010).

Although bevacizumab is remarkably well-tolerated by patients, a distinct pattern of adverse effects that are thought to be related to angiogenesis inhibition has emerged (Geiger-Gritsch et al., 2010; Hapani et al., 2010). The most concerning of these effects are hypertension, proteinuria, wound healing, venous and arterial thromboembolic events, gastrointestinal perforations, and congestive heart failure (Hapani et al., 2009; Ranpura et al., 2010; Choueiri et al., 2011). Neutropenic events, including febrile neutropenia, are generally characterized as side effects of chemotherapy. They have a significant negative impact on mortality, morbidity, and healthcare costs, often leading to treatment delays and interruptions. Recently, Ranpura et al. (2011) found that neutropenic events are the second most common cause of fatal adverse events in cancer patients treated with bevacizumab.

The reported overall incidence of neutropenic events, including febrile neutropenia, associated with

*[1]Department of Hepatobiliary and Pancreatic Surgery, the Second Affiliated Hospital of Nanchang University, [2]Jiangxi Provincial Center for Hepatobiliary Disease, Nanchang, China [&]Equal contributors *For correspondence: zhoufan.123@hotmail.com, nczhoufan@hotmail.com*

bevacizumab therapy has varied substantially among clinical trials (Escudier et al., 2007; Reck et al., 2009; Tebbutt et al., 2010). Therefore, an accurate quantification of this risk remains to be performed. While a recent meta-analysis of published clinical trials indicated that bevacizumab is associated with increased risks of neutropenic events (Schutz et al., 2011), the risk factors of neutropenic events were not identified. To better understand the overall risk of neutropenic events imparted by bevacizumab therapy and to identify the underlying risk factors, we conducted a meta-analysis of the published randomized controlled trials (RCTs) that had investigated bevacizumab combination treatment of cancer patients and occurrence of neutropenic events.

Materials and Methods

Data sources

We conducted an independent review of citations listed on PubMed between January 1, 1966, and December 31, 2011. The key words used were "bevacizumab", "Avastin" and "cancer", and the search was limited to RCTs. The search strategy also used the following text terms to identify additional relevant information: "neutropenia", "febrile neutropenia", "angiogenesis", and "VEGF". In addition, abstracts and virtual meeting presentations published by the American Society of Clinical Oncology conferences (http://www.asco.org/ASCO) between January 2000 and December 31, 2011 were searched using the terms "bevacizumab" and "Avastin". Independent searches of the EMBASE or Web of Science databases were performed to ensure that no clinical trials were missed. Each potentially relevant publication was downloaded for investigation of the full-text. If more than one publication was identified from the same clinical trial, only the most recent or complete report was selected. The updated manufacturer's package insert of bevacizumab was also reviewed to identify any additional relevant information.

Study selection

Only RCTs that directly compared cancer patients treated with and without bevacizumab were incorporated into the analysis. In addition, all clinical trials were required to meet the following criteria for inclusion in the meta-analysis: (1) prospective phase 2 or 3 trials involving patients with cancer; (2) random group assignment of participants to the bevacizumab treatment or control (placebo or best supportive care) in addition to concurrent therapy using a chemotherapeutic or biological agent; and (3) available data, including events or incidence of neutropenia and sample size, for analysis. Study quality was assessed by considering adequate blinding of randomization, completeness of follow-up, and objectivity of outcome measurements, as previously described (Meade et al., 1997).

Data extraction and clinical endpoints

Data extraction was conducted independently by two investigators (FZ and JHS) and according to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) statement (www.prisma-statement.org). Any discrepancies were resolved by consensus. For each study, the following information was extracted: first author's name, year of publication, trial phase, underlying malignancy, number of enrolled patients, treatment arms, number of neutropenic events in experimental and control arms, drug dose/schedule, median age, median follow-up, median treatment duration, and median progression-free survival.

The goal of this study was to determine whether bevacizumab contributes to the development of high-grade neutropenia (HGN) and/or high-grade febrile neutropenia (HGFN) in cancer patients. Therefore, the number of neutropenic and/or febrile neutropenic events reported in the safety profile section of each study was recorded. Only adverse events of grade 3 or higher (serious) according to the National Cancer Institute Common Toxicity Criteria (NCI-CTC, version 2 or 3; http://ctep.cancer.gov) were included in the analysis, as trials rarely report all-grade or low-grade neutropenia and febrile neutropenia.

Statistical analysis

All statistical analyses was performed by Review Manager (RevMan) version 5.0 (Copenhagen: Nordic Cochrane Centre, Cochrane Collaboration, 2008) or STATA 10SE statistical software (STATA, College Station, TX, USA). To calculate incidence, the number of patients with each adverse event and the number of patients receiving bevacizumab were used to derive the proportion of patients with adverse events and 95% confidence interval (CI) for each study. To calculate relative risk (RR), patients who received bevacizumab in combination with chemotherapy were compared with those in the same trial who received chemotherapy alone.

Both fixed-effects and random-effects models were considered in the meta-analyses. For each meta-analysis, the Cochran Q statistic and I^2 score were first calculated to assess heterogeneity among the proportions of the included trials. If the *P*-value was less than 0.1, the assumption of homogeneity was deemed invalid, and a random-effects model using the DerSimonian and Laird method was reported after exploring the causes of heterogeneity. Otherwise, results from the fixed-effects model were reported by using the inverse variance method. Statistical heterogeneity was evaluated using I^2 statistics, with values up to 25%, 25%–50%, and above 50% indicating low, moderate, and high levels of heterogeneity, respectively. A Chi-squared (χ^2) test for heterogeneity was performed, for which $P<0.1$ was considered statistically significant. Subgroup analyses was performed to identify risk factors for neutropenia and febrile neutropenia with bevacizumab-based therapy. To explore a dose-effect relationship, the bevacizumab therapy group was further divided into those receiving low-dose (2.5, 5, or 7.5 mg/kg schedule, equivalent to a weekly dose of 2.5 mg/kg) and high-dose (10 or 15 mg/kg schedule, equivalent to a weekly dose of 5 mg/kg), as previously described (Ranpura et al., 2011). Subgroup analyses were also performed for the year that the study was performed, the tumor types, and the chemotherapy regimens used. Q statistics were used for comparison of subgroup results. Publication bias was

Table 1. Characteristics of Randomized Controlled Clinical Trials Included in the Meta-analysis

Source (publication year)	Trial phase	Patients enrolled[a]	Patients analyzed[b]	Underlying malignancy	Duration of follow-up, months	Concurrent treatment	Bevacizumab dose, mg/kg/week[c]
Baar (2009)	2	49	49	breast cancer	NA	Docetaxel	5
Brufsky (2010)	3	684	679	breast cancer	NA	Carboplatin and paclitaxel	5
Miles (2010)	3	736	730	breast cancer	10.2	Docetaxel	2.5 or 5
Miller (2007)	3	722	711	breast cancer	25.9	Paclitaxel	5
Robert (2009)	3	1237	1220	breast cancer	19.2	Capecitabine, taxane, or anthracycline	5
Allegra (2009)	3	2710	2710	colorectal cancer	22·4	Fluorouracil, oxaliplatin, and leucovorin	2.5
Hurwitz (2005)	3	210	207	colorectal cancer	NA	Fluorouracil and leucovorin	2.5
Moehler (2009)	2	46	46	colorectal cancer	19.5	Capecitabine and irinotecan	2.5
Tebbutt (2010)	3	471	313	colorectal cancer	30.8	Capecitabine	5
Kang (2010)	3	774	767	gastric cancer	NA	Capecitabine and cisplatin	2.5
Okines (2010)	2/3	104	104	gastric cancer	NA	Epirrubicin	2.5
Karrison (2007)	3	108	108	mesothelioma	NA	Gemcitabine and cisplatin	5
Zalcman (2010)	2/3	111	94	mesothelioma	6	Pemetrexed and cisplatin	5
Sandler (2006)	3	878	867	NSCLC	19	Carboplatin and paclitaxel	5
Herbst (2007)	2	122	120	NSCLC	15.8	Docetaxel or pemetrexed	5
Reck (2009)	3	1043	986	NSCLC	NA	Gemcitabine and cisplatin	2.5 or 5
Burger (2010)	3	1873	1816	ovarian cancer	17.4	Carboplatin and paclitaxel	5
Kindler (2010)	3	602	540	pancreatic cancer	NA	Gemcitabine	5
Van Cutsem (2009)	3	607	583	pancreatic cancer	6.7	Gemcitabine and erlotinib	2.5
Kelly (2010)	3	1050	1050	prostate cancer	24	Docetaxel	5
Escudier (2007)	3	649	641	renal cell cancer	13.3	Interferon alfa	5
Rini (2010)	3	732	715	renal cell cancer	NA	Interferon alfa	5

NA, data not available; NSCLC, non–small cell lung cancer; [a]The number of patients recruited for the original study; [b]The number of patients actually exposed to the study drugs; [c]The dose schedule was converted from a milligrams-per-kilogram schedule

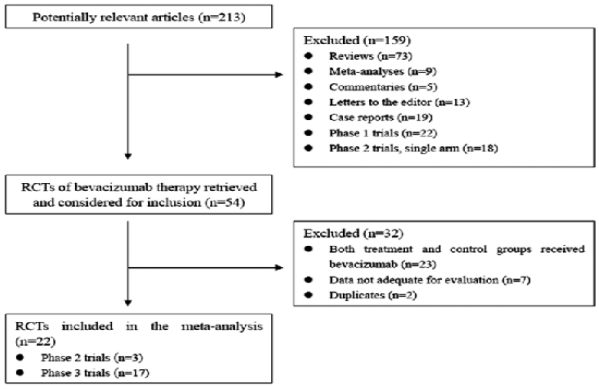

Figure 1. Selection Process for RCTs Included in the meta-analysis. A total of 213 potentially relevant studies of bevacizumab were identified, of which 159 were initially excluded for not meeting the inclusion criteria. An additional 32 trials were excluded for being duplicates, for having administering bevacizumab to both treatment and control groups, or not reporting adequate data for evaluation. Thus, 22 trials were selected for inclusion in the meta-analysis, including three phase 2 and 17 phase 3 studies

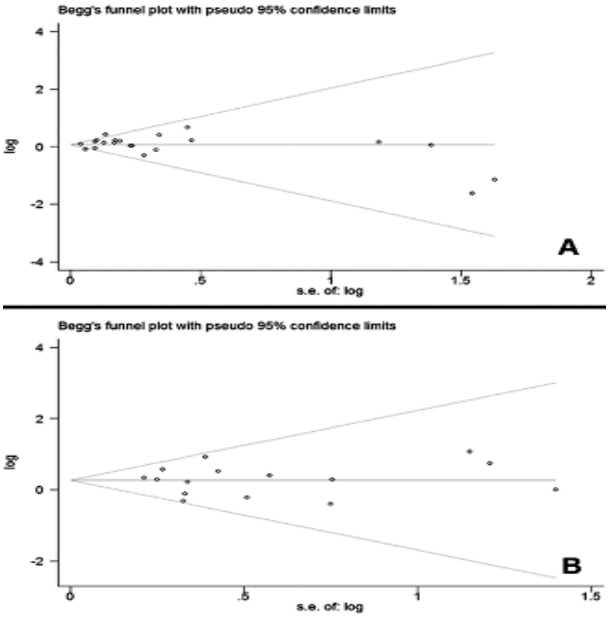

Figure 2. Begg's Test Assessment of Publication bias for the Primary Endpoint of Relative Risk of (A) High-grade Neutropenia and (B) High-grade Febrile Neutropenia Events. No evidence of publication bias was detected for the primary endpoint of this study

evaluated using funnel plots and quantified by Begg's test. A two-tailed *P*-value less than 0.05 was considered statistically significant.

Results

Search results and study quality

Our search yielded a total of 213 potentially relevant studies of bevacizumab, of which 159 were initially excluded for not meeting the inclusion criteria. After evaluating each remaining study, 22 RCTs were selected for the meta-analysis (Hurwitz et al., 2005; Sandler et al., 2006; Escudier et al., 2007; Herbst et al., 2007; Karrison et al., 2007; Miller et al., 2007; Allegra et al., 2009; Baar et al., 2009; Moehler et al., 2009; Reck et al., 2009; Robert et al., 2009; Van Cutsem et al., 2009; Brufsky et al., 2010; Burger et al., 2010; Kang et al., 2010; Kelly et al., 2010; Kindler et al., 2010; Miles et al., 2010; Okines et al., 2010; Rini et al., 2010; Tebbutt et al., 2010; Zalcman et al., 2010). The selection process is summarized in Figure 1.

Randomized treatment allocation sequences had been generated in all trials. Ten trials were double-blinded and

Table 2. Incidence and Relative Risk of High Grade Neutropenia with Bevacizumab According to Dose, Tumor Type, and Chemotherapy Type

Tumor type	No. of studies	No. of HGN events		Incidence, % (95% CI)		RR (95%CI)
		Bevacizumab/Total	Control/Total	Bevacizumab	Control	
Breast cancer	5	214/2163	85/1226	9.89 (8.70-11.23)	5.43 (2.07-13.49)	1.2 (0.95-1.52)
Colorectal cancer	3	392/1512	434/1494	25.93 (23.78-28.20)	29.05 (26.80-31.40)	0.9 (0.80-1.01)
Gastric cancer	2	148/439	155/432	33.83 (29.54-38.40)	35.96 (31.56-40.61)	0.94 (0.78-1.13)
Mesothelioma	2	36/100	41/102	36.22 (27.35-46.15)	40.2 (21.15-49.96)	0.91 (0.64-1.29)
NSCLC	3	366/1125	185/809	28.91 (19.77-40.17)	21.64 (12.36-35.10)	1.29 (1.11-1.50)
Ovarian cancer	1	769/1215	347/601	63.29 (60.54-65.96)	57.74 (53.75-61.63)	1.10 (1.01-1.19)
Prostate cancer	1	157/524	126/526	29.96 (26.19-34.02)	23.95 (20.5-27.79)	1.25 (1.02-1.53)
Pancreatic cancer	2	153/573	125/550	26.54 (16.53-39.72)	22.51 (12.98-36.13)	1.17 (0.95-1.43)
Renal cell carcinoma	2	48/699	38/651	7.26 (5.51-9.51)	6.91 (5.07-9.37)	1.17 (0.78-1.77)
Dose						
2.5 mg/kg/week	5	602/2090	636/2057	26.44 (20.84-32.92)	26.89 (19.64 -35.64)	0.93 (0.85-1.01)
5.0 mg/kg/week	14	1333/5102	756/3776	26.13 (24.94-27.35)	20.02 (18.78-21.33)	1.14 (1.07-1.21)
Chemotherapeutic agents (5.0 mg)						
Platinum or taxanes	9	1161/3152	629/2303	36.83 (35.17-38.53)	27.31 (25.53-29.17)	1.13 (1.06-1.22)
Non-platinum-taxanes	5	172/1950	127/1473	8.82 (7.64-10.16)	8.62 (7.29-10.17)	1.16 (0.95-1.43)
Overall	21	2283/8350	1536/6391	27.34 (26.40-28.31)	24.03 (23.00-25.10)	1.10 (1.02, 1.19)

Table 3. Incidence and Relative Risk of High Grade Febrile Neutropenia with Bevacizumab According to Dose, Tumor Type, and Chemotherapy Type

Tumor type	No. of studies	No. of HGFN events		Incidence, % (95% CI)		RR (95%CI)
		Bevacizumab/Total	Control/Total	Bevacizumab	Control	
Breast cancer	4	115/2139	39/1201	5.38 (4.50-6.42)	3.25 (2.38-4.41)	1.37 (0.97-1.94)
Colorectal cancer	3	23/1592	29/1575	1.53 (1.02-2.29)	1.91 (1.33-2.74)	0.78 (0.45-1.34)
Gastric cancer	2	32/439	29/432	7.29 (5.20-10.13)	6.71 (4.70-9.49)	1.05 (0.66-1.68)
Mesothelioma	2	3/100	2/102	3.11 (1.01-9.22)	1.97 (0.49-7.52)	1.52 (0.25-9.13)
NSCLC	3	36/1125	13/809	3.47 (1.53-7.66)	1.61 (0.94-2.75)	2.22 (1.20-4.12)
Ovarian cancer	1	56/1215	21/601	4.61 (3.56-5.94)	3.49 (2.29-5.3)	1.32 (0.81-2.16)
Prostate cancer	1	37/524	21/526	7.06 (5.16-9.59)	3.99 (2.62-6.05)	1.77 (1.05-2.98)
Pancreatic cancer	1	3/296	1/287	1.01 (0.33-3.09)	0.35 (0.00-2.43)	2.91 (0.30-27.80)
Renal cell carcinoma	1	0/362	4/347	0	1.15 (0.43-3.03)	0.11 (0.01-1.97)
Dose						
2.5 mg/kg/week	5	54/2170	56/2138	3.95 (3.02-5.16)	4.02 (3.09-5.23)	0.94 (0.65-1.34)
5.0 mg/kg/week	11	161/4464	73/3184	3.61 (3.10-4.20)	2.29 (1.83-2.87)	1.55 (1.18-2.05)
Chemotherapeutic agents (5.0 mg)						
Platinum or taxanes	8	133/3128	59/2278	4.76 (4.03-5.62)	2.59 (2.01-3.33)	1.59 (1.18-2.15)
Non platinum-taxanes	3	28/1336	14/906	2.1 (1.45-3.02)	1.58 (0.94-2.65)	1.36 (0.67-2.76)
Overall	18	305/7792	159/5880	3.91 (3.51-4.37)	2.70 (2.32-3.15)	1.31 (1.08, 1.59)

placebo-controlled, three other trials had placebo controls, and the remaining trials had active controls. Follow-up time was adequate for each trial. The quality of all the trials was acceptable.

No evidence of publication bias was detected for the primary endpoint of this study (RR of HGN/HGFN) by Begg's test (HGN, P=0.24 and HGFN, P=0.91; Figure 2).

Patient characteristics

A total of 15056 patients from the 22 clinical trials were included in the analysis. The trials (n) covered a variety of underlying malignancies: breast cancer (n=5), colorectal cancer (n=4), gastric cancer (n=2), mesothelioma (n=2), non-small cell lung cancer (n=3), ovarian cancer (n=1), pancreatic cancer (n=2), prostate cancer (n=1), and renal cell carcinoma (n=2). Five of the trials were phase II studies and 17 were phase III studies. The trial characteristics are presented in Table 1.

In general, the baseline Eastern Cooperative Oncology Group status of patients was between 0 and 1. According to the inclusion criteria of each trial, patients were required to have adequate hepatic, renal, and hematologic functions. The exclusion criteria reported for the studies included the following conditions: significant cardiovascular disease, peripheral vascular disease, uncontrolled hypertension, serious non-healing wounds, major surgery within the previous 28 days, pre-existing bleeding diathesis, brain metastasis, regular use of aspirin (>325 mg/d) or nonsteroidal anti-inflammatory drugs, pregnancy or lactation, and current use of oral or parenteral anticoagulants, with the exception of prophylactic anticoagulants to maintain patency of vascular device access. In all trials, randomization had been performed between the control and bevacizumab groups. The bevacizumab doses were 2.5 or 5 mg/kg/week.

Incidence of neutropenic events

There were 2283 HGF events reported for 8350 patients who received bevacizumab (Table 2). The highest incidence (63.29%; 95% CI: 60.54%-65.96%) was observed in an ovarian cancer trial. The lowest incidence (7.26%; 95% CI: 5.51%-9.51%) was seen in the trials of

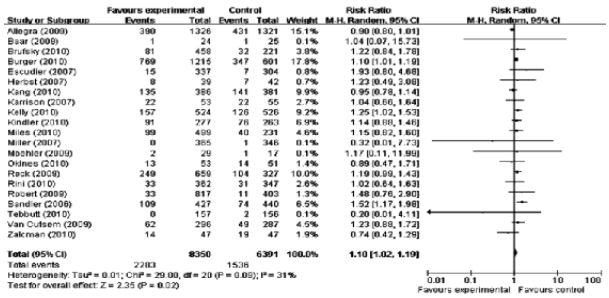

Figure 3. Relative Risk of High-grade Neutropenia (HGN) Associated with Bevacizumab Versus Control. The overall relative risk of developing HGN was greater in patients treated with bevacizumab (RR=1.10; 95% CI: 1.02-1.19; *P*=0.02). A moderate level of heterogeneity existed in these studies (*P*=0.09; I^2=31%)

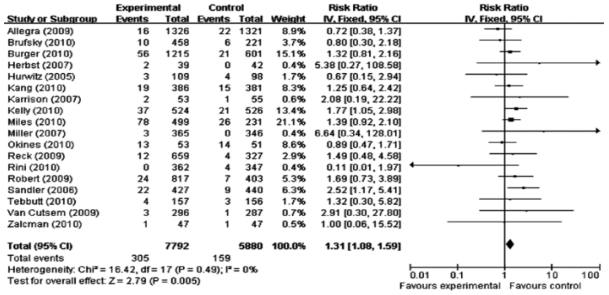

Figure 4. Relative Risk of High-grade Febrile Neutropenia Associated with Bevacizumab Versus Control. Among the 7792 patients in the 18 RCTs analyzed, the relative risk of HGFN was significantly increased in bevacizumab-treated patients (RR=1.31; 95% CI: 1.08–1.59; *P*=0.005). No significant heterogeneity was found among the included trials (Q=16.42; *P*=0.49; I^2=0.0%)

patients with renal cell cancer. Using a fixed-effects model, the summary incidence of HGF in patients receiving bevacizumab was 27.34% (95% CI: 26.40%-28.31%).

For HGFN, there were 305 events reported for 7792 patients, with the highest incidence seen in trials of patients with gastric cancer and the lowest incidence in trials of patients with renal cell cancer (Table 3). The summary incidence of HGFN in patients receiving bevacizumab was 3.91% (95% CI: 3.51%-4.37%).

Relative risk of neutropenic events

In order to assess the contribution of bevacizumab to the development of neutropenic events, including febrile neutropenia, we calculated the overall relative risk of HGN and HGFN. The overall RR of developing HGN in patients treated with bevacizumab versus those who did not receive bevacizuma was 1.10 (95% CI: 1.02-1.19; *P*=0.02) (Figure 3). A moderate level of heterogeneity existed in these studies (*P*=0.09; I^2=31%). Similarly, the RR of high-grade febrile neutropenia was also significantly increased in bevacizumab-treated patients (RR=1.31; 95% CI: 1.08–1.59; *P*=0.005) (Figure 4); however, no significant heterogeneity was found among the included trials (Q=16.42; *P*=0.49; I^2=0.0%).

Tumor type and risk of neutropenic events

To explore the relationship between tumor type and the risk of developing HGN and HGFN, we stratified patients by their underlying malignancy. RRs of HGN varied significantly by tumor type (*P*=0.005), suggesting that the association of bevacizumab with HGN may be different among these tumor types. In contrast, RRs of HGFN did not vary significantly by tumor type (*P*=0.16).

Bevacizumab dose and risk of neutropenic events

The two approved doses of bevacizumab are 2.5 mg/wk (low-dose) and 5.0 mg/wk (high-dose). Therefore, the RRs of HGN and HGFN with bevacizumab were determined for each bevacizumab dose. Among the clinical trials analyzed, data was available from five studies to calculate the RR of HGN in patients receiving low-dose bevacizumab and from 14 studies to calculate that in patients receiving the high-dose. The high-dose administration was found to be associated with a significantly increased risk of HGN (RR=1.14; 95% CI: 1.07-1.21; *P*=0.0001), while the low-dose administration showed no significant association with risk of HGN (RR=0.93; 95% CI: 0.85-1.02; *P*=0.14). Overall, a significant difference was found for the rate of HGN between the high- and low-doses of bevacizumab (*P*=0.006).

Five clinical trials provided data to calculate the RR of HGFN in patients receiving low-dose bevacizumab and 11 provided data to calculate that of high-dose bevacizumab. The high-dose administration was found to be associated with a significantly increased risk of HGFN (RR=1.55; 95% CI: 1.18-2.05; *P*=0.002). However, the low-dose administration was not found to be associated with an increased risk of HGFN (RR=0.94; 95% CI: 0.65-1.34; *P*=0.72). A significant difference was also found for the rate of HGFN between the high- and low-dose groups (*P*=0.03).

Concomitant chemotherapy regimen and risk of neutropenic events

Subgroup risk stratification analysis was carried out according to chemotherapy regimen (platinum- and taxane-based regimens versus non-platinum- and non-taxane-based regimens). Due to an inadequate number of low-dose trials for subgroup analysis, the RRs were only calculated for consistent high-dose administration of bevacizumab. Fourteen clinical trials provided data to calculate the RR of HGN and 11 provided data for calculation of that for HGFN. For HGN, the RR for bevacizumab with platinum- or taxane-based regimens was 1.13 (95% CI, 1.06-1.22), which was not significantly different from that for non-platinum- or non-taxane-based regimens (vs. RR=1.16; 95% CI, 0.95-1.43; *P*=0.81). Similar non-significant effects were observed for the risk of HGFN (*P*=0.69).

Discussion

Incidences of serious neutropenia, febrile neutropenia, and neutropenia-related infections have been reported as significantly increased in cancer patients treated with myelotoxic chemotherapy regimens plus bevacizumab, compared to patients treated with chemotherapy alone (Schutz et al., 2011). The results of our meta-analysis of 22 RCTs indicate that the risk of either HGN or HGFN was

significantly increased in bevacizumab-treated patients, compared to control patients. Given that neutropenic events are well-recognized major risk factors for the development of infections in cancer patients receiving chemotherapy (Aapro et al., 2006) the risk of neutropenia-related infections should be evaluated and prophylaxis medication should be considered for patients receiving bevacizumab therapy, especially when combined with chemotherapy.

Multiple mechanisms may be involved in the pathogenesis of bevacizumab-associated neutropenic events. It is possible that perturbed VEGF signaling may disrupt hematopoiesis in bevacizumab-treated patients. Inhibition of the VEGF receptor 1 (VEGFR1) has been shown to block hematopoietic stem cell cycling, differentiation, and recovery after bone marrow suppression (Rafii et al., 2003) Blockage of VEGFR1 or VEGFR2 signaling in mouse models was shown to inhibit the proliferation of hematopoietic progenitor cells and to impair repopulation of the hematopoietic compartment after myeloablation. When the mouse model was subjected to a combination of VEGF blockage and administration of cytotoxic drugs (including 5-fluorouracil, carboplatin and adriamycin), the risk of myelosuppression was increased and bone marrow recovery was delayed (Novitskiy et al., 2010) A meta-analysis performed by Schutz et al. (Schutz et al., 2011) indicated that sorafenib, a small molecule tyrosine kinase inhibitor targeting VEGFR, was also associated with an increased risk of neutropenia. Furthermore, PIGF, a member of the VEGF family, has been shown to restore hematopoiesis following bone marrow insult (Hattori et al., 2002; Rafii et al., 2003). Collectively, these data suggest that various forms of VEGF blockade, by inhibition of the receptors' tyrosine-kinase domains or through antibodies targeting the VEGF ligand, may induce myelosuppression and delay bone marrow recovery.

Risk factors for neutropenic events associated with bevacizumab are currently poorly understood. We, therefore, evaluated the association of bevacizumab with neutropenic events according to tumor type, bevacizumab dose, and chemotherapeutic agent. Our results showed that the incidence of neutropenic events with bevacizumab varied significantly among different tumor types. The RRs of HGN with bevacizumab varied significantly by tumor types, although no significant difference was found in the RRs of HGFN by tumor types. These findings suggest that the underlying tumor biology or associated treatment might affect the incidence of neutropenic events (Ranpura et al., 2011).

The increased risk of bevacizumab-associated neutropenic events appears to be dose-dependent, as the relative risk of either HGN or HGFN was found to be greater in the high-dose group than in the low-dose group. This result suggests that blockade of VEGF signaling might induce dose-dependent myelosuppression or delayed bone marrow recovery. Considering that no significant association was found between the RRs of bevacizumab-associated neutropenic events and chemotherapy agent, the interaction between bevacizumab and the concomitant chemotherapy agent might be minor.

Our study has several limitations that should be taken into consideration when interpreting the results. First, the studies included in this meta-analysis were conducted at various institutions by different investigators with patients of different nationalities/ethnicities, and these differences may have biased the reported incidences. Second, these studies were conducted at academic centers and large institutions using patients with adequate major organ function, which might not reflect the patient population in other communities or patients with organ dysfunction. Finally, since this study was designed as a meta-analysis, confounding factors at the patient level could not be assessed properly or incorporated into the analysis.

In conclusion, our study has shown that bevacizumab is associated with an increased risk of either HGN or HGFN in cancer patients receiving concurrent chemotherapy. Furthermore, the increased risk of bevacizumab-associated neutropenic events is dose-dependent. These findings provide insights into the risk of neutropenic events that accompanies bevacizumab therapy. It is important for physicians and patients to recognize the risks associated with bevacizumab treatment so that serious neutropenic events may be detected early and resolved by altering the therapeutic regimen or other means.

Acknowledgements

This work was supported by grants from the National Natural Science Foundation of China (No. 30960373) and the Youth Science Foundation of Jiangxi Provincial Education Department (No. GJJ10066) and Science and Technology Department (No. 20114BAB215009).

References

Aapro MS, Cameron DA, Pettengell R, et al (2006). EORTC guidelines for the use of granulocyte-colony stimulating factor to reduce the incidence of chemotherapy-induced febrile neutropenia in adult patients with lymphomas and solid tumours. *Eur J Cancer*, **42**, 2433-53.

Allegra CJ, Yothers G, O'Connell MJ, et al (2009). Initial safety report of NSABP C-08: A randomized phase III study of modified FOLFOX6 with or without bevacizumab for the adjuvant treatment of patients with stage II or III colon cancer. *J Clin Oncol*, **27**, 3385-90.

Baar J, Silverman P, Lyons J, et al (2009). A vasculature-targeting regimen of preoperative docetaxel with or without bevacizumab for locally advanced breast cancer: impact on angiogenic biomarkers. *Clin Cancer Res*, **15**, 3583-90.

Brufsky A, Rivera RR, Hurvitz SA, et al (2010). Progression-free survival (PFS) in patient subgroups in RIBBON-2, a phase III trial of chemotherapy (chemo) plus or minus bevacizumab (BV) for second-line treatment of HER2-negative, locally recurrent or metastatic breast cancer (MBC). *J Clin Oncol* **28**, 1021[meeting abstract].

Burger RA, Brady MF, Bookman MA, et al (2010). Phase III trial of bevacizumab (BEV) in the primary treatment of advanced epithelial ovarian cancer (EOC), primary peritoneal cancer (PPC), or fallopian tube cancer (FTC): a gynecologic oncology group study. *J Clin Oncol*, **28**, LBA1[meeting abstract].

Choueiri TK, Mayer EL, Je Y, et al (2011). Congestive heart failure risk in patients with breast cancer treated with

bevacizumab. *J Clin Oncol*, **29**, 632-8.
Escudier B, Pluzanska A, Koralewski P, et al (2007). Bevacizumab plus interferon alfa-2a for treatment of metastatic renal cell carcinoma: a randomised, double-blind phase III trial. *Lancet*, **370**, 2103-11.
Folkman J (2002). Role of angiogenesis in tumor growth and metastasis. *Semin Oncol*, **29**, 15-8.
Geiger-Gritsch S, Stollenwerk B, Miksad R, et al (2010). Safety of bevacizumab in patients with advanced cancer: a meta-analysis of randomized controlled trials. *Oncologist*, **15**, 1179-91.
Hapani S, Chu D, Wu S (2009). Risk of gastrointestinal perforation in patients with cancer treated with bevacizumab: a meta-analysis. *Lancet Oncol*, **10**, 559-68.
Hapani S, Sher A, Chu D, Wu S (2010). Increased risk of serious hemorrhage with bevacizumab in cancer patients: a meta-analysis. *Oncology*, **79**, 27-38.
Hattori K, Heissig B, Wu Y, et al (2002). Placental growth factor reconstitutes hematopoiesis by recruiting VEGFR1(+) stem cells from bone-marrow microenvironment. *Nat Med*, **8**, 841-9.
Herbst RS, O'Neill VJ, Fehrenbacher L, et al (2007). Phase II study of efficacy and safety of bevacizumab in combination with chemotherapy or erlotinib compared with chemotherapy alone for treatment of recurrent or refractory non small-cell lung cancer. *J Clin Oncol*, **25**, 4743-50.
Hurwitz HI, Fehrenbacher L, Hainsworth JD, et al (2005). Bevacizumab in combination with fluorouracil and leucovorin: an active regimen for first-line metastatic colorectal cancer. *J Clin Oncol*, **23**, 3502-8.
Kang Y, Ohtsu A, Van Cutsem E, et al (2010). AVAGAST: A andomized, double-blind, placebo-controlled, phase III study of first-line capecitabine and cisplatin plus bevacizumab or lacebo in patients with advanced gastric cancer (AGC). *J Clin Oncol*, **28**, LBA4007 [meeting abstract].
Karrison T, Kindler HL, Gandara DR, al e (2007). Final analysis of a multi-center, double-blind, placebo-controlled, randomized phase II trial of gemcitabine/cisplatin (GC) plus bevacizumab (B) or placebo (P) in patients (pts) with malignant mesothelioma (MM). *J Clin Oncol*, **25**, 7526 [meeting abstract].
Kelly WK, Halabi S, Carducci MA, et al (2010). A randomized, double-blind, placebo-controlled phase III trial comparing docetaxel, prednisone, and placebo with docetaxel, prednisone, and bevacizumab in men with metastatic castration-resistant prostate cancer (mCRPC): survival results of CALGB 90401. *J Clin Oncol*, **28**, LBA4511 [meeting abstract].
Kerbel RS (2008). Tumor angiogenesis. N Engl J Med, 358, 2039-49.
Kindler HL, Niedzwiecki D, Hollis D, et al (2010). Gemcitabine plus bevacizumab compared with gemcitabine plus placebo in patients with advanced pancreatic cancer: phase III trial of the Cancer and Leukemia Group B (CALGB 80303). *J Clin Oncol*, **28**, 3617-22.
Meade MO, Richardson WS (1997). Selecting and appraising studies for a systematic review. *Ann Intern Med*, **127**, 531-7.
Miles DW, Chan A, Dirix LY, et al (2010). Phase III study of bevacizumab plus docetaxel compared with placebo plus docetaxel for the first-line treatment of human epidermal growth factor receptor 2-negative metastatic breast cancer. *J Clin Oncol*, **28**, 3239-47.
Miller K, Wang M, Gralow J, et al (2007). Paclitaxel plus bevacizumab versus paclitaxel alone for metastatic breast cancer. *N Engl J Med*, **357**, 2666-76.
Moehler M, Sprinzl MF, Abdelfattah M, et al (2009). Capecitabine and irinotecan with and without bevacizumab for advanced colorectal cancer patients. *World J Gastroenterol*, **15**, 449-56.
Novitskiy SV, Csiki I, Huang Y, et al (2010). Anti-vascular endothelial growth factor treatment in combination with chemotherapy delays hematopoietic recovery due to decreased proliferation of bone marrow hematopoietic progenitor cells. *J Thorac Oncol*, **5**, 1410-5.
Okines AF, Langley R, Cafferty FH, et al (2010). Preliminary safety data from a randomized trial of perioperative epirubicin, cisplatin plus capecitabine (ECX) with or without bevacizumab (B) in patients (pts) with gastric or oesophagogastric junction (OGJ) adenocarcinoma. *J Clin Oncol*, **28**, 4019 [meeting abstract].
Rafii S, Avecilla S, Shmelkov S, et al (2003). Angiogenic factors reconstitute hematopoiesis by recruiting stem cells from bone marrow microenvironment. *Ann N Y Acad Sci*, **996**, 49-60.
Ranpura V, Hapani S, Wu S (2011). Treatment-related mortality with bevacizumab in cancer patients: a meta-analysis. *JAMA*, **305**, 487-94.
Ranpura V, Pulipati B, Chu D, et al (2010). Increased risk of high-grade hypertension with bevacizumab in cancer patients: a meta-analysis. *Am J Hypertens*, **23**, 460-8.
Reck M, von Pawel J, Zatloukal P, et al (2009). Phase III trial of cisplatin plus gemcitabine with either placebo or bevacizumab as first-line therapy for nonsquamous non-small-cell lung cancer: AVAil. *J Clin Oncol*, **27**, 1227-34.
Rini BI, Halabi S, Rosenberg JE, et al (2008). Bevacizumab plus interferon alfa compared with interferon alfa monotherapy in patients with metastatic renal cell carcinoma: CALGB 90206. *J Clin Oncol*, **26**, 5422-8.
Rini BI, Halabi S, Rosenberg JE, et al (2010). Phase III trial of bevacizumab plus interferon alfa versus interferon alfa monotherapy in patients with metastatic renal cell carcinoma: final results of CALGB 90206. *J Clin Oncol*, **28**, 2137-43.
Robert NJ, Dieras V, Glaspy J, et al (2009). RIBBON-1: Randomized,double-blind, placebo-controlled, phase III trial of hemotherapy with or without bevacizumab (B) for first-line reatment of HER2-negative locally recurrent or metastatic reast cancer (MBC). *J Clin Oncol*, **27**, 1005 [meeting abstract].
Sakurai T, Kudo M (2011). Signaling pathways governing tumor angiogenesis. *Oncology*, **81**, 24-9.
Sandler A, Gray R, Perry MC, et al (2006). Paclitaxel-carboplatin alone or with bevacizumab for non-small-cell lung cancer. *N Engl J Med*, **355**, 2542-50.
Schutz FA, Jardim DL, Je Y, Choueiri TK (2011). Haematologic toxicities associated with the addition of bevacizumab in cancer patients. *Eur J Cancer*, **47**, 1161-74.
Schutz FA, Je Y, Choueiri TK (2011). Hematologic toxicities in cancer patients treated with the multi-tyrosine kinase sorafenib: a meta-analysis of clinical trials. *Crit Rev Oncol Hematol*, **80**, 291-300.
Tebbutt NC, Wilson K, Gebski VJ, et al (2010). Capecitabine, bevacizumab, and mitomycin in first-line treatment of metastatic colorectal cancer: results of the Australasian Gastrointestinal Trials Group Randomized Phase III MAX Study. *J Clin Oncol*, **28**, 3191-8.
Van Cutsem E, Vervenne WL, Bennouna J, et al (2009). Phase III trial of bevacizumab in combination with gemcitabine and erlotinib in patients with metastatic pancreatic cancer. *J Clin Oncol*, **27**, 2231-7.
Zalcman G, Margery J, Scherpereel A, et al (2010). IFCT-GFPC-0701 MAPS trial, a multicenter randomized phase II/III trial of pemetrexed-cisplatin with or without bevacizumab in patients with malignant pleural mesothelioma. *J Clin Oncol*, **28**, 7020 [meeting abstract].