Report on the Deliberation Results

March 6, 2007

Evaluation and Licensing Division, Pharmaceutical and Food Safety Bureau

Ministry of Health, Labour and Welfare

[Brand name]	Avastin 100 mg/4 mL Intravenous Infusion
	Avastin 400 mg/16 mL Intravenous Infusion
[Non-proprietary name]	Bevacizumab (Genetical Recombination) (JAN*)
[Applicant]	Chugai Pharmaceutical Co., Ltd.
[Date of application]	April 21, 2006

[Results of deliberation]

In the meeting held on February 22, 2007, the Second Committee on New Drugs concluded that the product may be approved and this result was to be reported to the Pharmaceutical Affairs Department of the Pharmaceutical Affairs and Food Sanitation Council. It was decided that the product is classified as a biological product, the re-examination period is 8 years, and the drug substance and the drug product are both classified as a powerful drug.

The instruction to consider a more appropriate method for anti-bevacizumab antibody assay after market launch was given and a precautionary statement against mixing Avastin with dextrose solutions in “PRECAUTIONS” (“Precautions concerning Use”) of the package insert was to be rewritten to make it easier to understand.

**Japanese Accepted Name (modified INN)*

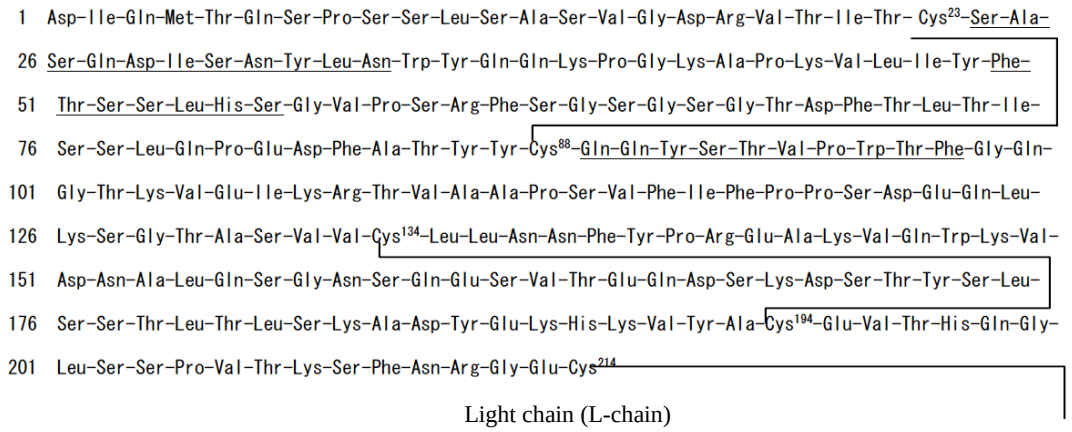
Review Report

February 14, 2007
Pharmaceuticals and Medical Devices Agency

The results of a regulatory review conducted by the Pharmaceuticals and Medical Devices Agency on the following pharmaceutical product submitted for registration are as follows.

[Brand name]	Avastin 100 mg/4 mL Intravenous Infusion Avastin 400 mg/16 mL Intravenous Infusion
[Non-proprietary name]	Bevacizumab (Genetical Recombination)
[Applicant]	Chugai Pharmaceutical Co., Ltd.
[Date of application]	April 21, 2006
[Dosage form/Strength]	Injection: each vial contains 100 or 400 mg of Bevacizumab (Genetical Recombination).
[Application classification]	Prescription drug (1) Drug with a new active ingredient

[Amino acid sequence]



Light chain (L-chain)

(Continued to the next page)

—: Disulfide bond, The underlined parts: The complementarity-determining regions

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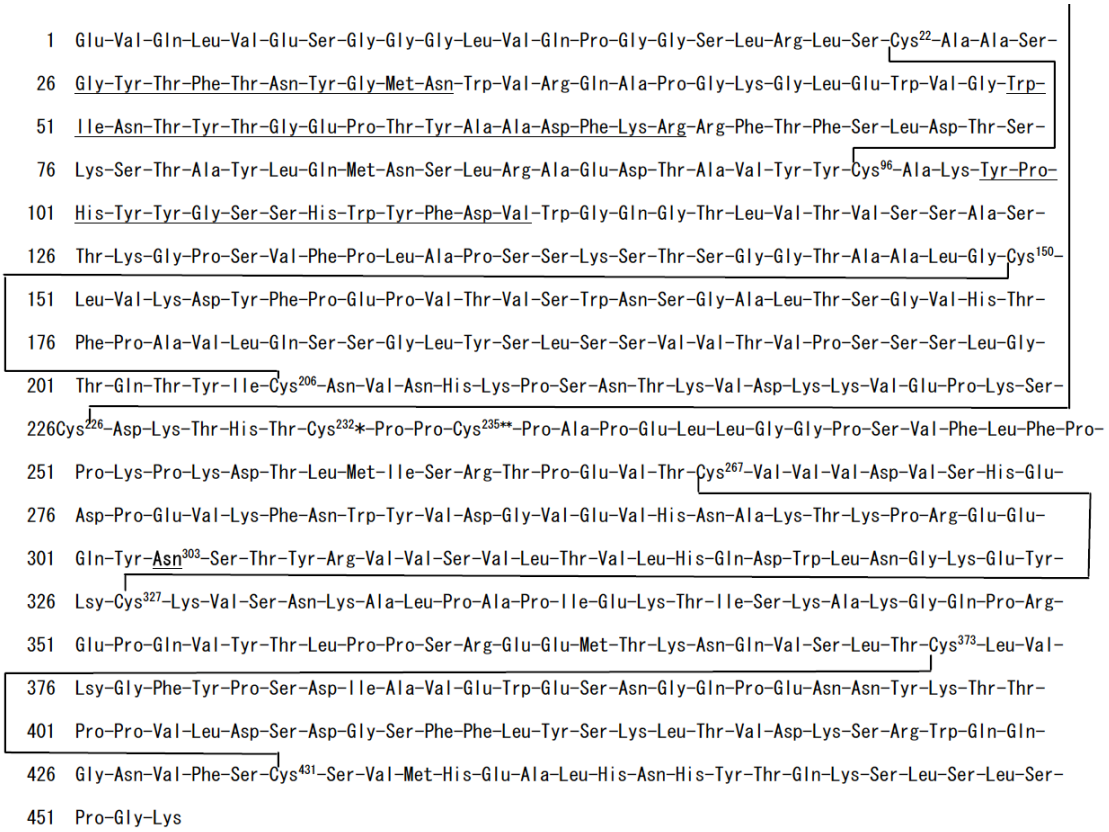
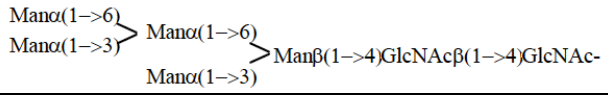
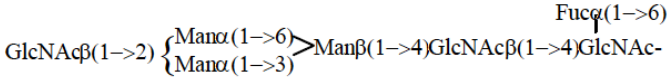
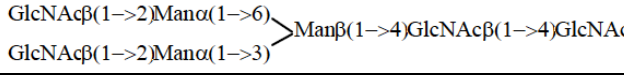
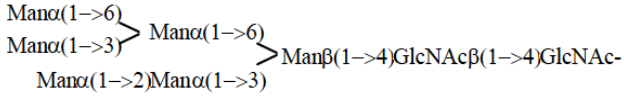
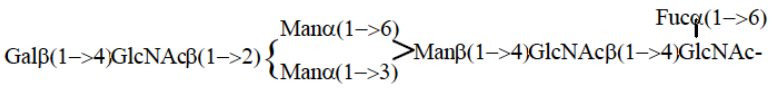
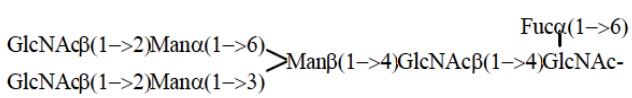


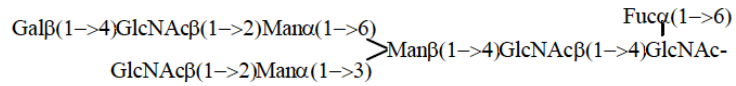
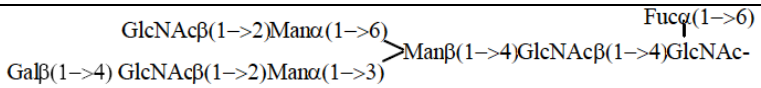
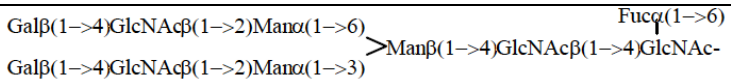
Heavy chain (H-chain)

—:Disulfide bond, *, **: Disulfide bond between the heavy chains, Asn[303]: N-linked glycosylation site

The underlined parts: The complementarity-determining regions

[Predicted carbohydrate structure]

Structure	Abbreviation
	Man5
	G-1
	G0-F
	Man6
	G1-1
	G0

	G1 (1-6)
	G1 (1-3)
	G2

Man: Mannose, Fuc: Fucose, Gal: Galactose, GlcNAc: N-acetylglucosamine

Molecular formula: $C_{6538}H_{10000}O_{2032}N_{1716}S_{44}$

Molecular weight: approximately 149,000 Da

Chemical Name:

Glycoprotein (molecular weight: ca.149,000) consisting of two molecules of light chain containing 214 amino acid residues ($C_{1034}H_{1591}N_{273}O_{338}S_6$; molecular weight : 23446.71), and two molecules of heavy chain containing 453 amino acid residues ($C_{2235}H_{3413}N_{585}O_{678}S_{16}$; molecular weight: 49838.57; including a molecule lacking a C-terminal lysine residue), produced in Chinese hamster ovary cells by expression of a humanized monoclonal antibody cDNA encoding complementarity determining regions from mouse anti-human vascular endothelial growth factor monoclonal antibody and a frame work region and a constant region derived from IgG1.

[Items warranting special mention]
Priority Review (PFSB/ELD Notification No. 0531004 dated May 31, 2006)

[Reviewing Office] Office of New Drug I

Results of Review

February 14, 2007

[Brand name]	Avastin 100 mg/4 mL Intravenous Infusion Avastin 400 mg/16 mL Intravenous Infusion
[Non-proprietary name]	Bevacizumab (Genetical Recombination)
[Applicant]	Chugai Pharmaceutical Co., Ltd.
[Date of application]	April 21, 2006
[Dosage form/Strength]	Injection: each vial contains 100 or 400 mg of Bevacizumab (Genetical Recombination).

[Results of Review]
The Pharmaceuticals and Medical Devices Agency (PMDA) judged that the submitted data has demonstrated the efficacy and safety of the product in "the treatment of unresectable advanced or recurrent colorectal cancer."
As a result of its regulatory review, the PMDA has concluded that the product may be approved for the following indications and dosage and administration, subject to the conditions for approval as described below.

[Indications]
Unresectable advanced or recurrent colorectal cancer

[Dosage and Administration]
The usual adult dosage is 5 mg/kg (body weight) or 10 mg/kg (body weight) of bevacizumab given as an intravenous infusion in combination with chemotherapy. The dosing interval should be ≥2 weeks.

[Conditions for approval]
Because of the very limited number of subjects treated in the Japanese clinical trials, conduct all-case investigation until the data from a certain number of patients are accumulated after market launch in order to identify the background of patients treated with Avastin and collect safety and efficacy data on Avastin early, and take necessary measures for the proper use of Avastin.

[Instructions]
1. Summarize the final data from the safety confirmation study promptly and publish the results.
2. Conduct studies with an appropriate design in order to further determine the pharmacokinetics of Avastin and publish the results.

Review Report (1)

January 25, 2007

I. Overview of the Product

[Brand name]	Avastin 100 mg/4 mL Intravenous Infusion Avastin 400 mg/16 mL Intravenous Infusion
[Non-proprietary name]	Bevacizumab (Genetical Recombination)
[Applicant]	Chugai Pharmaceutical Co., Ltd.
[Date of application]	April 21, 2006
[Dosage form/Strength]	Injection: each vial contains 100 or 400 mg of Bevacizumab (Genetical Recombination).

[Proposed indications]
Avastin in combination with chemotherapy is indicated for unresectable advanced or recurrent colorectal cancer

[Proposed Dosage and Administration]
1. Dosage and administration method
(1) Choose the Method A for patients with advanced colorectal cancer who are not candidates for curative resection and patients with recurrent colorectal cancer including those after post-operative adjuvant therapy, or the Method B for patients who have progressed after receiving cancer chemotherapy for advanced or recurrent colorectal cancer unsuitable for curative resection.

Method A: The usual adult dosage is 5 mg/kg (body weight) of bevasizumab given, as a general rule, every 2 weeks as an intravenous infusion.
Method B: The usual adult dosage is 10 mg/kg (body weight) of bevacizumab given, as a general rule, every 2 weeks as an intravenous infusion.

(2) Avastin therapy should be initiated in combination with chemotherapy.
(3) Refer to “Precautions for Dosage and Administration” for choosing the chemotherapy regimen to be combined with Avastin. Since the efficacy and safety of Avastin in combination with chemotherapy containing oral fluoropyrimidine in the treatment of advanced or recurrent colorectal cancer have not been established, Avastin should not be combined with oral fluoropyrimidine agents.
(4) If adverse reactions suspected to be associated with the chemotherapy that Avastin is combined with are noted, or further administration of the chemotherapy is considered unnecessary, the chemotherapy regimen should be changed or discontinued.

2. Preparing the infusion and infusion duration
(1) Withdraw the necessary volume of Avastin with a syringe to obtain the required dose and dilute in a total volume of approximately 100 mL of Isotonic Sodium Chloride Solution (JP). The initial dose should be delivered over 90 minutes as an intravenous infusion.
(2) If the first infusion is well tolerated, the second infusion may be administered over 60 minutes. If the 60-minute infusion is well tolerated, all subsequent infusions may be administered over 30 minutes.

Formula for the necessary volume to be withdrawn

$$\text{Volume to be withdrawn (mL)} = \text{Body weight (kg)} \times \frac{\text{Dose per administration (mg/kg)}}{25 \text{ (mg/mL)}}$$

Method	Dose per administration	Formula for the necessary volume to be withdrawn (mL)	Administration interval
A	5 mg/kg	Volume to be withdrawn (mL) =Body weight (kg)× 0.2 (mL/kg)	2 weeks
B	10 mg/kg	Volume to be withdrawn (mL)= Body weight (kg)× 0.4 (mL/kg)	2 weeks

II. Summary of the Submitted Data and Outline of Review by the Pharmaceuticals and Medical Devices Agency

With regard to this application, the data submitted by the applicant and the applicant's responses to the questions from the Pharmaceuticals and Medical Devices Agency (PMDA) are outlined below.

1. Origin or background of discovery and usage conditions in foreign countries etc.

1) Overview of the product

Following the identification of new cancer-associated antigens, a variety of antibody drugs targeting cancer-associated antigens that are specific to cancer or highly expressed in cancer have been studied. Therapy with such drugs has advantages of high specificity to the target and less effects on normal tissues. Along with advances in recombinant DNA technology, recombinant monoclonal antibodies such as trastuzumab (genetical recombination) targeting the human epidermal growth factor receptor type 2 known to be involved in tumor progression, have already been applied clinically.

Tumor tissues produce vascular endothelial growth factor (VEGF) for their growth. Upon binding to its receptors on vascular endothelial cells in the vicinity, VEGF promotes vascular endothelial cell proliferation, migration, tube formation, and microvascular permeability via intracellular signal transduction system, and induces angiogenesis. VEGF is overexpressed in a variety of tumors and is known to be a cytokine involved in tumor growth.

Bevacizumab (Genetical Recombination) (hereinafter referred to as bevacizumab) is a humanized monoclonal antibody targeting VEGF, derived from recombinant DNA technology. It is considered that bevacizumab blocks various signal transduction pathways involved in angiogenesis by binding to VEGF and inhibiting the binding of VEGF to its receptors, resulting

in the inhibition of tumor growth.

2) Background of development of bevacizumab

A cell line producing muMAb A4.6.1, murine anti-VEGF monoclonal antibody, was selected among the hybridoma cell lines generated from mice immunized with human VEGF containing 165 amino acid residues ($VEGF_{165}$) and its antibody gene was humanized by recombinant DNA technology. Bevacizumab is an anti-VEGF monoclonal antibody produced in the Chinese hamster ovary (CHO) G7 cell line transfected with the recombinant gene construct (approximately 93% of the overall amino acid sequence of bevacizumab is derived from human).

As a result of crystal structure analysis of the bevacizumab Fab fragment-VEGF complex, the set of VEGF amino acid residues important for binding to VEGF receptors (VEGFR-1 and -2) are not identical, but partially same to, those critical for binding to bevacizumab. Therefore, it is inferred that VEGF-receptor interaction is blocked sterically when bevacizumab binds to VEGF.

Genentech (the US) initiated a phase I clinical trial of bevacizumab in patients with advanced solid tumors in the US in 1997. In 1998, phase II studies to evaluate the efficacy of bevacizumab combined with 5-FU/LV regimen (fluorouracil (5-FU) + calcium folinate (LV)) or IFL regimen (irinotecan hydrochloride + 5-FU/LV) in patients with colorectal cancer were conducted. A phase III study of bevacizumab in combination with IFL regimen in patients with colorectal cancer was undertaken in September 2000. Based on these clinical study data, a new drug application for bevacizumab was filed in the US in September 2003. As a result, bevacizumab was approved in February 2004 for the following indication and dosage and administration: “AVASTIN, used in combination with intravenous 5-fluorouracil-based chemo-therapy, is indicated for first-line treatment of patients with metastatic carcinoma of the colon or rectum”; 5 mg/kg given every 2 weeks as an intravenous infusion.

The Eastern Cooperative Oncology Group (ECOG) conducted a phase III study evaluating bevacizumab in combination with FOLFOX4 regimen (oxaliplatin + 5-FU continuous IV infusion + LV) in the second-line or subsequent treatment of metastatic colorectal cancer. Genentech submitted the results of this study to the US Food and Drug Administration and the dose of 10 mg/kg (in combination with FOLFOX4) for colorectal cancer patients previously treated with chemotherapy was approved in June 2006.

In Europe, F. Hoffman-La Roche (hereinafter abbreviated as Roche) submitted a marketing authorization application for Bevacizumab, and in January 2005, the product was approved under the centralized procedure for the following indication: “Avastin (bevacizumab) in combination with intravenous 5-fluorouracil/folinic acid or intravenous 5-fluorouracil/folinic acid/irinotecan is indicated for first-line treatment of patients with metastatic carcinoma of the colon or rectum.”

In Japan, in November 2004 following the approval in the US, the applicant, Chugai

Pharmaceutical Co., Ltd. initiated a phase I study of single agent bevacizumab followed by bevacizumab in combination with 5-FU/levofolinate calcium (*l*-LV) regimen in patients with colorectal cancer. This study was originally planned as a phase I/II study and its phase II portion was intended to investigate bevacizumab in combination with 5-FU/*l*-LV. However, in March 2005, oxaliplatin was approved for "the treatment of patients with advanced or recurrent carcinoma of the colon or rectum who are not candidates for curative resection" and became available in clinical practice. Therefore, the study was terminated with the completion of the phase I portion in order to review the study plan of the phase II portion.

A new drug application for bevacizumab has been filed based on the data from the Japanese phase I study and foreign phase III studies of bevacizumab in combination with IFL or FOLFOX4.

The 5th Investigational Committee for Usage of Unapproved Drugs held on July 22, 2005 reported that "Clinical study data reported to date are all from phase III studies and it is considered that the clinical usefulness of bevacizumab has been confirmed. Therefore, early filing should be made for bevacizumab based on these clinical study data and Japanese phase I study data for which the primary evaluations have been completed" (http://www.mhlw.go.jp/shingi/2005/07/txt/s0727-3.txt), and a safety confirmation study of bevacizumab in combination with FOLFOX4 (In Japan, *l*-LV in the (S, S) form is used, instead of LV in the (R, S) form) is currently ongoing.

The doses of LV and *l*-LV are expressed in terms of folinate and levofolinate, respectively, in the following text.

2. Data relating to quality

Summary of the submitted data

Bevacizumab is a glycoprotein (approximately 149,000 Da) composed of two light chains, consisting of 214 amino acid residues ($C_{1034}H_{1591}N_{273}O_{338}S_6$, approximately 23,447 Da) and two 453 residue heavy chains ($C_{2235}H_{3413}N_{585}O_{678}S_{16}$, approximately 49,839 Da) (approximately 49,719 Da for a heavy chain lacking C-terminal Lys^{453}), produced in CHO cells transfected with cDNA coding for a humanized monoclonal antibody in which the six complementarity-determining regions (CDR) of a murine anti-human VEGF monoclonal antibody muMAb A4.6.1 were inserted into a human antibody framework which has a human κ globulin subgroup I light chain (VL-CL) and human γ globulin subgroup III (IgG1) heavy chain (VH-CH1). Like IgG1, bevacizumab contains intermolecular disulfide bonds.

1) Manufacturing process for the drug substance

(1) Establishment of cell banking system

Bevacizumab is a recombinant humanized monoclonal antibody, originally derived from muMAb A4.6.1, which was produced using hybridomas generated from mice immunized with the human $VEGF_{165}$ conjugated with keyhole limpet hemocyanin. The nucleic acid sequences of the six CDRs were determined from the muMAb A4.6.1-producing hybridoma A4.6.1. cell line.

The six CDRs of A4.6.1. were inserted in place of those of human VL-CL and VH-CH1 domains using the plasmid pEMX1 as template, by [redacted] to produce the gene of a humanized Fab fragment. Then, in order to enhance the binding capacity to VEGF, [redacted] framework VL residues and [redacted] framework VH residues outside the CDR regions were substituted to produce a humanized fragment, Fab-12. The VH and VL domains of Fab-12 were combined with human IgG1 heavy chain constant domains CH1-CH2-CH3 and human κ light chain constant domain CL, respectively, to produce the gene of bevacizumab, a humanized antibody. For the expression of bevacizumab in CHO cells, the heavy chain gene and light chain gene were introduced into an expression construct to produce pSVID5.ID.LLnspeV.xvegf36HC.LC. This expression construct was introduced into CHO DP-12 cells (derived from CHO DUX-B11, a CHO-K1 mutant dihydrofolate reductase deficient cell line) by lipofection, the cells were cultured in DMEM/Ham's F-12 containing methotrexate (MTX) and fetal bovine serum, and bevacizumab-producing CHO 107N cell line was selected in the presence of increasing concentrations of MTX and used for the production of the drug substance for foreign phase I and phase II clinical trials. This cell clone was cloned in the presence of further higher concentrations of MTX to obtain the more highly productive CHO G7 cell line. The CHO G7 cell line was expanded in serum-free medium containing MTX to prepare a master cell bank (MCB). A working cell bank (WCB) was prepared from the MCB.

(2) Characterization and control of cell banks

Characterization analysis and purity tests have been performed on the MCB, WCB, end of production (EOP) cells, and cells at the limit of *in vitro* cell age used for production (CAL).

The MCB, WCB, EOP cells, and CAL were characterized by the complete nucleotide sequence of the structural gene, Southern blotting, the gene copy number, peptide mapping, and isoenzyme. The complete nucleotide sequence of the MCB structural gene was identical to the nucleotide sequence of the heavy chain and light chain expression plasmid. A consistent banding pattern was observed by Southern blot analysis for the MCB and CAL and the gene copy number was determined to be about [redacted] copies per cell by quantitative PCR for both. The peptide map of bevacizumab derived from the MCB, WCB, and CAL was consistent with that of a product lot characterized for structure. Isoenzyme analysis of the MCB and WCB confirmed the bevacizumab cell banks as being of Chinese hamster origin.

In order to determine the purity of the MCB and WCB, sterility test, mycoplasma testing (culture method, indicator cell culture method), adventitious virus *in vitro* assay (inoculated into Vero, MRC-5, and CHO-K1 cells), rodent parvovirus test (inoculated into 324K cells), the mouse antibody production test, the hamster antibody production test, *in vivo* assay to reveal latent viruses (inoculated into adult mice, suckling mice, guinea pig, and embryonated eggs), and cocultivation assay (indicator cells: rhabdomyosarcoma cells, human lung epithelial carcinoma-derived A549 cells, and mink lung cells) were performed. The purity of CAL was determined by adventitious virus *in vitro* assay, *in vivo* assay to reveal latent viruses, the assessment of the ultrastructure of virus particles and observation of retrovirus-like particles by electron microscopy for pre-harvest cell culture fluids, mink S^+L^-focus assay, and

cocultivation assay. The purity of the EOP was determined by reverse transcriptase activity, mink $S^{+}L^{-}$focus assay, the assessment of the ultrastructure of virus particles and observation of retrovirus-like particles in pre-harvest cell culture fluids by electron microscopy, bioburden testing, mycoplasma testing, adventitious virus *in vitro* assay, and rodent parvovirus test. As a result, these were all shown to be free of detectable, infectious contaminants within the scope of the tests performed.

About [redacted] ampoules of the WCB are currently available and it is predicted that the current WCB can be used for [redacted] years. An additional WCB is to be prepared with a view to the anticipated annual usage, when there are less than [redacted] ampoules of the WCB. For a newly prepared WCB, peptide mapping, isoenzyme analysis, sterility test, mycoplasma testing (culture method, indicator cell culture method), virus tests (adventitious virus *in vitro* assay [inoculated into Vero, MRC-5, and CHO-K1 cells], rodent parvovirus test [inoculated into 324K cells], and *in vivo* assay to reveal latent viruses [inoculated into adult mice, suckling mice, guinea pig, and embryonated eggs]) are to be performed. In order to confirm the quality of the WCB during storage, [redacted] is to be measured when [redacted]. Since the current MCB is large enough to prepare more than 100 WCBs, preparing a new MCB is considered unnecessary and the procedure for preparing a new MCB is not provided.

(3) Fermentation process

After the thawing of an ampoule from the WCB ([redacted] or [redacted] mL), [redacted] culture is initiated in seed culture medium without MTX ([redacted] g/L DMEM/Ham's F-12 [containing [redacted], not containing [redacted], [redacted], [redacted], [redacted], or [redacted]], [redacted] g/L [redacted], [redacted] g/L [redacted], [redacted] g/L [redacted], [redacted] g/L [redacted], [redacted] mg/L [redacted], [redacted] mg/L human insulin [genetical recombination], [redacted] mL/L [redacted] solution, [redacted] mmol/L [redacted]). [redacted] is used for [redacted] mL-ampoule of the WCB and [redacted] is used for [redacted] mL-ampoule of the WCB. Then, [redacted] culture is conducted. Bevacizumab-producing cells are selectively cultured in seed culture medium with [redacted] μmol/L MTX ([redacted] g/L DMEM/Ham's F-12 [containing [redacted], not containing [redacted], [redacted], [redacted], [redacted], and [redacted]], [redacted] g/L [redacted], [redacted] g/L [redacted], [redacted] g/L [redacted], [redacted] g/L [redacted], [redacted] mg/L [redacted], [redacted] mg/L human insulin [genetical recombination], [redacted] mL/L [redacted] solution, [redacted] mmol/L [redacted]) in [redacted], or in seed culture medium with [redacted] μmol/L MTX ([redacted] g/L DMEM/Ham's F-12 [containing [redacted], not containing [redacted], [redacted], [redacted], [redacted], and [redacted]], [redacted] g/L [redacted], [redacted] g/L [redacted], [redacted] g/L [redacted], [redacted] g/L [redacted], [redacted] mg/L [redacted], [redacted] mg/L human insulin [genetical recombination], [redacted] mL/L [redacted] solution) in [redacted].

In the inoculum culture stage, MTX-free inoculum culture medium ([redacted] g/L DMEM/Ham's F-12 [containing [redacted] μmol/L [redacted], [redacted], [redacted], and [redacted], not containing [redacted], [redacted], and [redacted]], [redacted] g/L [redacted], [redacted] mmol/L [redacted], [redacted] g/L [redacted], [redacted] or [redacted] g/L [redacted], [redacted] mg/L [redacted], [redacted] g/L [redacted], [redacted] mg/L human insulin [genetical recombination], [redacted] g/L hydrolyzed peptone

derived from porcine stomach) is used to expand the cell population into 80-L, 400-L, and 2,000-L vessels. [redacted] g/L [redacted] is used for culture in [redacted] or [redacted] L [redacted] and [redacted] g/L [redacted] is used for culture in [redacted] L [redacted]. The timing of inoculation into the next bioreactor has been prescribed based on [redacted] ([redacted]) and [redacted].

In the production culture stage, the production culture medium ([redacted] g/L DMEM/Ham's F-12 [containing [redacted] μmol/L [redacted], [redacted], [redacted], and [redacted], not containing [redacted], [redacted], and [redacted]], [redacted] g/L [redacted], [redacted] mmol/L [redacted], [redacted] g/L [redacted], [redacted] g/L [redacted], [redacted] mg/L [redacted], [redacted] g/L [redacted], [redacted] mg/L human insulin [genetical recombination], [redacted] g/L hydrolyzed peptone derived from porcine stomach) is used and after cell culture for a certain period of time, [redacted] medium ([redacted] g/L DMEM/Ham's F-12 [containing [redacted] and [redacted], not containing [redacted] and [redacted]], [redacted] g/L [redacted], [redacted] g/L [redacted], [redacted] mL/L [redacted] solution, [redacted] g/L hydrolyzed peptone derived from porcine stomach) is added. If [redacted] in the cell culture fluid is [redacted] [redacted] g/L, [redacted] should be [redacted] to [redacted]. After the end of culture, the production cell culture fluid is ultrafiltrated ([redacted]) and normally stored at [redacted] - [redacted]℃, and the purification is initiated within [redacted] days. If the purification is not performed on the day of the completion of the cell culture, the purification should be initiated within [redacted] days under the storage condition of [redacted]℃ or within [redacted] days under the storage condition of [redacted]–[redacted]℃.

In the fermentation process, [redacted] step has been defined as a critical process step. Rodent parvovirus test (PCR) is conducted on [redacted] culture medium. As in-process controls for the harvest step, rodent parvovirus test (PCR, *in vitro*), bioburden testing, adventitious virus test (*in vitro*), and mycoplasma testing have been set for pre-harvest [redacted]. Before moving into the purification process, [redacted] in [redacted] ([redacted]) is measured.

In order to evaluate the fermentation process, the cell culture and harvest steps have been validated at a commercial scale (12,000 L) and at a small scale ([redacted] L).

Production culture of cells with a cell age of [redacted]–[redacted] days was performed for [redacted]–[redacted] days at a commercial scale and at a small scale. Then, the cell culture properties were assessed by measuring [redacted], pre-harvest [redacted], [redacted], and [redacted]. The physical property of the produced bevacizumab was assessed by the main peak % by cation exchange chromatography (IEC), the predominant oligosaccharide G0 % by capillary electrophoresis, peptide mapping, [redacted]% by size exclusion chromatography (SEC), and potency based on the ability to inhibit the proliferation of human umbilical vein endothelial cells (HUVEC). The assessments of the cell culture and physical properties at different culture scales confirmed that the production culture characteristics and the physical property of the produced bevacizumab are comparable between commercial and small scales. The following process evaluation data include test data from culture at a small scale or a commercial scale or those from culture at a commercial scale followed by a small scale.

In order to assess the effects of cell age on the cell culture characteristics and the physical

property of the produced bevacizumab, the production culture was performed for [redacted] days using cells with different cell ages (the cumulative numbers of days of culture from the thawing of the MCB to the end of culture [cell age] were [redacted] days). The production culture characteristics were assessed by measuring [redacted], pre-harvest [redacted], [redacted], and [redacted]. As a result, [redacted] and pre-harvest [redacted] were [redacted] with [redacted] while there were no changes in other parameters. The physical property of the produced bevacizumab was assessed by measuring the main peak % by IEC, the predominant oligosaccharide G0 % by capillary electrophoresis, peptide mapping, [redacted]% by SEC, and potency based on the ability to inhibit the proliferation of HUVEC, and there were no alterations for all parameters.

In addition, the production culture was performed for [redacted] days using cells cultured for cumulative [redacted] days (cell age), and the cell culture characteristics and the physical property with different culture durations were assessed by measuring [redacted], pre-harvest [redacted], [redacted], and [redacted].

The production culture characteristics showed [redacted] for [redacted], pre-harvest [redacted], and [redacted], whereas there were no differences in the bevacizumab-producing capacity. The physical property of the produced bevacizumab was assessed by measuring the main peak % by IEC, the predominant oligosaccharide G0 % by capillary electrophoresis, peptide mapping, [redacted]% by SEC, and potency based on the ability to inhibit the proliferation of HUVEC, and there were no differences in the physical property of the produced bevacizumab between different culture durations. The main peak % by IEC and G0 % by capillary electrophoresis showed that bevacizumab of consistent quality is yielded with production culture durations up to [redacted] days.

(4) Purification process

The harvested cell culture fluid is purified by affinity chromatography, and the eluate is treated with [redacted] (≤[redacted], ≥[redacted] minutes). The pH is adjusted to [redacted] followed by the purification by Q Sepharose anion exchange chromatography, which is treated with [redacted] ([redacted] mol/L [redacted], ≥[redacted] minutes and ≤[redacted] minutes). Then, the resulting solution is purified by CM Sepharose cation exchange chromatography and ultrafiltered/diafiltered (UF/DF) using [redacted]-pore-size [redacted], then added with a buffer solution for adjustment ([redacted] mmol/L sodium phosphate, [redacted] mg/mL trehalose, [redacted] w/v% polysorbate 20 [PS20], pH6.1) and sterile-filtered using a 0.2-μm-pore-size filter, and the resulting filtrate is the drug substance. The drug substance is stored in a 120 or 300 L, movable freeze/thaw tank at 2-8℃ or below－20℃. For transportation, the movable freeze/thaw tank is transferred to a dedicated freezer car maintained at [redacted]℃.

[redacted] step by [redacted], [redacted] step by [redacted], and [redacted] step have been defined as critical process steps. As in-process controls, bacterial endotoxins test and bioburden testing have been set for [redacted] and [redacted] chromatography load [redacted], bacterial endotoxins test, bioburden testing, and protein content have been set for

[redacted] pool, and CHO proteins has been set for [redacted] pool ([redacted] is added to [redacted] pool).

As refiltration may be carried out either before the storage of the drug substance or before sterile filtration in the formulation step, filter compatibility studies, extractables testing on the filters, and stability testing were conducted and it has been determined that refiltration may be repeated up to 5 times. Refiltration will be carried out if (a) [redacted], (b) [redacted] in [redacted] or [redacted] are identified, (c) Due to [redacted] or [redacted], [redacted], (d) Due to [redacted] or [redacted] etc., [redacted] ([redacted] or [redacted] L), (e) [redacted]. However, if the bioburden level exceeds the established limits, refiltration is not permitted as reprocessing.

The purification process was evaluated as follows: (a) Bevacizumab protein concentration, the yield of bevacizumab, cell substrate-derived impurities (CHO protein and DNA) and process-related impurities (MTX, gentamicin, human insulin [genetical recombination], protein A, medium component A, medium component B, medium component C, and medium component D), and aggregates were measured for each step, which confirmed the robustness and reproducibility of the process with respect to the yield and removal of impurities for each step. (b) Although the manufacturing process steps for the drug substance have been designed for continuous operation, it may become necessary to store the step pool before moving into the next step. Thus, validation was performed to establish the allowable holding time for buffer solutions used for different pools or manufacturing processes. [redacted], [redacted], [redacted]% in [redacted] and [redacted], the main peak %, and the anti-proliferative activity against HUVEC were measured and it was determined that the quality of the drug substance is not effected significantly under the conditions shown in the following table. It was also determined that there are no changes in [redacted] or [redacted] of the buffer solution used for adjustment or manufacture for up to [redacted] days at the environmental temperature. (c) In order to assess re-use of columns to be used for the manufacture of the drug substance (protein A resin, anion exchange resin, cation exchange resin), protein (bevacizumab, protein A, host cell protein) and DNA levels in the carry-over pool (the solution pool from the used and cleaned columns to check the carry-overs) were measured. As a result, the protein carry-over was <[redacted] ng/bevacizumab [redacted] mg and the DNA carry-over was <[redacted] ng per maximum dose, and it was confirmed that the cleaning procedures used in the purification process are capable of effectively cleaning the equipments and the columns that come in contact with the product. (d) In order to determine the maximum number of re-uses of the column resins, the yield, CHO protein, DNA and [redacted] were assessed, and in addition, medium component E, medium component A, and protein A were assessed for the protein A resin, protein A for the anion exchange resin, and aggregates for the cation exchange resin. As a result, it was confirmed that the protein A resin, the anion exchange resin, and the cation exchange resin are capable of removing cell-derived and process-related impurities for up to [redacted], [redacted], and [redacted] re-uses, respectively. In order to determine the maximum number of re-uses of [redacted] membrane, the total protein and DNA carryover were assessed for [redacted] membrane after [redacted]–[redacted] uses, which will be repeated periodically until the limit of re-uses is established. [redacted] and [redacted] analysis were performed on the sterile filter and [redacted]

pool, which confirmed that no extractables/leachables adversely affecting the safety or quality of the product are released.

Holding time and temperature for each pool

Pool	Temperature	Maximum allowable holding time
HCCF	[redacted]℃	[redacted] days
	[redacted]℃	[redacted] days
	[redacted]℃	[redacted] days
	[redacted]℃	[redacted] days
Affinity pool	[redacted]℃	[redacted] days
	[redacted]℃	[redacted] days
Anion exchange pool	[redacted]℃	[redacted] days
	[redacted]℃	[redacted] days
Cation exchange load	[redacted]℃	[redacted] hours
	[redacted]℃	[redacted] hours
Cation exchange pool	[redacted]℃	[redacted] days
	[redacted]℃	[redacted] days
UF/DF dilution pool	[redacted]℃	[redacted] days
	[redacted]℃	[redacted] days

(5) Safety evaluation of adventitious infectious agents

The following bio-derived materials are used in the manufacturing process for bevacizumab: yeast-derived human insulin (genetical recombination) during the preparation of the MCB and WCB and in the fermentation process for the drug substance; L-cysteine and L-tyrosine derived from human hair or avian feather, as medium components for the preparation of the MCB and WCB; and hydrolyzed peptone derived from porcine stomach in the fermentation process for the manufacture of the drug substance. However, purity tests were performed on the established MCB, WCB, EOP, and CAL for the detection of adventitious and endogeneous infectious agents, which all confirmed the absence of adventitious and endogeneous infectious agents [See "2. Data relating to quality 1) (2) Characterization and control of cell banks"]. The pre-harvest cell culture fluid was tested for the detection of rodent parvovirus (PCR, *in vitro*), bioburden, adventitious viruses (*in vitro*) and mycoplasma, which confirmed the absence of these agents. Furthermore, bacterial endotoxins test and bioburden testing are performed as in-process controls of the drug substance, and sterility test is performed as in-process controls of the drug product, and it has been confirmed that the manufacturing process of bevacizumab is adequately controlled against non-viral adventitious infectious agents.

The ability of the purification process to clear viruses was evaluated by cell-based or quantitative PCR using murine leukemia virus (X-MuLV), which is recommended as a model virus of type C retrovirus, by the ICH Guideline Q5A (PMSB/ELD Notification No. 329 dated February 22, 2000 "Viral Safety Evaluation of Biotechnology Products Derived From Cell Lines of Human or Animal Origin") and it was confirmed that hamster retrovirus-like particles are virtually inactivated/removed.

Retrovirus clearance in the purification process (Log10)

Purification process step	Virus reduction factor
Affinity pool ([redacted] treatment, [redacted] minutes)	[redacted] *1
Cation exchange load ([redacted] mol/L [redacted] treatment, [redacted] minutes)	≧[redacted] *1
Affinity fraction	[redacted] *2,*3
Anion exchange pool	[redacted] *2,*3
Cation exchange pool	[redacted] *2,*3
Overall virus reduction factor	≧[redacted] *4

*1 TCID50, *2 pRNA copy number, *3 Virus reduction factor of a new resin or a reused resin, whichever lower, *4 The sum of individual reduction factors

The capacity to clear adventitious viruses was also evaluated. As model viruses, minute virus of mice (MMV), which is a non-enveloped single stranded DNA virus and SV40, which is a non-enveloped, double-stranded DNA virus, were used and quantitative PCR assay was performed. As a result, it was confirmed that the viruses are removed primarily by anion exchange chromatography.

Clearance of adventitious viruses in the bevacizumab purification process (Log10)

Virus	Purification process step	Virus reduction factor*1	Overall virus reduction factor*2
MMV	Affinity fraction	[redacted]	6.9
	Anion exchange pool	[redacted]	
SV40	Affinity fraction	[redacted]	6.1
	Anion exchange pool	[redacted]	

*1 Virus reduction factor of an unused resin or a reused resin, whichever lower,
*2 The sum of individual reduction factors

(6) Manufacturing process development (comparability)

In the manufacturing process for the drug substance for foreign phase I clinical trials, cells derived from the 107N cell line were cultured in medium with [redacted] nmol/L MTX and, in the purification process, virus inactivation treatment and affinity, anion exchange, and cation exchange chromatographies were used (Manufacturing Method A). The formulation for the drug substance contained 10 mg/mL bevacizumab, 10 mmol/L histidine (pH5.5), 100 mg/mL trehalose, and 0.02% PS20 (Formulation A).

During the manufacture of the drug substance for foreign phase II trials, in order to increase the stability, the composition of the study drug was changed to 10 mg/mL bevacizumab, 51 mmol/L sodium phosphate (pH 6.2), [redacted] mg/mL trehalose, and [redacted]% [redacted] (Formulation B). Only a [redacted]L scale culture system was used in the Manufacturing Method A while the production scales of [redacted] and [redacted]L were used in the Manufacturing Method B (Manufacturing Method B).

From the first quarter of 1999, in order to increase the yield of bevacizumab, cells derived from the G7 cell line were cultured in the medium containing [redacted] nmol/L [redacted] and a medium component was changed from bovine peptone to peptone derived from porcine stomach (Manufacturing Method C). [redacted] was about [redacted]% with the Manufacturing Method B, whereas it was [redacted]% after the introduction of the Manufacturing Method C. In the

Manufacturing Method C, only the bevacizumab concentration was changed to 25 mg/L with the same excipients as the Formulation B, in order to supply high concentration drug substance for foreign phase II and phase III trials (Formulation C).

Then, in the manufacture of the drug substance for foreign phase II and phase III trials, [redacted] treatment concentration was changed in order to increase the robustness of the process step and the type of the cation exchange column was changed from [redacted] to [redacted] in order to improve the ability to remove aggregates (Manufacturing Method D). The change of the cell line from 107N to G7 resulted in an increase of aggregates in the drug substance in the Manufacturing Method C, which was reduced in the Manufacturing Method D.

In the manufacture of the drug substance for foreign phase II and phase III trials, overseas marketing, and validation, direct inoculation into [redacted] was also allowed when using [redacted] mL ampoule from the WCB (Manufacturing Method E). The Formulation C was used for these drug substances. Differently charged impurities and [redacted] varied slightly depending on the time of production, and the percentage of acidic variants was [redacted]% with the Manufacturing Method B, [redacted]% with the Manufacturing Method D, and [redacted]% with the Manufacturing Method E. The percentage of G1 was [redacted]-[redacted]% with the Manufacturing Methods B, C, and D vs. [redacted]% with the Manufacturing Method E. There were no variations in the potency.

The drug substance for Japanese clinical trials and the drug product to be marketed in Japan is produced by the Manufacturing Method E using the Formulation C. Based on the results of quality assessments of the drug substances produced by different manufacturing methods and the results of non-clinical and clinical studies, it has been determined that there are no effects on the quality of the product due to the changes in the manufacturing process.

2) Drug substance

(1) Structure/Composition

The drug substance has been characterized by peptide mapping (confirmation of the amino acid sequence), N-terminal amino acid sequence analysis, C-terminal amino acid sequence analysis, sulfhydryl groups, disulfide bonds, carbohydrate structure analysis (matrix-assisted laser desorption/ionization time-of-flight [MALDI-TOF] mass spectrometry and [redacted]-labeled capillary electrophoresis [[redacted]-labeled CE]), glycosylation site analysis (tryptic peptide mapping, [redacted]-cleaved Lys-C peptide mapping, and reduced/S-carboxymethylated [CM] Lys-C peptide mapping), glycosylation rate (reducing SDS-capillary electrophoresis [CE-SDS], tryptic peptide mapping, and mass spectrometry of reduced bevacizumab), sialic acid content, molecular weight, glycation (analysis of glycation site, the relative amount of glycated bevacizumab), oxidation rate, deamidation, isoelectric point (capillary isoelectric focusing [cIEF], gel isoelectric focusing), ultraviolet spectrum, SEC, IEC before and after CpB digestion, reducing/non-reducing SDS-PAGE, reducing/non-reducing CE-SDS, the potency of related substances based on the ability to inhibit the proliferation of HUVEC, antigen specificity, and biological properties (VEGF binding activity, inhibition of activation of kinase receptor, anti-proliferative activity against HUVEC).

- [redacted]-cleaved or CM Lys-C peptide mapping and the LC/MS results were identical to those expected from the amino acid sequence, and the identified amino acid residues accounted for [redacted]% of the expected residues. Molecular weight was also determined by MS/MS.
- No absence or heterogeneity was detected in the heavy- and light-chain N-terminal and light-chain C-terminal amino acid sequences. Heavy chain C-terminal Gly^{452} and Lys^{453} residues were detected. The Lys^{453} content was about [redacted]% for the initial reference material produced from the 107N cell line and [redacted]% for the current reference material produced from the G7 cell line and validation lots.
- Of the 32 Cys residues, all of [redacted] pairs of disulfide bonds, which are distinguishable based on molecular symmetry, were identified.
- Using MALDI-TOF-MS, the structure of carbohydrates released by digestion with N-glycosidase F (PNGaseF) was predicted and then relative glycoform abundances were calculated by [redacted]-labeled CE. The predominant glycoform was of fucosylated complex biantennary structure with two non-reducing terminal N-acetylglucosamine (GlcNAc) residues (G0), followed by G1 (1-6) and G1 (1-3), i.e. the structures with one non-reducing terminal galactose (Gal) residue. Also, the G2 glycoform, i.e. the structure with two non-reducing terminal Gal residues, was also slightly detected in more than one lot. Furthermore, [redacted] type ([redacted]), [redacted] type ([redacted]) and [redacted] type ([redacted], [redacted]) were slightly detected.
- According to tryptic, [redacted]-cleaved, and CM Lys-C peptide maps, the glycosylation site was located only at Asn^{303} of the heavy chain. The percentage of the glycosylated heavy chain was determined to be [redacted]–[redacted]% by reducing CE-SDS, [redacted]–[redacted]% by tryptic peptide mapping, and [redacted]–[redacted]% by mass spectrometry of reduced bevacizumab.
- The sialic acid content was determined to be [redacted]–[redacted]mol/mol bevacizumab by [redacted] derivatization.
- Electrospray ionization mass spectrometry (ESI-MS) yielded a main peak of [redacted]Da, which agreed well with [redacted]Da, i.e. the theoretical mass for one molecule of G0 attached to each of the two 452 residues heavy chains (G0/G0). Other minor peaks of [redacted]Da and [redacted]Da appeared to represent G0/G1 and G1/G1, respectively. More minor peaks of [redacted]Da and [redacted]Da appeared to represent [redacted] and [redacted] type, respectively.
- The components unretained and retained by [redacted] chromatography ([redacted]) were fractionated and de-glycosylated, and then analyzed by ESI-MS. As a result, it was confirmed that about [redacted]% of the component retained by [redacted] are glycated components. The unretained and retained components were analyzed by tryptic peptide mapping. As a result, a total of [redacted] glycation sites, [redacted] in the light chain and [redacted] in the heavy chain, were confirmed. No glycation of the CDR region was observed. The percentage of glycated bevacizumab was [redacted]–[redacted]%.
- [redacted]-cleaved Lys-C peptide map analysis showed partial oxidation of Met residues (the percentage of the oxidized form of Met[redacted] and Met[redacted] was [redacted]–[redacted]% and [redacted]–[redacted]%, respectively).
- Reduced/CM, and trypsin-digested bevasizumab was analyzed by LC/MS and Edman

degradation, and deamidation of [redacted] and [redacted] was confirmed.

- The pI of bevacizumab was estimated at [redacted] by cIEF and gel isoelectric focusing.
- SDS-PAGE (silver staining) yielded [redacted] bands under non-reducing conditions and [redacted] bands under reducing conditions, which were all confirmed to be related to bevacizumab by immunoblotting using [redacted] anti-human IgG antibody and mass spectrometry after in-gel tryptic digestion. CE-SDS can analyze Related substance A and Related substance B and a total of 7 lots were analyzed. As a result, the percentage of unglycosylated bevacizumab was [redacted]–[redacted]% and its specific activity (relative potency) was [redacted]% of the reference material.
- muMAb A4.6.1, from which bevacizumab was originally derived, showed no crossreactivity with PDGF, acid FGF, EGF, NGF, or HGF (*Growth Factors* 1992; 7: 53-64) and exhibited crossreactivity with human $VEGF_{165}$ and a weak crossreactivity with rabbit VEGF in an interspecies crossreactivity study. $VEGF_{121}$, $VEGF_{165}$, $VEGF_{189}$, and $VEGF_{206}$ isoforms exist and the Kd value of bevacizumab to soluble $VEGF_{165}$, which is the predominant isoform in most tissues and tumor, was 1.1 nmol/L.
- The VEGF binding activity of bevacizumab was measured by sandwich ELISA, which confirmed that bevacizumab binds to VEGF in a concentration-dependent manner. bevacizumab did not exhibit complement dependent cytotoxicity (CDC) activity or antibody-dependent cell-mediated cytotoxicity (ADCC) activity.
- The inhibitory activity on kinase receptor activation was measured by ELISA detecting VEGF-induced receptor phosphorylation in KDR (kinase insert domain receptor)-transfected CHO cells. As a result, it was confirmed that bevacizumab inhibits receptor phosphorylation in a concentration-dependent manner.
- The relative potencies of bevacizumab-related substances were determined by measuring the anti-proliferative activity against HUVEC. As a result, the relative potencies of the oxidized form, the glycated form, the unglycosylated form, SEC main peak, and IEC main peak were [redacted]%, [redacted]%, [redacted]%, [redacted]%, and [redacted]%, respectively, and these were considered the desired product and product related substances. The relative potencies of dimers+high molecular weight aggregates, truncated bevacizumab, IEC acidic variants, and IEC basic variants were [redacted]%, below the quantification limit, [redacted]%, and [redacted]%, respectively and these were considered product-related impurities [See “2. Data relating to quality 2) (2) Impurities”].

(2) Impurities

Substance-related impurities and process-related impurities have been assessed.
Substance-related impurities include truncated forms of bevacizumab, aggregates, and differently charged impurities (acidic variants and basic variants by IEC).

Sample under the non-denaturing condition was separated by SEC, which identified high molecular weight aggregates, dimers, monomers, and multiple peaks of low molecular weight bevacizumab. The multiple low molecular weight peaks separated by SEC were analyzed by ESI-MS, which identified multiple truncated forms of bevacizumab. The content of truncated forms (Related substance D, Related substance E, Related substance A) excluding Related substance C which was overlapped with the main peak on the SEC chromatogram was ≤[redacted]%.

Dissociative non-[redacted] aggregates are present, which are composed of Related substance F and Related substance G. The total amount of Related substance F and Related substance G is assessed by neat sample ([redacted] mg/mL) and the amount of Related substance G is mainly assessed by a [redacted]-fold diluted sample ([redacted] mg/mL). Although the amount of aggregates differs depending on the dilution of neat sample, monomers are in equilibrium with the respective aggregates. Aggregates are all composed of dimers, trimers, and high molecular weight multiples and the major component is dimers. The content of Related substance F is sensitive to [redacted], [redacted], [redacted], and [redacted]. On the other hand, compared to Related substance F, Related substance G is insensitive to them. Specification tests have been set for truncated forms, Related substance F, and Related substance G.

Differently charged impurities were assessed by IEC. It was confirmed that the acidic variants include truncated forms (Related substance E, Related substance A), Related substance H, Related substance I, and Related substance J and the basic variants include Related substance K, Related substance L, and Related substance M. Since the specific activity of the basic and acidic variants was [redacted]% and [redacted]%, respectively, specification tests have been set for acidic and basic variants.

In order to assess process-related impurities, cell substrate-derived impurities (CHO protein, DNA), fermentation process-related impurities (gentamicin, MTX, insulin), purification process-related impurities (medium component F, medium component B, medium component G) and residual solvents were measured using an UF/DF dilution pool, and medium component A and protein A were measured using an affinity pool. These process validation studies confirmed the removal of process-related impurities.

(3) Specifications

The proposed specifications for the drug substance include description, identity test (peptide mapping), osmolarity, pH, purity test (CE-SDS, IEC, SEC), bacterial endotoxins test (chromogenic technique or gel-clot techniques), PS20, quantity (protein assay), and potency assay (anti-proliferative activity against HUVEC).

(4) Stability of the drug substance

Four lots of the drug substance produced at the 12,000 L manufacturing scale were stored in a 120 L stainless steel tank (Lot No. A), which is to be used for commercial production, at －20 ±5℃ for 34 cumulative months (1031 days) and at 5±3℃ for 70 cumulative days, including a total of 5 freeze/thaw cycles (long-term testing in a 120 L stainless steel tank) or in 55 mL stainless steel tanks (Lot No. B, C, and D) at －20±5℃ for 24 months (long-term testing in a 55 mL stainless steel tank) or at 5±3℃ for 90 days (accelerated testing). The stability was assessed based on description, pH, quantity, purity (CE-SDS, IEC, SEC), and potency (anti-proliferative activity against HUVEC).

In the 55 mL and 120 L long-term studies, there were no changes from baseline for all lots.

In the accelerated testing, for all three lots, there was a decrease in the IEC main peak from baseline, which was correlated with a trend towards increases in acidic and basic variant peaks, but there were no changes in other attributes tested.

Based on the above results, the proposed storage time of the drug substance is 24 months when stored at －20±5℃ in a stainless, movable freeze/thaw tank.

3) Drug product

(1) Formulation development

The drug product is a liquid formulation and each vial is filled with 25 mg/mL of bevacizumab, an active ingredient. Based on various investigations [See "2. Data relating to quality 1) (6) Manufacturing process development (comparability)"], 60 mg/mL trehalose as an isotonizing agent, 51 mmol/L sodium phosphate (pH 6.2) as a buffer, and 0.04 w/v% PS20 as a stabilizer have been selected. The composition of the sodium phosphate buffer is sodium dihydrogen phosphate monohydrate and sodium phosphate dibasic anhydrous. No overages are used.

(2) Drug product formulation process

The drug substance (the isotonizing agent, buffer, and stabilizer have already been added during the manufacture of the drug substance [See "2. Data relating to quality 1) (4) Purification process"]) is thawed and then sterile filtered using a 0.22-μm-pore-size [redacted] filter, and filled into colorless glass vials. A cleaned and sterilized butyl rubber stopper is seated in each vial and the vials are capped and screw-closed with aluminium caps. [redacted] step and [redacted] step have been defined as critical process steps. As in-process controls, [redacted] has been set for [redacted] step and [redacted] has been set for [redacted] step. 100% of screw-closed vials are visually inspected.

(3) Specifications

The proposed specifications for the drug product include description, identity test, capillary zone ectrophoretic (CZE), osmolarity, pH, purity test (IEC and SEC), bacterial endotoxins test, test for extractable volume, foreign insoluble matter test, insoluble particulate matter test, sterility test, quantity, and potency assay.

(4) Stability of the drug product

Long-term testing (2-8℃, 24 months) was performed with 5 lots of the 100 mg/4 mL vials (Lot No. E, F, G, I, and J), 1 lot of the 400 mg/16 mL vials (Lot No. K), and 3 lots of the 1,000 mg/40 mL vials (Lot No. L, M, and N), and description, pH, insoluble particulate matters, quantity, purity (CE-SDS, IEC, SEC), potency, and the airtightness of the container were measured (The attributes tested are common among the stability studies of the drug product). Although only one lot of the 400 mg/16 mL vials was tested, its expiration dating has been proposed based on the data from the stability studies of the 100 mg/4 mL and 1,000 mg/40 mL vials, applying bracketing, in accordance with the ICH-Q5C (PMSB/ELD Notification No. 6 dated January 6, 1998 "Stability Testing of Biotechnological/Biological Products").

In the long-term testing of the 100 mg/4 mL vials, there was a decrease in the IEC main peak, which was correlated with a trend towards increases in acidic and basic variant peaks. The stability of the drug product (Lot No. G) manufactured from the drug substance refiltrated 5 times (Lot No. P) was also similar to the stability of the drug product manufactured from the drug substance not refiltrated, according to the data up to 18 months. The test results of the 400 mg vials and the 1,000 mg vials were also similar to those of the 100 mg vials.

Accelerated testing (28-35℃, 3 months) was conducted with 5 lots of the 100 mg/4 mL vials, 1 lot of the 400 mg/16 mL vials, and 3 lots of the 1,000 mg/40 mL vials. For all fills, a decrease in IEC main peak, which was correlated with increases in acidic and basic variant peaks, a decrease in SEC monomer peak, and a reduction in the potency were observed. It was concluded that there are no differences in the stability among different vial sizes (100 mg/4 mL in 5-mL vials, 400 mg/16 mL in 20-mL vials, 1,000 mg/40 mL in 50 mL-vials) or different fills.

In stress testing, the stability following exposure to the storage conditions envisaged during transportation and the photostability were assessed. In order to assess the stability under the storage conditions envisaged during transportation, the 100 mg/4 mL vials and the 400 mg/16 mL vials were stored at 2-8℃ for 3-4 months, at 28-35℃ for 15 days (vibrated for 1 day), at 38-42℃ for 1 day, and at －90 to －50℃ for 3 days, and then stored at 2-8℃ for 24 months. As a result, a decrease in IEC main peak, which was correlated with increases in acidic and basic variant peaks, a decrease in SEC monomer peak, and a reduction in the potency were observed. Photostability testing was conducted using the 100 mg/4mL vials, the 400 mg/16 mL vials, and the 1,000 mg/40 mL vials before the label was attached, exposed to light providing an overall illumination of 1.28 million lux･h and an integrated near ultraviolet energy of 522 W･h/m^2. As a result, (a) CE-SDS patterns were different from those of the reference material. (b) A decrease in IEC main peak was observed, which was correlated with increases in acidic and basic variant peaks. (c) SEC showed an increase in aggregates and a decrease in monomer peak. (d) Titers became unmeasurable after light exposure. Therefore, it was determined that long-term exposure to strong light should be avoided.

Based on the above results, the proposed shelf life for the drug product is 24 months when stored at 2-8℃, protected from light, and the proposed temperature during transportation is 2-8℃, protected from light.

In addition to the above studies, as bevacizumab is mixed with a diluent and then administered via an IV bag for intravenous infusion, the compatibility with physiological saline or 5w/v% dextrose solution, i.e. diluents that are likely to be used, and the compatibility with polyvinyl chloride or polyolefin IV bags were determined.

The compatibility with physiological saline was determined as follows:
Bevacizumab was mixed with physiological saline to make bevacizumab concentrations of [redacted] mg/mL and [redacted] mg/mL. Then, 100-mL Viaflex IV bags manufactured by Baxter were used and the stability after 48 hours at 2-8℃ and 30℃ was assessed. As a result, there were no

changes in bevacizumab concentration, pH, IEC, SEC or potency.

The compatibility with 5 w/v% dextrose solution was determined as follows:
Bevacizumab was mixed with 5w/v% dextrose solution to make bevacizumab concentrations of approximately [redacted] mg/mL and approximately [redacted] mg/mL. Then, 100-mL Viaflex IV bags manufactured by Baxter were used and the stability after 48 hours at 2-8℃ and 30℃ was assessed. As a result, the sample of lower concentration ([redacted] mg/mL) stored at 30℃ showed a decrease in the proportion of IEC main peak.

In order to assess the compatibility with polyvinyl chloride or polyolefin IV bags, physiological saline was injected into polyvinyl chloride and polyolefin IV bags to make bevacizumab concentrations of approximately [redacted] mg/mL, approximately [redacted] mg/mL, and approximately [redacted] mg/mL. Then, the stability when stored at 30℃ was assessed. As a result, there were no changes in bevacizumab concentration, pH, IEC, SEC or potency.

The above results confirmed that there are no interactions with physiological saline or IV bags. “Precautions concerning Use” of the package insert includes the following statements: Use Isotonic Sodium Chloride Solution (JP) only for preparing the infusion; As mixing Avastin with dextrose solutions may cause a decrease in potency, do not mix Avastin with dextrose solutions; and Do not use the same line as dextrose-containing infusions.

4) Reference material
The reference standard material used in the early phase of development was antivegf898-1, which was prepared from 3 lots of the drug substance produced at a [redacted] L scale from the 107N cell line (Lot No. Q, R, and S). It contained [redacted] mmol/L sodium phosphate (pH[redacted]), [redacted] mg/mL trehalose, and [redacted]w/v%[redacted] besides [redacted] mg/mL bevacizumab, and was stored at [redacted] to [redacted]℃. The current reference standard material is antivegf801-2, which was prepared from 2 lots of the drug substance produced at a 12,000 L scale from the G7 cell line (Lot No. T and A). It contains [redacted] mmol/L [redacted] (pH[redacted]), [redacted] mg/mL trehalose, and [redacted]w/v%[redacted] besides [redacted] mg/mL bevacizumab, and is stored at [redacted] to [redacted]℃. Antivegf801-2 has been characterized for description, identity (CZE, peptide mapping), purity (CE-SDS, IEC, SEC), potency, quantity (protein content), PS20, and osmolarity. The current reference standard material, antivegf801-2, has a lower content of [redacted] compared to the initial reference standard material, antivegf898-1, but there are no differences in other attributes.

The proposed specifications and test methods for a newly prepared reference material include description, identity test (CZE, peptide mapping), osmolarity, pH, purity test (CE-SDS, IEC, SEC), PS20, quantity (protein content), and potency assay. And, the identity (CZE, peptide mapping) and purity (CE-SDS) of the newly prepared reference material are to be compared to those of the current reference material.

The outline of review by the PMDA for the submitted quality data is presented in the Review Report (2).

3. Non-clinical Data

As non-clinical data, the results from pharmacology studies, pharmacokinetic studies, and toxicity studies conducted by Genentech (the US) were submitted.

3.1 Pharmacology studies

Summary of the submitted data

Twenty-two reports on primary pharmacodynamics (8 reports as Evaluation Data, 14 reports as Reference Data) and 5 reports on pharmacodynamic drug interactions (all Reference Data) were submitted.

1) Primary pharmacodynamics

(1) Characterization of a murine monoclonal antibody, muMAb A4.6.1 (*Growth Factors* 7: 53-64, 1992)

Mice were immunized with recombinant human $VEGF_{165}$ conjugated with keyhole limpet hemocyanin, which is an antigen-stimulating carrier protein. Hybridomas were generated by fusing spleen cells from the mice with murine myeloma cells P3X63Ag8U.1 and IgG1 type muMAb A4.6.1 was selected among the murine anti-human VEGF antibodies produced by these polyclones, based on the results of assessments of the binding activity to human VEGF isoforms, the binding specificity to human VEGF, and the inhibitory activity on VEGF-induced proliferation of vascular endothelial cells. The results on muMAb A4.6.1 among 4 candidate antibodies tested are shown below.

The antigen specificity of muMAb A4.6.1 was assessed by immunoprecipitation. muMAb A4.6.1 recognized all of $VEGF_{121}$, $VEGF_{165}$, and $VEGF_{189}$, which are major isoforms of human VEGF, but did not exhibit binding activity to other growth factors tested (PDGF, EGF, acid-FGF, NGF, and HGF). muMAb A4.6.1 inhibited human VEGF-induced proliferation of bovine adrenal cortex vascular endothelial cells.

In order to assess the inhibitory activity of muMAb A4.6.1 on increased vascular permeability induced by VEGF, a pre-reacted mixture of human $VEGF_{165}$ and muMAb A4.6.1 was intradermally administered to guinea pigs pretreated with Evans blue dye. The subcutaneous permeability of dye induced by human $VEGF_{165}$ 20 ng/site was completely inhibited by a tenfold molar ratio of muMAb A4.6.1.

In order to assess the inhibitory activity of muMAb A4.6.1 on VEGF-induced angiogenesis, methylcellulose disks containing $VEGF_{165}$ and muMAb A4.6.1 were inoculated on the top of the chick embryo chorioallantoic membrane and angiogenesis around each disk was observed. As a result, angiogenesis induced by human $VEGF_{165}$ 400 ng was inhibited by muMAb A4.6.1 16 μg (at a molar ratio of muMAb A4.6.1 to $VEGF_{165}$ of 10:1).

(2) Characterization of bevacizumab (*Cancer Res* 57: 4593-9, 1997 (Reference Data), Study [redacted]-0348-1753, Study 1756-582-RPT-1_0)

cDNA that encodes the antibody gene from the muMAb A4.6.1-producing cell line was generated and six complementarity determining regions were determined. These six complementarity determining regions were inserted in place of those of human κ globulin subgroup I light chain (VL-CL) and human γ globulin subgroup III (IgG1) heavy chain (VH-CH1) to produce the gene of a humanized Fab fragment. Then, in order to enhance the binding capacity to VEGF, the nucleotide sequence was modified for substitutions of amino acid residues outside the complementarity determining regions to produce a humanized fragment, Fab-12. The VH and VL domains of Fab-12 were combined with human IgG1 heavy chain constant domains CH1-CH2-CH3 and human κ light chain constant domain CL, respectively, to produce the gene of bevacizumab, a humanized antibody. Bevacizumab is produced in CHO cells transfected with this recombinant gene [See "2. Data relating to quality"].

In order to compare the anti-VEGF activity between bevacizumab and muMAb A4.6.1, bovine adrenal cortex vascular endothelial cells were incubated in the presence of human $VEGF_{165}$ 3 ng/mL and each antibody and the anti-proliferative activity (IC_{50}) of each antibody was calculated based on the number of cells after incubation. As a result, the IC_{50} values of bevacizumab and muMAb A4.6.1 were 50 and 48 ng/mL, respectively (*Cancer Res* 57: 4593-9, 1997). When human umbilical vein endothelial cells were incubated in the presence of human $VEGF_{165}$ 30 ng/mL and each antibody, the IC_{50} values of bevacizumab and muMAb A4.6.1 calculated based on the number of cells after incubation were 0.89 and 0.61 nmol/L, respectively (Study [redacted]-0348-1753).

In order to compare the anti-tumor growth activity *in vivo* between bevacizumab and muMAb A4.6.1, nude mice (10 mice/group) were inoculated with human rhabdomyosarcoma A673 cells and intraperitoneally administered bevacizumab (0.5 or 5 mg/kg) or muMAb A4.6.1 (0.5 or 5 mg/kg) twice weekly from the following day of the inoculation, and the tumor weights were measured at 4 weeks post-inoculation. The tumor weights in the bevacizumab 0.5 and 5 mg/kg groups were reduced by 90% and 95%, respectively, compared to the control group, and the tumor weights in 0.5 and 5 mg/kg groups of the muMAb A4.6.1 were reduced by 85% and 93%, respectively, compared to the control group (*Cancer Res* 57: 4593-9, 1997).

In order to assess the inhibitory effect of bevacizumab on major human VEGF isoforms, human umbilical vein endothelial cells were incubated for 4 days in the presence of bevacizumab and 10 ng/mL of either human $VEGF_{165}$, $VEGF_{121}$, or $VEGF_{110}$ and then the anti-proliferative activity against each isoform was determined based on viable cell count. As a result, the IC_{50} of bevacizumab for inhibiting $VEGF_{165}$, $VEGF_{121}$, and $VEGF_{110}$-induced proliferation was 60, 86, and 32 ng/mL, respectively (Study 1756-582-RPT-1_0).

(3) Species specificity (Study [redacted]-0317-1753, Study [redacted]-0318-1753)

The binding affinity of bevacizumab to human $VEGF_{165}$, rabbit VEGF, and mouse VEGF was

assessed, using Biacore (surface plasmon resonance). The dissociation constants (Kd values) for human $VEGF_{165}$ and rabbit VEGF were 1.1±0.8 and 8.0±5.1 nmol/L, respectively. On the other hand, bevacizumab did not exhibit a selective binding activity to mouse VEGF even at 650 nmol/L.

(4) Crystal structure analysis of the bevacizumab Fab fragment-VEGF complex (*Structure* 6: 1153-67, 1998)

In order to identify VEGF amino acid residues that are critical for the binding between bevacizumab and human VEGF, alanine scanning analysis of the amino acid residues that form the contacts was performed. As a result, 25 amino acid residues of the bevacizumab molecule formed the interface with VEGF and of which, 8 amino acid residues directly involved in binding were substituted by alanine, resulting in a reduction of the binding capacity to $\leq$ 1/150. Also, 19 amino acid residues of the VEGF molecule were involved in the binding to the Fab fragment of bevacizumab, and of which, 6 residues localized to β5-β6 of the four-stranded beta-sheet forming the center of the VEGF molecule (Met^{81}, Arg^{82}, Ile^{83}, Gly^{88}, Gln^{89}, and Gly^{92}) were substituted by alanine, resulting in a reduction of the binding capacity to 1/22-1/107. Although the 19 amino acid residues on the VEGF molecule involved in the binding to bevacizumab are not identical with the VEGF amino acid residues important for binding to VEGF receptors (VEGFR-1 and -2), since some of the 19 residues are on the binding sites with the receptor, it is considered that VEGF interaction with its receptor is blocked sterically when bevacizumab binds to VEGF. The binding activities of VEGF and bevacizumab-VEGF complex to the VEGF family receptors other than VEGFR-1 and -2 are being checked with the applicant.

(5) Effects on the growth of rhabdomyosarcoma, glioblastoma multiforme, and leiomyosarcoma cells (*Nature* 362: 841-4, 1993)

Beige-nude mice (10 mice/group) were subcutaneously inoculated with 1×10^6 cells of human rhabdomyosarcoma A673 cells, glioblastoma multiforme G55 cells, or leiomyosarcoma SK-LMS-1 cells, and intraperitoneally administered muMAb A4.6.1 10-400 μg/mouse twice weekly. Then, the tumor weights were measured at 4 weeks after the start of treatment for the mice inoculated with A673 or G55 cells and at 10 weeks after the start of treatment for the mice inoculated with SK-LMS-1 cells to assess the anti-tumor growth activity. In the mice inoculated with A673 or G55 cells, muMAb A4.6.1 inhibited tumor growth at doses $\geq$10 μg/mouse and the tumor weights were reduced by 96 and 80%, respectively, at 4 weeks after the start of treatment in the 100 μg group compared to the control group. In the mice inoculated with SK-LMS-1 cells, the tumor weights were reduced by 70% at 100 μg. In the mice inoculated with A673 cells, the vascular density in the area around the inoculated tumor was measured by immunohistological staining, using an anti-factor VIII-related antibody. As a result, the vascular density in the tumor tissue was reduced in the muMAb A4.6.1 groups compared to the control group.

On the other hand, in *in vitro* cell culture, A673, G55, and SK-LMS-1 cells released VEGF into the culture medium, but the addition of muMAb A4.6.1 0.2-20 μg/mL to the culture medium did not affect cell growth and VEGF 0.5-20 ng/mL also did not affect cell growth.

Based on the above results, it is discussed that the anti-tumor growth activity of muMAb A4.6.1 observed *in vivo* is associated with the inhibition of an angiogenesis factor produced by tumor cells, instead of direct tumor growth inhibition or the inhibition of VEGF autocrine action.

(6) Pharmacodynamics and pharmacokinetics (*Toxicol Pathol* 27: 14-21, 1999)

Beige-nude mice (24 mice/group [14 mice were used for pharmacokinetic assessment only]) were subcutaneously inoculated with 2×10^6 cells of human rhabdomyosarcoma A673 cells and intraperitoneally administered muMAb A4.6.1 (0.05-5 mg/kg) twice weekly for 4 weeks from 24 hours post-inoculation, and the effects of muMAb A4.6.1 on tumor growth and plasma muMAb A4.6.1 concentrations were assessed. The anti-tumor growth activity was similar between the 2.5 mg/kg and 5 mg/kg groups among the muMAb A4.6.1-treated groups. The mean plasma muMAb A4.6.1 concentration (calculated using a complementary exponential function of plasma concentrations and time at 4 days after the last dose) in the 2.5 mg/kg group was 30.6 μg/mL (range: 12.47-56.93 μg/mL).

(7) Others

Concerning the expression of VEGF and its receptors in human colorectal cancer tissues and human colorectal cancer-derived cells, and the effects of muMAb A4.6.1 on tumor growth and metastases, accumulation of ascites fluid, the vascular density in tumor tissue, the number of vascular smooth muscle cells/the vascular diameter, the distribution of vascular smooth muscle, vascular permeability, and interstitial pressures/local oxygen partial pressures in nude mice inoculated with human tumor cells (including colorectal cancer), the following 13 published articles were submitted as Reference Data. The applicant explains that whether anti-VEGF antibody used for *Neoplasia* 2: 306-14, 2000 was muMAb A4.6.1 or not is unknown.

J Clin Invest 95: 1789-97, 1995, *Am J Pathol* 153: 1249-56, 1998, *J Pediatr Surg* 38: 308-14, 2003, *Neoplasia* 2: 306-14, 2000, *Prostate* 35: 1-10, 1998, *Cancer Res* 56: 4032-9, 1996, *J Urol* 161: 960-3, 1999, *J Pediatr Surg* 35: 30-3, 2000, *Proc Natl Acad Sci USA* 93: 14765-70, 1996, *Am J Obstet Gynecol* 183: 956-63, 2000, *J Magn Reson Imaging* 15: 233-40, 2002, *Cancer Invest* 16: 225-30, 1998, *Cancer Res* 60: 5565-70, 2000

Based on the above study results (including the published articles), the applicant discusses the mechanism of action of bevacizumab and muMAb A4.6.1 as follows:

As muMAb A4.6.1 and bevacizumab share common amino acid sequence of the antigen-binding site and their binding properties to VEGF, etc. are very similar, their pharmacological activities are equivalent.

Bevacizumab (or muMAb A4.6.1) inhibits the binding of the ligand to VEGF receptors expressed on vascular endothelial cells by selectively binding to human VEGF (VEGF-A) [Note by the PMDA: The binding activity of bevacizumab or muMAb A4.6.1 to the VEGF family other than VEGF-A is being checked with the applicant]. Consequently, VEGF signal transduction is blocked, resulting in the inhibition of VEGF-induced angiogenesis and the inhibition of increased vascular permeability in tumor tissue and then the suppression of tumor

exacerbation/metastases.

In tumor tissue, the inhibition of VEGF signal transduction by bevacizumab (or muMAb A4.6.1) results in the inhibition of the formation of abnormal vasculature and an increase in the ratio of blood vessels with normal structure. Normalization of vasculature and reduced vascular permeability associated with the blockage of VEGF signaling are also considered to contribute to a decrease of increased interstitial pressures in tumor tissue.

2) Secondary pharmacodynamics
As bevacizumab inhibits angiogenesis by blocking human VEGF signal transduction, epiphyseal dysplasia and the effects on wound healing and female reproductive function etc., due to its pharmacological effects, were observed [See "3.3 Toxicology studies"].

3) Safety pharmacology
In repeat-dose toxicity studies of bevacizumab in cynomolgus monkeys, the effects of Bevacizumab on the central nervous system, cardiovascular system, respiratory system, and renal system were investigated. Following 4-week, 13-week, and 26-week repeat-dose administration, there were no effects on general condition/behaviour, rectal body temperature, blood pressure, ECG, respiratory rate, or urinalysis (urine volume, urine pH, etc.) at doses up to 50 mg/kg (administered once or twice weekly) [See "3.3 Toxicology studies"].

4) Pharmacodynamic drug interactions
Concerning the effects of muMAb A4.6.1 in combination with paclitaxel (PTX), topotecan, doxorubicin hydrochloride (DXR), docetaxel hydrate, or radiation therapy as measured by tumor growth and angiogenesis etc., the following 5 published articles were submitted as Reference Data.

Am J Pathol 161: 1917-24, 2002, *Clin Cancer Res* 8: 3226-31, 2002, *J Pediatr Surg* 36: 1177-81, 2001, *Anticancer Res* 19: 4203-14, 1999, *Cancer Res* 61: 3369-72, 2001

The applicant discusses the mechanism of the add-on effect of muMAb A4.6.1 in combination with other antineoplastic agents reported in the above published articles, as follows:
Interstitial pressures in tumor tissue are high, which is considered to restrict the tissue penetration of chemotherapy agents. It has been reported that muMAb A4.6.1 improves the penetration of irinotecan etc. into tumor tissue (*Br J Cancer* 88: 1979-86, 2003), and decreased tumor interstitial pressures following the administration of muMAb A4.6.1 as observed in mice inoculated with human tumor would be one of the mechanisms of the add-on effect.

Outline of review by the PMDA
PMDA considers that the applicant's discussion on the mechanism of inhibition of tumor growth with bevacizumab alone is acceptable because:
In foreign studies where single agent bevacizumab was administered in patients with solid tumors, the response rate was 0/23 subjects in Study AVF0737, 5/75 subjects in Study

AVF0776g, and 0/15 subjects in Study AVF0775g, and the proportions of subjects with tumor shrinkage following single agent bevacizumab administration were low in clinical studies. Meanwhile, *in vivo* pharmacology studies demonstrated the inhibition of tumor growth by bevacizumab alone, except for a model of inoculation of tumor with a low level of VEGF mRNA expression.

As to the add-on effect of bevacizumab in combination with other antineoplatic agents, the dosing schedule was different from that in a study showing the effect of muMAb A4.6.1 on tumor interstitial pressures and interstitial pressures were not assessed in the coadministration studies. Thus, whether the effect of muMAb A4.6.1 on interstitial pressures contributes to the add-on effect can not be determined.

PMDA reviewed the submitted pharmacology data as follows.

1) Pharmacokinetics of muMAb A4.6.1 and bevacizumab

PMDA asked the applicant to explain differences in the pharmacokinetics between a humanized antibody, bevacizumab and a murine antibody, muMAb A4.6.1, in mice, since muMAb A4.6.1 was mainly used in *in vivo* studies.

The applicant explained as follows:

The blood elimination half-life ($t_{1/2}$) of murine IgG1 in immunodeficient mice has been reported to be 6.5 days (*Cancer Res* 52: 1916-23, 1992) and it is considered that a murine monoclonal antibody, muMAb A4.6.1 also behaves in the body in a similar way to murine IgG1. Meanwhile, the $t_{1/2}$ following the administration of bevacizumab to non-tumor-bearing immunodeficient mice was 6.8-7.2 days and the $t_{1/2}$ of bevacizumab and muMAb A4.6.1 in immunodeficient mice are similar. The major pathway of clearance for bevacizumab and muMAb A4.6.1 is uptake by the reticuloendothelial system (*Drug Discov Today* 11:81-8, 2006) and it has been reported that human and murine IgG1 show similar affinity for murine FcRn which mediates this pathway (*Int Immunol* 13: 1551-59, 2001). Therefore, it is inferred that clearance by the reticuloendothelial system is comparable for the two antibodies in mice. The following pharmacology study data obtained after the regulatory submission (Study PHM[redacted]-0173S conducted by the applicant) have confirmed that bevacizumab inhibits the proliferation of human colorectal cancer *in vivo*.

Nude mice (5-6 mice/group) were subcutaneously inoculated with 5×10^6 cells of human colorectal cancer COLO205 cells, a tumor tissue (about 2 mm square) of human colorectal cancer CXF280, or a tumor tissue (about 2 mm square) of human colorectal cancer COL-16-JCK, and the anti-tumor growth activity of bevacizumab administered intraperitoneally twice weekly for a total of 6 doses was evaluated. In addition, in the nude mice inoculated with a tumor tissue of COL-16-JCK, the effects of coadministration of bevacizumab (4.0 mg/kg, twice weekly, for a total of 6 doses by intraperitoneal administration) with capecitabine (359 or 180 mg/kg (in combination with oxaliplatin), once daily for 14 consecutive days by oral administration) and oxaliplatin (5 mg/kg intravenous administration) were evaluated. The results are presented below.

Dose (mg/kg)	TGI (%)		
	COLO205	CXF280	COL-16-JCK
Bevacizumab 0.4	—	22	—
Bevacizumab 1.2	33	40	46
Bevacizumab 2.5	41	47	59
Bevacizumab 4.0	44	—	55

TGI＝[1－(mean tumor volume in the test group)÷ (mean tumor volume in the untreated group)]×100

Drug	TGI (%)	Drug	TGI (%)
Bevacizumab	35	Bevacizumab	44
Capecitabine (359 mg/kg)	52	Capecitabine (180 mg/kg)	38
Bevacizumab/Capecitabine	80	Oxaliplatin	23
		Capecitabine/Oxaliplatin	70
		Bevacizumab/Capecitabine/Oxaliplatin	86

TGI＝[1－(mean tumor volume in the test group)÷ (mean tumor volume in the untreated group)]×100

2) Animal species used

The species specificity of muMAb A4.6.1 has been studied using human $VEGF_{165}$, rabbit VEGF, and mouse VEGF. PMDA asked the applicant to explain the binding activity of bevacizumab to cynomolgus monkey VEGF, which was used in secondary pharmacodynamic and safety pharmacology studies of bevacizumab.

The applicant responded as follows:

The amino acid sequence of human VEGF is identical to that of cynomolgus monkey VEGF (*Invest Ophthalmol Vis Sci* 37: 1334-40, 1996), and the epitope on a VEGF molecule involved in the binding to bevacizumab (79th to 94th amino acids from the N terminus) is conserved in cynomolgus monkey VEGF as well. Thus, bevacizumab is considered to bind to cynomolgus monkey VEGF, too. The binding affinity of bevacizumab to rabbit VEGF is lower compared to human VEGF and furthermore, anti-bevacizumab antibodies are formed during treatment. Taking also account of these points, the test conditions were set. And, it is necessary to consider the possibility that some findings similar to those in rabbits attributable to the pharmacological effects of bevacizumab may occur in humans, at a lower exposure than the corresponding level in rabbits.

PMDA accepted the response, judging that the safety pharmacology of bevacizumab can be evaluated in cynomolgus monkeys. PMDA considers that it is necessary to advise caution about the findings observed in cynomolgus monkeys and rabbits (delayed healing of wound, epiphyseal dysplasia, effects on female reproductive function and embryo/fetus).

3) Mechanism of the development of thromboembolism and hypertension

See “3.3 Toxicology studies.”

4) Mechanism of the development of reversible posterior leukoencephalopathy syndrome

PMDA asked the applicant to explain the association between the pharmacological effect of bevacizumab (inhibition of vascular permeability increase induced by VEGF) and reversible posterior leukoencephalopathy syndrome (RPLS) reported in clinical use.

The applicant explained as follows:

RPLS is characterized by vascular edema in the posterior half of the cerebrum and it is presumed that vascular edema develops due to increased permeability/breakage of the blood-brain barrier resulting from an acute increase of cerebral blood flow, dysfunction of cerebral vascular autoregulation caused by vascular spasm etc., or damage to vascular endothelial cells (*Brain and Nerve* 57: 767-777, 2005). Brain edema occurs also in diseases other than RPLS, and it has been inferred that increased vascular permeability induced by VEGF is involved in these diseases (*Brain* 125: 2549-57, 2002, *J Neuroimmunol* 160: 170-7, 2005), and it is expected that brain edema will be suppressed by inhibiting VEGF. However, while it has been reported that the administration of VEGF-neutralizing antibody improves brain edema in a murine hypoxia model (*Brain* 125: 2549-57, 2002), even the administration of bevacizumab did not improve brain edema in a rabbit bacterial meningitis model (*J Neuroimmunol* 160: 170-7, 2005), and no consistent results have been available. One of the reasons for that is presumably that the main permeability-enhancing factor of cerebral blood vessels differs depending on the mechanism of inducing brain edema. It is thought that there are multiple mechanisms of the development of RPLS as well, and how VEGF and the inhibition of VEGF are involved in the onset of RPLS/the development of the pathological condition has not been elucidated. However, since it has been inferred that bevacizumab inhibits VEGF-induced nitric oxide (NO) production in vascular endothelial cells, leading to changes in blood pressure, and hypertension is known to be one of the causes of the development of RPLS, the possibility that changes in blood pressure associated with bevacizumab induced RPLS also can not be ruled out.

PMDA instructed the applicant to continue to collect information on the association between RPLS and the pharmacological effect of bevacizumab, including a literature search.

3.2 Pharmacokinetic studies

Summary of the submitted data

The pharmacokinetics (PK) of bevacizumab was evaluated in mice, rats, New Zealand white rabbits, and cynomolgus monkeys, pharmacokinetic interactions with other antineoplastic agents were assessed in cynomolgus monkeys, and the clearance of bevacizumab-VEGF complexes and the comparability of different lots of Bevacizumab used in nonclinical and clinical studies were investigated in rats.

1) Absorption

(1) Single-dose administration

Following single intravenous administration of 0.8 or 8.5 mg/kg of bevacizumab in mice, the clearance (CL) of bevacizumab was 34.1 and 14.8 mL/day/kg, respectively, and the half-life

($t_{1/2\lambda z}$) was 3.1 and 7.2 days, respectively. The bioavailability (BA) calculated from the ratio of $AUC_{0\text{-}17d}$ following single intraperitoneal administration of 0.8 or 8.5 mg/kg of bevacizumab was 94 and 96%, respectively.

Following single intravenous administration of 9.3 mg/kg of bevacizumab in mice, the CL of bevacizumab was 15.7 mL/day/kg, volume of distribution of the central compartment (Vc) and at steady-state (Vss) were 53.0 mL/kg and 152 mL/kg, respectively, and the initial half-life ($t_{1/2\alpha}$) and terminal half-life ($t_{1/2\beta}$) were 1.2 h and 6.8 days, respectively. The BA calculated from the ratio of AUC following single subcutaneous administration of 9.3 mg/kg of bevacizumab was 110%.

Following single intravenous administration of 0.664 or 10.1 mg/kg of bevacizumab in rats [Note by the PMDA: the nominal dose was 1 or 10 mg/kg, respectively], the CL was 8.37 and 4.83 mL/day/kg, respectively, and the $t_{1/2\beta}$ was 5.42 and 12.3 days, respectively. The Vc and Vss were 25.0 and 58.8 mL/kg, respectively in the 0.664 mg/kg group, and 30.8 and 79.5 mL/kg, respectively in the 10.1 mg/kg group. The BA calculated from the ratio of AUC following single subcutaneous administration of 10.1 mg/kg of bevacizumab was 69%.

Following single intravenous administration of 0.5 mg/kg of bevacizumab in rabbits, the CL of bevacizumab was 14.0 mL/day/kg, the $t_{1/2\beta}$ was 3.88 days, and the Vc and Vss were 41.8 and 69.5 mL/kg, respectively.

Single intravenous doses of 2, 10, or 50 mg/kg of bevacizumab or a single subcutaneous dose of 10 mg/kg of bevacizumab were administered to cynomolgus monkeys. The AUC and C_{max} of serum bevacizumab after intravenous administration increased in an almost dose-proportional manner and the CL, Vc, Vss, $t_{1/2\alpha}$, $t_{1/2\beta}$, and mean residence time (MRT) were similar across the three doses (See the table below).

Parameters	2 mg/kg, i.v.	10 mg/kg, i.v.	50 mg/kg, i.v.	10 mg/kg, s.c.
AUC (μg·day/mL)	430±72	1810±140	8800±1400	1770±260
C_{max} (μg/mL)	68±6.2	290±29	1400±210	120±3
CL (mL/day/kg)	4.76±0.88	5.56±0.46	5.78±0.84	5.74±0.85
Vc (mL/kg)	30.1±2.0	36.3±2.4	36.8±4.9	77.6±11
Vss (mL/kg)	64.0±16	66.8±8.3	73.9±11	NA
$t_{1/2\alpha}$ (h)	11.5±5.0	10.9±2.4	19.2±9.5	–
$t_{1/2\beta}$ (day)	9.88±.9	8.75±0.84	10.3±3.1	9.39±0.46
MRT (day)	13.4±2.2	12.0±1.0	13.1±3.5	13.5±0.66
bioavailability (%)	–	–	–	98

NA: not applicable, mean±SD, n=4

(2) Repeat-dose administration

Bevacizumab 10 mg/kg was intravenously administered to rabbits on Days 1, 4, 8, and 11, and serum bevacizumab concentrations were measured after the last dose. Compared to the PK parameters following a single intravenous dose of 0.5 mg/kg of bevacizumab [See "3.2 Pharmacokinetic studies, *Summary of the submitted data* 1) (1) Single-dose administration"], the Vc and Vss were similar (39.9 and 62.9 mL/kg, respectively), whereas the CL (8.13

mL/day/kg) was smaller and the $t_{1/2\beta}$ □(5.52 days) was prolonged. The applicant infers that the differences in CL and $t_{1/2\beta}$ were attributable to a difference in the dose.

Bevacizumab 2, 10, or 50 mg/kg was intravenously administered twice weekly (4-week and 13-week, repeat-dose studies) or once weekly (26-week repeat-dose study, once weekly and twice weekly for the 10 mg/kg group only) to cynomolgus monkeys and serum bevacizumab concentrations were measured. Serum bevacizumab concentrations were increased in an almost dose-proportional manner. The PK parameters following 4-week and 13-week repeat administration of bevacizumab 50 mg/kg are presented in the following table. Since the AUC, CL, Vc, Vss, and $t_{1/2\beta}$ were similar to those after single intravenous administration of bevacizumab 50 mg/kg [See "3.2 Pharmacokinetic studies, *Summary of the submitted data* 1) (1) Single-dose administration"], the applicant infers that there is little accumulation of the drug after multiple dosing. With respect to about 30% lower CL in the 26-week repeat-dose study compared to the other two studies, the applicant discusses as follows: This was very likely to be errors due to the small number of animals studied (2 males and 2 females) and a higher variability between males and females in the 26-week repeat-dose study compared to the 4-week and 13-week studies, etc. and was unlikely to be a change associated with a prolonged duration of treatment.

Dose	50 mg/kg		
Parameters	4 weeks, twice weekly	13 weeks, twice weekly	26 weeks, once weekly
AUC (μg·day/mL)	9900±760	8070±860	13000±2600
CL (mL/day/kg)	5.07±0.40	6.25±0.71	3.98±0.808
Vc (mL/kg)	38.5±4.8	46.9±5.3	33.1±3.22
Vss (mL/kg)	68.4±5.5	84.3±2.5	64.7±9.61
$t_{1/2\alpha}$ (h)	14.1±4.2	28.2±34	77.0±30.3
$t_{1/2\beta}$ (day)	9.89±1.2	11.4±3.8	20.4±8.67
MRT (day)	13.6±1.6	13.6±1.4	16.5±2.59

Mean±SD, n=4 (2 males and 2 females)

2) Distribution

A single intravenous dose of ^{125}I-labeled bevacizumab (22.2-24.1 MBq/kg, the total protein dose of 4.8-5.2 μg/kg) was administered to rabbits and the tissue distribution of radioactivity was studied.

At both 2 hours and 48 hours post-dose, in all tissues including plasma, the distribution of total radioactivity was similar to the distribution of trichloroacetic acid (TCA)-precipitable radioactivity.

At 2 hours post-dose, total radioactivity was localized primarily in plasma with limited distribution in other tissues. The level of TCA-precipitable radioactivity in plasma (0.499% of dose/g tissue) was about 10-fold higher than those in other tissues. The organs that exhibited the highest levels of TCA-precipitable radioactivity were, in decreasing order, kidney > testis > spleen > heart > lung > thymus (0.069%-0.018% of dose/g tissue).

The level of TCA-precipitable radioactivity was still highest in plasma at 48 hours post-dose,

which was about 1/2 of the value at 2 hours post-dose (0.246% of dose/g tissue). The decreases in TCA-precipitable radioactivity were minimal in other tissues.

There were no differences in the pattern of tissue distribution between bevacizumab and the control IgG1 antibody (^{125}I-labeled rhuMAb E25 [omalizumab]) and the applicant discusses that at the doses used in this study, the distribution pattern of bevacizumab was representative and consistent with the tissue distribution of a humanized IgG1 monoclonal antibody (*Arzneimittelforschung* 44: 890-8, 1994, *J Pharmacol Exp Ther* 279: 1000-8, 1996).

Rabbits were intravenously administered bevacizumab 10 or 100 mg/kg on gestation day 18 and necropsied on gestation day 21 (the single-dose group) or intravenously administered bevacizumab 10, 30, or 100 mg/kg on gestation days 6, 9, 12, 15, and 18 and necropsied on gestation day 29 (the repeat-dose group), and bevacizumab concentrations in maternal serum, fetal serum, and amniotic fluid were measured.

In the single-dose group, maternal serum bevacizumab concentrations were increased in a dose-proportional manner, while the mean ratio of fetal serum bevacizumab concentrations between the doses was about 3 though the ratio of the doses was 10, and the ratio of fetal serum concentration to maternal serum concentration was lower in the 100 mg/kg group compared to the 10 mg/kg group. The variability in bevacizumab concentrations in fetal serum and amniotic fluid was higher than that in maternal serum concentrations. In the repeat-dose group, although bevacizumab concentrations in maternal serum and amniotic fluid were largely dose-proportional at 10-100 mg/kg, the ratio of fetal serum bevacizumab concentrations between the doses was less than the ratio of the doses. The ratio of fetal serum concentration to maternal serum concentration of bevacizumab and the ratio of amniotic fluid concentration to maternal serum concentration were both higher in the repeat-dose group than in the single-dose group.

3) Metabolism/Excretion

A single intravenous dose of ^{125}I-labeled bevacizumab (22.2-24.1 MBq/kg, the total protein dose of 4.8-5.2 μg/kg) was administered to rabbits, and degradation products in plasma, urine, and tissues were determined by SDS-PAGE autoradiography. Intact ^{125}I-labeled bevacizumab was predominantly detected in plasma at 48 hours post-dose with very weak bands for degradation products. Although small amounts of low molecular weight degradation products were detected in the tissues of the kidney, testis, spleen, heart, and lung, the degradation pattern varied from tissue to tissue. Similar results were obtained for the control IgG1 antibody, omalizumab. These results are consistent with low catabolism of IgG antibodies, and the applicant discusses that it has been suggested that bevacizumab shows a metabolism profile consistent with that of a typical humanized IgG1 monoclonal antibody.

As for urinary excretion at 2 hours and 48 hours after the administration of ^{125}I-labeled bevacizumab and control IgG1 antibody, TCA-precipitable radioactivity represented only 6 to9% [Note by the PMDA: it seems to refer to the percentage of TCA-precipitable radioactivity

in total radioactivity in the urine] and unchanged bevacizumab was not detected in the urine at 48 hours post-dose. Similar results were obtained for the control IgG1 antibody and the applicant discusses that the pattern of urinary excretion of bevacizumab is similar to that of the control IgG1 antibody.

4) Pharmacokinetic drug interactions

(1) Bevacizumab in combination with cisplatin/paclitaxel regimen

Cynomolgus monkeys were intravenously administered bevacizumab 10 mg/kg on Days 1, 4, 8, 11, 15, and 18 and cisplatin (CDDP) 1 mg/kg and paclitaxel (PTX) 4 mg/kg on Day 18, and serum concentrations of each agent were assessed. Multiple doses of bevacizumab had no effects on the PK of either CDDP or PTX and concurrent CDDP/PTX had no effects on the PK of bevacizumab.

(2) Bevacizumab in combination with IFL regimen

Plasma concentrations of irinotecan and 5-FU were measured when bevacizumab in combination with IFL regimen was administered to cynomolgus monkeys. Bevacizumab 10 mg/kg, irinotecan hydrochloride 125 mg/m^2, 5-FU 500 mg/m^2, and LV 20 mg/m^2 were intravenously administered on Days 1 and 8. The CL of irinotecan and 5-FU was 25.0 and 8.33 L/h/m^2, respectively, which was not different from the CL without bevacizumab (26.9 L and 9.72 L/h/m^2, respectively), and the PK parameters other than CL were also similar to those without bevacizumab. Thus, the applicant discusses that the PK of irinotecan or 5-FU is not altered by concurrent bevacizumab. In this study, the impact of the IFL regimen on the PK of bevacizumab was not assessed due to a limitation on the volume of blood that could be collected from the animals.

The serum bevacizumab concentrations in the above 2 studies encompassed the range above those observed in clinical studies (207±60.7 μg/mL, the maximum concentration reached following 7 doses of bevacizumab 5 mg/kg given at 2-week intervals).

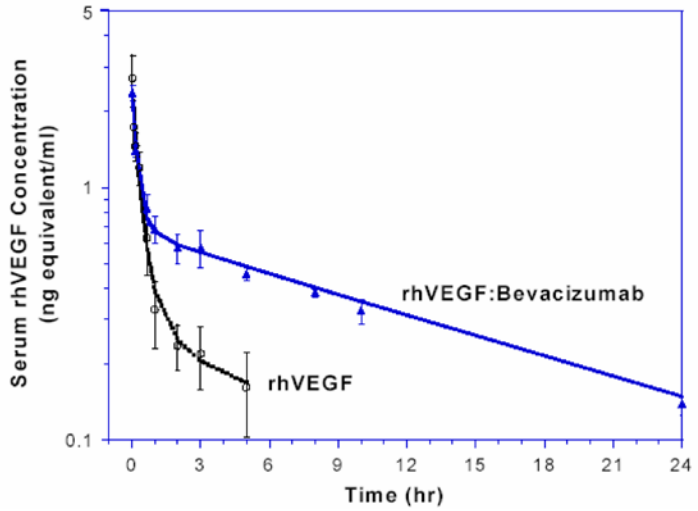
Based on the above, the applicant infers that there are no pharmacokinetic problems with the addition of bevacizumab to chemotherapy in humans as well.

5) Investigation of clearance of bevacizumab-VEGF complexes

In foreign clinical trials, following bevacizumab administration, total VEGF concentrations in blood (bevacizumab-VEGF complex and free VEGF) were shown to increase over time [See "4.2 Clinical pharmacology"]. This increase was attributed to a decrease in VEGF CL associated with complexation of VEGF with bevacizumab. This hypothesis was tested in rats.

After complexes of ^{125}I-labeled human VEGF$_{165}$ and bevacizumab (1:10 molar ratio) were formed, single intravenous doses of the solution containing 25.7 μg/kg (1.12 MBq/kg) of VEGF or the solution containing 1 mg/kg of bevacizumab were administered and serum concentrations of human VEGF and bevacizumab were measured (See the figure below). The CL of human VEGF was 65.9 mL/h/kg when combined with bevacizumab, which was about 1/3 of the CL

with human VEGF alone (225 mL/h/kg). On the other hand, the CL of bevacizumab was 7.83 mL/day/kg when combined with human VEGF and 8.97 mL/day/kg with bevacizumab alone, showing no differences. The applicant discusses that this was because the ratio of bevacizumab forming complexes with VEGF was small compared to the ratio of unchanged bevacizumab. The applicant also discusses that decreased CL of human VEGF when combined with bevacizumab is consistent with a report where administration of anti-IgE antibody, omalizumab resulted in an increase in blood concentrations of the target antigen, IgE (*J Allergy Clin Immunol* 95 (1 Pt 2): 356, 1995).



6) Lot-to-lot comparability

Using two lots of bevacizumab (the lot used in the 4-week and 13-week repeat-dose studies in cynomolgus monkeys [Lot No. AC] and the lot used in the 26-week repeat-dose study in cynomolgus monkeys [Lot No. AD]), the PK following the intravenous administration of 10 mg/kg to rats was evaluated.

The CL of Lot No. AC was similar to the CL of Lot No. AD, i.e. 6.35 mL/day/kg and 6.70 mL/day/kg, respectively and the ratio of $AUC_{0\text{-}11}$ was 0.944 (90% CI [confidence interval], 0.89-1.00). Therefore, there were no differences in the PK of bevacizumab between the two lots, and the applicant states that it has been confirmed that lower CL observed in the 26-week repeat-dose study compared to the 4-week and 13-week repeat-dose studies [See "3.2 Pharmacokinetic studies, *Summary of the submitted data* 1) (2) Repeat-dose administration"] was not attributable to lot-to-lot variations.

Using (a) the lot from a new cell line G7 used in foreign phase III trials (Lot No. X) and the control lot used in foreign phase II trials (Lot No. Z) and (b) the lot used in foreign phase III trials (Lot No. AB) and the lot intended for marketing (Lot No. AA), the PK was compared in rats. As a result, the applicant explains that the PK parameters were similar between the lots and the exposure was almost the same.

7) The applicant's discussion

Bevacizumab binds to cynomolgus monkey VEGF and rabbit VEGF, but does not bind to rodent VEGF. In all animal species tested, bevacizumab was slowly eliminated from serum with limited tissue penetration. Thus, it has been confirmed that bevacizumab is distributed primarily in plasma and the PK of bevacizumab is unaffected regardless of binding to endogenous VEGF.

It has been strongly suggested that neonatal Fc receptor (FcRn), also known as the IgG1 salvage receptor, is responsible for the small volume of distribution and slow elimination from serum of IgG1 antibody, and the major sites of expression and mechanism of action of FcRn are being elucidated (*Eur J Immunol* 26: 690-6, 1996, *J Exp Med* 180: 2377-81, 1994, *Ann Rev Immunol* 18: 739-66, 2000, *Int Immunol* 10: 1289-98, 1998, etc.). Serum bevacizumab has a long elimination half life, and like other IgG antibodies, bevacizumab is inferred to bind to FcRn and be catabolized in the lysosome.

Furthermore, using pharmacokinetic data on single intravenous dose in mice, rats, cynomolgus monkeys, and humans, the relationship between body weight and the CL of bevacizumab was evaluated by allometry. As a result, there was a linear relationship between body weight and the CL of bevacizumab [CL (mL/day)＝6.1605×Body weight$^{0.8179}$ (kg), R^2＝0.9881], indicating that the CL of bevacizumab is similar across animal species.

Outline of review by the PMDA

Based on the submitted data and the following review, PMDA judged that the applicant's discussion on the absorption, distribution, metabolism, excretion and pharmacokinetic interactions of bevacizumab in a non-clinical setting is largely acceptable. However, PMDA recommends that study data supporting the applicant's discussion on the CL of bevacizumab should continue to be collected and basic information on the PK of bevacizumab and the mechanism should be further examined and discussed.

1) CL in single-dose studies in mice and rats

PMDA asked the applicant to discuss the reason for a trend towards increased CL of bevacizumab in the low dose group in the single-dose studies in mice and rats.

The applicant responded as follows:

In the single-dose study in mice, it was impossible to assess serum bevacizumab concentrations over time by repeated blood sampling from one animal and serum drug concentrations were measured by taking whole blood from different animals at different time points (n=2). Thus, an inter-animal variability was likely to occur due to its study design and actually, the inter-animal variability in the measurements on and after 5 days post-dose for the 0.80 mg/kg group (the low dose group) was particularly high. Therefore, the trend towards increased CL of bevacizumab in the low dose group appears to be attributable to a high variability in the data, especially on and after 5 days post-dose, which affected the calculation of CL.

In the single-dose study in rats, serum bevacizumab concentrations were measured up to 14

days post-dose, which was well longer than the $t_{1/2\beta}$ (5.42 days) in the low dose group (0.664 mg/kg), but was similar to the $t_{1/2\beta}$ (12.3 days) in the high dose group (10.1 mg/kg). Meanwhile, in a comparability study of different lots in rats (the dose: 10 mg/kg), serum bevacizumab concentrations were analyzed up to 21 to 29 days post-dose, which was well longer than the $t_{1/2\beta}$ (5.96-11.6 days). As a result, the CL values were 6.35 to 14.7 mL/day/kg, which were similar to the CL value in the low dose group of the single dose study in rats (8.37 mL/day/kg). Therefore, it appears that a failure to adequately cover the elimination phase was one of the reasons for low CL in the high dose group of the single-dose study in rats and if the sampling duration is well longer than the $t_{1/2\beta}$, the CL values should be constant, independent of the dose.

As to the elimination of IgG antibody from blood, two mechanisms have been reported: CL by the formation of an immune complex with antigen (CL_{target}) and nonspecific CL by the reticuloendothelial system (CL_{RES}). If the antigen does not exist in the body, there will be no decrease in CL associated with the saturation of CL_{target} and CL is presumably constant at any dose (*Drug Discov Today* 11: 81-8, 2006). Since bevacizumab does not bind to mouse or rat VEGF, only CL_{RES} can contribute to the elimination of bevacizumab in these animal species and the CL values are presumed to be constant.

Based on the above, the trend towards increased CL in the low-dose group in the two studies was due to errors in experimental technique and nonlinear CL does not occur in mice or rats.

PMDA considers as follows:
Although the reasons discussed by the applicant are possible, as there is no study data supporting their discussion, the possibility that factors other than the sampling duration and the variability in the data were involved in the differences in the CL value between the doses in these two studies can not be ruled out.

2) CL after multiple doses
The applicant discusses that the CL value was lower in the 26-week repeat-dose study compared to the 4-week and 13-week repeat-dose studies in cynomolgus monkeys due to the small number of animals studied and the high inter-animal variability [See "3.2 Pharmacokinetic studies, *Summary of the submitted data* 1) (2) Repeat-dose administration"].

PMDA asked the applicant to reexamine the differences in the CL value among the studies, including the reason for a higher inter-animal variability in the 26-week repeat-dose study compared to the 4-week and 13-week repeat-dose studies.

The applicant responded as follows:
Individual CL values of animals in these 3 studies using cynomolgus monkeys were checked. As a result, the CL was almost constant among the females and the overall mean CL value of the three studies was 5.26 mL/kg/day and the CV value was 13.0%. On the other hand, among the males, 1 animal in the 13-week repeat-dose study had a high CL value (7.24 mL/kg/day) and 2 animals in the 26-week repeat-dose study had a low CL value (3.27 and 3.32 mL/kg/day) as

compared to the mean CL value in the 4-week repeat-dose study, and the CV of the overall mean CL for the males was 30.8%, showing a high variability. Since the half-life of bevacizumab is about 10 days, elimination after the first dose can be better compared among individual animals in the 26-week repeat-dose study where bevacizumab was administered once weekly, than the other two studies where bevacizumab was administered twice weekly. Thus, in the 26-week repeat-dose study, serum concentrations over time after the first dose were compared among 2 males and 2 females. As a result, the elimination of serum bevacizumab was slower in the males compared to the females.

Based on the above, the reason for lower CL in the 26-week repeat-dose study compared to the other two studies seems an inter-animal variability associated with low CL in the 2 males.

PMDA understands that it is difficult to adequately compare different studies due to the number of animals in each study and an inter-animal variability, but considers that it is unclear whether the differences in the CL value between the studies in cynomolgus monkeys can be explained by a variability alone.

PMDA considers as follows:
Although the above issues on the CL of bevacizumab in mice, rats and cynomolgus monkeys would not affect the approvability of bevacizumab, in view of clinical study data [See "4.3 Clinical efficacy and safety"], as there is no study data directly supporting the applicant's discussion, it is recommended that study data supporting the applicant's discussion should be obtained if there is an opportunity for the PK of bevacizumab to be assessed in a non-clinical study with a more appropriate design, i.e. appropriate number of animals and sampling duration.

3.3 Toxicology studies

Summary of the submitted data

Bevacizumab binds to VEGF and blocks the binding of the ligand to VEGF receptors, resulting in the inhibition of VEGF signal transduction. Since the amino acid sequence of cynomolgus monkey VEGF is predicted to be identical to that of human VEGF and VEGF binding assay showed that bevacizumab binds rabbit VEGF, although with a lower affinity than for human VEGF [See "3.1 Pharmacology studies"], cynomolgus monkeys and New Zealand white rabbits were selected for toxicity studies.

Although rodents are commonly used for toxicity studies, as bevacizumab does not bind to mouse VEGF and is predicted not to bind to rat VEGF based on its homology to the amino acid sequence of mouse VEGF [See 3.1 Pharmacology studies"], these animal species were not used in toxicity studies of bevacizumab.

1) Single-dose toxicity

No single-dose toxicity studies were performed because only the 10 mg/mL formulation of bevacizumab was available at the time of initiating a toxicity study in the early phase of development and the maximum single dose of bevacizumab that could be physically

administered was considered to be 50 to 100 mg/kg, which made it difficult to evaluate the single-dose toxicity adequately.

In a 4-week repeat-dose study in cynomolgus monkeys, the Cpeak after the first dose in the 50 mg/kg group (males and females: 1200 μg/mL) was about 6-fold the Cpeak at the recommended clinical dose of bevacizumab (5 mg/kg) for the first-line treatment of colorectal cancer (Steady-state in a phase II clinical study AVF0780g: 207 μg/mL), but there were no deaths or abnormalities in general symptoms. In a rabbit study to determine the deposition of bevacizumab in the kidney, rabbits were given two doses of bevacizumab 100 mg/kg and there were no deaths or changes in general symptoms.

2) Repeat-dose toxicity (including toxicokinetics)

(1) 4-week repeat-dose study in cynomolgus monkeys

Vehicle or bevacizumab 2, 10, or 50 mg/kg were intravenously administered twice weekly for 4 weeks to 2- to 7-year-old male or female cynomolgus monkeys. Of which, 2 males and 2 females from each of the control and highest-dose groups were used for a 4-week recovery study after treatment [See “3.2 Pharmacokinetic studies” for toxicokinetics].

No animals died and there were no abnormalities in general symptoms, body weight, food consumption, blood pressure, physiological examinations (rectal body temperatures and respiration rates), ECG, ophthalmic examinations, electroretinograms, laboratory tests (blood biochemistry, hematology, urinalysis), organ weights, or gross pathology.

Histopathologically, epiphyseal dysplasia of the distal femur was noted in males treated with 10 or 50 mg/kg, which was characterized by thickened growth plate cartilage (epiphyseal cartilage), clusters of hyperplasic chondrocytes, subchondral bony plate formation, inhibition of vascular invasion of the growth plate, and degeneration of the cartilage matrix. The severity of epiphyseal dysplasia was slight to moderate in males treated with 10 mg/kg and moderate to severe in males treated with 50 mg/kg, while epiphyseal abnormalities were not observed in females at any dose level. Epiphyseal dysplasia was present in the two males of the high-dose recovery group after the 4-week recovery period, but its severity was minimal and severe, respectively, indicating a trend towards recovery upon drug withdrawal. The animal with severe epiphyseal dysplasia also after the recovery period had minimal diffuse degeneration and necrosis of the metaphyseal bone marrow. There were no other abnormal histopathologic findings associated with the administration of bevacizumab.

Anti-bevacizumab antibodies were not detected at any timepoint (Days 1, 15, 22, 29, and 56 [recovery animals only]).

The no observed adverse effect level from this study was determined to be 2 mg/kg.

(2) 13-week repeat-dose study in cynomolgus monkeys

Vehicle or bevacizumab 2, 10, or 50 mg/kg were intravenously administered twice weekly for

13 weeks to 3- to 6-year-old male or female cynomolgus monkeys. Of which, 2 males and 2 females from each of the control and highest-dose groups were used for a 4-week recovery study after treatment [See "3.2 Pharmacokinetic studies" for toxicokinetics].

No animals died and there were no abnormalities in general symptoms, body weight, food consumption, blood pressure, ECG, physical examinations (rectal body temperatures and respiration rates), ophthalmic examinations, laboratory tests (hematology, coagulation test, urinalysis), or gross pathology.

One male in the 50 mg/kg group had marked decreases in serum total protein and albumin and an increase in cholesterol at Week 13 and was histopathologically diagnosed with membranoproliferative glomerulonephritis. It has been reported that idiopathic glomerulonephritis naturally occurs in cynomolgus monkeys, and this finding is also inferred to be a naturally occurring change. In repeat-dose studies of up to 26 weeks in duration, a total of 92 male or female cynomolgus monkeys were treated with 2 to 50 mg/kg once or twice weekly, but none of the animals had glomerulonephritis except for the above-mentioned animal.

Ovarian and uterine weights were reduced in females in the 10 and 50 mg/kg groups. These changes coincided with a reduction in number or an absence of corpora lutea. In the 50 mg/kg group, a reduction in the number of corpora lutea persisted even after the 4-week recovery period, but there were no reductions in ovarian or uterine weights, suggesting that the effect of bevacizumab on female reproductive function tends to be reversible upon cessation of treatment.

As with a 4-week repeat-dose administration, the severity and incidence of epiphyseal dysplasia were dose-dependent and an additional finding of linear fissuring of the cartilaginous growth plate was occasionally observed in this study. Epiphyseal dysplasia was noted in all males treated with bevacizumab and the severity was minimal to severe in the 2 mg/kg group and slight to severe in the 10 and 50 mg/kg groups. On the other hand, minimal to slight epiphyseal dysplasia was present in all females in the 10 and 50 mg/kg group, but none in the 2 mg/kg group. Also in the 4-week recovery group, epiphyseal dysplasia was present, but less severe as compared to the severity at the end of treatment, suggesting a trend towards recovery. The applicant discusses that the incidence of epiphyseal dysplasia was higher in the males than in the females because more females were mature than males in this study, and this was not a gender effect.

Anti-bevacizumab antibodies were not detected at any timepoint (Days －6, 11, 15, 22, 53, 92, and 119 [recovery animals only]).

The no observed adverse effect level from this study was determined to be less than 2 mg/kg.

(3) 26-week repeat-dose study in cynomolgus monkeys

Vehicle or bevacizumab 2, 10, or 50 mg/kg were intravenously administered once weekly or 10

mg/kg was intravenously administered twice weekly for 26 weeks to 4- to 7-year-old male or female cynomolgus monkeys. Of which, 2 males and 2 females from each of the control and high-dose groups were used for a 12-week recovery study after treatment [See “3.2 Pharmacokinetic studies” for toxicokinetics].

No animals died and there were no abnormalities in physical examinations (rectal body temperatures and respiration rates), ECG, blood pressure, radiograms, ophthalmic examinations, laboratory tests (hematology, coagulation test, urinalysis), or gross pathology.

Compared to the control group, the mean body weights (adjusted for baseline body weight as a covariate) were decreased by 5 to 10% in males of the 10 mg/kg (twice weekly) group beginning at Week 24 and by 8 to 13% in males of the 50 mg/kg group beginning at Week 14, which were both significant. One male in the 50 mg/kg group had emaciation at Week 24, which coincided with decreased food consumption. The mean body weights were decreased slightly by 5 to 7% in the 2 and 10 mg/kg (once weekly) groups compared to the control group. The males receiving 10 mg/kg (twice weekly) and those 50 mg/kg showed decreased food consumption, which coincided with decreased body weight. After the 12-week recovery period, there were no effects of bevacizumab on body weight or food consumption.

At $\geq$10 mg/kg, regardless of dosing frequency, abnormal menstrual cycles (decreased number of menstrual cycles) and lower uterine weight and reduced endometrial proliferation were observed in females. In addition, treatment with 10 mg/kg (twice weekly) or 50 mg/kg inhibited follicular maturation at the early Graafian follicle stage and resulted in an absence of corpora lutea. Following the 12-week recovery period, 1 of the 2 females in the 50mg/kg group demonstrated an absence of corpora lutea.

The severity and incidence of epiphyseal dysplasia were increased dose-dependently and additionally, linear fissuring was noted in 1 male in the 10 mg/kg (twice weekly) group and 1 male in the 50 mg/kg group. The severity was minimal to slight in males treated with 2 mg/kg, minimal to moderate in males treated with 10 mg/kg (once weekly), and slight to moderate in males treated with 50 mg/kg. All males treated with 10 mg/kg (twice weekly) had moderate dysplasia. On the other hand, the severity was minimal in 1 of 4 females treated with 2 mg/kg, and slight in 2 of 4 females treated with 10 mg/kg (once weekly) and slight in 2 of 4 females treated with 50 mg/kg. Only 1 female in the 10 mg/kg (twice weekly) group had moderate epiphyseal dysplasia. Epiphyseal dysplasia was severer in the males of the 10 mg/kg (twice weekly) group and of the 50 mg/kg group with marked changes in body weight, as compared to the males of the 2 and 10 mg/kg (once weekly) groups with no marked body weight changes and the females of all dose groups. Reductions in body weight in males of the 10 mg/kg (twice weekly) and 50 mg/kg groups may have been associated with growth retardation due to epiphyseal dysplasia. Epiphyseal dysplasia was not present following the 12-week recovery period.

Since membranoproliferative glomerulonephritis was noted in the 50 mg/kg group in the

13-week repeat-dose study, the kidneys of the male and female control groups, the 10 mg/kg (twice weekly) group, and the 50 mg/kg group were closely examined by periodic acid-Schiff-methenamine silver staining. As a result, no abnormalities were detected.

Test for anti-bevacizumab antibodies was positive for 1 animal of the control group on Day 15 and 1 animal of the 50 mg/kg group on Day 183, which were both positive responses just above the limit of detection. The animal in the control group with a positive result was tested negative at all other timepoints (Weeks 4, 13, 27, and 39), suggesting that the result produced on Day 15 (Week 3) may have been false-positive.

The no observed adverse effect level was determined to be less than 2 mg/kg.

(4) Bevacizumab in combination with IFL regimen

The toxicity of the IFL chemotherapy regimen consisting of irinotecan hydrochloride, 5-FU, and LV, in combination with bevacizumab was assessed in cynomolgus monkeys.

Two- to three-and-half-year-old male cynomolgus monkeys were treated intravenously with irinotecan hydrochloride 100 or 125 mg/m^2, 5-FU 500 mg/m^2, and LV 20 mg/m^2 on Days 1 and 8 and bevacizumab 10 mg/kg on Days 1 and 8 (the dose of irinotecan hydrochloride when combined with bevacizumab was 125 mg/m^2 only), and necropsied on Day 15.

No death occurred in any group. Administration of IFL resulted in diarrhoea, body weight loss, and decreased food consumption and changes in hematologic and blood biochemistry test values such as decreases in white blood cell count/platelet count, anaemia, and decreases in cholesterol/total protein/potassium. A small thymus with corresponding lymphoid depletion was observed in 2 of 7 animals treated with IFL alone and in 2 of 5 animals treated with IFL+bevacizumab. In the sternal bone marrow of most animals, erythroid hyperplasia or myeloid hypoplasia was mainly observed. The alterations observed in the IFL+bevacizumab group were similar to those in the IFL group and the coadministration of bevacizumab did not enhance the toxic effects.

(5) Bevacizumab in combination with cisplatin/paclitaxel regimen

The toxicity of cisplatin (CDDP) and paclitaxel (PTX) in combination with bevacizumab was assessed in cynomolgus monkeys.

Four- to ten-year-old male cynomolgus monkeys were intravenously administered CDDP 1 mg/kg and PTX 4 mg/kg on Day 18 and bevacizumab 10 mg/kg on Days 1, 4, 8, 11, 15 and 18.

No death occurred in any group. In the single agent bevacizumab group, there were no effects on general symptoms, body weight, food consumption, or laboratory tests (hematology, blood biochemistry, urinalysis). In the single agent bevacizumab group, the CDDP/PTX group, and the CDDP/PTX+bevacizumab group, there were no effects on physical examinations (rectal body temperatures and respiration rates) or ECG, either. In the CDDP/PTX group and the

CDDP/PTX+bevacizumab group, vomiting occurred and decreased body weight and transient decreases in white blood cell and neutrophil counts compared to the control group were also noted. The alterations observed in the CDDP/PTX+bevacizumab group were similar to those in the CDDP/PTX group and the coadministration of bevacizumab did not enhance the toxic effects.

3) Genotoxicity
In vitro or *in vivo* genotoxicity studies were not conducted.

4) Carcinogenicity
No carcinogenicity studies were conducted.

5) Reproductive and developmental toxicity (including toxicokinetic assessment)
(1) Study of fertility and early embryonic development to implantation
No studies of this type were conducted.

(2) Rabbit study for effects on embryo-fetal development (a dose-ranging study)
Five- to five-and-half-month-old pregnant rabbits were treated intravenously with 10, 30, or 100 mg/kg of bevacizumab during the period of organogenesis (Day of gestation 6-18, hereinafter abbreviated as DG), on DG 6, 9, and 12 or on DG 12, 15, and 18. The dosing regimen was selected to maintain an average serum bevacizumab concentration approximately equivalent to the human clinical exposure and to minimize the exposure to anti-bevacizumab antibodies and antigen-antibody complexes during the period of organogenesis.

The 100 mg/kg group (dosed on DG 12, 15, and 18) showed decreases in maternal body weight and body weight gain throughout the gestation period and a decrease in food consumption as well during the post-treatment period (DG 19-29). The average fetal body weight was reduced in the 100 mg/kg group (dosed on DG 12, 15, and 18). At caesarean sectioning, 47% of the animals treated with bevacizumab on DG 6, 9, and 12 and 33% of the animals treated with bevacizumab on DG 12, 15, and 18 had developed antibodies to bevacizumab.

(3) Rabbit study for effects on embryo-fetal development
Five- to six-month-old pregnant rabbits were treated intravenously with vehicle or bevacizumab 10, 30, or 100 mg/kg on DG 6, 9, 12, 15, and 18 and were Caesarean-sectioned on DG 29 (the toxicity study group). Separately, satellite groups for toxicokinetic sampling (5 animals/group) were used. Animals assigned to satellite groups were administered bevacizumab 10 or 100 mg/kg on DG 18 and necropsied on DG 21 (the satellite A groups) or administered bevacizumab 10 or 100 mg/kg on DG 6, 9, 12, 15, and 18 and necropsied on DG 29 (the satellite B groups). The fetuses in the satellite groups were not to be observed.

No death occurred and there were no abnormalities attributable to the administration of bevacizumab. A decrease in maternal body weight gain was observed in the 30 and 100 mg/kg groups on DG 6 to 7, but no effects were present at other timepoints and there were no changes

in food consumption. In the 100 mg/kg group, the total number of fetal resorptions, the number of litters with resorptions, and the percent dead or resorbed conceptuses per litter were increased, which was primarily attributed to an increase in the number of late resorptions.

Fetal body weights were dose-dependently decreased and the number of litters with any fetal alteration was increased in the 30 mg/kg and 100 mg/kg groups. In the 100 mg/kg group, the total number of fetuses with any alterations increased, as well as the percentage of fetuses per litter with any alterations. Fetal external and skeletal abnormalities were increased in the 100 mg/kg group, the number of ossification sites for metacarpals was reduced in the 10 mg/kg and higher dose groups, and the mean number of ossification sites for caudal vertebrae and fore- and hind-limb phalanges was also reduced in the 100 mg/kg group.

Antibodies to bevacizumab were detected in maternal serum in 1/20 rabbits in the vehicle group, 1/20 rabbits in the 10 mg/kg group, 4/20 rabbits in the 30 mg/kg group, and 2/20 rabbits in the 100 mg/kg group of the toxicity study. While antibodies were not detected in maternal serum of the satellite A groups at any timepoint (DG 18, 19, 20, 21), antibodies were detected in one animal each for the satellite B groups on DG 29. Overall, anti-bevacizumab antibodies were detected in maternal serum of 12% (9/73 rabbits) of the rabbits treated with bevacizumab and in fetal serum of 13% (9/71 rabbits) of the rabbits treated with bevacizumab and of 5% (1/19 rabbits) of the vehicle group. Antibodies to bevacizumab were detected in the amniotic fluid of 10% (7/73 rabbits) of the animals treated with bevacizumab and of 5% (1/19 rabbits) of the vehicle group. Although one animal in the vehicle group with a positive antibody result may have been false-positive, its detailed cause is unknown.

The no observed adverse effect level was determined to be 10 mg/kg for maternal general toxicity and less than 10 mg/kg for fetal development.

(4) Study for effects on pre-and postnatal development, including maternal function
No studies of this type were conducted.

6) Local tolerance study
No local tolerance studies were performed. However, in repeat-dose intravenous studies in cynomolgus monkeys, there were no drug-related findings at the injection sites and bevacizumab is considered to be well tolerated locally.

7) Other toxicity studies
(1) Hemolytic potential and blood compatibility testing
Hemolytic potential and blood compatibility tests were performed on bevacizumab using human and cynomolgus monkey whole blood and serum/plasma.

The hemolytic potential was evaluated by measuring the concentration of hemoglobin in the supernatant plasma of a mixture of vehicle or bevacizumab (at a final concentration of 5 mg/mL) and human or cynomolgus monkey whole blood. The presence or absence of

precipitation or coagulation in a mixture of bevacizumab or vehicle and human or cynomolgus monkey serum or plasma was observed macroscopically.

Vehicle or bevacizumab did not cause hemolysis and precipitation or coagulation reaction did not occur.

(2) Cross reactivity of biotinylated bevacizumab with tissues of various animal species

The tissue specificity of bevacizumab against a panel of normal rabbit, cynomolgus monkey, and human tissues was determined via immunohistochemical staining using 10 and 400 μg/mL of biotinylated bevacizumab. As a result, in 9 rabbit tissues, 30 cynomolgus monkey tissues, or 36 human tissues stained, no specific positive reactions were observed.

(3) Timing of antibody development in the rabbit

Although bevacizumab has binding activity for rabbit VEGF, rabbits produce antibodies to bevacizumab after exposure. The time course of antibody development was thus studied in the rabbit in order to find out the time period during which bevacizumab can be administered without antibody development.

A total of 4 intravenous doses of vehicle or bevacizumab 10 mg/kg were administered to male rabbits weighing 2.5 to 2.8 kg on Days 1, 4, 8, and 11 and blood antibodies were measured during the treatment period and up to Day 50. Anti-bevacizumab antibodies were not detected in any group on Day 8, but were detected in all animals treated with bevacizumab (2/2 rabbits) on Day 11. Antibodies were quantifiable until Day 36 for one rabbit and until Day 50 for the other rabbit. The data indicated that anti-bevacizumab antibodies develop between 8 and 11 days after the initiation of dosing in rabbits.

(4) Investigation of epiphyseal dysplasia in rabbits

Epiphyseal dysplasia secondary to the inhibition of blood vessel formation in long bone growth plate, associated with the pharmacological effects of bevacizumab was observed in repeat-dose studies in cynomolgus monkeys. The suitability of rabbits for further study of epiphyseal dysplasia was assessed.

Six-week-old female rabbits were intravenously administered vehicle or bevacizumab 10, 50, or 75 mg/kg on Days 1, 4, 7, and 10 and necropsied on Day 14.

No animals died and there were no effects on general symptoms or body weight. Animals that received bevacizumab showed a slight thickening of the growth plate cartilage. In contrast to the effect noted in cynomolgus monkeys, bevacizumab did not inhibit vascular invasion or induce subchondral bony plate formation in rabbits at any dose level. Since anti-bevacizumab antibodies were shown to develop between 8 and 11 days after the initiation of dosing in rabbits, the duration of dosing was shortened compared to the studies in cynomolgus monkeys. Epiphyseal changes in long bone were mild in rabbits because this short exposure period may have been insufficient.

(5) Effect of bevacizumab on ovarian function in rabbits

Decreases in ovarian/uterine weights and the number of corpora lutea were observed in the repeat-dose studies in cynomolgus monkeys, indicating an effect of bevacizumab on female reproductive function. Therefore, the effect of bevacizumab on ovarian function in rabbits was closely examined based on body weight, serum progesterone, uterine/ovarian weights, and the presence or absence of corpora lutea.

Study 1　The ability to produce luteal progesterone:

Human chorionic gonadotropin (hCG) was intravenously administered to about 6-month-old female rabbits in order to induce ovulation (Day 1). A total of 4 intravenous doses of vehicle or bevacizumab 50 mg/kg were given on Days －3, 1, 4, and 7, and 4 rabbits from each group were necropsied on Day 8 and the remaining 4 rabbits from each group (the recovery group) underwent a recovery period of about 1 month followed by readministration of hCG, and were necropsied on Day 48.

Following hCG administration, serum progesterone levels were elevated, which was reduced by bevacizumab. A necropsy on Day 8 showed that ovarian and uterine weights and the numbers of surface follicles and corpora lutea were reduced in animals treated with bevacizumab compared to controls. Following hCG stimulation after the recovery period of about one month, serum progesterone levels were elevated to a similar extent in the control and bevacizumab groups. A necropsy on Day 48 showed changes in the uterine weights, while there were no abnormalities in histopathological findings of the uterus and uterine cervix.

Based on the above, four doses of bevacizumab 50 mg/kg given every 3 days inhibited the ovarian function in rabbits, which recovered upon drug withdrawal and were reversible changes. These trends were consistent with the findings in cynomolgus monkeys.

Study 2　Dose response:

The dose-response of the effect of bevacizumab on ovarian function was investigated based on serum progesterone, ovarian weights, and the presence or absence of corpora lutea.

hCG was intravenously administered to induce ovulation (Day 1), and a total of 3 intravenous doses of vehicle or bevacizumab 2, 10, or 50 mg/kg were given on Days －4, 1, and 5, and necropsy was performed on Day 7.

There were no changes in serum progesterone levels in the 2 and 10 mg/kg groups, while serum progesterone levels were markedly lowered in the 50 mg/kg group compared to the vehicle group. Ovarian weights were decreased dose-dependently and the number of corpora lutea was reduced in the 50 mg/kg group compared to the control group.

The results of this study are consistent with the effect of bevacizumab on female reproductive function observed in the cynomolgus monkey and indicate that a high-dose of bevacizumab

inhibits luteinization and subsequent progesterone secretion.

(6) Effect of bevacizumab on wound healing

VEGF is involved in wound healing process and because of the concern that bevacizumab may delay wound healing in patients undergoing surgery, the effect of bevacizumab on wound healing was investigated using a linear-incision model and a model of a circular wound that mimics an ulcerative lesion and is thus distinct from a linear incision.

Effect of bevacizumab on wound healing in a linear incision model:

Full-thickness linear incisions (2.5 cm) were made on the back of about 6-week-old female rabbits and closed with sutures (Day 1). A total of 3 intravenous doses of vehicle or bevacizumab 0.5, 1, or 2 mg/kg were given on Days －2, 1, and 3, and necropsy was performed 5 days after the wound was made. While there were no marked changes in general symptoms or body weight, the tensile strength of the wounds was decreased dose-dependently in animals treated with bevacizumab, indicating that bevacizumab interferes with wound healing.

The applicant explains the reason for a major difference in the dose of bevacizumab between the linear incision skin wound and the following circular wound as follows:

Different endpoints were used for the wounds, and in the study investigating the effect of bevacizumab on linear incision wounds, low doses that would delay wound healing and would not fully heal the wound before the day of necropsy were used for the purpose of comparing bevacizumab with [redacted]VEGF[redacted].

Effect of bevacizumab on wound healing in a circular wound model:

A circular wound with a diameter of 8 mm was produced on the inside of the ear of male rabbits (Day 1), and a total of 5 intravenous doses of vehicle or bevacizumab 50 mg/kg were given on Days 1, 3, 5, 7, and 10. Methylprednisolone, which is known to delay wound healing was used as a positive control and a total of 6 intramuscular doses of 35 mg/kg were given on Days －2, 1, 3, 5, 7, and 11, and necropsy was performed on Day 12.

One rabbit in the methylprednisolone group died due to escherichia coli enterocolitis on Day 12 but all other rabbits survived until necropsy. There were no changes in general symptoms or body weight attributable to the administration of bevacizumab. By Day 12, the wounds in the vehcle group were 78% closed (determined based on the area calculated from the diameter of the wound), while in the methylprednisolone and bevacizumab groups, the wounds were 33% and 46% closed, respectively. The re-epithelialization rate by histopathological examination was 67% in the vehcle group, 50% in the methylprednisolone group, and 17% in the bevacizumab group. The above results indicate that bevacizumab 50 mg/kg impairs wound healing in the circular wound model in the rabbit.

The dose-response of the effect of bevacizumab in a circular wound model:

A circular wound with a diameter of 8 mm was made on the inside of the ear of rabbits (Day 1), and a total of 4 intravenous doses of vehicle or bevacizumab 2 or 10 mg/kg were given on Days

1, 4, 8, and 11, and necropsy was performed on Day 21 after a 10-day drug withdrawal.

By Day 12, the wounds were 69% closed in the control group, 47% in the 2 mg/kg group, and 36% in the 10 mg/kg group. The circular wound was completely closed upon cessation of dosing in both 2 and 10 mg/kg groups, but the time to wound closure was 3-5 days longer compared to the control group.

Effect of bevacizumab on wound healing in cynomolgus monkeys:
Full-thickness linear incisions (2 cm) were made in the scapular region of cynomolgus monkeys (Day 1) and a total of 4 intravenous doses of bevacizumab 0.5 or 2 mg/kg were given on Days －2, 1, 3, and 5 and necropsy was performed on Day 7.

As in the rabbit study, there were the effects of bevacizumab on wound healing, such as a decrease in the tensile strength of the wounds. However, the inter-animal variability was high and no clear dose response was observed. One of the reasons for the high inter-animal variability is considered as follows: The rabbits were comparable with respect to age (month-old) and body weight while the cynomolgus monkeys varied as to age and body weight.

(7) Investigation of thrombosis
The incidence of thromboembolism was increased in subjects treated with bevacizumab in clinical trials. In contrast, there were no marked changes in hematology or coagulation parameters in the repeat-dose studies in cynomolgus monkeys. To investigate the effect of bevacizumab on the occurrence of thrombosis, a rabbit model of acute thrombosis was used.

Rabbits were given vehicle or bevacizumab 75 mg/kg intravenously daily for 8 days. Following the 8th dose, a thrombus was induced by clamping the jugular vein and causing mild damage. The effect of bevacizumab on thrombus formation was assessed by measuring the time to clot formation and clot weights. Furthermore, cuticle bleeding time, hematology and coagulation parameters (prothrombin time, activated partial thromboplastin time, blood cell counts, D-dimer concentrations, whole blood recalcification time, platelet aggregation, activated clotting time) were measured.

There were no marked changes in hematology and coagulation tests, time to clot formation, clot weight, or cuticle bleeding time, and it was determined that the administration of bevacizumab does not exacerbate thrombosis when a thrombus has been induced by mechanical manipulation.

(8) Renal effect and deposition of bevacizumab in the kidney
The incidence of proteinuria was increased in subjects treated with bevacizumab in clinical trials. In contrast, no alterations in blood biochemistry or urinalysis parameters suggestive of impaired renal function were observed in the repeat-dose studies in cynomolgus monkeys. An exploratory study on the deposition of bevacizumab in the kidney was conducted to examine the effect of bevacizumab on renal function.

Study in normal rabbits:
Rabbits were intravenously administered vehicle or bevacizumab 2, 10, or 100 mg/kg on Days 1 and 3 and necropsied on Day 5. Examination of the kidneys by light and electron microscopy indicated no histopathological differences between control and treated animals and no deposition of bevacizumab in the kidney was noted also by immunohistochemical staining for anti-bevacizumab antibodies.

Study in a CDDP-induced model of renal dysfunction:
The effect of bevacizumab on kidney damage was investigated in rabbits with renal injury induced by CDDP known to have nephrotoxic effects on the proximal tubule.

A total of 6 intravenous doses of CDDP 1 mg/kg or saline were given on Days 1, 3, 5, 8, 10, and 12 to rabbits. At Week 2, vehicle or bevacizumab 50 mg/kg was intravenously administered on Days 8, 10, and 12. Necropsy was performed on Day 14.

No changes in general symptoms associated with CDDP or bevacizumab were noted and there were no marked changes in urinary protein concentrations or hematology.

There were no changes in body weight, blood urea nitrogen (BUN), creatinine, or urine specific gravity in the bevacizumab group. Body weights were reduced in the CDDP group and the CDDP+bevacizumab group compared to the control group (vehicle/saline). In addition, BUN and creatinine were elevated and urinary specific gravity values were lower than baseline and control values. However, there were no significant differences in any parameter between the CDDP group and the CDDP+bevacizumab group.

Histopathological examination revealed renal lesions (dilated medullary tubules, scattered interstitial inflammatory changes) in the CDDP group and the CDDP+bevacizumab group. The incidence and severity of the lesions were similar between the two groups and these changes were consistent with renal lesions associated with CDDP treatment.

The above results suggest that the administration of 50 mg/kg of bevacizumab does not exacerbate renal injury induced by CDDP in this rabbit model.

Bovine serum albumin overload model of renal dysfunction:
The effect of bevacizumab on renal damage was further examined, using bovine serum albumin (BSA) overload model in the rabbit, as renal damage induced by protein overload, a different mechanism from CDDP-induced renal injury.

Study 1:
Rabbits were intravenously administered BSA 5 or 20 mg/kg 5 days a week from Week 1 to Week 2 and daily from Week 3 to Week 6, and given a total of 4 intravenous doses of bevacizumab 50 mg/kg every other day during Week 6 (the BSA+bevacizumab group). This

study also included the bevacizumab group: no treatment for 5 weeks after the initiation of the study followed by a total of 4 intravenous doses of bevacizumab 50 mg/kg given every other day during Week 6, and the BSA group: intravenous administration of BSA 20 mg/kg from Week 1 to Week 6 followed by no treatment during Week 6. There were no clear differences in body weight among the groups and no consistent changes in urine specific gravity or urinary protein levels were observed and the degree of the changes was minimal. Treatment with BSA (the BSA group and the BSA+bevacizumab group) induced mild renal dysfunction with increased BUN and creatinine although the data was variable. In two out of the 8 rabbits in the BSA+bevacizumab group, BUN and creatinine levels were increased to 4 to 7 fold the baseline levels, which were 2 to 3 times the normal range, and moderate glomerulonephritis was noted. However, as death due to anaphylactic reaction to BSA occurred and the number of animals studied in each group was small, no definitive conclusion on the effect of bevacizumab on BSA-induced renal damage was drawn from this study.

Study 2:
Male rabbits were intravenously administered BSA 20 mg/kg or saline daily for 6 weeks, untreated during Week 7, and given a total of 4 intravenous doses of vehicle or bevacizumab 50 mg/kg every other day during Week 8 (the BSA group, the BSA+bevacizumab group).

Two rabbits died due to anaphylaxis etc. during treatment with BSA. Although eruption on ear occurred in 50% of the animals treated with BSA (the BSA group and the BSA+bevacizumab group), this symptom had existed before the administration of bevacizumab and was inferred to be caused by a high-dose of antihistamine administered to prevent anaphylactic reactions to BSA. Following the administration of bevacizumab to BSA-untreated rabbits, there were no abnormalities in urinary protein levels or histopathology of the kidney throughout the study period.

Treatment with BSA, with or without bevacizumab, resulted in an increase in urinary protein, but the interanimal variability was high. Treatment with BSA also induced renal injury characterized as mild glomerulonephritis with slight changes in BUN and creatinine, but there were no effects of bevacizumab on any parameter.

In this study, BSA induced mild renal injury, but 50 mg/kg of bevacizumab did not exacerbate the injury produced by BSA. There was no initiation of renal disorder by treatment with bevacizumab 50 mg/kg alone.

Outline of review by the PMDA
1) Reversibility
PMDA asked the applicant to explain the association between “dysplasia” and “necrosis” since epiphyseal dysplasia of the distal femur was observed after treatment and necrosis of the metaphyseal bone marrow was present after the recovery period in the repeat-dose study in cynomolgus monkeys.

The applicant responded as follows:
In the 4-week repeat-dose study in cynomolgus monkeys, the inhibition of angiogenesis, consistent with the pharmacological effect of bevacizumab, persisted also during the recovery period and epiphyseal dysplasia, characterized by inhibition of vascular invasion of the growth plate, thickened growth plate cartilage (epiphyseal cartilage), clusters of hyperplasic chondrocytes, and subchondral bony plate formation, was observed in males in the high dose group. The animal with severe epiphyseal dysplasia had also minimal diffuse degeneration and necrosis of the metaphyseal bone marrow. “Necrosis of the bone marrow” is necrosis found in the bone marrow stroma beneath the growth plate and the details of its mechanism of development is unknown. Since necrosis was found beneath the growth plate where angiogenesis occurs, it is considered attributable to micro-environmental changes in bone marrow tissues associated with the angiogenesis inhibitory effect of bevacizumab. The animal with minimal necrosis of bone marrow stroma after the recovery period had also severe epiphyseal dysplasia, and it is inferred that necrosis of bone marrow stroma may occur if epiphyseal dysplasia develops very severely.

PMDA judged as follows:
Although it is difficult to determine the association between epiphyseal dysplasia and necrosis of the metaphyseal bone marrow since only 2 animals were tested for recovery, the applicant’s explanation is largely acceptable.

Then, PMDA asked the applicant to explain the reversibility of decreased body weights and the effects on female reproductive function observed in the 26-week repeat-dose study in cynomolgus monkeys, and the applicant responded as follows:

The reversibility of decreased body weights in males:
In this study, compared to the control group, the mean body weights during the treatment period were significantly decreased by 5 to 10% in the 10 mg/kg group (twice weekly) and by 8 to 13% in the 50 mg/kg group ($p \leq 0.05$), but the two recovery animals in the 50 mg/kg group tended to recover upon drug withdrawal. Reductions in body weight gain observed in the animals treated with bevacizumab in the 26-week repeat-dose study are considered attributable to growth retardation due to epiphyseal dysplasia, and epiphyseal dysplasia also tended to be reversible upon cessation of treatment.

The effects on female reproductive function and the reversibility:
At $\geq$10 mg/kg, regardless of dosing frequency, abnormal menstrual cycles (decreased number of menstrual cycles) were noted and lower uterine weights and reduced endometrial proliferation were observed at the end of treatment. In addition, treatment with 10 mg/kg (twice weekly) or 50 mg/kg inhibited follicular maturation at the early Graafian follicle stage and resulted in an absence of corpora lutea. Angiogenesis is deeply involved in the development of ovarian follicles/corpora lutea, and periodic angiogenesis occurs in the theca interna of an ovarian follicle and is controlled by VEGF. It is inferred that the observed effects of bevacizumab on the ovarian function resulted from the inhibition of signal transduction via VEGF in the tissues

where angiogenesis was occurring actively. At necropsy after the recovery period, the uterine weights of the two animals in the control group were 6.3 and 14.1 g, in contrast to 5.4 and 6.7 g for the two animals of the 50 mg/kg group. Also, menstrual cycles (the number of menstrual cycles) were 1 and 2 in the two animals of the control group and 0 and 1 in the two animals of the 50 mg/kg group, showing no major differences between the groups. Histopathological examination at the end of treatment showed an absence of corpora lutea and inhibited follicular maturation in all females of the 50 mg/kg group, but at the end of the recovery period, the two animals did not show the inhibition of follicular maturation and corpora lutea was absent in one animal. The above findings indicate that the effects of bevacizumab on female reproductive function tend to be reversible upon drug withdrawal.

PMDA considers as follows:
Although reduced body weight gain in male cynomolgus monkeys tended to be reversible upon drug withdrawal, there is little evidence for concluding that reduced body weight gain was caused by growth retardation associated with epiphyseal dysplasia as only one animal was studied. As to the effects on female reproductive function, menstrual cycles in the control group at the time of measuring the uterine weight are unknown and whether zero menstrual cycles mean the cessation or delay of menstrual cycles is unclear. Thus, it is difficult to draw a conclusion on the reversibility.

Therefore, PMDA judged that although ovarian/uterine weights and hormones (the ability to produce progesterone in the rabbit) tend to recover upon drug withdrawal, the reversibility of the effects on reproductive function is unknown.

2) The effect of bevacizumab on vascular endothelium
Since PMDA had advised the applicant to carefully examine the effect of bevacizumab on vascular endothelial cells based on existing toxicity study data at a face-to-face consultation held in [redacted], PMDA asked the applicant to explain the content and results of the examination and the applicant responded as follows:

The effect on blood vessels (including vascular endothelial cells) in toxicity studies:
As to the results of histopathological examinations in the 4-week, 13-week, and 26-week repeat-dose studies in cynomolgus monkeys, the effect of bevacizumab on the aorta and blood vessels (including vascular endothelial cells) in different organs was reexamined. As a result, there were no changes considered associated with the effect of bevacizumab in any study.

A literature review concerning the effect of VEGF inhibition on blood vessels:
VEGF regulates blood vessel formation in normal process and pathologic process associated with tumor growth and promotes cell division of vascular endothelial cells and is a survival factor for vascular endothelial cells. There have been a number of reports highlighting the importance of angiogenesis in normal fetal development, and embryonic death in early pregnancy, abnormal vascular system, dysplasia of hematopoietic cells, and abnormalities in vascular endothelial cells have been reported in VEGF knockout mice (VEGF−/− and

VEGF+/−) and VEGF receptor knockout mice (Flt-1−/−, KDR−/−). On the other hand, although it has been suggested that the response to a VEGF inhibitor (the extent of effect) is different between growing and mature blood vessels (*Development* 126: 1149-59, 1999, *Circ Res* 94: 984-92, 2004, *J Biol Chem* 274: 31047-54, 1999, *Cell Mol Life Sci* 61: 2224-43, 2004), the cause for no damage observed in vascular endothelial cells of normal cynomolgus monkeys treated with bevacizumab has not been elucidated so far. However, studies on VEGF have been conducted actively and if findings are accumulated and the role of VEGF in mature blood vessels is determined in future, it will become possible to discuss the cause for no damage in vascular endothelial cells of normal cynomolgus monkeys.

PMDA considers as follows:
Although the effect of VEGF is different between mature vessels and neovessels, the reason for no injurious changes in mature blood vessels of normal cynomolgus monkeys is unknown at present and awaits a future investigation.

3) Thromboembolism and hypertension
PMDA asked the applicant to explain the mechanism of the development of thromboembolism and hypertension reported in clinical use of bevacizumab and the reason why these findings did not occur in cynomolgus monkeys.

The applicant responded as follows:
Although the mechanism of the development of adverse events in humans and differences in the response between humans and normal cynomolgus monkeys are unknown, we discuss as follows, at present.

The mechanism of the development of hypertension:
Placental secretion of sFlt1 (soluble VEGFR-1) is increased in the early phase of preeclampsia, blood VEGF and placenta-derived growth factor (PlGF) levels are decreased in patients with preeclampsia, and following the administration of sFlt1 to the rat, blood pressure is elevated and proteinuria occurs (*J Clin Invest* 111: 649-58, 2003). Thus, it is inferred that increased blood pressure and proteinuria are induced by the inhibition of VEGF and PlGF by sFlt1. Hypertension as an adverse drug reaction, has been reported also with another angiogenesis inhibitor that blocks VEGF signal transduction pathway (vatalanib) (*Curr Opin Oncol* 17: 578-83, 2005), suggesting that the inhibition of this pathway is related to the development of hypertension. Another mechanism has also been postulated: VEGF induces nitric oxide (NO) production in vascular endothelial cells, which relaxes the vascular smooth muscle and dilates the blood vessels (*Am J Physiol Cell Physiol* 280: C1375-86, 2001). VEGF-induced, NO production in human umbilical vein endothelial cells was inhibited by bevacizumab (*Angiogenesis* 7: 335-45, 2004). Such inhibition of VEGF-induced NO production in vascular endothelial cells can be one of the causes of hypertension associated with bevacizumab.

Thromboembolism:
Vascular endothelial cells produce both antithrombotic and prothrombotic factors. In the normal

state, the production of antithrombotic factors surpasses the production of prothrombotic factors, suppressing blood coagulation. However, cancer patients have concomitant thrombosis and it is presumed that the activity of their coagulation system is increased. The activity of prothrombotic factors produced in cancer cells, such as tissue factor (TF) and cancer procoagulant (CP), is enhanced by IL-8, VEGF, TNF-α, and IL-1β, and as a result, normally antithrombotic vascular endothelium becomes prothrombotic with an increased activity of coagulation system (*Thromb Res* 102: V215-24, 2001). VEGF is prothrombotic by inducing the expression of TF and von Willebrand factor etc. while it exerts even an antithrombotic effect through the inhibition of platelet aggregation by the induction of the production of NO and PGI2, and the expression and activation of urokinase-type plasminogen activator and tissue-type plasminogen activator. Therefore, the inhibition of VEGF appears to affect both the coagulation and fibrinolysis systems. A clinical study of SU5416, which inhibits VEGF signals (inhibition of VEGFR-1 and -2), has reported that, in patients with thromboembolism, the activation of vascular endothelial cells and an increased activity of coagulation system had been noted at baseline, which were further enhanced after the administration of SU5416 (*Arterioscler Thromb Vasc Biol* 22: 1500-5, 2002). In a foreign clinical study of bevacizumab (Study AVF0780g), 9 subjects were tested for thrombin time and activated partial thrombin time, α2 antiplasmin, D-dimer, and platelet function and there were no abnormalities in any parameter (*J Clin Oncol* 21: 60-5, 2003, *J Clin Oncol* 21: 3542-6, 2003). However, due to the small number of subjects studied (9 subjects), no definitive conclusion on the effect of bevacizumab on coagulation system has been drawn. Based on the above, the activity of the coagulation system is often increased in cancer patients and VEGF is involved in both prothrombotic and antithrombotic states, but how bevacizumab acts on the coagulation and fibrinolysis systems is unknown and the mechanism of increased thromboembolic events associated with bevacizumab has not been elucidated.

No occurrence of thromboembolism or hypertension in cynomolgus monkeys:
It is presumed that the inhibition of the proliferation of vascular smooth muscle cells, the maintenance of survival of vascular endothelial cells, the suppression of thrombus formation, and anti-inflammation by VEGF (*Am J Physiol Cell Physiol* 280: C1375-86, 2001) are primarily mediated by NO and PGI2 produced in vascular endothelial cells, which is induced by VEGF. bevacizumab inhibited VEGF-induced NO production in human umbilical vein endothelial cells *in vitro*. Meanwhile, in the repeat-dose toxicity studies in cynomolgus monkeys, bevacizumab was administered at doses 20 to 40 fold the recommended clinical weekly dose, but there were no changes in blood pressure or no effects on the coagulation system such as platelet count, prothrombin time, and activated partial thrombin time. The mechanism of the development of hypertension and thromboembolism associated with bevacizumab has not been elucidated and the reason for no effects on blood pressure or the coagulation system in normal cynomolgus monkeys, is also unclear. Many of the patients who developed hypertension or thromboembolism in clinical trials of bevacizumab had previous illnesses or risk factors and one possibility is that the effects of the inhibition of VEGF on blood pressure and the coagulation system, which can be regulated in the normal state, are more likely to become apparent in these patients.

PMDA considers that it is necessary to continue to investigate the mechanism of the development of thromboembolism and hypertension associated with bevacizumab, including a non-clinical investigation.

4) Gender differences

In the 13-week repeat-dose study in cynomolgus monkeys, epiphyseal dysplasia occurred at ≥ 2 mg/kg in males and at ≥10 mg/kg in females and the severity was higher in males. The applicant discusses that there were gender differences because more females were mature than males in this study. PMDA asked the applicant to explain the development of epiphyseal dysplasia, taking account of age, sexual maturation, and ovarian function etc., also including the data from the 4-week repeat-dose study where similar gender differences were observed.

The applicant responded as follows:

In the 4-week and 13-week repeat-dose studies, epiphyseal dysplasia of the femur or the humerus occurred, which was characterized by histopathological changes such as inhibition of vascular invasion of the growth plate, thickened growth plate cartilage (epiphyseal cartilage), clusters of hyperplasic chondrocytes, and subchondral bony plate formation, and its severity and incidence were increased with increasing dose and the severity was higher in males than in females. However, there were no gender differences in the trough levels of blood bevacizumab in either study, and the gender differences in epiphyseal dysplasia were not considered attributable to differences in exposure.

Sex hormones are involved in growth plate closures and it has been reported that the growth plates of the femur and humerus in cynomolgus monkeys close at a mean age of 6 years for males and at a mean age of 4 years 9 months for females (*Exp Anim* 27: 387-97, 1978). Bevacizumab binds to VEGF and inhibits vascular invasion of the growth plate (angiogenesis inhibition) and blocks endochondral ossification, and animals with open growth plates at baseline are considered to be more susceptible to epiphyseal dysplasia. The mean age of females in each group in the 4-week and 13-week repeat-dose studies was 3.8 to 4.8 years, which was similar to the age of closure of growth plates of the femur and the humerus. On the other hand, the mean age of males in each group was 2.5 to 4 years, younger than the age of growth plate closure. Although the age was different between males and females in the studies, the body weights were similar.

With respect to sexual maturation of the animals used, histopathological examination in the 4-week repeat-dose study showed “immaturity” of the testis in many animals treated and it seems that most male animals were sexually immature and had open growth plates at baseline. Meanwhile, in the 13-week repeat-dose study, “immaturity” of the testis was noted in only two animals in the high dose group and the age of males at baseline in this study was slightly higher than that in the 4-week repeat-dose study, but not a major difference, and it is considered that the maturity stage of the testis at baseline of animals used in the two studies did not differ. Menstruation is one of the indicators of sexual maturation of female animals. However, (a)

Bevacizumab affected female reproductive function (26-week repeat-dose study: reduced uterine weights, reduced endometrial proliferation, inhibited follicular maturation, an absence of corpora lutea, decreased number of menstrual cycles, etc.) and there were effects on female reproductive function also in the 13-week repeat-dose study (reductions in ovarian and uterine weights, a reduction in number of corpora lutea), and it is presumed that menstruation cycles became irregular (decreased menstruation), (b) The estrous cycle of the control group in the 26-week repeat-dose study occurred every 1-3 months and the 4-week repeat-dose study was too short to accurately monitor the presence or absence of menstruation. Therefore, it was difficult to accurately grasp the presence or absence of menstruation in female animals used in the 4-week and 13-week repeat-dose studies.

PMDA considers as follows:
It is inadequate to judge the maturity stage based only on body weight and age and draw a conclusion on gender differences in the effect of bevacizumab on bones. It is necessary to collect information on the development of epiphyseal dysplasia associated with bevacizumab or drugs of the same class in mature male cynomolgus monkeys, the effects of estrogen on it, and the functional reversibility of damage in female reproductive organs etc., for determining the presence or absence of gender differences in the development of epiphyseal dysplasia.

4. Clinical Data

4.1 Data of biopharmaceutic studies and associated analytical methods

Summary of the submitted data

1) Bevacizumab assay

Bevacizumab in human serum was measured by an ELISA using solid-phased rhVEGF (recombinant human VEGF) and goat anti-human IgG Fc-HRP (detection reagent).

2) Assay for anti-bevacizumab antibody

Anti-bevacizumab antibodies in human serum were measured by two methods. One is an ELISA detecting antibodies against the Fab region of bevacizumab, which was used in foreign clinical studies initiated before the start of a Japanese phase I study, JO18157. The other is an electrochemiluminescence assay (ECLA) detecting antibodies against the entire bevacizumab including the Fc region, which was employed for Japanese and foreign clinical studies initiated after Japanese Study JO18157. This ECLA method produces false positive results if serum VEGF concentrations are $\geq$1 ng/mL, due to a reaction between solid-phased bevacizumab and VEGF in the sample.

3) VEGF assay

Endogenous VEGF in human serum or plasma was measured by an ELISA. In the Japanese clinical study, plasma concentrations of VEGF unbound to bevacizumab were measured. In all foreign clinical studies where VEGF was measured, total VEGF concentrations in serum or plasma (VEGF bound to bevacizumab + free VEGF) were measured. VEGF assay used in foreign clinical studies underestimates the true VEGF levels in the case where the sample contains $\geq$100 μg/mL of bevacizumab.

Outline of review by the PMDA

1) Effects of bevacizumab on VEGF assay

PMDA asked the applicant to explain whether VEGF concentrations have been assessed appropriately based on the bevacizumab concentrations in the samples for VEGF assay from Japanese and foreign clinical trials as the true VEGF concentrations are underestimated in the presence of bevacizumab ≥100 μg/mL coexisting in the sample and Foreign Study AVF0737g showed that an increase in the plasma VEGF concentration following the administration of bevacizumab was not dose-related.

The applicant responded as follows:

The assay for total VEGF concentrations used in foreign clinical studies underestimated the total VEGF concentration by 20-30% in the presence of bevacizumab at 100 μg/mL coexisting in the sample. Therefore, it is presumed that the presence of bevacizumab affected the VEGF assay and the true VEGF concentration was underestimated for the samples containing ≥100 μg/mL of bevacizumab (all or part of the samples from the 10 mg/kg groups and the ≥ 3 mg/kg multiple dose groups) among those collected from foreign clinical studies.

On the other hand, a validation study of a commercial assay kit to measure free VEGF unbound to bevacizumab (manufactured by [redacted], [redacted]), used in the Japanese clinical study, confirmed that the measurements are below the lower limit of quantification in the presence of bevacizumab ≥3 μg/mL coexisting in the sample. However, despite that the bevacizumab concentrations in Japanese Study JO18157 (the trough level at the lowest dose of 3 mg/kg: ≥7.07 μg/mL) exceeded 3 μg/mL, which was supposed to affect the assay for free VEGF, the measurements were not below the lower limit of quantification of 15.6 pg/mL (23.81-52.87pg/mL in the 3 mg/kg group, 27.00-67.44 pg/mL in the 5 mg/kg group, and 30.17-45.28 pg/mL in the 10 mg/kg group), which were inconsistent with the results of the validation study. Thus, for the samples collected from the Japanese clinical study, not only free VEGF, but also bevacizumab-VEGF complexes may have been detected and it is considered that the true free VEGF concentrations were not obtained.

The applicant concluded that bevacizumab-VEGF complexes in the sample were also detected and the measurements did not reflect the true free VEGF concentrations since the results were inconsistent with the validation data on the assay kit manufactured by [redacted], used in the Japanese clinical study. However, there are little grounds for the applicant's discussion and their failure to fully examine this phenomenon is a problem. Moreover, the applicant quantitatively compared/examined VEGF concentrations over time among different dose groups in foreign clinical studies submitted, but explained in the response to a set of questions from the PMDA that the measurement results are not true values, fundamentally denying the relevant comparison/discussion in the applicant dossier [See "4.2 Clinical pharmacology"]. Therefore, the applicant's response is contradictory.

PMDA recognized that it is difficult at present to quantitatively assess the relationship

between the dose of bevacizumab administered (or the blood bevacizumab concentration) and the blood VEGF concentration based on the submitted data. However, the applicant needs to provide a solid discussion on the inconsistent data obtained and continue to investigate this issue thoroughly. Since blood VEGF concentrations were measured as a pharmacodynamic marker of bevacizumab in clinical studies, the applicant, as the developer of bevacizumab, is required to ensure that the relationship between the blood VEGF concentration over time after the administration of bevacizumab and the dose of bevacizumab administered (or the blood bevacizumab concentration) can be assessed quantitatively when the pharmacodynamics is further investigated in future clinical studies etc. after an assay for VEGF in blood samples is established.

4.2 Clinical pharmacology

Summary of the submitted data

The human pharmacokinetics of bevacizumab when given alone or in combination with chemotherapy was investigated in patients with various solid tumors or those with colorectal cancer in Japanese and foreign clinical studies. Data on the pharmacokinetics of bevacizumab in patients with breast cancer, non-small cell lung cancer, or prostate cancer were submitted as Reference Data.

1) Japanese clinical studies

(1) Japanese phase I/II study of bevacizumab in combination with 5-FU/*l*-LV regimen (Study Number JO18157)

Serum bevacizumab concentrations following the intravenous administration of 3, 5, or 10 mg/kg alone were assessed in 18 patients with advanced or recurrent colorectal cancer. From 3 weeks after the administration of bevacizumab alone, 5-FU/*l*-LV regimen (5-FU 500 mg/m^2 and *l*-LV 250 mg/m^2 intravenously administered once weekly for 6 weeks, followed by a 2-week rest) was initiated in combination with bevacizumab, which was intravenously administered every 2 weeks at the same dose as when given alone, and the serum bevacizumab concentrations were assessed. As the AUC_{inf} following the administration of bevacizumab alone was increased in a dose-proportional manner and the clearance (CL) was constant (See the table below), the applicant discusses that a linearity was observed. The ratios of the CL and volume of distribution of the central compartment (Vc) of bevacizumab for bevacizumab in combination with 5-FU/*l*-LV／single agent bevacizumab (estimates) were 0.899 and 1.093, respectively [Note by the PMDA: According to the pharmacokinetic analysis report submitted after application filing, the estimates were 0.863 and 1.118, respectively], and the applicant discusses that when combined with 5-FU/*l*-LV, the CL of bevacizumab tends to be decreased and the Vc tends to be increased, but the degrees are small and the effects on the PK are small.

	3 mg/kg	5 mg/kg	10 mg/kg
AUC_{inf} (μg･day/mL)	852.3±237.4	1387.2±426.9	2810.9±344.8
Vd (mL/kg)	62.50±11.10	73.47±18.34	60.26±8.93
CL (mL/day/kg)	3.80±1.20	3.94±1.34	3.61±0.48
$t_{1/2}$ (day)	12.33±4.52	13.40±2.82	11.68±1.74

Mean±SD, n=6, AUC_{inf}: AUC to infinity

2) Foreign clinical studies

(1) Foreign phase I study of single agent bevacizumab (Study Number AVF0737g)

Serum bevacizumab concentrations were assessed when 25 patients with advanced solid tumors received 0.1, 0.3, 1, 3 or 10 mg/kg of intravenous bevacizumab on Days 0, 28, 35, and 42. Serum bevacizumab was eliminated monophasically or biphasically, and the serum bevacizumab concentrations in the two dose groups, i.e. the 0.1 mg/kg and 0.3 mg/kg groups declined rapidly compared to the 1, 3, and 10 mg/kg groups (See the table below). The mean and standard deviation of the CL of bevacizumab were higher in the two dose groups, i.e. the 0.1 and 0.3 mg/kg groups than in the 1, 3, and 10 mg/kg groups, the Vc was similar to the serum volume, and the volume of distribution at steady state (Vss) and Vc were almost constant, being independent of the dose of bevacizumab. There were no changes in CL or Vc at doses of 1 to 10 mg/kg and linearity was observed. As the differences in the PK parameters between the first-dose data and all available data up to Day 72 were small, the applicant discusses that the multiple dose PK is equivalent to the single dose PK and can be predicted from the single dose PK.

	0.1 mg/kg, N=5	0.3 mg/kg, N=5	1 mg/kg, N=5	3 mg/kg, N=4	10 mg/kg, N=5
CL (mL/day/kg)	9.29±7.07	5.07±2.39	3.27±0.81	3.65±2.10	2.75±0.47
Vc (mL/kg)	48.0±17.4	48.6±13.0	37.9±7.77	41.4±12.0	43.5±12.6
Vss (mL/kg)	50.1±17.0	60.3±7.30	60.4±18.8	53.4±12.0	53.0±10.9
$t_{1/2}$ initial (day)[a]	NA	1.9	1.30±0.535	0.844	2.17
$t_{1/2}$ terminal (day)[b]	5.21±2.41	10.4±5.34	14.7±6.92	12.8±6.60	14.2±3.36
MRT (day)	7.40±3.44	13.9±6.11	19.9±9.25	18.1±9.36	19.3±3.18

The results of analyses using all available data up to Day 72, Mean±SD

[a]: Calculated with only the patients analyzed using a 2-compartment model (n=1-4)

[b]: $t_{1/2}$ was used for the patients analyzed using a 1-compartment model and $t_{1/2\beta}$ was used for the patients analyzed using a 2-compartment model.

(2) Foreign phase I study of bevacizumab in combination with chemotherapy (Study Number AVF0761g)

Serum bevacizumab concentrations were assessed when bevacizumab 3 mg/kg was intravenously administered once weekly for 8 weeks in combination with chemotherapy in 12 patients with advanced solid tumors. Concomitantly used cancer chemotherapy agents were (a) doxorubicin hydrochloride (DXR) (intravenous administration of 50 mg/m^2 on Days 0 and 28), (b) CBDCA/PTX (intravenous administration of CBDCA AUC=6 mg･min/mL and PTX 175 mg/m^2 on Days 0 and 28) or (c) 5-FU/LV regimen (intravenous administration of 5-FU 500 mg/m^2 and LV 20 mg/m^2 once weekly for 6 weeks). The PK parameters of serum bevacizumab were almost equivalent for all groups and based on a pooled analysis of PK data from this study, the mean±SD of the CL, Vc, and MRT was 3.11±0.792 mL/day/kg, 56.8±12.2 mL/kg, and 18.8±3.98 days, respectively, which were similar to the results of Study AVF0737g where single agent bevacizumab was administered [See "4.2 Clinical pharmacology, *Summary of the submitted data* 2) (1) Foreign phase I study of single agent bevacizumab"]. Therefore, the applicant discusses that there was no clear difference in the pharmacokinetics of bevacizumab in combination with DXR, CBDCA/PTX, or 5-FU/LV compared to single agent bevacizumab.

There were no major changes in the plasma concentration of each chemotherapy agent on the day of the first dose, Day 28, or Day 35, and the applicant discusses that the PK of these

chemotherapy agents is not altered by the administration of bevacizumab.

(3) Foreign phase II study of bevacizumab in combination with 5-FU/LV regimen (Study Number AVF0780g)

Serum bevacizumab concentrations were assessed when bevacizumab 5 or 10 mg/kg was intravenously administered every 2 weeks in combination with 5-FU/LV regimen (5-FU 500 mg/m^2 and LV 500 mg/m^2 were intravenously administered once weekly for 6 weeks followed by a 2-week rest) in 104 patients with metastatic colorectal cancer (62 patients were included in pharmacokinetic analysis). The trough concentration of bevacizumab increased after multiple dosing and reached a steady-state by Day 100. Regardless of the dose of bevacizumab, the CL and Vc were constant (2.78-2.79 mL/day/kg and 45.4-46.1 mL/kg, respectively) and the applicant states that there were no major differences between this study data and AVF0737g data [See "4.2 Clinical pharmacology, *Summary of the submitted data* 2) (1) Foreign phase I study of single agent bevacizumab"].

(4) Foreign phase III study of bevacizumab in combination with IFL regimen (Study Number AVF2107g)

Bevacizumab 5 mg/kg was intravenously administered every 2 weeks in combination with IFL or 5-FU/LV regimen to 923 patients with metastatic colorectal cancer (214 patients were included in the pharmacokinetic analysis of bevacizumab, 67 patients and 68 patients were included in the pharmacokinetic analysis of irinotecan hydrochloride and its metabolite, respectively) and population pharmacokinetic (PPK) analysis of serum bevacizumab concentrations was performed. The IFL regimen was irinotecan hydrochloride 125 mg/m^2, 5-FU 500 mg/m^2, and LV 20 mg/m^2 intravenously administered once weekly for 4 weeks followed by a 2-week rest. The 5-FU/LV regimen was 5-FU 500 mg/m^2 and LV 500 mg/m^2 intravenously administered once weekly for 6 weeks, followed by a 2-week rest.

No. of patients	214
Population mean [CL (mL/h)]	10.2 (2.80)
Population mean [Vc (mL)]	3230 (2.00)
Inter-individual variability [ωCL (%)]	30.8 (10.5)
Inter-individual variability [ωVc (%)]	18.7 (30.1)
Intraindividual variability [σProp (%)]	12.4 (38.4)
Intraindividual variability [σAdd (μg/mL)]	16.7 (18.3)

In the case of body weight of 80 kg (the median body weight of the patients included in the population pharmacokinetic analysis was 78.0 kg), the CL and Vc of serum bevacizumab were 3.06 mL/kg/day and 40.3 mL/kg, respectively, which were consistent with the results from Study AVF0780g in which bevacizumab was combined with the same 5-FU/LV regimen (5 mg/kg group: 2.79 mL/kg/day and 45.4 mL/kg, respectively). The trough serum bevacizumab concentration (Mean±SD) when bevacizumab was combined with IFL or 5-FU/LV was 28.6±10.6 and 32.5±18.8 μg/mL, respectively, on Day 14, and 83.6±31.4 and 77.0±26.9 μg/mL, respectively, on Day 84. Serum bevacizumab concentrations were similar between the IFL+bevacizumab group and the 5-FU/LV+bevacizumab group and the applicant discusses that

the addition of irinotecan hydrochloride to the regimen of 5-FU/LV＋bevacizumab does not affect the pharmacokinetics of bevacizumab.

Plasma concentrations of irinotecan and its active metabolite (SN-38) were assessed in the patients who received IFL regimen. The ratio of the AUC_{0-t} for the IFL+bevacizumab group and the IFL group (28 patients in the IFL+bevacizumab group/36 patients in the IFL group, corrected for the dose of irinotecan hydrochloride) was 1.10 for irinotecan and 1.33 for SN-38. The applicant discusses that it is unknown at present whether higher AUC of SN-38 in the IFL+bevacizumab group was due to bevacizumab administration [Note by the PMDA: The results of a foreign clinical study of bevacizumab in combination with FOLFIRI regimen were presented in the response to a set of questions from the PMDA. See "4.2 Clinical pharmacology, *Outline of review by the PMDA"*].

In a foreign phase III study in patients with metastatic breast cancer (Study AVF2119g), which was submitted as Reference Data, the pharmacokinetics of capecitabine and its metabolites when given with bevacizumab were investigated, but there was a high variability in the data and no definitive conclusion on pharmacokinetic interactions could be drawn.

(5) Population pharmacokinetic analysis (Study Numbers: AVF0737g, AVF0761g, AVF0780g, AVF2107g, AVF0755g, AVF0776g, AVF0757g, AVF2119g)

PPK analysis was performed based on the pooled data sets from 8 foreign clinical studies involving 491 cancer patients who received intravenous bevacizumab 1 to 20 mg/kg weekly, every 2 weeks, or every 3 weeks, and covariates were explored. As a result, gender, body weight, albumin, alkaline phosphatase, AST, and combination chemotherapy were associated with CL. Gender, body weight, and albumin had impact on Vc.

The CL of bevacizumab was 19.3% higher in patients with low serum albumin (29 g/L: 5 percentile) and 23.0% higher in patients with high alkaline phosphatase (483 IU/L: 95 percentile), compared to patients with median laboratory values. The CL was 26.4% higher in men than in women. The CL was 0.207 and 0.262 L/day, respectively for a female and male patient with the assumed median covariate value and the Vc was 2.66 and 3.25 L, respectively. The CL of bevacizumab was equivalent for the bevacizumab alone group and the bevacizumab+IFL group, whereas it was 17.4% lower when combined with 5-FU/LV, CBDCA/PTX, capecitabine, or DXR as compared to bevacizumab alone.

3) Pharmacodynamic assessment using VEGF concentrations as a marker

Bevacizumab binds to VEGF and inhibits the binding of the ligand to its receptors, resulting in the inhibition of angiogenesis and tumor growth. Thus, total serum/plasma VEGF concentrations were investigated as a biomarker [Note by the PMDA: The applicant indicated in the response to a set of questions from PMDA that the VEGF assays used in Japanese and foreign clinical studies are not fully validated ones (See "4.1 Data of biopharmaceutic studies and associated analytical methods")].

(1) Foreign clinical studies

In Foreign Study AVF0737g evaluating single agent bevacizumab, total serum VEGF concentrations in the 0.1 and 0.3 mg/kg groups were almost constant throughout the study period while in the 1 to 10 mg/kg groups, total serum VEGF concentrations increased on Day 7 after the initital administration and then decreased [Note by the PMDA: Total serum VEGF concentrations increased more greatly in the 1 mg/kg group than in the 3 mg/kg group]. Subsequent intravenous doses of bevacizumab on Days 28, 35, and 42 after the initial administration further increased total serum VEGF concentrations. Following weekly administration of bevacizumab 10 mg/kg, serum VEGF concentrations reached about 10-fold the baseline levels. Total serum VEGF concentrations declined after the last dose, but did not return to the baseline levels at the end of a follow-up period (72 days after the first dose, 30 days after the last dose). The applicant discusses that the increase in total serum VEGF concentration is attributable to a decrease in CL associated with the formation of bevacizumab-VEGF complexes. The phenomenon that the CL of VEGF is decreased due to the formation of bevacizumab-VEGF complexes was also observed in a non-clinical study in rats [See "3.2 Pharmacokinetic studies"].

In Studies AVF0761g and AVF0780g evaluating bevacizumab in combination with chemotherapy agents, the mean total serum VEGF concentrations following the intravenous administration of bevacizumab 3 to 10 mg/kg in combination with DXR, CBDCA/PTX or 5-FU/LV were also higher than the baseline levels.

(2) Japanese clinical studies

The application data does not contain the results of measurement of plasma VEGF concentrations in Japanese Study JO18157. In the PK analysis report on this study submitted as a response to a set of questions from the PMDA after the regulatory submission, plasma VEGF concentrations are described as follows.

In Study JO18157, plasma VEGF concentrations following the administration of bevacizumab were below the lower limit of quantification up to 8 hours after the first dose in the bevacizumab 3 mg/kg group and up to 24 hours after the first dose in the 5 and 10 mg/kg groups, and then tended to return to the baseline levels gradually. Also, following the administration of bevacizumab at 3 weeks after the first dose, plasma VEGF concentrations over time were similar to those after the first dose. With increasing number of doses of bevacizumab, subjects with a plasma VEGF concentration below the lower limit of quantification were decreased gradually. There were no major differences in plasma VEGF concentration over time among any groups. Although the apparent plasma VEGF concentrations over time following the administration of bevacizumab in Study JO18157 were different from those in foreign clinical studies, despite that the mean serum bevacizumab concentrations tended to increase until 5 doses, the degree of decrease in plasma VEGF concentration was smaller with increasing number of doses. Thus, like foreign clinical study data, total plasma VEGF concentrations may have been increased with increasing number of doses.

4) Test for anti-bevacizumab antibodies

In Japanese Study JO18157, 3/18 subjects (5 mg/kg group: 1 subject, 10 mg/kg group: 2 subjects) at baseline and 4/4 subjects (2 subjects each in the 3 mg/kg group and the 5 mg/kg group) at the end of the study (3 weeks after the last dose of bevacizumab) were tested positive for serum anti-bevacizumab antibodies (Data cutoff: [redacted] 20[redacted]).

There were no major differences in serum bevacizumab concentration over time between the subjects with a positive antibody reaction at baseline (Case No. X1, X2, X3) and those with a negative antibody reaction. Also, the PK parameters obtained by a non-compartmental analysis based on the time course of bevacizumab concentrations from the first dose until immediately before the second dose (CL, Vd, $t_{1/2}$, and MRT) were similar regardless of the results of antibody test at baseline. Since these 3 subjects had never received bevacizumab at the start of the study; there were no major differences between the PK parameters after the first dose and the multiple-dose PK parameters; There were no clear differences in the PK between these subjects and those with a negative anti-bevacizumab antibody test; And, in spite of receiving multiple doses of bevacizumab during the study period, these subjects did not develop adverse events clearly suggestive of hypersensitivity reactions during or after treatment with bevacizumab, the applicant discusses that the positive antibody test results in the Japanese clinical study were very likely to be associated with false positive reactions, caused by detecting substances that bind to bevacizumab nonspecifically.

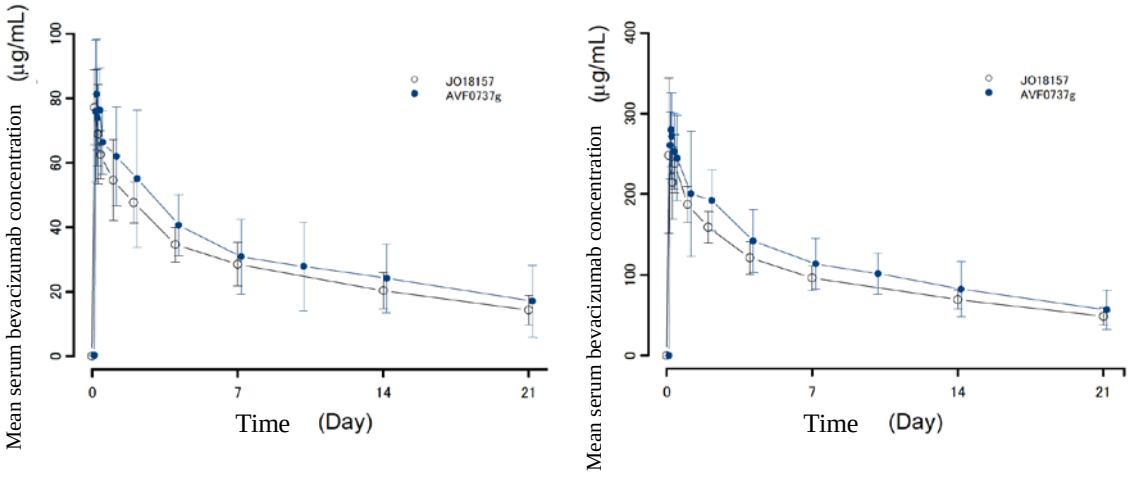
On the other hand, of the subjects with a positive antibody reaction at 3 weeks after the last dose of bevacizumab (Case No. X4, X5, X6, X1), 3 subjects with a negative antibody reaction at baseline (Case No. X4, X5, X6) showed little differences in PK parameters, CL and Vc, between single-dose and multiple-dose, except for CL in 1 subject (Case No. X6) [Note by the PMDA: In Case No. X6, the multiple-dose CL was reduced to about 1/2 of the single-dose CL]. Even in 1 subject with positive antibody test results at both baseline and 3 weeks after the last dose (Case No. X1), the difference between the first-dose and multiple dose CL was 7.79% and the difference in Vc was −4.02%, showing no major changes. Also for these 4 subjects, there were no reports of adverse events clearly suggestive of hypersensitivity reactions during or after treatment with bevacizumab. Given that the test produces false positive results when serum VEGF concentrations are high and that bevacizumab delays the elimination of VEGF, resulting in a rise in plasma VEGF concentration, it is unclear whether or not the antibody test performed at 3 weeks after the last dose detected anti-bevacizumab antibodies. Taking account of the above situation, the 4 subjects with positive antibody test results at 3 weeks after the last dose of bevacizumab are scheduled to undergo the antibody test again at $\geq$3 months after the last dose by when bevacizumab will have fully been eliminated from serum and VEGF concentrations will have declined to the baseline levels.

Based on the above, the applicant discusses that there are no major differences in the PK of bevacizumab between subjects with a positive antibody reaction and those with a negative antibody reaction. Different test methods for anti-bevacizumab antibodies were used in Japanese and foreign clinical studies [See “4.1 Data of biopharmaceutic studies and associated analytical

methods"] and it is difficult to simply compare the test results, but in foreign clinical studies, 4/837 subjects were tested positive at baseline and none of the 494 subjects tested after treatment with bevacizumab had positive results.

5) The applicant's discussion on PK in Japanese and foreigners

The mean serum bevacizumab concentration over time following the administration of single agent bevacizumab tended to be lower in Japanese subjects (Study JO18157) than in American subjects (Study AVF0737g), but the variability in the Japanese data was small, which was within the variation range of the data from American subjects (See the figures below). Many of the measured serum bevacizumab concentrations in Japanese Study JO18157 fell within the 5 to 95 percentiles of Monte Carlo simulation in which the background factors of the subjects in the 5 mg/kg group of Japanese Study JO18157 were imputted to foreign PPK analysis results. The applicant discusses that it has been suggested that there are no major differences in the PK of bevacizumab between Japanese and foreigners although only a small number of Japanese subjects were studied.



Serum bevacizumab concentration over time following the administration of single agent bevacizumab in Japanese and foreign clinical studies
Left figure: 3 mg/kg, Right figure: 10 mg/kg, Mean±SD

Outline of review by the PMDA

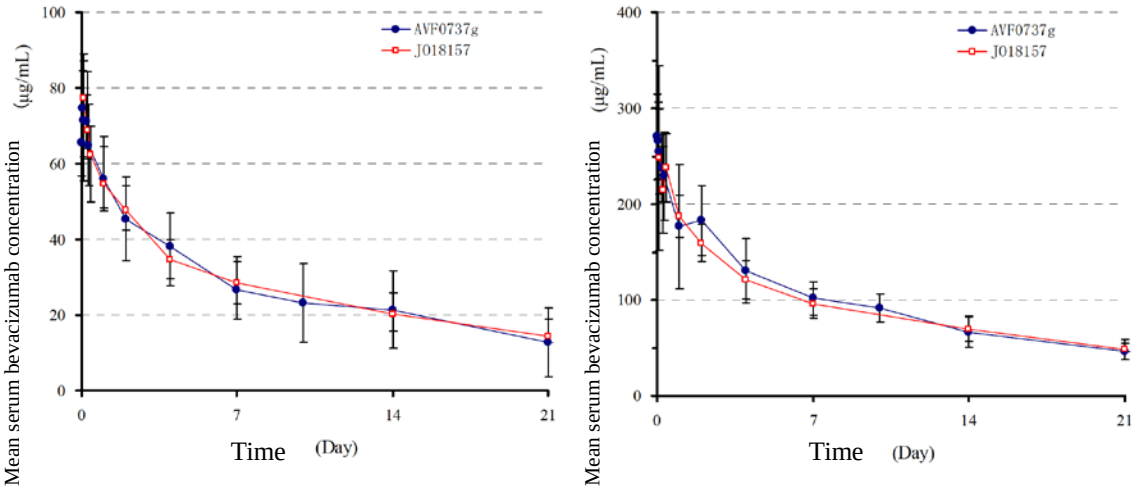
1) PK of bevacizumab in Japanese and foreign clinical studies

PMDA asked the applicant to discuss the reason for lower serum bevacizumab concentrations over time in Japanese subjects than in American subjects.

The applicant responded as follows:

Since the variability in serum bevacizumab concentrations following the administration of bevacizumab in Japanese Study JO18157 was small, which was within the variation range of the data from the US Study AVF0737g [See "4.2 Clinical pharmacology, *Summary of the submitted data* 5) The applicant's discussion on PK in Japanese and foreigners"], the interindividual variability in the data was compared between the two studies. As a result, 1 subject in the 3 mg/kg group and 1 subject in the 10 mg/kg from Study AVF0737g (Case No. X7 and X8) had

higher serum bevacizumab concentrations compared to the other 4 subjects treated with the same dose, respectively, and when these subjects with high values were excluded from the groups and the mean value calculated from the remaining 4 subjects in each group was compared, there were little differences between the Japanese and foreign studies (See the figures below). Therefore, serum bevacizumab concentrations tended to be lower in Japanese subjects than in American subjects because the impact of individuals with high serum bevacizumab concentrations in the US clinical study was strongly reflected on the mean value due to the small number of subjects in both studies.



Serum bevacizumab concentration over time following the administration of single agent bevacizumab in Japanese and foreign clinical studies (when 1 subject with high serum bevacizumab concentrations is excluded from each group)

Left figure: 3 mg/kg, Right figure: 10 mg/kg, Mean±SD

PMDA considers as follows:

Due to the small number of subjects in the Japanese and foreign clinical studies, it is difficult to draw a conclusion on ethnic differences in the PK of bevacizumab, but there seem no clear differences in the PK of bevacizumab between Japan and overseas. However, as only a small number of subjects have been compared to date, it is necessary to collect comparable information with regard to ethnic differences in the PK of bevacizumab, including published articles, and take appropriate action, e.g. provide information based on such data.

2) Multiple-dose PK

The applicant discussed as follows:

When the PK parameters estimated from the first-dose data and those from all first- and multiple-dose data in Foreign Study AVF0737g are compared, the multiple-dose PK of bevacizumab is equivalent to the single-dose PK.

PMDA asked the applicant to explain the reason for judging that it is appropriate to use all available data including the first-dose data for examining the impact of multiple dosing on PK.

The applicant responded as follows:
In Study AVF0737g, a blood sample for pharmacokinetic analysis was collected at 24 timepoints following the administration of bevacizumab, which include 13 timepoints from the first dose until immediately before the second dose and 7 timepoints after the last dose (the 4th dose). When PK is assessed in a multiple-dose study, usually, the impact of multiple dosing is examined using the first-dose data and the steady-state data or the data after the last-dose. However, in the case of a model-independent analysis, if blood sampling is sparse and a steady-state has not been reached like the condition after the last dose in this study, it is possible to calculate the terminal half-life using the slope of multiple dosing, but CL or Vd can not be estimated. Furthermore, if a compartment model analysis is performed using only the data after the last dose obtained with a sparse sampling, calculation will not converge and PK parameters can not be estimated for many subjects, which makes adequate evaluation difficult. Therefore, in order to estimate more accurate multiple-dose PK parameters in Study AVF0737g by compartment model analysis, the first-dose data was included in the analysis. The appropriateness of this analysis is thought to have been confirmed because the terminal half lives after the first dose and multiple doses, calculated using a model-independent analysis, were similar and the measured values after multiple dosing were similar to the estimates obtained by compartment analysis of the first-dose data.

PMDA largely accepted the response, considering that there are no clear changes in the PK profile following multiple doses of bevacizumab based on the data from Japanese and foreign clinical studies including Study AVF0737g. However, since information on the multiple-dose PK of bevacizumab was not available at the time of initiating Study AVF0737g, PMDA considers that the appropriateness of the above analysis was presented after the fact.

3) Effects of anti-bevacizumab antibodies
The applicant discussed that there are no major differences in the PK of bevacizumab between subjects tested positive for anti-bevacizumab antibodies and those tested negative [See “4.2 Clinical Pharmacology, *Summary of the submitted data* 4) Test for anti-bevacizumab antibodies”].

PMDA considers as follows:
The applicant’s discussion on anti-bevacizumab antibodies also mentions that there is a problem with the assay system. Although 4/4 subjects had positive anti-bevacizumab antibodies at the end of the study in Japanese Study JO18157, the potential for anti-bevacizumab antibodies to act as neutralizing antibodies, affecting the PK of bevacizumab, has not adequately been examined.

PMDA asked for the applicant’s view on the necessity of investigating the effects of anti-bevacizumab antibodies on the PK of bevacizumab after market launch.

The applicant responded as follows:
In Japanese Study JO18157, the test for anti-bevacizumab antibodies by ECLA was positive in

3/18 subjects at baseline and 7/7 subjects at the end of the study [Note by the PMDA: The data at the end of the study include additional data that became available after the NDA filing]. It was revealed in February 2006 that, in a foreign clinical study that was initiated at the same time as Japanese Study JO18157, the test for anti-bevacizumab antibodies by ECLA was positive for the samples of 33/35 subjects collected after the treatment with bevacizumab. Taking account of the above test results, Genentech (the US) judged that the patients with positive antibody titers by ECLA may have been false-positive due to the assay system and suspended the antibody test by ECLA, and is currently reevaluating the ECLA, including changing the threshold value for positivity and is considering to develop a new antibody assay method. In planned or ongoing clinical studies in and outside Japan, an antibody test using the new criteria for positivity or by a new assay method is to be performed and the PK of bevacizumab is to be assessed. In future, it is expected that the effects of anti-bevacizumab antibodies on the PK of bevacizumab will be determined based on clinical study results. Therefore, we intend to decide whether to conduct a post-marketing investigation of anti-bevacizumab antibodies and the PK of bevacizumab, taking account of the results of the above clinical studies.

Regarding the subjects who were scheduled to undergo antibody test again at $\geq$3 months after the last dose of bevacizumab, the test result is available for only 1 subject (Case No. X4, positive), at present. The test results for the other subjects will be submitted as soon as the results of test by a new antibody assay method become available.

PMDA considers as follows:
The effects of anti-bevacizumab antibodies on the PK of bevacizumab is unclear at present. Since the possibility that the efficacy of bevacizumab is diminished due to the development of anti-bevacizumab antibodies can not be ruled out and the safety of bevacizumab is not necessarily high [See “4.3 Clinical efficacy and safety” and “4.4 Adverse events etc. reported in clinical studies”], it is important to evaluate the effects of anti-bevacizumab antibodies on the PK of bevacizumab for the appropriate use of bevacizumab. The applicant needs to establish an assay for anti-bevacizumab antibodies as soon as possible, further investigate the effects of the development of anti-bevacizumab antibodies on the PK, efficacy and safety of bevacizumab, and determine the presence or absence or the extent (clinical significance) of such effects.

As the applicant responded that the effects of anti-bevacizumab antibodies on the PK of bevacizumab will be assessed in ongoing or planned clinical studies of bevacizumab, PMDA is asking the applicant to explain about its concrete plan, schedule, and the current status of reevaluation of the ECLA.

4) Pharmacokinetic interactions
In Foreign Study AVF2107g, the mean AUC of SN-38, the active metabolite of irinotecan, was 33% higher in the IFL chemotherapy+bevacizumab group compared to the IFL group and the increased exposure to SN-38 may have been associated with a higher incidence of adverse events $\geq$Grade 3 (diarrhoea, etc.) in the IFL+bevacizumab group than in the IFL group. Thus, PMDA asked the applicant to explain the mechanism of pharmacokinetic interactions between

bevacizumab and SN-38.

The applicant responded as follows:
Study AVF2107g was inadequate to investigate the pharmacokinetic interactions between bevacizumab and irinotecan or SN-38 since the interindividual variability in irinotecan and SN-38 plasma concentrations was high, different patients were studied between the groups due to its parallel-group design, and plasma concentrations were measured only up to 5 hours post-dose. Thus, Study AVF3135g was conducted overseas in order to evaluate the PK of irinotecan and SN-38 when bevacizumab was combined with the FOLFIRI regimen consisting of irinotecan hydrochloride and infusional 5-FU/LV. In this study, 36 patients with solid tumor received the FOLFIRI regimen (intravenous administration of irinotecan hydrochloride 180 mg/m^2) on Day 0, bevacizumab 5 mg/kg on Day 2, and the FOLFIRI regimen + bevacizumab 5 mg/kg on Days 16 and 30, and plasma concentrations of irinotecan and SN-38 were measured on Days 0 and 30. As a result, no differences in irinotecan or SN-38 plasma concentrations over time were observed with or without bevacizumab and the ratio of $AUC_{0\text{-last}}$ (with bevacizumab/without bevacizumab) was 0.99 (90% CI, 0.93-1.07) and 1.01 (90% CI, 0.92-1.11), respectively and bevacizumab did not affect the PK of irinotecan or SN-38.

Therefore, in Study AVF2107g, higher AUC of SN-38 in the IFL+bevacizumab group than in the IFL group may have been causally related to an increased incidence of adverse events ≥Grade 3. However, we think that the AUC of SN-38 in the IFL+bevacizumab group was higher in this study due to the interindividual variability and sparse sampling for the measurement of plasma concentrations, not pharmacokinetic interactions with bevacizumab.

PMDA considers as follows:
In view of the applicant's response that SN-38 concentrations following the FOLFIRI regimen were unaffected by concurrent bevacizumab in the foreign clinical study, clear pharmacokinetic interactions between bevacizumab and SN-38 are unlikely. However, many studies have been conducted to examine the metabolism of irinotecan at the gene level and as ethnic differences in genetic polymorphisms in UGT1A1, a metabolizing enzyme of SN-38, have also been noted (*Drug Metab Dispos* 33: 458-65, 2005, etc.), it is recommended that potential interactions between irinotecan (or its active metabolite, SN-38) and bevacizumab in Japanese subjects should be investigated also at the molecular level.

In a foreign clinical study, the XELOX regimen containing capecitabine, an oral fluoropyrimidine agent, in combination with bevacizumab, has been evaluated. After the market launch of bevacizumab in Japan, the concomitant use of bevacizumab with not only 5-FU injection but also capecitabine (brand name: Xeloda Tablets 300), as fluoropyrimidine agents, can be envisaged in future [Note by the PMDA: capecitabine has not been approved for colorectal cancer], but at present, the effects of concurrent bevacizumab on the PK profile of capecitabine and its metabolites are unclear. Therefore, it should be noted that pharmacokinetic interactions between bevacizumab and capecitabine are unclear and it is necessary to provide information on the results of Japanese and foreign clinical studies evaluating the PK of

capecitabine and its metabolites (Study JO19380, Study NP18587) to medical practice settings promptly.

4.3 Clinical efficacy and safety

As Evaluation Data on efficacy and safety, results of a total of 6 studies including one Japanese phase I study, two foreign phase I studies, two foreign phase II studies, and one foreign phase III study were submitted. As Reference Data, results of one Japanese safety confirmation study, two foreign studies conducted by the Eastern Cooperative Oncology Group (ECOG), two foreign extension studies, and four foreign clinical studies in patients with types of cancer other than colorectal cancer were submitted.

The 5th Investigational Committee for Usage of Unapproved Drugs held on July 22, 2005 reported that "Clinical study data reported to date are all from phase III studies and it is considered that the clinical usefulness of bevacizumab has been confirmed. Therefore, early filing should be made for bevacizumab based on the data from these clinical studies and a Japanese phase I study (the primary evaluations have been completed)" (http://www.mhlw.go.jp/shingi/2005/07/txt/s0727-3.txt), and a safety confirmation study of bevacizumab in combination with FOLFOX4 was initiated and a new drug application was filed while a foreign phase III study in patients with colorectal cancer sponsored by Roche (Study NO16966) was ongoing. The analysis results of this phase III study were submitted after the NDA filing.

Summary of individual clinical studies and the major efficacy results are shown below.

Region	Study Number	Phase	Data category	Study population	Dosage regimen	No. of cases	Major endpoints	Major results
Japan	JO18157	I	Evaluation	Previously treated or untreated advanced or recurrent colorectal cancer	5-FU/l-LV+bev. 3, 5, or 10 mg/kg (3 dose levels), on and after Day 22, 5-FU/*l*-LV+bev. 3, 5, or 10 mg/kg (every 2 weeks)	18 cases	Initial safety, tumor response	There were no adverse drug reactions leading to discontinuation. PR: 2/18 cases SD:14/18 cases PD: 1/18 cases NE: 1/18 cases
	JO18158	#	Reference	Initial treatment of advanced or recurrent colorectal cancer	FOLFOX4+bev. 5 mg/kg (every 2 weeks)	15 cases (as of March 31, 2006)	Safety	Ongoing
Overseas	AVF0737g	I	Evaluation	Various advanced solid tumors	Bev. 0.1, 0.3, 1, 3, or 10 mg/kg (5 dose levels), on and after Day 28, weekly administration	25 cases	Initial safety	No DLT was observed up to 10 mg/kg after weekly administration
	AVF0761g	I		Advanced solid tumors eligible for chemotherapy with either DXR or CBDCA/PTX or 5-FU/LV	DXR, CBDCA/PTX, or 5-FU/LV+bev. 3 mg/kg (weekly administration)	12 cases	Initial safety	11 subjects completed the study except for 1 subject withdrawn due to progressive disease. There were no adverse events leading to discontinuation.

	AVF0780g	II		First-line treatment of metastatic colorectal cancer	5-FU/LV+bev. 5 or 10 mg/kg (every 2 weeks)	5-FU/LV group: 36 cases, 5-FU/LV+bev. 5 mg/kg group: 35 cases, 5-FU/LV+bev. 10 mg/kg group: 33 cases	PFS, RR	PFS assessed by IRF/Investigator Stratified HR: 5 mg/kg group: 0.44, p=0.005, 10 mg/kg group: 0.69, p=0.217
	AVF2192g	II		First-line treatment of metastatic colorectal cancer considered inappropriate for treatment with irinotecan hydrochloride	5-FU/LV±bev. 5 mg/kg (every 2 weeks, up to 96 weeks)	5-FU/LV group: 105 cases, 5-FU+bev. group: 104 cases	OS, PFS	5-FU+ bev. group: Median PFS 9.17 months, HR 0.496, Median OS 16.56 months, HR 0.766
	AVF2107g	III		First-line treatment of metastatic colorectal cancer	IFL±bev. 5 mg/kg (every 2 weeks, up to 96 weeks)	IFL group: 411 cases, IFL+bev. group: 402 cases	OS, PFS	IFL+ bev. group: Median PFS 10.58 months, HR 0.577, Median OS 20.37 months, HR 0.714
	NO16966	III	Reference	First-line treatment of metastatic colorectal cancer	FOLFOX4±bev. 5 mg/kg (every 2 weeks), XELOX±bev. 7.5 mg/kg (every 3 weeks)	2,035 cases	PFS	XELOX (with or without bev.) vs. FOLFOX4 (with or without bev.): HR 1.05 Superiority test for PFS: chemotherapy vs. chemotherapy+bev. HR 0.83 (97.5%CI; 0.72, 0.95), p=0.0023
Overseas	E3200	III	Reference	Advanced or metastatic colorectal cancer previously treated with a fluoropyrimidine and an irinotecan-based regimen used either alone or in combination	FOLFOX4±bev. 10 mg/kg (every 2 weeks, until disease progression)	FOLFOX4 group: 292 cases, FOLFOX +bev. group: 293 cases	OS, PFS	FOLFOX4+bev. group: Median PFS 7.5 months, HR 0.518, Median OS 13.0 months, HR 0.751
	AVF0778g	Extension study		Extension study of phase I and II trials (AVF0737g, AVF0757g, AVF0761g, AVF0775g, AVF0776g, AVF0780g)		56 cases	Long-term safety	The risk of hypertension and proteinuria persisted during the treatment period
	AVF2540g	Extension study		Extension study of phase II and III trials (AVF2107g, AVF2192g, AVF2119g)		105 cases	Long-term safety	The risk of hypertension and proteinuria persisted during the treatment period
	E2200	II		Previously untreated advanced, metastatic colorectal cancer	IFL+ bev. 10 mg/kg (every 2 weeks)	92 cases	PFS	Median PFS: 10.0 months
	AVF0757g	II	Reference / other types of cancer	Non-small cell lung cancer	CBDCA/PTX±bev. 7.5 or 15 mg/kg (every 3 weeks)	99 cases	TTP	TTP (a) CBDCA/PTX group: 129 days (b) CBDCA/PTX+bev. 7.5 mg/kg group: 131 days (c) CBDCA/PTX+bev. 15 mg/kg group: 225 days, (a) vs (c) : p = 0.0234
	AVF0775g	II		Hormone-refractory prostate cancer	bev. 3 or 10 mg/kg (every 2 weeks)	15 enrolled cases	RR	Of the 15 subjects, 5 subjects completed treatment and 10 subjects did not complete treatment: 1 subject was withdrawn due to his/her convenience and 9 subjects were withdrawn due to progressive disease.
	AVF0776g	II		Relapsed metastatic breast cancer after chemotherapy	bev. 3-20 mg/kg (every 2 weeks)	75 cases	RR	Response 5/75 cases MST 10.2 months TTP 2.4 months

	AVF2119g	III		Metastatic breast cancer unresponsive to anthracycline/taxane based chemotherapy	Capecitabine±bev. 15 mg/kg (every 3 weeks)	462 cases (capecitabine alone: 230 cases, capecitabine+bev.: 232 cases)	PFS	Capecitabine group: 4.17 months, capecitabine+bev. group: 4.86 months, HR 0.98, p=0.857

#: Safety confirmation study

5-FU: fluorouracil, LV: calcium folinate, *l*-LV: calcium levofolinate, DXR: doxorubicin hydrochloride, CBDCA: carboplatin, PTX: paclitaxel, HR: Hazard Ratio, OS: Overall Survival, PFS: Progression Free Survival, TTP: Time to Progression, RR: Response ratio

1) Japanese phase I study of bevacizumab in combination with 5-FU/*l*-LV regimen (Study Number JO18157, No publication, Study Period: November 2004 to [redacted] 20[redacted] [Note by the PMDA: The data cutoff date was [redacted] 20[redacted] at the time of regulatory submission])

A multi-center, open-label, dose escalation study was conducted at 3 centers in Japan in order to confirm the initial safety and pharmacokinetics and estimate the recommended clinical dose of bevacizumab when used alone or in combination with 5-FU/*l*-LV in patients with advanced or recurrent colorectal cancer (target number of cases: 6 cases per group, 18 cases in total).

Bevacizumab at a designated dose (3, 5, or 10 mg/kg) was to be administered alone on Day 1 and after 3 weeks (from Day 22), the designated dose of bevacizumab was to be administered every 2 weeks in combination with 5-FU/*l*-LV (5-FU 500 mg/m^2 IV bolus, *l*-LV 250 mg/m^2 IV infusion). During combination therapy, bevacizumab was to be administered every 2 weeks and 5-FU/*l*-LV was to be administered once weekly for 6 weeks, followed by a 2-week rest, per 8-week cycle. Each designated dose was to be tested in 6 patients.

Six patients each in the bevacizumab 3, 5, and 10 mg/kg groups were assessed. Tumor response assessed by the investigator as of the cutoff date ([redacted] 20[redacted], 7 weeks after the last patient in the 10 mg/kg group started study treatment) is shown below.

Tumor response in Study JO18157 (as of the cutoff date)

Dose	CR	PR in	SD	PD	NE	Total
3 mg/kg	–	1	4	1	–	6
5 mg/kg	–	–	6	–	–	6
10 mg/kg	–	1	4	–	1	6
Total	–	2	14	1	1	18

In this study, no death occurred during the study period as of [redacted] 20[redacted] and no patients died up to [redacted] 20[redacted]. The main adverse events other than deaths observed in this study are described in "4.4 Adverse events etc. reported in clinical studies."

2) Japanese safety confirmation study of bevacizumab in combination with FOLFOX4 regimen (Study Number JO18158, No publication, Study Period: [redacted] 2005－Ongoing)

A multi-center, open-label, safety confirmation study to evaluate the safety of bevacizumab in combination with FOLFOX4 in patients with advanced or recurrent colorectal cancer is ongoing at [redacted] centers in Japan (target number of cases: 50).

One cycle consisted of IV infusion of bevacizumab 5 mg/kg (initial treatment) or 10 mg/kg

(previously treated patients) on Day 1, followed by FOLFOX4 (Day 1: IV infusion of oxaliplatin 85 mg/m^2 and *l*-LV 100 mg/m^2 and 5-FU 400 mg/m^2 IV bolus followed by 600 mg/m^2 continuous IV infusion, Day 2: IV infusion of *l*-LV 100 mg/m^2 and 5-FU 400 mg/m^2 IV bolus followed by 600 mg/m^2 continuous IV infusion), and this combination therapy was to be performed every 2 weeks.

At the time of interim data collection (data cutoff date: March 31, 2006), the number of enrolled patients was 15, and out of whom 14 patients (8 patients with colon cancer, 6 patients with rectal cancer) excluding 1 untreated patient (Case No. X9) were included in efficacy and safety analysis. The doses of bevacizumab in the treated cases were all 5 mg/kg.

Among the 14 treated patients, 13 patients had target lesions. The lesions were observed after the start of study treatment in 12 (11 patients with target lesions) out of the 14 treated patients, and 8 patients had PR and 3 patients had SD according to the latest overall assessment by the investigator before the data cutoff date.

In this study, no death occurred as of the data cutoff date (March 31, 2006) and none of the 38 patients treated with the study drug died as of the second data cutoff date ([redacted]) [Note by the PMDA: Later, one patient died due to "renal impairment" on [redacted]]. The main adverse events other than deaths observed in this study are described in "4.4 Adverse events etc. reported in clinical studies."

3) Foreign phase I, dose-escalation study (Study Number AVF0737g, *J Clin Oncol* 2001; 19: 843-50, Study Period: [redacted] 1997 to [redacted] 1997)

A multi-center, open-label, dose-escalation study was conducted at 3 centers overseas in order to evaluate the safety and pharmacokinetics of a single dose and multiple doses of single agent bevacizumab in patients with various advanced solid tumors. This study consisted of a screening period, a treatment period (Days 0-42), and a follow-up period (Days 43-72).

Bevacizumab (0.1, 0.3, 1, 3, or 10 mg/kg) was to be administered on Days 0, 28, 35, and 42.

In this study, no death occurred during the treatment or follow-up period. The main adverse events other than deaths observed in this study are described in "4.4 Adverse events etc. reported in clinical studies."

4) Foreign phase I, dose-escalation study (Study Number AVF0761g, *J Clin Oncol* 2001; 19: 851-6, Study Period: [redacted] 1997 to [redacted] 1998)

A multi-center, open-label, dose-escalation study was conducted at 2 centers overseas in order to evaluate the safety and pharmacokinetics of multiple doses of bevacizumab in combination with chemotherapy in patients with various advanced solid tumors who were eligible for chemotherapy with either DXR or CBDCA/PTX or 5-FU/LV.

In this study, no death occurred during the treatment or follow-up period. The main adverse

events other than deaths observed in this study are described in "4.4 Adverse events etc. reported in clinical studies."

5) Foreign phase II study of bevacizumab in combination with 5-FU/LV regimen (Study Number AVF0780g, *J Clin Oncol* 2003; 21: 60-5, Study Period: June 1998 to [redacted] 19[redacted])

A multi-center, randomized, open-label clinical study was conducted at 8 centers overseas in order to evaluate the efficacy and safety of 5-FU/LV and 5-FU/LV in combination with bevacizumab (5 or 10 mg/kg, every 2 weeks) in chemotherapy-naive patients with metastatic colorectal cancer (target number of cases: 30 cases per group, 90 cases in total).

After 5-FU/LV was administered (5-FU 500mg/m^2 IV bolus and LV 500 mg/m^2 IV infusion, once weekly for 6 weeks, followed by a 2-week rest, per 8-week cycle), bevacizumab 5 or 10 mg/kg was to be administered every 2 weeks for up to a total of 24 doses or until evidence of disease progression. In this study, patients randomized to the 5-FU/LV group were allowed to receive bevacizumab 10 mg/kg upon disease progression.

One hundred four randomized patients (5-FU/LV group: 36 patients, 5-FU/LV+bevacizumab 5 mg/kg group: 35 patients, 5-FU/LV+bevacizumab 10 mg/kg group: 33 patients) were included in the efficacy analysis and 102 patients (5-FU/LV group: 35 patients, 5-FU/LV+bevacizumab 5 mg/kg group: 35 patients, 5-FU/LV+bevacizumab 10 mg/kg group: 32 patients) excluding 2 patients who did not receive study treatment due to progressive disease (1 patient each for the 5-FU/LV group and the 5-FU/LV+bevacizumab 10mg/kg group) were included in the safety analysis. In addition, twenty-two patients in the 5-FU/LV group received bevacizumab (20 patients had progressive disease, 2 patients had no assessment of progression).

The primary efficacy endpoint was Progression Free Survival (hereinafter referred to as PFS) and the median PFS (assessed by the investigator) was 5.4 months in the 5-FU/LV group (control group), 6.8 months in the 5-FU/LV+bevacizumab 5 mg/kg group, and 8.4 months in the 5-FU/LV+bevacizumab 10 mg/kg group, showing a significant prolongation in both the 5-FU/LV+bevacizumab 5 mg/kg group and the 5-FU/LV+bevacizumab 10 mg/kg group, compared to the 5-FU/LV group (control group vs 5 mg/kg group: stratified hazard ratio 0.58, p=0.043, control group vs 10 mg/kg group: stratified hazard ratio 0.53, p=0.027, log-rank test). Whereas, based on the independent review facility (IRF)/investigator assessment (besides 93 cases assessed by the IRF, if data was unavailable for review by the IRF, investigator assessment was used), a significant prolongation was noted in the 5-FU/LV+bevacizumab 5 mg/kg group only (control group vs 5 mg/kg group: stratified hazard ratio 0.44, p=0.005, control group vs 10 mg/kg group: stratified hazard ratio 0.69, p=0.217, log-rank test). The response rates are presented below. IRF assessment was impossible for 3 out of the 96 patients (5 mg/kg group: Case No. X10 and X11, 10 mg/kg group: Case No. X12), since the films for efficacy assessment were not submitted.

	5-FU/LV(Control group) N=36	5-FU/LV+bev. 5 mg/kg N=35	5-FU/LV+bev.10 mg/kg N=33
IRF/Investigator			
Responders	6	14	8
Response rate (95% CI)	17% (7%-34%)	40% (24%-58%)	24% (12%-43%)
p value (χ^2 test)	—	0.03	0.43
Investigator			
Responders	7	12	12
Response rate (95% CI)	19% (9%-37%)	34% (20%- 52%)	36% (21%-55%)
p value (χ^2 test)	—	0.16	0.12

The secondary endpoint was Overall Survival (OS), and the median OS was 13.6 months in the control group, 17.7 months in the 5-FU/LV+bevacizumab 5 mg/kg group, and 15.2 months in the 5-FU/LV+bevacizumab 10 mg/kg group, showing no significant prolongation in the 5 mg/kg group or the 10 mg/kg group compared to the control group (control group vs 5 mg/kg group: stratified hazard ratio 0.52, p=0.073, control group vs 10 mg/kg group: stratified hazard ratio 1.01, p=0.978, log-rank test). All patients were observed for at least 1 year for survival.

Regarding the safety, 50 out of the 104 randomized patients (control group: 19/36 patients, 5 mg/kg group: 12/35 patients, 10 mg/kg group: 19/33 patients) died and the causes of death were progressive disease in 18 patients (including 2 untreated patients) and mucositis/diarrhoea/neutropenia in 1 patient in the control group, progressive disease in 11 patients and respiratory distress in 1 patient in the 5 mg/kg group, and progressive disease in 18 patients and pulmonary embolism in 1 patient in the 10 mg/kg group. It was determined by the investigator that deaths in the control group (cause of death: mucositis/diarrhoea/neutropenia) and the 10 mg/kg group (cause of death: pulmonary embolism) were not caused by the primary disease. Fourteen patients died within 4 weeks after study discontinuation (6 patients in the control group, 4 patients in the 5 mg/kg group, 4 patients in the 10 mg/kg group). The main adverse events other than deaths observed in this study are described in "4.4 Adverse events etc. reported in clinical studies."

6) Foreign phase II study of bevacizumab in combination with 5-FU/LV regimen (Study Number AVF2192g, *J Clin Oncol* 2005; 23: 3697-705, Study Period: August 2000 to [redacted] 20[redacted])

A multi-center, randomized, double-blind, active-controlled, comparative clinical study was conducted at 60 centers overseas in order to evaluate the efficacy and safety of 5-FU/LV vs. bevacizumab in combination with 5-FU/LV in chemotherapy-naive patients with metastatic colorectal cancer who were considered inappropriate for treatment with irinotecan (target number of cases: 100 cases per group, 200 cases in total).

Bevacizumab (5 mg/kg IV infusion, every 2 weeks) or placebo was to be administered for up to a total of 48 doses or until evidence of disease progression, in combination with 5-FU/LV (LV 500 mg/m^2 IV infusion and 5-FU 500 mg/m^2 IV bolus, once weekly for 6 weeks, followed by a 2-week rest per 8-week cycle) (Bevacizumab or placebo was given after 5-FU/LV was administered).

A total of 214 patients were enrolled into the study and of whom, 209 patients excluding 5 patients with violations such as false reporting from the medical institution, were included in the efficacy analysis and 204 patients excluding 5 patients who did not receive study drug (the judgment of the investigator for 2 patients, the patient's wish for 2 patients, unknown reason for 1 patient) were included in the safety analysis.

The primary efficacy endpoint was OS and the median OS as of the data cutoff date ([redacted] 20[redacted]) was 13.24 months in the 5-FU/LV group and 16.56 months in the 5-FU/LV+bevacizumab group, showing no significant prolongation compared to the 5-FU/LV group (hazard ratio 0.766 [95% CI, 0.56-1.05], p=0.0942, log-rank test). The secondary endpoint was Progression Free Survival (PFS) and the median PFS was 5.52 months in the 5-FU/LV group and 9.17 months in the 5-FU/LV＋bevacizumab group, showing a significant prolongation compared to the 5-FU/LV group (hazard ratio 0.496 [95% CI, 0.34-0.73], p=0.0002, log-rank test). The median time to deterioration in QOL as measured by a major QOL scale (FACT-C Colorectal Cancer Subscale score) was 3.02 months in the 5-FU/LV group and 3.12 months in the 5-FU/LV＋bevacizumab group, which demonstrated no between-treatment difference (hazard ratio 0.813 [95% CI, 0.58-1.15], p=0.2381, log-rank test).
Regarding the safety, in this study, 86/104 patients (82.7%) in the 5-FU/LV group and 77/100 patients (77.0%) in the 5-FU/LV＋bevacizumab group died during the treatment period or the follow-up period. The causes of death in the 5-FU/LV group were progressive disease in 71 patients, cardiac disease in 5 patients, infection in 3 patients, diarrhoea in 2 patients, pulmonary embolism in 1 patient, respiratory arrest in 1 patient, suicide in 1 patient, and unknown in 2 patients. The causes of death in the 5-FU/LV+bevacizumab group were progressive disease in 71 patients, haemorrhage in 1 patient, cardiac disease in 1 patient, infection in 2 patients, respiratory failure in 1 patient, and unknown in 1 patient. Serious adverse events with a fatal outcome occurred in 11 patients, which include sepsis in 2 patients, and asthenia, myocardial infarction, atrial fibrillation, hypotension, pulmonary embolism, diarrhoea, haemorrhage, and apnoea, one case each, for the 5-FU/LV group, and sepsis, abscess, peritonitis, myocardial infarction, haemorrhage, and apnoea, one case each for the 5-FU/LV+bevacizumab group. The main adverse events other than deaths observed in this study are described in "4.4 Adverse events etc. reported in clinical studies."

7) Foreign phase III study of bevacizumab in combination with IFL regimen (Study Number AVF2107g, *N Engl J Med* 2004; 350: 2335-42, Study Period: September 2000 to [redacted] 20[redacted] [Data other than survival] or [redacted] 20[redacted] [Survival data])
A multicenter, randomized, comparative study was conducted at 163 centers overseas in order to evaluate the efficacy and safety of IFL and bevacizumab in combination with IFL or 5-FU/LV in the first-line treatment of chemotherapy-naive patients with metastatic colorectal cancer (target number of cases: 400 cases each for Arm 1 and Arm 2 and 100 cases for Arm 3, Total 900 cases).

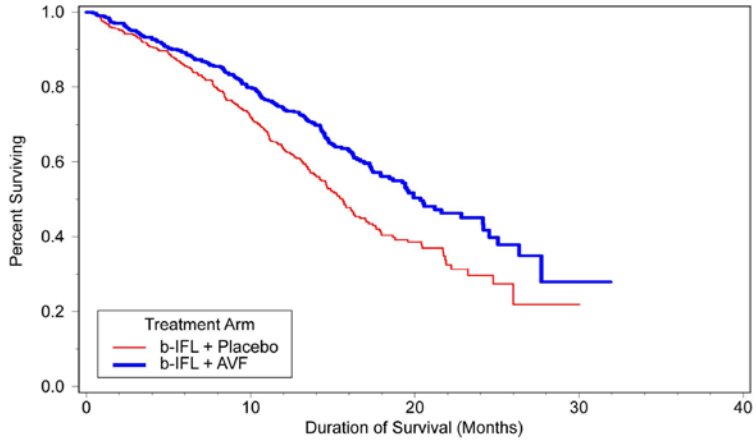
The IFL regimen consisted of irinotecan hydrochloride 125 mg/m^2 IV infusion, 5-FU 500 mg/m^2 IV bolus, and LV 20 mg/m^2 IV bolus, weekly for 4 weeks followed by 2 weeks of rest,

per 6-week cycle. 5-FU/LV was administered based on the Roswell-Park regimen: LV 500 mg/m^2 IV infusion and 5-FU 500 mg/m^2 IV bolus, weekly for 6 weeks followed by 2 weeks of rest, per 8-week cycle. Bevacizumab 5 mg/kg or placebo was to be given by IV infusion every 2 weeks after IFL or 5-FU/LV was administered. The duration of treatment with bevacizumab was up to 96 weeks or until evidence of disease progression. Patients were randomly assigned to one of the treatment groups (Arm 1: IFL+placebo, Arm 2: IFL+bevacizumab, Arm 3: 5-FU/LV+bevacizumab) and the efficacy and safety of Arm 1 and Arm 2 were evaluated in a double-blind manner.

As the safety of bevacizumab in combination with IFL had not been investigated in previous clinical studies, the Independent Data Monitoring Committee (IDMC) instantly evaluated deaths, serious adverse events, and diarrhoea, haemorrhage, and thrombosis ≥Grade 3 in an unblinded fashion until the number of randomized patients in this study reached 300 (100 patients/group). When the number of randomized patients reached 300, the IDMC reviewed all adverse events, the patients' conditions, study drug administration, clinical laboratory values, and vital signs and performed safety interim analysis. As a result, the IDMC judged that the safety of Treatment Arm 2 was acceptable, discontinued enrollment to Arm 3 in accordance with the protocol, and recommended that patient recruitment should be continued until the total number of enrolled patients reach 400 each for Arm 1 and Arm 2. Then, when 193 deaths had been reported, safety and efficacy interim analysis was performed. By the time of analysis, 925 patients had been randomized. Interim analysis showed a trend towards overall survival prolongation in Arm 2 compared to Arm 1 (p=0.003, stratified log-rank test), which exceeded p=0.0018, i.e. the early stopping criterion for efficacy. As a result of safety and efficacy review, the IDMC recommended that this study should be continued until the planned final analysis without protocol amendments.

A total of 925 patients were enrolled. Of whom, 923 patients (Arm 1: 411 patients, Arm 2: 402 patients, Arm 3: 110 patients) excluding 2 patients with violations such as false reporting from the medical institution (Case No. X13 and X14) were randomized and included in the efficacy analysis, and 898 patients (Arm 1: 397 patients, Arm 2: 392 patients, Arm 3: 109 patients) excluding 25 patients who did not receive first-line treatment (Arm 1: 14 patients, Arm 2: 10 patients, Arm 3: 1 patient) were included in the safety analysis. The primary endpoint was OS, and survival time for patients surviving at the time of analysis was censored at the last date they were confirmed to be alive. The median follow-up for Arm 1 (IFL+placebo) and Arm 2 (IFL+bevacizumab) was 21 months.

Regarding the efficacy, the median OS as of the data cutoff date ([redacted] 20[redacted]) was 15.6 months in Arm 1 and 20.3 months in Arm 2, showing a significant prolongation compared to Arm 1 (stratified hazard ratio 0.660 [95% CI, 0.54-0.81], p<0.0001, log-rank test). Additionally submitted data (cutoff date: [redacted] 20[redacted]) also showed that the median OS was 15.80 months in Arm 1 and 20.37 months in Arm 2, which confirmed that IFL in combination with bevacizumab results in significant prolongation as compared to IFL alone (hazard ratio 0.714 [95% CI, 0.61-0.84], p<0.0001, log-rank test).



Overall survival in Study AVF2107g including chemotherapy-naive patients with metastatic colorectal cancer (data cutoff date: [redacted] 20[redacted], prepared by the applicant, AVF: Bevacizumab)

The secondary endpoint was PFS and the median PFS was 6.24 months in Arm 1 and 10.55 months in Arm 2 (stratified hazard ratio 0.544 [95% CI, 0.45-0.66], p<0.0001, log-rank test), the response rate was 34.8% and 44.8%, respectively (p=0.0036, χ^2 test), and the duration of response was 7.06 months and 10.35 months, respectively (p＝0.0014, log-rank test). However, the time to deterioration in QOL as measured by Colorectal Cancer Subscale (CCS) during first-line treatment was 2.73 months in Arm 1 and 2.89 months in Arm 2 (hazard ratio 0.916 [95% CI, 0.67-1.25], p=0.5807, log-rank test).

According to additionally submitted data (cutoff date: [redacted] 20[redacted]), the PFS was 6.28 months vs. 10.58 months (hazard ratio 0.577 [95% CI, 0.48-0.69], p<0.0001, log-rank test), the response rate was 34.8% vs. 45.0% (p=0.0029, χ^2 test), and the duration of response was 7.13 months vs. 10.84 months (p＝0.0016, log-rank test).

As to the safety, 307/396 patients (77.3%) in Arm 1, 268/392 patients (68.4%) in Arm 2, and 86/109 patients (78.9%) in Arm 3 died during the study period or the follow-up period. The causes of death were progressive disease in 283 patients, infection in 8 patients, others in 5 patients, unknown in 5 patients, cardiac disease in 3 patients, and pulmonary embolism in 3 patients for Arm 1, progressive disease in 247 patients, infection in 7 patients, others in 6 patients, cardiac disease in 3 patients, unknown in 3 patients, haemorrhage in 1 patient, and pulmonary embolism in 1 patient for Arm 2, progressive disease in 75 patients, cardiac disease in 3 patients, others in 3 patients, infection in 2 patients, unknown in 2 patients, and pulmonary embolism in 1 patient for Arm 3. Deaths within 30 days after the last dose occurred in 27/396 patients (6.8%) of Arm 1, 29/392 patients (7.4%) of Arm 2, and 12/109 patients (11.0%) of Arm 3 and the causes of death were progressive disease in 16 patients, infection in 5 patients, cardiac disease in 3 patients, pulmonary embolism in 1 patient, others in 1 patient, and unknown in 1 patient for Arm 1, progressive disease in 19 patients, infection in 4 patients, cardiac disease in 2 patients, haemorrhage in 1 patient, pulmonary embolism in 1 patient, others in 1 patient, and unknown in 1 patient for Arm 2, progressive disease in 5 patients, cardiac disease in 3 patients, others in 2 patients, pulmonary embolism in 1 patient, and unknown in 1 patient for Arm 3.

Serious adverse events with a fatal outcome occurred in 11 patients of Arm 1 (sepsis 3 cases, peritonitis 1 case, myocardial infarction 2 cases, pulmonary embolism 2 cases, congestive heart failure 1 case, heart failure 1 case, hypotension 1 case, diarrhoea 1 case, leukopenia 1 case) and 13 patients of Arm 2 (sepsis 3 cases, fungal infection 1 case, infection [necrotising peritonitis developed after gastrointestinal perforation] 1 case, myocardial infarction 2 cases, pulmonary embolism 1 case, cerebrovascular accident 1 case, cardiac arrest 1 case, subarachnoid haemorrhage 1 case, diarrhoea 1 case, intestinal necrosis 1 case, intestinal obstruction 1 case, large intestine perforation 1 case, hypovolaemia 1 case). The main adverse events other than deaths observed in this study are described in "4.4 Adverse events etc. reported in clinical studies."

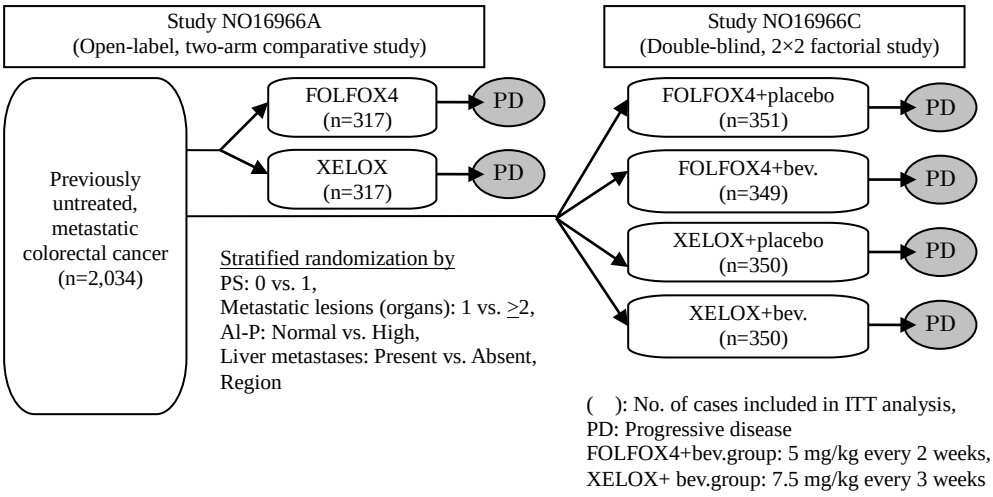
8) Foreign phase III study of bevacizumab in combination with FOLFOX4 regimen or XELOX regimen (Study Number NO16966, No publication, Study Period: [redacted] 2003－Ongoing)

A multinational, randomized, comparative study is ongoing at 218 centers overseas in order to evaluate the efficacy and safety of FOLFOX4, bevacizumab in combination with FOLFOX4, XELOX, and bevacizumab in combination with XELOX, in chemotherapy-naive patients with metastatic colorectal cancer. Although this study was originally initiated as a randomized, open-label study evaluating the efficacy and safety of FOLFOX4 vs. XELOX in chemotherapy-naive patients with metastatic colorectal cancer (Study NO16966A), the protocol was later amended and a randomized, 2x2 factorial, double-blind study (Study NO16966C) evaluating the efficacy and safety of FOLFOX4+either bevacizumab or placebo and XELOX+either bevacizumab or placebo was added. The primary objective of this study was to confirm the following as measured by PFS: (a) Non-inferiority of all groups containing XELOX (Overall XELOX group: XELOX group, XELOX+placebo group and XELOX+bevacizumab group) over all groups containing FOLFOX4 (Overall FOLFOX4 group: FOLFOX4 group, FOLFOX4+placebo group and FOLFOX4+bevacizumab group) and (b) Superiority of the treatment groups with bevacizumab (XELOX+bevacizumab group and FOLFOX4+bevacizumab group) over the treatment groups without bevacizumab (XELOX+placebo group and FOLFOX4+placebo group). A total of 6 treatment groups were included in the study and the dosing schedules in these groups are shown below.

Regimen	Dosing schedule
XELOX (XELOX group)	Oxaliplatin 130 mg/m^2 given as an IV infusion on Day 1 (every 3 weeks), capecitabine 1000 mg/m^2 orally administered twice daily (a 2-week treatment followed by a 1-week rest) One cycle is 3 weeks, up to 16 cycles (48 weeks)
FOLFOX4 (FOLFOX4 group)	Oxaliplatin 85 mg/m^2 given as an IV infusion on Day 1 (every 2 weeks), LV 200 mg/m^2 given as an IV infusion followed by 5-FU 400 mg/m^2 IV bolus and 600 mg/m^2 given as an IV infusion on Days 1 and 2 One cycle is 2 weeks, up to 24 cycles (48 weeks)
XELOX+placebo (XELOX+P group)	Placebo given as an IV infusion, oxaliplatin 130 mg/m^2 given as an IV infusion on Day 1 (every 3 weeks), capecitabine 1000 mg/m^2 orally administered twice daily (a 2-week treatment followed by a 1-week rest) One cycle is 3 weeks, up to 16 cycles (48 weeks)
FOLFOX4+placebo (FOLFOX4+P group)	Placebo given as an IV infusion, oxaliplatin 85 mg/m^2 given as an IV infusion on Day 1 (every 2 weeks), LV 200 mg/m^2 given as an IV infusion followed by 5-FU 400 mg/m^2 IV bolus and 600 mg/m^2 given as an IV infusion on Days 1 and 2

	One cycle is 2 weeks, up to 24 cycles (48 weeks)
XELOX+bevacizumab (XELOX+bevacizumab group)	Bevacizumab 7.5 mg/m^2 given as an IV infusion, oxaliplatin 130 mg/m^2 given as an IV infusion on Day 1 (every 3 weeks), capecitabine 1000 mg/m^2 orally administered twice daily (a 2-week treatment followed by a 1-week rest) One cycle is 3 weeks, up to 16 cycles (48 weeks)
FOLFOX4+bevacizumab (FOLFOX4+ bevacizumab group)	Bevacizumab 5 mg/m^2 given as an IV infusion, oxaliplatin 85 mg/m^2 given as an IV infusion on Day 1 (every 2 weeks), LV 200 mg/m^2 given as an IV infusion followed by 5-FU 400 mg/m^2 IV bolus and 600 mg/m^2 given as an IV infusion on Days 1 and 2 One cycle is 2 weeks, p to 24 cycles (48 weeks)

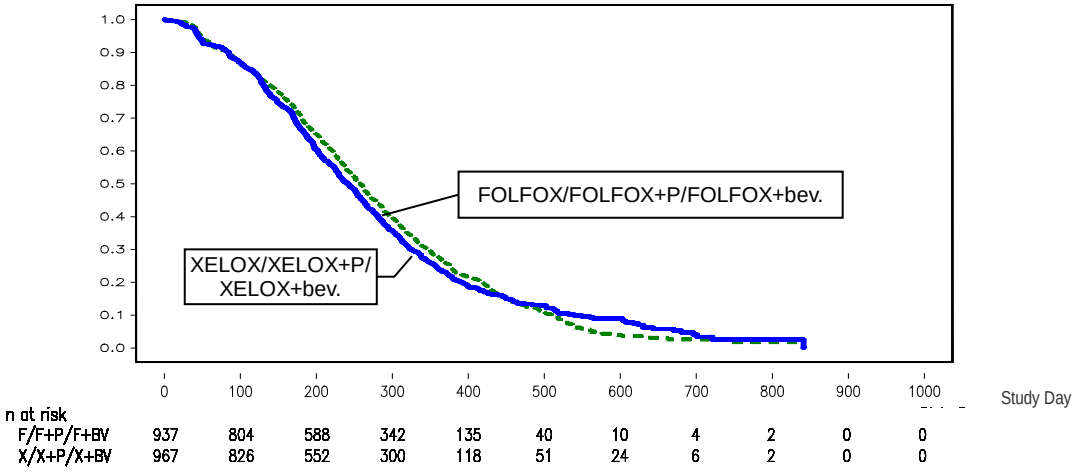
In Study NO16966A (hereinafter referred to as Study A), 317 patients were randomized to the XELOX group and 317 patients to the FOLFOX4 group. In Study NO16966C (hereinafter referred to as Study C), 350 patients were randomized to the XELOX+placebo group, 351 patients to the FOLFOX4+placebo group, 350 patients to the XELOX+bevacizumab group, and 349 patients to the FOLFOX4+bevacizumab group (total 2,034 patients).



Regarding the efficacy, the median PFS in the overall XELOX group (with or without bevacizumab) and the overall FOLFOX4 group (with or without bevacizumab) was 241 days and 259 days, respectively, and the hazard ratio in the overall XELOX group relative to the overall FOLFOX4 group was 1.05 (97.5% CI, 0.94-1.18). Since the upper limit of the 97.5% confidence interval was less than the predefined non-inferiority margin of 1.23, the non-inferiority of XELOX (with or without bevacizumab) over FOLFOX4 (with or without bevacizumab) was demonstrated. PFS data and a Kaplan-Meier plot for the overall XELOX group (with or without bevacizumab) and the overall FOLFOX4 group (with or without bevacizumab) are shown below.

Results of non-inferiority test for PFS

Positioning of analysis	Treatment group	Median PFS (No. of events)	Hazard ratio (97.5% CI)
Primary analysis	Overall FOLFOX4 group (with or without bevacizumab) [FOLFOX4 group, FOLFOX4+placebo group and FOLFOX4+bevacizumab group]	259 days (768)	HR=1.05 (0.94-1.18)
	Overall XELOX group (with or without bevacizumab) [XELOX group, XELOX+placebo group and XELOX+bevacizumab group]	241 days (779)	
Secondary analysis	FOLFOX4+placebo group (without bevacizumab)	241 days (530)	HR=1.06 (0.92-1.22)
	XELOX +placebo group (without bevacizumab)	220 days (529)	
Exploratory analysis	FOLFOX4+bevacizumab group	285 days (238)	HR=1.04 (0.84-1.27)
	XELOX+bevacizumab group	281 days (250)	

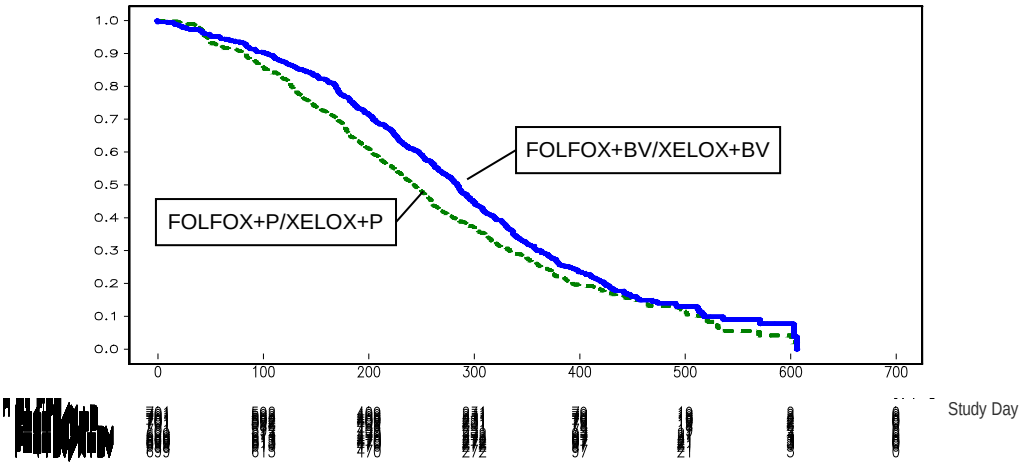


PFS (XELOX group [with or without bevacizumab] and FOLFOX4 group [with or without bevacizumab])
(prepared by the applicant, P: placebo)

The median PFS in the chemotherapy (FOLFOX4 or XELOX)+bevacizumab group and the chemotherapy (FOLFOX4 or XELOX)+placebo group was 285 days and 244 days, respectively, the hazard ratio in the chemotherapy+bevacizumab group relative to the chemotherapy+placebo group was 0.83 (97.5% CI, 0.72-0.95; p=0.0023; log-rank test), and the superiority of the chemotherapy+bevacizumab group over the chemotherapy+placebo group was demonstrated. PFS data and a Kaplan-Meier plot for the chemotherapy+placebo group and the chemotherapy+bevacizumab group are shown below. Subgroup analysis did not show the superiority of the FOLFOX4+bevacizumab group over the FOLFOX4+placebo group (hazard ratio 0.89 [97.5% CI, 0.73-1.08; p=0.1871; log-rank test]).

Results of superiority test for PFS

Positioning of analysis	Treatment group	Median PFS (No. of events)	Hazard ratio [97.5% CI] p value (log-rank test)
Primary analysis	Chemotherapy+placebo group [FOLFOX4+P and XELOX+P]	244 days (547)	HR=0.83 [0.72-0.95] p=0.0023
	Chemotherapy+bevacizumab group [FOLFOX4+bevacizumab and XELOX+bevacizumab]	285 days (513)	
Secondary analysis	XELOX+placebo group	225 days (270)	HR=0.77 [0.63- 0.94] p=0.0026
	XELOX+bevacizumab group	282 days (258)	
Secondary analysis	FOLFOX4+placebo group	261 days (277)	HR=0.89 [0.73-1.08] p=0.1871
	FOLFOX4+bevacizumab group	286 days (255)	



PFS (chemotherapy +bevacizumab group vs. chemotherapy+placebo group)
(prepared by the applicant, P: placebo, BV: bevacizumab)

In this study, treatment-related death within 28 days after the last dose occurred in 5/336 patients (1.5%) in the FOLFOX4+placebo group, 6/341 patients (1.8%) in the FOLFOX4+bevacizumab group, 5/339 patients (1.5%) in the XELOX+placebo group, and 8/353 patients (2.3%) in the XELOX+bevacizumab group.

	FOLFOX4+placebo N= 336 n, (%)	FOLFOX4+bevacizumab N=341 n, (%)	XELOX+placebo N=339 n, (%)	XELOX+bevacizumab N=353 n, (%)
Treatment-related death	7 (2.1%)	7 (2.1%)	6 (1.8%)	8 (2.3%)
Death within 28 days after the last dose	7 (2.1%)	14 (4.1%)	9 (2.7%)	19 (5.4%)
Treatment-related death within 28 days after the last dose	5 (1.5%)	6 (1.8%)	5 (1.5%)	8 (2.3%)

The main adverse events other than deaths observed in this study are described in "4.4 Adverse events etc. reported in clinical studies."

9) Foreign phase II study of bevacizumab in combination with IFL regimen (Study Number E2200, *Ann Oncol* 17: 1399-403, 2006, Study Period: November 2000 to February 2002)

A multicenter, open-label, clinical study was conducted at 16 centers overseas in order to evaluate the efficacy and safety of bevacizumab in combination with IFL in patients with previously untreated advanced, metastatic colorectal cancer (target number of cases: 55).

Although the Saltz regimen of IFL was initially employed (irinotecan hydrochloride 125 mg/m^2 IV infusion, 5-FU 500 mg/m^2 IV bolus, and LV 20 mg/m^2 IV bolus administered once weekly for 4 weeks followed by 2 weeks of rest per 6-week cycle), the protocol was amended due to a safety problem reported from other clinical studies using the Saltz regimen and after [redacted]

20[redacted] during the study, the doses of irinotecan hydrochloride and 5-FU were to be reduced (irinotecan hydrochloride 100 mg/m^2, 5-FU 400 mg/m^2 and LV 20 mg/m^2) and bevacizumab 10 mg/kg was to be administered concurrently every 2 weeks.

A total of 92 patients were enrolled into the study and of whom, 87 patients excluding 5 untreated patients were included in the safety analysis and 81 patients excluding 6 ineligible patients were included in the efficacy analysis. The first 20 patients received the Saltz regimen of IFL and 72 patients enrolled after the protocol amendment received reduced dose IFL.

Recurrence or progressive disease was observed in 53 out of the 81 patients, and the median PFS (the primary endpoint) was 10.0 months (95% CI, 8.4 months-12.2 months). As of the date of preparation of the report ([redacted] 20[redacted]), overall survival (median) has not been available.

Regarding the safety, one treatment-related death due to cerebrovascular ischemia was reported and its causal relationship to bevacizumab could not be denied. In addition, one death due to bowel perforation was reported. The main adverse events other than deaths observed in this study are described in "4.4 Adverse events etc. reported in clinical studies."

10) Foreign phase III study of bevacizumab in combination with FOLFOX4 regimen (Study Number E3200, No publication, Study Period: [redacted] 20[redacted] to [redacted] 20[redacted])

A randomized, open-label, clinical study was conducted at 220 centers overseas by the Eastern Cooperative Oncology Group (ECOG) in order to evaluate the efficacy and safety of FOLFOX4, FOLFOX4 in combination with bevacizumab, and bevacizumab alone in patients with previously treated (a fluoropyrimidine and an irinotecan hydrochloride-based regimen used either alone or in combination), advanced or metastatic colorectal cancer (target number of cases: approximately 293 cases per group, total 880 cases).

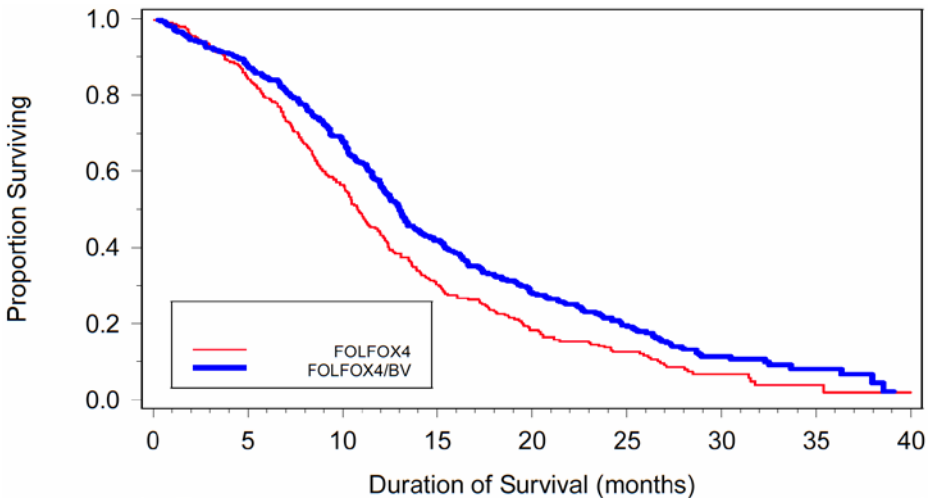
FOLFOX4 (Oxaliplatin 85 mg/m^2 on Day 1, LV 200 mg/m^2 IV infusion and 5-FU 400 mg/m^2 IV bolus followed by 600 mg/m^2 continuous IV infusion on Days 1 and 2) was to be administered every 2 weeks and bevacizumab 10 mg/kg was to be given as an IV infusion every 2 weeks.

In order to evaluate the initial safety, the enrollment was suspended when 50 patients had been entered into each group, as per the predefined rule. The initial safety was assessed by the ECOG Independent Data Monitoring Committee (ECOG IDMC) and as a result, no specific protocol amendments were required and case accrual was resumed. Prior to the 1st interim efficacy analysis, the ECOG IDMC pointed out that the OS in the bevacizumab alone group may be shorter compared to the other treatment groups and enrollment to the bevacizumab alone group was discontinued. Then, when 327 deaths had been reported, the 1st interim efficacy analysis was performed and the ECOG IDMC recommended the continuation of the study. Furthermore, when 416 deaths had been reported, the second interim analysis was performed and the FOLFOX4+bevacizumab group had a significantly longer OS than the FOLFOX4 group (hazard ratio 0.74, p=0.0024, log-rank test). The p-value was less than 0.0097, the predefined

criterion.

In the end, efficacy assessments were performed for 292 patients in the FOLFOX4 group, 293 patients in the FOLFOX4+bevacizumab group, and 244 patients in the bevacizumab alone group.

The primary efficacy endpoint was OS and the median OS was 10.8 months in the FOLFOX4 group and 13.0 months in the FOLFOX4+bevacizumab group, showing a significant prolongation compared to FOLFOX4 (stratified hazard ratio 0.751 [95% CI, 0.632-0.893], p=0.0012, log-rank test). The median OS in the bevacizumab alone group was 10.2 months. Regardless of baseline risk factors (radiation therapy, age, gender, race, ECOG Performance Status, the number of metastatic organs, baseline CEA value, a sum of the longest diameters of all target lesions), there was a trend towards a longer OS in the FOLFOX4+bevacizumab group compared to the FOLFOX4 group.



Overall survival in Study E3200 (prepared by the applicant, BV: bevacizumab)

The median PFS (the secondary endpoint) was 4.5 months in the FOLFOX4 group and 7.5 months in the FOLFOX4+bevacizumab group, showing that combination with bevacizumab resulted in a significant prolongation (stratified hazard ratio 0.518 [95% CI, 0.42-0.65], p<0.0001, log-rank test). The median PFS in the bevacizumab alone group was 2.5 months.

The response rate (the percentage of patients who had a complete response or a partial response as their best response) was 3.3% in the bevacizumab alone group, 8.6% in the FOLFOX4 group, and 22.2% in the FOLFOX4+bevacizumab group, whereas the duration of response was 5.7 months, 6.0 months, and 6.2 months, respectively.

Regarding the safety, 259/285 patients (90.9%) in the FOLFOX4 group, 254/287 patients (88.5%) in the FOLFOX4+bevacizumab group, and 211/234 patients (90.2%) in the bevacizumab alone group died during the study period or the follow-up period. The causes of death were progressive disease in 208 patients, others in 8 patients, unknown in 7 patients, and

undocumented in 36 patients for the FOLFOX4 group, progressive disease in 197 patients, progressive disease and sepsis syndrome/febrile neutropenia associated with study drug in 1 patient, others in 7 patients, unknown in 7 patients, and undocumented in 42 patients for the FOLFOX4+bevacizumab group, and progressive disease in 161 patients, others in 7 patients, unknown in 7 patients, and undocumented in 36 patients for the bevacizumab alone group.

Death within 30 days after the protocol-specified administration occurred in 11/285 patients (3.9%) in the FOLFOX4 group, 18/287 patients (6.3%) in the FOLFOX4+bevacizumab group, and 20/234 patients (8.5%) in the bevacizumab alone group.

Adverse events with a fatal outcome for which a causal relationship to the study drug could not be denied were not noted in the FOLFOX4 group, were FEV_1 decreased, pneumonitis/lung infiltration, and CNS haemorrhage, one case each, in the FOLFOX4+bevacizumab group, and hypoxia, CNS haemorrhage, myocardial ischaemia, wound infection, and bilirubin increased in the bevacizumab alone group. The main adverse events other than deaths observed in this study are described in “4.4 Adverse events etc. reported in clinical studies.”

The following two foreign extension studies of bevacizumab were submitted as Reference Data.

11) Foreign extension study (Study Number AVF2540g, No publication, Study Period: [redacted] 20[redacted] to [redacted] 20[redacted])

A multicenter, open-label, extension study was conducted at 56 centers overseas in order to evaluate the safety of an optional extended treatment with bevacizumab. Patients who completed bevacizumab therapy in a randomized comparative clinical study (AVF2107g, AVF2192g, or AVF2119g, hereinafter referred to as the parent study) or patients who received placebo in Study AVF2107g or Study AVF2192g were eligible for inclusion in this study.

Subjects received either the same dose as the parent protocol or a pharmacologically comparable dose (in mg/kg/week), based on the pretreatment screening body weight in the current study. The duration of treatment was up to 2 years.

All of the 104 treated subjects were included in the safety analysis. In this study, 20 subjects died and their causes of death were all progressive disease. The main adverse events other than deaths observed in this study are described in “4.4 Adverse events etc. reported in clinical studies.”

12) Foreign extension study (Study Number AVF0778g, *Proc. Soc. Oncol.* 2002; 21: 9a, Study Period: [redacted] 19[redacted] to [redacted] 20[redacted])

A multicenter, open-label, extension study was conducted at 12 centers overseas in order to provide an optional extension of bevacizumab treatment and evaluate the safety of long-term treatment with bevacizumab. Fifty-six subjects who received bevacizumab therapy in a phase I or phase II study (AVF0737g, AVF0757g, AVF0761g, AVF0775g, AVF0776g, or AVF0780g) and who did not show evidence of disease progression at completion of the parent study were included in the study.

The dose of bevacizumab administered was that given in the parent study or a pharmacologically comparable dose (equivalent on a mg/kg/week basis). Treatment with bevacizumab was divided into two 1-year periods (Treatment Periods 1 and 2), separated by an observation period of 6 months, during which no bevacizumab was administered.

All of the 56 subjects enrolled into the study were included in the analysis. In this study, 7 subjects died. Their causes of death were progressive disease in 6 subjects and sepsis associated with neutropenia in 1 subject. The subject who developed sepsis associated with neutropenia and one of the 6 subjects with progressive disease died within 30 days after the last dose of bevacizumab. The main adverse events other than deaths observed in this study are described in "4.4 Adverse events etc. reported in clinical studies."

Data of the following four foreign studies in patients with types of cancer other than colorectal cancer were submitted as Reference Data.

13) Foreign phase III study of bevacizumab in combination with capecitabine in patients with previously treated metastatic breast cancer (Study Number AVF2119g, *J Clin Oncol* 2005; 23: 792-9, Study Period: November 2000 to [redacted] 2002)

A multicenter, open-label, randomized, comparative clinical study in 462 patients with previously treated metastatic breast cancer was conducted at 96 centers overseas in order to evaluate the safety of multiple doses of bevacizumab (15 mg/kg every 3 weeks) in combination with capecitabine compared with capecitabine alone.

In this study, 11/215 patients (5.1%) in the capecitabine group and 11/229 patients (4.8%) in the capecitabine+bevacizumab group died. The causes of death were progressive disease in 9 patients and adverse events in 2 patients (cardiac arrest, presumably pulmonary embolism) for the capecitabine group and progressive disease in 10 patients and adverse event in 1 patient (sepsis) for the capecitabine+bevacizumab group. In the capecitabine+bevacizumab group, 8/70 patients who had progressed and continued the study with bevacizumab alone or other chemotherapy died during the continuation of the study and their causes of death were all progressive disease. The main adverse events other than deaths observed in this study are described in "4.4 Adverse events etc. reported in clinical studies."

14) Foreign phase II study of bevacizumab in combination with CBDCA/PTX in patients with advanced, non-small cell lung cancer (Study Number AVF0757g, *J Clin Oncol* 2004; 22: 2184-91, Study Period: [redacted] 19[redacted] to [redacted] 19[redacted])

A multicenter, open-label, randomized, comparative clinical study in 98 patients with locally advanced or metastatic (Stage IIIb/IV) or recurrent non-small cell lung cancer was conducted at 12 centers overseas in order to evaluate the efficacy and safety of multiple doses of bevacizumab (7.5 or 15 mg/kg every 3 weeks) in combination with CBDCA/PTX compared with CBDCA/PTX alone.

Ninety-nine patients were enrolled into the study and 98 patients excluding 1 patient who was found to have brain metastases before the start of treatment and did not receive study drug were included in the safety analysis.

During the study period, 9 deaths due to adverse events that were not directly related to disease progression (CBDCA/PTX group: 1/32 patients, CBDCA/PTX+bevacizumab 7.5 mg/kg group: 4/32 patients, CBDCA/PTX+bevacizumab 15 mg/kg group: 4/34 patients) occurred and a causal relationship to bevacizumab could not be denied for 5 out of the 8 patients treated with bevacizumab (Case No. X15, X16, X17, X18, X19) and these 5 deaths were all associated with haemorrhage. The main adverse events other than deaths observed in this study are described in "4.4 Adverse events etc. reported in clinical studies."

15) Foreign phase II study in patients with relapsed metastatic breast cancer (Study Number AVF0776g, No publication, Study Period: [redacted] 19[redacted] to [redacted] 20[redacted])

A multicenter, open-label, clinical study in 75 patients with previously treated metastatic breast cancer was conducted at 96 centers overseas in order to evaluate the efficacy and safety of single agent bevacizumab (3, 10, or 20 mg/kg every 2 weeks).

All of the 75 patients enrolled into the study were included in the safety analysis (3 mg/kg group: 18 patients, 10 mg/kg group: 41 patients, 20 mg/kg group: 16 patients). There were no deaths related to study drug. Forty-seven patients (63%) died due to progressive disease (3 mg/kg group: 12 patients, 10 mg/kg group: 24 patients, 20 mg/kg group: 11 patients). The main adverse events other than deaths observed in this study are described in "4.4 Adverse events etc. reported in clinical studies."

16) Foreign phase II study in patients with prostate cancer unresponsive to endocrine therapy (Study Number AVF0775g, No publication, Study Period: [redacted] 19[redacted] to [redacted] 19[redacted])

An open-label clinical study (designed as a multicenter study) in 15 patients with prostate cancer unresponsive to endocrine therapy was conducted at one center overseas in order to evaluate the efficacy and safety of single agent bevacizumab (3, 10, or 20 mg/kg every 2 weeks). Nine out of the 15 patients were discontinued due to disease progression. No death occurred during the study period. The main adverse events other than deaths observed in this study are described in "4.4 Adverse events etc. reported in clinical studies."

Outline of review by the PMDA

1) The efficacy in patients with advanced or recurrent colorectal cancer who are not candidates for curative resection

PMDA reviewed the results of clinical studies submitted as follows and as a result, judged that the clinical efficacy of the addition of bevacizumab to a conventional chemotherapy regimen in patients with advanced or recurrent colorectal cancer who are not candidates for curative resection has been demonstrated. However, a further investigation is needed to determine the optimal chemotherapeutic regimen to be combined with bevacizumab in first-line treatment.

(1) Previously untreated patients

PMDA considers that among the clinical studies involving previously untreated patients with advanced or recurrent colorectal cancer who are not candidates for curative resection (first-line treatment), the key studies showing the efficacy of bevacizumab are Foreign Studies AVF2107g and NO16966.

At the time of the NDA filing, Study NO16966 was ongoing and the results of analysis of this study were later submitted as "a prompt report on the final analysis results" on [redacted] 20[redacted].

(a) Study AVF2107g

This study was the primary evidence for the efficacy of bevacizumab in the first-line treatment of unresectable advanced or recurrent colorectal cancer, for filing a NDA for bevacizumab in the US and Europe.

The study demonstrated the add-on effect of bevacizumab in combination with IFL in terms of OS and PFS (OS: stratified hazard ratio 0.660, $p<0.0001$; PFS: stratified hazard ratio 0.544, $p<0.0001$).

PMDA checked the status of second-line treatment for interpreting OS data from the study since the proportion of subjects who entered second-line treatment during the study period was smaller in the IFL+placebo group than in the IFL+bevacizumab (IFL+placebo group: 12.9%, IFL+bevacizumab group: 35.6%). As a result, it was found that many of the subjects who had progressed in the IFL+bevacizumab group received second-line treatment on study (the study period) in order to continue treatment with bevacizumab while many of the subjects who had progressed in the IFL+placebo group received second-line treatment off study (the follow-up period) requiring less observations/examinations compared to on study. It was confirmed that the proportion of subjects who received any chemotherapy as second-line treatment irrespective of on study or off study, i.e. during the study period or the follow-up period, was similar between the two groups (IFL+placebo group: 64.5% [265/411 subjects], IFL+bevacizumab group: 69.4% [279/402 subjects]).

PMDA judged that the results of this study has demonstrated the add-on effect of bevacizumab in combination with IFL and the efficacy in first-line treatment.

(b) Study NO16966

PMDA judged that the results of this study has demonstrated the add-on effect of bevacizumab in combination with chemotherapy (FOLFOX4 or XELOX), as measured by PFS, in the first-line treatment of unresectable advanced or recurrent colorectal cancer.

However, subgroup analysis assessing the add-on effect of bevacizumab in combination with each chemotherapy regimen showed that the add-on effect of bevacizumab in combination with XELOX was significant while the add-on effect of bevacizumab in combination with FOLFOX4 was not significant (See the table below).

The results of superiority test for PFS

Positioning of analysis	Treatment group	Median PFS (No. of events)	Hazard ratio [97.5% CI] p value (log-rank test)
Primary analysis	Chemotherapy+placebo group [FOLFOX4+placebo/XELOX+placebo]	244 days (547)	HR=0.83 [0.72-0.95] p=0.0023
	Chemotherapy+bevacizumab group [FOLFOX4+bevacizumab/XELOX+bevacizumab]	285 days (513)	
Secondary analysis	XELOX+placebo group	225 days (270)	HR=0.77 [0.63-0.94] p=0.0026
	XELOX+bevacizumab group	282 days (258)	
	FOLFOX4+placebo group	261 days (277)	HR=0.89 [0.73-1.08] p=0.1871
	FOLFOX4+bevacizumab group	286 days (255)	

In light of the results of the subgroup analysis, PMDA asked for the applicant's view on the add-on effect of bevacizumab in combination with FOLFOX4.

The applicant performed the following additional analyses for Study NO16966 and explained that the most preferred chemotherapeutic regimen to be combined with bevacizumab is FOLFOX4.

i) The proportion of subjects who had been continuously treated with bevacizumab also during one month prior to confirming PD was 77% in Study AVF2107g compared to 45% in this study, which is one of the reasons why bevacizumab could not exert its full effect in this study.
ii) When the definition of an event for PFS for this study (first progression [or death] from the initiation of study treatment) was changed to the definition of an event for Study AVF2107g (progressive disease [or death] within 28 days from the last dose of study drug), the hazard ratio was 0.63 ($p<0.0001$).
iii) Within this study, the FOLFOX4 group from Study NO16966A (an open-label, comparative study of XELOX vs. FOLFOX4) and the FOLFOX4+placebo group from Study NO16966C (a multinational, 2x2 factorial, randomized, double-blind study of FOLFOX4+placebo, FOLFOX4+bevacizumab, XELOX4+placebo, and XELOX4+bevacizumab) combined were compared with the FOLFOX4+bevacizumab group based on PFS. As a result, the hazard ratio was 0.82 ($p=0.008$), showing a significant add-on effect of bevacizumab.
iv) The median PFS in 85 patients with "post-operative adjuvant chemotherapy" in the FOLFOX4+placebo group was 335 days, which was exceptionally high. In order to find out its cause, the patient background was examined. The background factors of patients with "post-operative adjuvant chemotherapy" were compared among the different groups. As a result, the time from the initial diagnosis of colorectal cancer to study entry and the time from the

completion of post-operative adjuvant chemotherapy to study entry were about 5 months longer in the FOLFOX4+placebo group compared to the FOLFOX4+bevacizumab group. Therefore, it seemed that patients with a good prognosis were randomized to the FOLFOX4+placebo group and there was an imbalance in the patient background between the groups. Then, reanalysis was performed excluding the 88 patients with "post-operative adjuvant chemotherapy" from the FOLFOX4+bevacizumab group and the 85 patients with "post-operative adjuvant chemotherapy" from the FOLFOX4+placebo group. As a result, the hazard ratio was 0.72 (p=0.0009), demonstrating a significant add-on effect.

PMDA considers the add-on effect of bevacizumab in combination with FOLFOX4 as follows:
Although the results of Study NO16966C showed the add-on effect of bevacizumab in combination with chemotherapy in first-line treatment, a subgroup analysis suggested that the add-on effect of bevacizumab varies depending on the chemotherapeutic regimen to be combined with bevacizumab.

PMDA considers as follows:
It can not be concluded that "there is no add-on effect of bevacizumab in combination with FOLFOX4" because of the results of subgroup analysis that the add-on effect of bevacizumab in combination with FOLFOX4 was not "significant." It is unclear whether the add-on effect of bevacizumab in combination with FOLFOX4 is truly lower than that in combination with XELOX or the influence of other factors resulted in a failure to demonstrate a "significant" add-on effect. It is necessary to define the most preferred chemotherapeutic regimen to be combined with bevacizumab in first-line treatment and the clinical positioning of the combination of bevacizumab with FOLFOX4 in future.

PMDA considers that the above additional analyses i)-iv) performed by the applicant to examine the add-on effect of bevacizumab in combination with FOLFOX4 are not pre-planned analyses and there are also problems with the content of the analyses.

The clinical positioning of bevacizumab for first-line treatment in Japan is described in "4.3 Clinical efficacy and safety, *Outline of review by the PMDA* 3) Clinical positioning of bevacizumab."

(c) The results from other clinical studies
The efficacy of bevacizumab in other clinical studies is as follows.

In Study AVF2192g, a foreign phase II study comparing 5-FU/LV to 5-FU/LV+bevacizumab in patients considered inappropriate for irinotecan-based therapy, although there was no add-on effect of bevacizumab based on the primary endpoint, OS (hazard ratio 0.766, p=0.0942), the add-on effect of bevacizumab was demonstrated based on PFS, the secondary endpoint (hazard ratio 0.496, p=0.0002).

In Study AVF0780g, a foreign phase II study of bevacizumab in combination with 5-FU/LV, the

add-on effect of bevacizumab 5 mg/kg in combination with 5-FU/LV was demonstrated based on the primary endpoint PFS (stratified hazard ratio 0.44, log-rank test, p=0.005).

A pooled analysis of 249 patients in the 5-FU/LV+bevacizumab groups and 241 patients in the control groups from foreign clinical studies in first-line treatment, i.e. Study AVF0780g (5-FU/LV+bevacizumab 5 mg/kg group: 35 patients, 5-FU/LV group: 36 patients), Study AVF2192g (5-FU/LV+bevacizumab 5 mg/kg group: 104 patients, 5-FU/LV+placebo group: 105 patients), and Study AVF2107g (110 patients in Arm 3: 5-FU/LV+bevacizumab 5 mg/kg group, 100 patients in Arm 1: IFL+placebo group enrolled concurrently with Arm 3) was performed (*J Clin Oncol* 23: 3706-12, 2005). As a result, the median OS was 14.59 months in the control group (bolus 5-FU/LV and IFL) and 17.94 months in the bolus 5-FU/LV+bevacizumab group, showing an OS prolongation (stratified hazard ratio 0.74, p=0.0081; log-rank test).

The above results are consistent with the results of Study AVF2107g and Study NO16966 where bevacizumab was combined with chemotherapy in first-line treatment, and PMDA considers that these results supplement the efficacy data of bevacizumab (the add-on effect in combination with chemotherapy).

(2) Second-line or subsequent treatment

The results of the second-line or subsequet treatment of unresectable advanced or recurrent colorectal cancer (previously treated patients) have been reported by Study E3200 conducted by the ECOG.

This study evaluated the add-on effect of bevacizumab 10 mg/kg in combination with FOLFOX4 in patients with advanced or recurrent colorectal cancer previously treated with a fluoropyrimidine and an irinotecan-based regimen.

The median OS (the primary endpoint) was 10.8 months in the FOLFOX4 group and 13.0 months in the FOLFOX4+bevacizumab 10 mg/kg group, demonstrating a significant add-on effect (stratified hazard ratio 0.751, p=0.0012; log-rank test). The median PFS (the secondary endpoint) was 4.5 months in the FOLFOX4 group and 7.5 months in the FOLFOX4+bevacizumab group, demonstrating a significant add-on effect (stratified hazard ratio 0.518, p<0.0001, log-rank test). The response rate was 8.6% (25/292 patients) in the FOLFOX4 group and 22.2% (65/293 patients) in the FOLFOX4+bevacizumab group (p<0.0001; Cochran-Mantel-Haenszel test).

PMDA considered that the efficacy of bevacizumab shown in Study E3200 may vary depending on the content of the prior therapy (the regimen or objectives of previous chemotherapy) and asked the applicant to explain the details of the prior therapy.

The applicant responded that although the details of the prior therapy were unknown, 96.4% (453 patients) of the patients enrolled into this study were patients with advanced or recurrent colorectal cancer who were not candidates for curative resection [Note by the PMDA:

According to the eligibility criteria for Study E3200, “patients who relapsed within 6 months after post-operative adjuvant chemotherapy” were allowed to enter the study as “previously treated patients”]. The applicant analyzed these 453 patients for OS. As a result, the OS was 10.8 months in the FOLFOX4 group and 13.3 months in the FOLFOX4+bevacizumab group, demonstrating a significant add-on effect (hazard ratio 0.743, p=0.0030; log-rank test).

PMDA judged that the efficacy of the addition of bevacizumab to FOLFOX4 has been demonstrated in those who have progressed or relapsed after a fluoropyrimidine and an irinotecan-based regimen among patients with advanced or recurrent colorectal cancer who are not candidates for curative resection, although the details of the prior therapy in these 453 patients are unknown.

(3) Histology

PMDA asked the applicant to explain the relationship between the histology of colorectal cancer and the efficacy.

The applicant presented the median OS, the survival rate at 24 months, the median PFS, and the response rate by histological type for the patients enrolled into Study AVF2107g, Study AVF2192g, and Study AVF0780g, and then responded that a conclusion could not be drawn with respect to the relationship between the histology and the efficacy because the majority of the patients had adenocarcinoma (adenocarcinoma: 637 patients, mucinous adenocarcinoma: 42 patients, adenosquamous carcinoma: 1 patient, others: 3 patients).

PMDA accepted the above response.

(4) Serum VEGF concentrations and the efficacy

Foreign Studies AVF0737g and AVF0761g showed that serum VEGF concentrations were increased dose-dependently following the administration of bevacizumab. On the other hand, among the 18 subjects in Japanese Study JO18157, the mean VEGF concentration was lower in the 10 mg/kg group than in the 5 mg/kg group and there were no concentration-dependent increases.

The applicant explained a difference in the dose response based on VEGF concentrations between the Japanese and foreign clinical studies as follows:

Due to the small number of subjects studied in each group, a conclusion can not be drawn on the impact of the variability in serum bevacizumab concentrations in each group and of differences in the concomitant medications. Since different assay methods for blood VEGF concentrations were used between Japan and overseas (overseas: total serum/plasma VEGF concentrations [the total concentration of VEGF bound to bevacizumab and free VEGF unbound to bevacizumab], Japan: serum bevacizumab concentrations [free VEGF unbound to bevacizumab]), the relationship between blood VEGF concentrations over time and the efficacy can not be examined between foreign and Japanese studies.

PMDA considers that this issue can not be discussed as the applicant has responded that there are problems with the assay for blood VEGF concentrations in both Japanese and foreign clinical studies and it is difficult to assess the measurement data [See "4.1 Data of biopharmaceutic studies and associated analytical methods"]. It is recommended that the relationship between blood VEGF concentrations and the efficacy should be clarified by a pharmacological approach after market launch.

(5) Cases of brain metastases

In Foreign Study AVF0737g including patients with solid tumors, cerebral haemorrhage was reported by a hepatocellular carcinoma patient with a brain metastasis and patients with brain metastases were excluded from subsequent clinical studies. Thus, the efficacy of bevacizumab against brain metastases has not been evaluated.

2) Safety

PMDA considers as follows:

Adverse events uniquely associated with bevacizumab include gastrointestinal perforation, delayed healing of wound, haemorrhage, thromboembolism, hypertension, reversible posterior leukoencephalopathy syndrome (RPLS), proteinuria, cardiotoxicity (congestive heart failure), and infusion reactions, and an adequate attention should be paid to a possible occurrence of these events during the use of bevacizumab. It is also necessary to concentrate on all of these adverse events when collecting information via post-marketing surveillance.

In addition, it is necessary to promptly provide newly available data, e.g. safety information being collected by Genentech and Roche in accordance with the US and European regulatory authorities' instructions, to the Japanese clinical practice as well.

In the following sections, the content of a review is presented by each adverse event. The description of Study NO16966 is based on the data submitted on [redacted] as "a prompt report on the final analysis results" after the NDA filing.

(1) Gastrointestinal perforation

The incidences of gastrointestinal perforation in major clinical studies overseas are as follows.

Study Number	Treatment group	No. of cases with gastrointestinal perforation (%)
Study AVF2107g	IFL+bevacizumab	8/392 (2.0%)
	IFL+placebo	0/397 (0%)
Study AVF2192g	5FU/LV+bevacizumab	2/100 (2.0%)
	5FU/LV+placebo	0/104 (0%)
Study E3200	FOLFOX4+bevacizumab	5/287 (1.7%)
	FOLFOX4	0/285 (0%)
	Bevacizumab alone	4/234 (1.7%)
Study NO16966	FOLFOX4+bevacizumab	1/341 (0.3%)
	FOLFOX4+placebo	0/336 (0%)
	XELOX+bevacizumab	3/353 (0.8%)
	XELOX+placebo	2/339 (0.6%)

All of the above studies indicate the potential for an increased incidence of gastrointestinal perforation in the bevacizumab group. The applicant performed an integrated analysis of safety in bevacizumab-treated patients from 4 studies: Study AVF2107g, Study AVF2192g, Study AVF0780g, and Japanese Study JO18157 (data cutoff: [redacted] 20[redacted]). As a result, 11 (1.6%) of the 708 patients had gastrointestinal perforation. According to foreign post-marketing data, 203 cases of "gastrointestinal perforation (including suspected gastrointestinal perforation)" (of which, 48 patients died) have been reported as of [redacted] 20[redacted] (The second PSUR appendix 8h p.1445-p.1525).

Gastrointestinal perforation has also been reported among patients with non-colorectal cancer (lung cancer, breast cancer, etc.) and 17 events of gastrointestinal perforation have been reported among 2,530 patients exposed to bevacizumab (the incidence is around 0.67%) as of [redacted] 20[redacted]. Moreover, in a clinical study in patients with ovarian cancer or primary peritoneal carcinoma (Study AVF2949g), 5 out of the 44 subjects (11%) experienced gastrointestinal perforation and patient enrollment was discontinued.

Based on the above, PMDA considers as follows:

The risk of gastrointestinal perforation associated with bevacizumab has been shown by multiple data and according to the above overseas data, the incidence of gastrointestinal perforation is estimated at up to around 2%.

On the other hand, in Japan, Study JO18158 is ongoing as a safety confirmation study and 1 case of gastrointestinal perforation (a 4[redacted] year-old male patient with colon cancer) (Case No. X20) has been reported (Case Report [redacted]). At present, as to the incidence of gastrointestinal perforation in Japanese patients, no definitive conclusion can be drawn. It is necessary to identify the incidence etc. of gastrointestinal perforation in Japan after market launch.

Then, PMDA asked the applicant to discuss the risk factors associated with the development of

gastrointestinal perforation following the administration of bevacizumab since gastrointestinal perforation is a clinically significant adverse reaction, which is likely to lead to a fatal outcome.

The applicant responded as follows:
Although intra-abdominal inflammatory process (gastric ulcer, tumour necrosis, diverticulitis, or colitis associated with cancer chemotherapy, etc.) is suggested, its causal relationship to gastrointestinal perforation is unclear. The latest safety analysis of bevacizumab (Study BRiTE [Note by the PMDA: an US post-marketing observational study under routine drug uses for the first-line treatment of unresectable advanced or recurrent colorectal cancer]) has reported that the incidence of gastrointestinal perforation was slightly increased in patients with intact primary tumor, patients who underwent colonoscopy within 1 month prior to the administration of bevacizumab, and patients who received post-operative radiotherapy (ASCO 2006 abstract #3535), factors predicting a high-risk population are unclear at present.

In order to prevent serious gastrointestinal perforation, it is important to detect gastrointestinal perforation early and take appropriate measures. Therefore, we will include warning and caution statements in the package insert and ensure information provision.

PMDA largely accepted the applicant's response that currently available information including the results of Study BRiTE will be provided to the medical practice. PMDA considers that it is necessary to continue to investigate the risk factors associated with the development of gastrointestinal perforation and to examine the risk factors for gastrointestinal perforation in Japanese patients via post-marketing surveillance.

(2) Delayed healing of wound
The incidences of delayed healing of wound (including post-operative haemorrhage) in major clinical studies overseas are as follows.

Study Number	Treatment group	No. of cases with delayed healing of wound
Study AVF2107g	IFL+bevacizumab	5/60 (8.3%)
	IFL+placebo	1/44 (2.3%)
Study AVF2192g	5FU/LV+bevacizumab	5/15 (33.3%)
	5FU/LV+placebo	0/3 (0%)
Study E3200 *	FOLFOX4+bevacizumab	–
	FOLFOX4 alone	–
	Bevacizumab alone	–
Study NO16966 **	FOLFOX4+bevacizumab	9/341 (2.6%)
	FOLFOX4+placebo	4/336 (1.2%)
	XELOX+bevacizumab	3/353 (0.8%)
	XELOX+placebo	3/339 (0.9%)

*: The details are unknown and are currently being checked.
**: Information on the proportion of patients with wound or those who underwent surgery is being sought.

The applicant explained as follows:
Concerning this adverse event in foreign post-marketing experience, no reference data exists and 3 out of the 18 patients had "post procedural haemorrhage" (<Grade 3 in all cases) in Japanese Study JO18157 and there was no case of delayed healing of wound in Study JO18158

(data cutoff: March 31, 2006).

The applicant described as follows:
Based on the above results, it is recommended that an adequate interval between the last dose of bevacizumab and subsequent surgery should be allowed, taking into consideration that the half-life of serum bevacizumab is about 20 days. We will include the relevant warning statement in the package insert and ensure information provision.

PMDA considered that the applicant's response is appropriate and accepted it.

In order to fulfill a post-marketing commitment instructed by the European regulatory authority, diabetic patients, who are likely to develop delayed healing of wound, have been examined using the data from Study AVF2107g, Study AVF2192g, and Study AVF0780g, and PMDA is currently asking about the details.

(3) Thromboembolism
The incidences of thrombosis in major clinical studies overseas are as follows. PMDA decided to distinguish between arterial thrombosis and venous thrombosis wherever possible, because the etiology and clinical signs are different between arterial thrombosis and venous thrombosis.

	No. of cases with arterial thromboembolism		No. of cases with venous thromboembolism	
	Bevacizumab group	Control group	Bevacizumab group	Control group
Study AVF0780g	0/35 (0%) (5 mg group)	1/36 (2.8%)	9/35 (25.7%) (5 mg group)	2/36 (5.6%)
	2/33 (6.1%) (10 mg group)		2/33 (6.1%) (10 mg group)	
Study AVF2107g	14/392 (3.6%)	5/397 (1.3%)	68/392 (17.3%)	62/397 (15.6%)
Study AVF2192g	10/100 (10.0%)	5/104 (4.8%)	9/100 (9.0%)	14/104 (13.5%)
Study E3200 *	3/287 (1.0%) (FOLFOX+bevacizumab group)	1/285 (0.4%)	10/287 (3.5%) (FOLFOX+bevacizumab group)	7/285 (2.5%)
	2/234 (0.9%) (Single agent bevacizumab group)		1/234 (0.4%) (Single agent bevacizumab group)	
Study NO16966C	17/694 (2.4%)	10/675 (1.5%)	92/694 (13.3%)	64/675 (9.5%)

*: ≥Grade 3 for Study E3200

The following data from pooled analyses performed by the applicant were submitted.

- A pooled analysis of bevacizumab-treated patients in 4 studies: Study AVF2107g, Study AVF2192g, Study AVF0780g, and Study JO18157 (data cutoff: [redacted] 20[redacted]) was performed. As a result, arterial thromboembolic events ≥Grade 3 occurred in 7.8% (55/708 patients) of the patients included in the pooled analysis [Note by the PMDA: This analysis did not mention venous thromboembolism]. According to this pooled analysis, adverse events relevant to thromboembolism occurring in ≥1% of the patients were syncope (3.0%), myocardial infarction (1.7%), cerebrovascular accident (1.1%), and chest pain (1.0%).
- A pooled analysis of a total of 1,745 patients enrolled into 5 studies: Study AVF2107g,

Study AVF2192g, Study AVF0780g, Study AVF2119g, and Study AVF0757g was performed. As a result, the incidence of arterial thromboembolic events was 4.9% (49/1,004 patients) in the chemotherapy+bevacizumab group and 2.3% (17/741 patients) in the chemotherapy alone group. Analysis of the risk factors for the development of arterial thromboembolic events showed that bevacizumab therapy, ≥65 years old, and a history of arterial thrombosis (transient ischaemic attack, cerebrovascular disorder, myocardial infarction, etc.) represent high risk factors.

- In Study AVF2192g, Study AVF2107g, and Study E3200, the incidence of arterial thromboembolism ≥Grade 3 was higher in the chemotherapy+bevacizumab group (chemotherapy alone group or chemotherapy+placebo group: 1.4% [11/786 patients] vs. chemotherapy+bevacizumab group: 2.6% [26/1,013 patients]).
- In the Japanese studies, there was no arterial or venous thromboembolism.

The applicant discussed as follows:
The above results suggest that both arterial thromboembolism and venous thrombosis tend to be more likely to occur with chemotherapy in combination with bevacizumab as compared to chemotherapy alone.

The applicant also stated as follows:
Since it has been suggested that elderly patients or a history of arterial thromboembolism are risk factors, warning and caution statements will be included in the package insert.

PMDA considered that the applicant's response is appropriate and accepted it for the following reasons:
Although the mechanism whereby bevacizumab induces thromboembolism is unknown at present, (a) The incidence of thromboembolism may be increased with the use of bevacizumab, (b) Clinical studies indicate that arterial thromboembolic events can occur at any timepoint during treatment with bevacizumab, (c) It has been reported that the incidence of venous thromboembolic events is relatively high in patients with colorectal cancer as well as those with breast or lung cancer (*Circulation* 107: I17-21, 2003).

However, whether INR (International Normalized Ratio) measurements can predict thromboembolism (and haemorrhage) following treatment with bevacizumab is currently being investigated overseas, using the data from Study NO16966. PMDA is asking the applicant about currently available data from this investigation and the status of use of anticoagulant therapy in this study. PMDA considers that it is also necessary to conduct a domestic investigation in Japanese patients after market launch in accordance with this investigation in Europe/the US.

(4) Haemorrhage

The incidences of haemorrhage ≥Grade 3 in major clinical studies overseas are as follows.

Study Number	Treatment group	No. of cases with haemorrhage and incidence
Study AVF2107g	IFL+bevacizumab	13/392 (3.3%)
	IFL+placebo	10/397 (2.5%)
Study AVF2192g	5FU/LV+bevacizumab	5/100 (5.0%)
	5FU/LV+placebo	3/104 (2.9%)
Study E3200	FOLFOX4+bevacizumab	11/287 (3.8%)
	FOLFOX4 alone	1/285 (0.4%)
	Bevacizumab alone	7/234 (3.0%)
Study NO16966	FOLFOX4+bevacizumab	7/341 (2.1%)
	FOLFOX4+placebo	2/336 (0.6%)
	XELOX+bevacizumab	6/353 (1.7%)
	XELOX+placebo	6/339 (1.8%)

According to a pooled analysis of bevacizumab-treated patients in 4 studies: Study AVF2107g, Study AVF2192g, Study AVF0780g, and Study JO18157 (data cutoff: [redacted] 20[redacted]) performed by the applicant, the incidence of haemorrhage ≥Grade 3 was 4.8% (34/708 patients).

In foreign post-marketing experience, 283 cases of haemorrhage (excluding haemoptysis and pulmonary haemorrhage) has been reported as of [redacted] 20[redacted], which include gastrointestinal haemorrhage (67.1%: 190 patients) and intracranial haemorrhage (13.4%: 38 patients) etc. Thirty-three of the 39 patients with haemoptysis had lung lesion (lung cancer in 19 patients, lung metastases or concomitant lung disease in 14 patients). Also in a foreign phase II study in patients with non-small cell lung cancer (Study AVF0757g), fatal pulmonary haemorrhage was reported in 6/66 patients (9.1%).

In Japanese Study JO18157, among the 18 patients, 9 patients had epistaxis, 3 patients had anal haemorrhage, and 3 patients had post procedural haemorrhage, but none of them had haemorrhage ≥Grade 3. Also in Study JO18158, haemorrhage such as epistaxis was reported in 7/14 patients (50%), but none of them were ≥Grade 3.

The applicant discussed as follows:
Although the risk factors for haemorrhage are unclear at present, the common site of haemorrhage is the gastrointestinal tract because tumor is located in the gastrointestinal tract.

PMDA asked the applicant to discuss the relationship between intracranial haemorrhage and brain metastases and to explain the applicant's view on the use of bevacizumab in patients with brain metastases.

The applicant responded as follows:
In the light of a report that the incidence of brain metastases from large intestine carcinoma is ≤2% (*J Clin Oncol* 22: 2865-72, 2004), the proportion of patients with brain metastases is considered to be about 2%. Bevacizumab has been used in a limited number of patients with brain metastases even overseas and a causal relationship between bevacizumab and cerebral haemorrhage in patients with brain metastases is unclear. According to foreign post-marketing data, among 54,000 patients presumably exposed to bevacizumab (including non-colorectal

cancer), 38 patients were reported to have intracranial haemorrhage and 21 of them had colorectal cancer. Of the 21 patients, 4 patients were considered as having tumor-associated haemorrhage, and it seems that the occurrence of intracranial haemorrhage associated with brain metastases from colorectal cancer is infrequent.

The applicant explained as follows:
Although the package insert in Europe states that bevacizumab is contraindicated in patients with untreated central nervous system (CNS) metastases, since there is no positive reason for bevacizumab to be contraindicated in patients with brain metastases in Japan, patients with brain metastases should not be excluded uniformly from treatment with bevacizumab and it is important to determine the necessity of treatment with bevacizumab for each patient, weighing the benefits against the risks carefully. Therefore, as with the US labeling (the September 30, 2005 edition), including it in the WARNINGS section of the package insert to call attention is appropriate.

PMDA considers as follows:
In a foreign phase I study conducted in the early phase of development of bevacizumab, i.e. AVF0737g, a hepatocellular carcinoma patient with a brain metastasis developed serious cerebral haemorrhage. Thus, patients with brain metastases were excluded from subsequent clinical trials. Also in Japanese Study JO18157, patients with brain tumor or brain metastases on an imaging study, etc. were excluded and the information on the safety and efficacy of bevacizumab in patients with brain metastases is extremely limited. Based on the above, although the use of bevacizumab in patients with brain metastases should essentially be avoided especially from a safety point of view, its use should be acceptable only when there is no other therapeutic option. After market launch, it is necessary to advise caution about this issue and collect information on the relationship between the site of haemorrhage and the location of tumor in Japanese patients.

As mentioned in the above “(3) Thromboembolism,” whether INR measurements can predict (thromboembolism and) haemorrhage following treatment with bevacizumab is currently being investigated overseas, using the data from Study NO16966. PMDA is asking the applicant about currently available data from this investigation. PMDA considers that it is also necessary to conduct a domestic investigation in Japanese patients after market launch in accordance with this investigation in Europe/the US.

(5) Hypertension

The incidences of hypertension of all Grades in major clinical studies overseas are as follows.

Study Number	Treatment group	No. of cases with hypertension and incidence
Study AVF2107g	IFL+bevacizumab	96/392 (24.5%)
	IFL+placebo	34/397 (8.6%)
Study AVF2192g	5FU/LV+bevacizumab	32/100 (32.0%)
	5FU/LV+placebo	5/104 (4.8%)
Study E3200 (≥Grade3)	FOLFOX4+bevacizumab	18/287 (6.3%)
	FOLFOX4 alone	5/285 (1.8%)
	Bevacizumab alone	17/234 (7.3%)
Study NO16966	FOLFOX4+bevacizumab	70/341 (20.5%)
	FOLFOX4+placebo	27/336 (8.0%)
	XELOX+bevacizumab	61/353 (17.3%)
	XELOX+placebo	16/339 (4.7%)

The following data from pooled analyses performed by the applicant were submitted.

- A pooled analysis of bevacizumab-treated patients in 4 studies: Study AVF2107g, Study AVF2192g, Study AVF0780g, and Study JO18157 (data cutoff: [redacted] 20[redacted]) was performed. As a result, 30.2% (214/708 patients) of the bevacizumab-treated patients had hypertensive events and the incidence of hypertension ≥Grade 3 was 16.5% (117/708 patients), and one case of Grade 4 hypertension (hypertensive crisis) was reported in Study AVF2107g.
- A pooled analysis of 3 studies: Study AVF2107g, Study AVF2192g, and Study AVF2119g was performed. As a result, the incidence of hypertension (systolic blood pressure >150 mmHg or diastolic blood pressure >100 mmHg) was 61.5% (507/824 patients) in the bevacizumab-treated patients and 38.5% (270/701 patients) in the control group. Analysis of the risk factors showed that patients with a Performance Status 0 and those with diabetes at the start of the study are at high risk.
- In foreign post-marketing experience (as of [redacted] 20[redacted]), 4 cases of hypertensive encephalopathy were reported. All of them were women (59-76 years old) and two of them had a fatal outcome.

In Japanese Study JO18157, hypertension was reported by 7/18 patients (38.9%) and of whom, 1 patient had Grade 3 hypertension. In this study, there was a trend towards increased systolic/diastolic blood pressure from baseline in all dose groups and this trend was especially pronounced for diastolic blood pressure. The trend towards increased blood pressure was stronger in the 10 mg/kg group compared to the 3 mg/kg group and the 5 mg/kg group, showing a dose-dependent tendency. In Japanese Study JO18158, hypertension was reported by 4/14 patients (28%), but there was no hypertension ≥Grade 3.

The applicant explained as follows:

Although hypertension occurring following treatment with bevacizumab can be controlled with oral antihypertensive drugs, since a complete follow-up to determine whether hypertension is reversible or not has not been performed, the reversibility is unknown.

PMDA asked the applicant to examine changes in systolic blood pressure over time after the

start of treatment with bevacizumab in Foreign Study AVF2107g by subgrouping the patients into those with hypertension ≥Grade 3 and the others.

The applicant responded as follows:
Many of the patients with hypertension ≥Grade 3 following treatment with bevacizumab had high blood pressure at baseline. However, when other risk factors for hypertension associated with bevacizumab were explored, no definitive conclusion could be drawn.

PMDA considers that it is necessary to call attention to the following points:
(a) Serious hypertension including hypertensive crisis may occur. (b) Patients with concomitant hypertension or a history of hypertension at the start of treatment with bevacizumab need to be carefully monitored, especially for the occurrence of hypertension. (c) Both systolic and diastolic blood pressures are increased following treatment with bevacizumab. (d) Hypertension associated with the concurrent use of bevacizumab can occur at any timepoint during treatment.

The mechanism, risk factors, and reversibility of hypertension associated with bevacizumab are unknown at present, and a further investigation is required.

(6) Cardiotoxicity (Congestive heart failure)
In clinical studies in patients with colorectal cancer, the incidence of heart failure associated with bevacizumab was 2.0% (2/100 patients) in the bevacizumab group of Foreign Study AVF2192g and 1.0% (4/392 patients) in Study AVF2107g. Of the 568 patients in the chemotherapy+bevacizumab groups from clinical studies involving patients with colorectal cancer conducted by the applicant and Genentech to date, 7 patients (1.2%) experienced heart failure. As of [redacted] 20[redacted], 37 cases of congestive heart failure, 10 cases of left ventricular failure, 9 cases of cardiomyopathy, and 1 case of heart failure have been reported overseas, including post-marketing reports.

The applicant explained as follows:
In clinical studies that evaluated the use of bevacizumab in breast cancer (Study AVF0776g, Study AVF2119g), congestive heart failure and cardiomyopathy were reported and analysis of these cases suggests that prior anthracyclines or prior chest wall irradiation are risk factors.

PMDA considers as follows:
It is necessary to advise caution about a possible occurrence of cardiotoxicity when bevacizumab is used in patients with colorectal cancer and to continue to collect information on the incidence and severity of cardiotoxicity in Japanese patients and the reversibility of cardiotoxicity, after market launch.

(7) Proteinuria

The incidences of proteinuria in major clinical studies overseas are as follows.

Study Number	Treatment group	No. of cases with proteinuria and incidence
Study AVF2107g	IFL+bevacizumab	113/392 (28.8%)
	IFL+placebo	89/397 (22.4%)
Study AVF2192g	5FU/LV+bevacizumab	38/100 (38.0%)
	5FU/LV+placebo	20/104 (19.2%)
Study E3200 (≥Grade3)	FOLFOX4+bevacizumab	2/287 (0.7%)
	FOLFOX4 alone	0/285 (0%)
	Bevacizumab alone	0/234 (0%)
Study NO16966 (≥Grade3)	FOLFOX4+bevacizumab	21/341 (6.2%)
	FOLFOX4+placebo	19/336 (5.7%)
	XELOX+bevacizumab	14/353 (4.0%)
	XELOX+placebo	11/339 (3.2%)

The following data from pooled analyses performed by the applicant were submitted.

- A pooled analysis of 3 studies: Study AVF2107g, Study AVF2192g, and Study AVF2119g was performed. As a result, the incidence of proteinuria was 24.8% (199/802 patients) in the bevacizumab-treated patients and 11.1% (155/674 patients) in the control group and the incidence of proteinuria ≥Grade 3 was 0.9% and 0.1%, respectively.
- A pooled analysis of bevacizumab-treated patients in 4 studies: Study AVF2107g, Study AVF2192g, Study AVF0780g, and Study JO18157 (data cutoff: [redacted] 20[redacted]) was performed. As a result, 32.6% (231/708 patients) experienced proteinuria and 74.9% (173/231 patients) of them had Grade 1 proteinuria.

The applicant described as follows:

The results of Study AVF2107g and the results of a pooled analysis of the above 4 studies indicate a trend towards an increased incidence of proteinuria in patients with a history of hypertension and those with an adverse event of hypertension.

According to foreign post-marketing data, a total of 101 cases including 87 cases of renal impairment (renal failure NOS, acute renal failure, chronic renal failure, hydronephrosis, pollakiuria) (of which, 17 patients had a fatal outcome) and 14 cases of nephrotic syndrome have been reported as of [redacted] 20[redacted] (the second PSUR appendix 8i p.1526-p.1617).

In Japanese Studies JO18157 and JO18158, proteinuria occurred in 38.9% (7/18 patients) and 21.4% (3/14 patients), respectively, and there was no proteinuria ≥Grade 3 in either study.

PMDA considers as follows:

It is necessary to perform urinalysis at an appropriate frequency during treatment with bevacizumab and pay attention to a possible occurrence of proteinuria. Especially, since the analysis performed by the applicant indicated that the risk factor for the development of proteinuria associated with bevacizumab is hypertension, special caution is required for blood pressure control in the event of hypertension associated with bevacizumab and the use of bevacizumab in patients with a history of hypertension.

(8) Infusion reactions

According to the second PSUR, 8 patients experienced "anaphylactic reaction" [Note by the PMDA: 1 patient was untreated with bevacizumab and all of the other 7 patients did not develop anaphylactic reaction after rechallenge] and 2 patients had "fatal hypersensitivity" among approximately 67,500 patients exposed to bevacizumab as of [redacted] 20[redacted]. PMDA is currently asking the applicant about patients who experienced infusion reactions in the Japanese clinical studies.

The applicant explained as follows:

Based on the results of clinical studies and foreign post-marketing data, infusion reactions are of no special concern for bevacizumab, whereas bevacizumab is a humanized murine monoclonal antibody and the possibility of causing anaphylactic shock (including anaphylactoid symptoms) can not be excluded. Thus, a caution about a possible occurrence of these events will be included in the package insert and information provision will be ensured.

PMDA accepted the applicant's response.

(9) Reversible posterior leukoencephalopathy syndrome

Two adverse reaction reports about reversible posterior leukoencephalopathy syndrome (RPLS) following treatment with bevacizumab have been published (*N Engl J Med* 354: 980-1, 2006). A 59 year-old woman with metastatic rectal cancer received 7 doses of bevacizumab and was then hospitalized due to tonic-clonic convulsion at 8 days after the last dose and was found to have cortical blindness. A 52 year-old female patient with metastatic rectal cancer had visual acuity reduced in both eyes, headache, and convulsion at 16 hours after the first dose of bevacizumab. Both patients were hypertensive at the time of onset of RPLS.

PMDA asked the applicant to explain about patients who developed RPLS following treatment with bevacizumab to date.

The applicant responded as follows:

Genentech and Roche assessed for RPLS, which revealed that there have been 4 cases of RPLS (3 females), 3 cases of suspected RPLS (all females), and 7 cases (5 females) for which the possibility of RPLS can not be ruled out as of [redacted] 20[redacted] [Note by the PMDA: These cases include the two published cases]. Hypertension is known to cause RPLS and blood pressure control is important. Warning and caution statements will be included in the package insert and information provision will be ensured.

PMDA considers as follows:

If RPLS is suspected, appropriate tests such as MRI need to be performed promptly in order to establish a diagnosis. Thus, the applicant's response that they will inform the medical practice about a possible occurrence of RPLS following treatment with bevacizumab, is appropriate. It is necessary to continue to collect information on the incidence of RPLS following treatment with

bevacizumab and the association between RPLS after treatment with bevacizumab and hypertension, also in Japan after market launch.

(10) Anti-bevacizumab antibodies

In foreign clinical studies, 4 out of the 837 patients who underwent antibody test (Study AVF2107g: 3 patients, Study AVF2119g: 1 patient) had positive results at baseline. In Japanese Study JO18157, 3 out of the 18 patients (5 mg/kg group: 1 patient, 10 mg/kg group: 2 patients) had positive results at baseline.

PMDA asked the applicant to explain the clinical significance of positive antibody test results at baseline.

The applicant responded as follows:

Positive antibody test results obtained in Japanese Study JO18157 were very likely to be false positive reactions, caused by detecting substances that bind to bevacizumab nonspecifically because (a) these patients did not develop adverse events clearly suggestive of hypersensitivity reactions during or after treatment with bevacizumab, and (b) In the Japanese study JO18157, 4 out of the 4 patients (3 mg/kg group: 2 patients, 5 mg/kg group: 2 patients) who underwent antibody test at 3 weeks after the last dose of bevacizumab had positive results, and even in 3 of them with negative antibody test results at baseline, the pharmacokinetics with bevacizumab showed no changes during treatment.

In addition, the applicant explained as follows:

As it is unknown whether the positive antibody test results at 3 weeks after the last dose of bevacizumab in the 4 patients truly detected anti-bevacizumab antibodies or not, another antibody test is scheduled $\geq$3 months after the last dose of bevacizumab. Genentech is reevaluating the existing ECLA, including changing the criteria for positivity and is developing a new antibody assay method [Note by the PMDA: Information on the up-to-date status is being sought (See “4.2 Clinical pharmacology”)].

PMDA asked the applicant to explain whether there are any differences in the efficacy and safety between patients with positive antibody test results and those with negative results.

The applicant responded as follows:

There were no noteworthy points as to the efficacy and safety in patients with positive results at baseline compared to those with negative results at baseline or the overall study population.

PMDA considers as follows:

Regarding anti-bevacizumab antibody formation, the positive antibody rate was higher in the Japanese study compared to the foreign clinical studies, but since different antibody test methods were used between Japan and overseas (Fab-ELISA in the foreign studies, ECLA in the Japanese study) and there is room for a review of the antibody assay, the relationship between antibody formation and the efficacy and safety can not adequately be discussed at present based

on the currently available data. It is necessary to continue to investigate the relationship between anti-bevacizumab antibody formation and the efficacy and safety [See "4.2 Clinical pharmacology"].

(11) Adverse events associated with long-term treatment

Taking into account Study AVF2540g, which is an extension study of foreign phase II and phase III trials (AVF2107g, AVF2192g, and AVF2119g), PMDA asked for the applicant's view on the safety of long-term treatment with bevacizumab.

The applicant responded as follows:

There were no adverse events ≥Grade 3 occurring at a ≥5% higher incidence in Study AVF2540g compared to the parent studies (AVF2107g, AVF2192g, and AVF2119g), and there is little safety concern with a long-term treatment. With respect to the outcomes of patients with continued hypertension or proteinuria in Study AVF2540g, none of the 26 patients with continued hypertension at the start of this study had a worse Grade during the study period or were discontinued from the study due to hypertension. While the event resolved before the end of the study in 9 patients, hypertension persisted even at the end of the study in the remaining 17 patients. Of these 17 patients, 3 patients completed a follow-up survey at 4 months after the end of the study and their hypertensive event persisted. Of the 20 patients with continued proteinuria at the start of this study, 3 patients had a worse Grade during the study period (worsening from Grade 1 to Grade 2), whereas there were no study discontinuations due to proteinuria. While proteinuria resolved before the end of the study in 5 patients, proteinuria persisted even at the end of the study in the remaining 15 patients and 2 of these 15 patients reported the outcome after the end of the study and their proteinuria persisted.

PMDA largely accepted the applicant's response, but considers that it is necessary to collect information on the profile and outcome of adverse events associated with a long-term treatment in Japanese patients also after market launch.

(12) The safety in Asian patients

In the major clinical studies submitted at the regulatory application, a total of 22 Asian patients were included in the safety analysis as the bevacizumab group of foreign clinical studies: 15 patients in Study AVF2107g, 3 patients in Study AVF2192g, and 4 patients in Study AVF0780g. As Asian patients enrolled into clinical studies were extremely limited compared to non-Asian patients (a total of 646 patients in Study AVF2107g, Study AVF2192g, and Study AVF0780g), although the results of a pooled analysis submitted have shown no noteworthy major differences in serious adverse events for Asian patients, PMDA is asking the applicant to examine the safety in Asian patients, incorporating the latest safety data obtained from Japanese patients, including an ongoing safety confirmation study.

(13) The safety in second-line or subsequent treatment

PMDA judged as follows:

Foreign Study E3200 is the only study that evaluated second-line or subsequent treatment.

When the results of this study are compared to the results of other foreign clinical studies in first-line treatment, there have been no major differences to be noted in the safety profile at the time of preparation of the Review Report (1).

In an ongoing Japanese Study JO18158, for second-line or subsequent treatment (FOLFOX+bevacizumab 10 mg/kg), the first patient for Step 1 was enrolled on [redacted] and is currently on study. PMDA confirmed that no serious adverse events have been reported as of [redacted] and is asking the applicant to present the update of the safety confirmation study.

3) Clinical positioning of bevacizumab

PMDA judged as follows:

Study NO16966, etc. have demonstrated the add-on effect of bevacizumab in combination with chemotherapy in first-line treatment while the information on the results of subgroup analysis of Study NO16966 should be provided appropriately to the clinical practice. In second-line or subsequent treatment, the add-on effect of bevacizumab in combination with chemotherapy is recognized based on the results of Study E3200.

(1) First-line treatment

PMDA judged that the results of foreign clinical studies in first-line treatment submitted have demonstrated the add-on effect of bevacizumab in combination with chemotherapy as measured by OS or PFS. However, PMDA asked for the applicant's view on the clinical positioning of bevacizumab in Japan (preferred chemotherapeutic regimens to be combined with bevacizumab) for the following reasons: (a) It has been suggested that the add-on effect of bevacizumab tends to differ between different chemotherapeutic regimens, (b) At present, the add-on effect of bevacizumab in combination with FOLFOX4, which is considered a standard first-line therapy, is not definite, (c) Although the add-on effect of bevacizumab in combination with IFL as measured by OS has been demonstrated with a significant difference (Study AVF2107g), IFL is not currently used as a standard first-line therapy in Japan due to its adverse reactions.

The applicant responded as follows:

When selecting a first-line therapy, we need to discuss "patients who tolerate intensive chemotherapy" and "patients who can not tolerate intensive chemotherapy" separately. Although there are no established definitions of such patients, "patients who can not tolerate intensive chemotherapy" in Study AVF2192g were defined as those who meet any of the following conditions: (a) Age $\geq$65 years, (b) Performance Status is 1 or 2, (c) Albumin $\leq$3.5g/dL, (d) Prior radiotherapy to abdomen or pelvis. Using this definition, we describe preferred chemotherapeutic regimens as follows.

The primary analysis of Study NO16966 showed that the addition of bevacizumab to chemotherapy (FOLFOX4 or XELOX) resulted in a significant prolongation of PFS. Subgroup analysis demonstrated a significant prolongation of PFS with the addition of bevacizumab to XELOX, but failed to show a significant prolongation of PFS with the addition of bevacizumab

to FOLFOX4. However, based on the results of additional analyses [See "4.3 Clinical efficacy and safety, *Outline of review by the PMDA* 1) The efficacy in patients with advanced or recurrent colorectal cancer who are not candidates for curative resection (1) (b) Study NO16966"], the add-on effect of bevacizumab can be expected also with FOLFOX4. Based on these results, bevacizumab in combination with FOLFOX4 is recommended for "first-line treatment of patients who can tolerate intensive chemotherapy."

Although Study AVF2107g showed the add-on effect of bevacizumab in combination with IFL, it is difficult to say that IFL is used frequently in Japan and moreover, it has been reported that FOLFOX4 is significantly superior to IFL in terms of PFS and OS (*J Clin Oncol* 22: 23-30, 2004). Thus, IFL is considered a preferred chemotherapeutic regimen to be combined with bevacizumab, after FOLFOX4.

The usefulness of FOLFIRI regimen, which consists of irinotecan hydrochloride and infusional 5-FU/LV, is almost equivalent to that of FOLFOX4, but a randomized, phase III, comparative study confirming the efficacy of bevacizumab in combination with FOLFIRI has not been conducted. Thus, when combined with bevacizumab, FOLFIRI seems less preferred compared with FOLFOX4. A foreign phase II study evaluating bevacizumab in combination with FOLFIRI (Study AVIRI) is currently ongoing, and according to currently available interim data, the response rate is 44% (of the 209 cases, 4 CR cases and 88 PR cases) and the progression free survival rate at 6 months is 82% (*J Clin Oncol* 24: Abstract3544, 2006).

Based on the above, as preferred chemotherapeutic regimens to be combined with bevacizumab for "first-line treatment of patients who can tolerate intensive chemotherapy," FOLFOX4 comes first, followed by IFL and FOLFIRI.

On the other hand, as to "first-line treatment of patients who can not tolerate intensive chemotherapy," a preferred chemotherapeutic regimen can be determined based on the results of Study AVF2192g. This study was conducted in the first-line treatment of patients considered inappropriate for treatment with irinotecan. The median OS (the primary endpoint) was 13.24 months in the 5-FU/LV group and 16.56 months in the 5-FU/LV+bevacizumab group, showing no significant difference (hazard ratio 0.766 [95% CI, 0.56-1.05], p=0.0942; log-rank test), while the median PFS (the secondary endpoint) was 5.52 months in the 5-FU/LV group and 9.17 months in the 5-FU/LV+bevacizumab group, showing a significant difference (hazard ratio 0.496 [95% CI, 0.34-0.73], p=0.0002; log-rank test).

Based on the above, a preferred chemotherapeutic regimen to be combined with bevacizumab for "first-line treatment of patients who can not tolerate intensive chemotherapy" is 5-FU/LV.

PMDA reviewed the clinical positioning of bevacizumab in the first-line treatment of unresectable advanced or recurrent colorectal cancer as follows:
For patients with advanced or recurrent disease who are not candidates for curative resection (metastatic colorectal cancer), chemotherapy-based treatment intended to confer a survival

benefit is indicated. Conventionally, 5-FU/LV (a fluoropyrimidine anticancer agent, 5-FU plus LV) was used as a standard chemotherapeutic regimen for these patients, but now, the usefulness of FOLFOX, FOLFIRI, and IFL, etc. is recognized and these chemotherapeutic regimens are used as standard treatment overseas.

The US National Comprehensive Cancer Network (NCCN) Clinical Practice Guideline in Oncology is one of the guidelines that are used as a reference by clinical oncologists in Japan and overseas for diagnosis and treatment. This guideline (Clinical Practice Guideline Version 2. 2006) recommends FOLFOX+bevacizumab, FOLFIRI+bevacizumab, IFL+bevacizumab, 5FU/LV+bevacizumab, and XELOX+bevacizumab [Note by the PMDA: The XELOX regimen and the CAPOX regimen are the same] as initial therapy for patients who can tolerate intensive chemotherapy [Note by the PMDA: "IFL+bevacizumab" is not included in the recommended regimens of initial therapy for patients who can tolerate intensive chemotherapy in the Clinical Practice Guideline Version 1. 2007]. As initial therapy for patients who can not tolerate intensive chemotherapy, 5-FU/LV+bevacizumab, 5-FU/LV, and capecitabine are recommended [Note by the PMDA: The Clinical Practice Guideline Version 1. 2007 recommends 5-FU/LV+bevacizumab, Capecitabine+bevacizumab, 5-FU/LV, and capecitabine as initial therapy for patients who can not tolerate intensive chemotherapy].

Against such background, (a) Study AVF2107g showed that the addition of bevacizumab to IFL resulted in a significant prolongation of OS compared to IFL, (b) A pooled analysis of Study AVF0780g, Study AVF2192g, and Study AVF2107g (*J Clin Oncol* 23: 3706-12, 2005) suggested a longer OS with the addition of bevacizumab to 5-FU/LV compared to the control group (5-FU/LV or IFL), (c) Study NO16966 showed that the addition of bevacizumab to chemotherapy (FOLFOX4 or XELOX) provided a significant prolongation of PFS compared to the chemotherapy alone. Therefore, PMDA judged that the add-on effect of bevacizumab in combination with fluoropyrimidine-based chemotherapy has been demonstrated [See "4.3 Clinical efficacy and safety, *Outline of review by the PMDA* 1) (1) Previously untreated patients"].

Meanwhile, PMDA understands the current situation of chemotherapy for colorectal cancer in Japan as follows:
(a) FOLFOX4 is most widely used, (b) Capecitabine is unapproved for colorectal cancer, (c) Study AVF2107g confirmed the add-on effect of bevacizumab in combination with IFL, but IFL is not very widely used in Japan, mainly for safety concerns.

Therefore, PMDA considers that, when selecting a chemotherapeutic regimen to be combined with bevacizumab, it is necessary to take into account the results of subgroup analysis of Study NO16966, which suggests that the add-on effect of bevacizumab in combination with FOLFOX4 was not "significant". (Concerning the results of bevacizumab in combination with IFL, i.e., the results of Study AVF2107g, a published article [*N Engl J Med* 350: 2335-42, 2004] and a textbook [*Cancer: Principles and Practice of Oncology.* 7th edition. Lippincott Williams & Wilkins] describe that it is the first phase III study confirming the efficacy of the therapy

based on the concept of angiogenesis inhibitory activity in treatment schemes for metastatic solid tumor, but it has not been confirmed whether bevacizumab in combination with regimens other than IFL produces a similar clinical benefit.)

However, PMDA considers that bevacizumab can be positioned as a first-line therapy in clinical practice in Japan for the following reasons.
・Multiple clinical studies have demonstrated the add-on effect of bevacizumab in combination with chemotherapy in first-line treatment as measured by OS or PFS.
・The primary objective of Study NO16966 was not to confirm the add-on effect of bevacizumab in combination with FOLFOX4, and at present, it is appropriate to interpret the results as follows: “the add-on effect of bevacizumab in combination with FOLFOX4 in first-line treatment has not been established.”
・The add-on effect of bevacizumab in combination with IFL or 5-FU/LV is useful information for patients who can not use platinum-based anticancer agents.
・Given that the treatment of patients with advanced or recurrent large intestine carcinoma who are not candidates for curative resection has so far been evaluated in a number of clinical studies (mainly overseas) and has been modified repeatedly based on the results of studies, further clinical studies may be conducted for choosing the optimum regimen to be combined with bevacizumab.

Based on the above, PMDA judged as follows:
Although the add-on effect can be expected depending on the therapeutic regimen to be combined with bevacizumab, the optimum regimen to be combined with bevacizumab is unknown at present. Thus, it is necessary to conduct a post-marketing drug utilization study in Japan to identify chemotherapeutic regimens combined with bevacizumab and discuss therapeutic regimens to be studied in future based on the results of the study. In addition, as to the results of subgroup analysis of Study NO16966, it is necessary to provide the information on the results of subgroup analysis accurately, instead of providing information inferred from the results of additional analyses performed by the applicant.

(2) Second-line or subsequent treatment
Study E3200 evaluated the add-on effect of bevacizumab in combination with FOLFOX4 in patients with previously treated (a fluoropyrimidine- and an irinotecan-based regimen used either alone or in combination) advanced or metastatic colorectal cancer.

PMDA asked the applicant to explain their view on preferred chemotherapeutic regimens to be combined with bevacizumab in second-line treatment (second-line or subsequent treatment).

The applicant responded as follows:
Study E3200 demonstrated the add-on effect of bevacizumab in combination with FOLFOX4 in patients with advanced colorectal cancer who had previously treated with a fluoropyrimidine- and irinotecan-based regimen, either alone or in combination. In addition to the above-mentioned prior chemotherapy, eligible patients were required to have adequate

renal/hepatic/bone marrow functions and a good performance status, i.e. “patients who can tolerate intensive chemotherapy.” Therefore, bevacizumab in combination with FOLFOX4 is recommended for previously treated patients who can tolerate intensive chemotherapy. Meanwhile, since a clinical study including “patients who can not tolerate intensive chemotherapy” has not been performed, no chemotherapy regimen to be combined with bevacizumab is recommended for this patient population.

Based on the above, we will provide information, making it clear that there is no clinical evidence for second-line treatment using bevacizumab in combination with regimens other than FOLFOX4. The US labeling (approved on October 11, 2006) mentions that the overall response rate was 1% (95% CI, 0%-5.5%) in a study that evaluated the activity of bevacizumab in combination with bolus or infusional 5-FU/LV in the third-line treatment of patients with disease progression following both irinotecan- and oxaliplatin-based chemotherapy regimens (Study TRC-0301). A caution statement that “if bevacizumab in combination with 5-FU/LV is used for third-line treatment, the response rate will be very low and the efficacy can not be expected,” will be included also in the Japanese package insert.

PMDA considers the add-on effect of bevacizumab in combination with chemotherapy in second-line treatment (second-line or subsequent treatment) as follows:
Study E3200 demonstrated the add-on effect of bevacizumab in combination with FOLFOX4 in patients with advanced colorectal cancer who had failed both prior 5-FU- and irinotecan hydrochloride-based therapy (The median OS in the FOLFOX4 group and FOLFOX4+bevacizumab group was 10.8 months and 13.0 months, respectively, stratified hazard ratio 0.751, p=0.0012; log-rank test).

According to the US NCCN Clinical Practice Guideline Version 2. 2006, (a) Depending on the regimen of the initial therapy, FOLFOX, FOLFIRI, irinotecan hydrochloride, and cetuximab (genetical recombination) [Note by the PMDA: unapproved in Japan] are recommended as second-line or third-line therapy for patients who can tolerate intensive chemotherapy [Note by the PMDA: The Clinical Practice Guideline Version 1. 2007 recommends FOLFOX, XELOX, FOLFIRI, irinotecan hydrochloride, FOLFIRI+cetuximab (genetical recombination), irinotecan hydrochloride+cetuximab (genetical recombination), cetuximab (genetical recombination), and panitumumab (genetical recombination, unapproved in Japan)], (b) There is no standard second-line or third-line therapy for patients who can not tolerate intensive chemotherapy. Other publications etc. report that 5-FU-based chemotherapy is used for palliation of symptoms (NCI-PDQ, *N Engl J Med* 330: 1136-42, 1994, *Curr Opin Oncol* 13: 275-86, 2001).

Based on the above, PMDA judged that bevacizumab can be positioned as a second-line or subsequent therapy in clinical practice, in view of the prognosis of patients on second-line or subsequent treatment, since Study E3200 demonstrated the add-on effect of bevacizumab in combination with the 5-FU-based FOLFOX4. The way of advising caution proposed by the applicant is appropriate and adequate information provision/cautioning are necessary.

4) Indications

Taking account of its review in "4.3 Clinical efficacy and safety, *Outline of review by the PMDA* 3) Clinical positioning of bevacizumab," PMDA judged that the efficacy of bevacizumab is recognized when combined with chemotherapy in patients with advanced or recurrent colorectal cancer who are not candidates for curative resection and concluded that the indication of "the treatment of unresectable advanced or recurrent colorectal cancer" is appropriate.

PMDA judged that it is necessary to advise caution about the following: At present, (a) The use of bevacizumab in a preoperative or postoperative adjuvant chemotherapy setting has not been evaluated and the efficacy and safety in such setting is unknown. (b) It has been shown that the add-on effect of bevacizumab in combination with FOLFOX4 in first-line treatment was not definite. (c) The efficacy and safety of bevacizumab in combination with regimens other than FOLFOX4 in second-line or subsequent treatment are unknown.

[Note by the PMDA: Other cautions are described in "4.3 Clinical efficacy and safety, *Outline of review by the PMDA* 5) Dosage and administration"]

5) Dosage and administration

As a result of its review as shown below, PMDA concluded that the appropriate dosage regimen should be "the usual adult dosage is 5 mg/kg (body weight) given every 2 weeks as an intravenous infusion" for first-line treatment and "the usual adult dosage is 10 mg/kg (body weight) given every 2 weeks as an intravenous infusion" for second-line or subsequent treatment. However, it is necessary to adequately caution about the content and scope of currently available evidence.

(1) The dose of bevacizumab

Based on the results of clinical trials submitted at the time of regulatory submission, the applicant proposed the following dosage regimen:

"5 mg/kg (body weight) is given, as a general rule, every 2 weeks as an intravenous infusion" for first-line treatment.

"10 mg/kg (body weight) is given, as a general rule, every 2 weeks as an intravenous infusion" for second-line or subsequent treatment.

PMDA judged that "5 mg/kg (body weight) is given every 2 weeks as an intravenous infusion" for first-line treatment is appropriate, because (a) the dose of bevacizumab was 5 mg/kg in Study AVF2107g and Study NO16966 which were major clinical studies demonstrating the efficacy of bevacizumab in first-line treatment, and (b) 5 mg/kg was chosen for use in Study AVF2107g based on the results of a phase II study (Study AVF0780g) including the doses of 5 mg/kg and 10 mg/kg.

Second-line or subsequent treatment was evaluated in Study E3200. A dose of 10 mg/kg that was tolerated in Study AVF0780g was chosen for this study, expecting a higher therapeutic effect, because patients to be enrolled had more advanced disease requiring second-line or subsequent treatment. At present, Study E3200 is the only study that evaluated second-line or

subsequent treatment and PMDA judged that “10 mg/kg (body weight) is given every 2 weeks as an intravenous infusion” for second-line or subsequent treatment is appropriate.

PMDA also judged as follows:
Multiple clinical studies have demonstrated the efficacy of bevacizumab and the chemotherapy regimens combined with bevacizumab were different between different studies, and the optimum chemotherapeutic regimen to be combined with bevacizumab is still under investigation. Thus, in the dosage and administration section, chemotherapy regimens to be combined with bevacizumab should not be specified and information on clinical study data obtained to date should be provided appropriately. Specifically, the following information need to be noted.

・The content of bevacizumab and combined chemotherapeutic regimens in major clinical studies (Study AVF2107g, Study NO16966, and Study E3200) that evaluated the efficacy and safety of bevacizumab to date
・Bevacizumab needs to be used in combination with chemotherapy and the usefulness of single agent bevacizumab is unknown.
・Data is available only for bevacizumab in combination with 5-FU-containing regimens.
・In first-line treatment, the add-on effect of bevacizumab in combination with FOLFOX4 as compared to FOLFOX4 alone was not definite.
・There is no clinical evidence for bevacizumab in combination with regimens other than FOLFOX4 in second-line or subsequent treatment.
・In a study of bevacizumab in combination with bolus or infusional 5-FU/LV as a third-line treatment (Study TRC-0301), the overall response rate was 1% (95% CI, 0%-5.5%).

In currently ongoing Japanese Study JO18158 assessing the safety of bevacizumab in combination with FOLFOX4, all of the 14 patients received 5 mg/kg as of the data cutoff date: March 31, 2006 and PMDA confirmed that (a) 8 patients achieved PR, (b) According to currently available data, there have been no notable major differences in serious adverse events as compared to foreign data. In this study, after the data cutoff date, an investigation of a dose of 10 mg/kg was initiated and the applicant presented the information that 12 patients have been enrolled into the 10 mg/kg group as of [redacted] 20[redacted]. PMDA is currently checking the latest safety data from this study.

(2) Single agent bevacizumab
In the clinical studies submitted, except for a foreign phase I study, the efficacy and safety of bevacizumab were evaluated in combination with other anti-cancer agents (chemotherapy). In 4 studies (AVF2107g, AVF2192g, AVF2540g, and AVF0778g) among these studies, there were patients treated with single agent bevacizumab after the concurrent use of bevacizumab with other anti-cancer agents. When single agent administration is defined as “at least 3 doses of bevacizumab alone administered,” 72 patients were treated with single agent bevacizumab.

Based on the above, PMDA asked for the applicant’s view on the positioning of single agent

bevacizumab.

The applicant responded as follows:
Although some patients were treated with single agent bevacizumab after concurrent use of bevacizumab with chemotherapy in clinical studies, such data is insufficient for evaluating the efficacy of single agent bevacizumab and single agent bevacizumab administration can not be recommended from an efficacy point of view. Also, in Study E3200 in second-line or subsequent treatment, enrollment to the single agent bevacizumab group was discontinued during the study due to a lack of efficacy. Thus, single agent bevacizumab administration from the start of treatment is not recommended. Therefore, regardless of previously treated or untreated patients, single agent bevacizumab administration can not be recommended. However, in clinical practice, single agent bevacizumab administration should not be excluded in the following cases: (a) while no disease progression is noted with bevacizumab in combination with chemotherapy, chemotherapy can not be continued due to its toxicities, (b) CR has been achieved with bevacizumab in combination with chemotherapy and further continuation of chemotherapy is considered unnecessary.

PMDA judges that the applicant's discussion that the use of bevacizumab alone can not be recommended based on the results of clinical studies is appropriate, but considers that the usage of single agent bevacizumab in clinical practice as described by the applicant is a claim based on inference. Especially, as to (b), although it is possible that the occurrence of adverse drug reactions may require interruption or discontinuation of the chemotherapy bevacizumab was combined with, the clinical significance of continued treatment with bevacizumab alone after CR is achieved is unclear, and if the applicant makes such claim, they need to evaluate it by conducting an appropriate study.

Based on the above, PMDA concluded that single agent bevacizumab administration is not recommended at present.

(3) The number of doses
The maximum number of doses specified for major clinical studies (a Japanese study and foreign phase III studies) was as follows:
・Until PD: Study JO18157, Study E3200
・Until PD or up to 48 weeks (up to 24 doses): Study NO16966
・Until PD or up to 96 weeks (up to 48 doses): Study AVF2107g

As patients in Study AVF2107g could continue to receive bevacizumab after entering Study AVF2540g, PMDA asked the applicant about the maximum number of doses of bevacizumab and chemotherapy administered in patients enrolled into this study. As a result, the maximum number of doses administered was 54 (one case each) for both the IFL+bevacizumab group and 5-FU/LV+bevacizumab group.

The appropriate number of cycles of bevacizumab in combination with chemotherapy for

first-line treatment or second-line or subsequent treatment is not clear from the present data submitted. Bevacizumab in combination with chemotherapy is expected to be continued as long as there is no problem with tolerability and no disease progression is noted. PMDA considers that it is necessary to collect safety data on long-term treatment after market launch in Japan, in addition to providing information on the number of doses per patient administered in clinical studies.

6) Post-marketing investigations

(1) Dose

Study E3200 has demonstrated the add-on effect of bevacizumab at a dose of 10 mg/kg in combination with FOLFOX4 in second-line or subsequent treatment. Meanwhile, in first-line treatment, the add-on effect of bevacizumab at a dose of 5 mg/kg in combination with chemotherapy has been demonstrated, and Study NO16966 has suggested that as measured by the median PFS (the primary endpoint), the add-on effect of bevacizumab 5 mg/kg given every 2 weeks in combination with FOLFOX4 (FOLFOX+placebo group: 261 days, FOLFOX4+bevacizumab group: 286 days) may be lower than the add-on effect of Bevacizumab 7.5 mg/kg given every 3 weeks in combination with XELOX (XELOX+placebo group: 225 days, XELOX+bevacizumab group: 282 days). Based on the above, PMDA asked for the applicant's view on the following post-marketing plans: an investigation of a dose of 10 mg/kg of bevacizumab in first-line treatment, expecting an enhanced add-on effect of bevacizumab in combination with FOLFOX4 and an investigation of a dose of 5 mg/kg since a dose response of bevacizumab has not been studied in second-line treatment.

The applicant responded as follows:

In first-line treatment, Study AVF2107g at a dose of 5 mg/kg has demonstrated a survival benefit and the PFS results from Study E2200 at a dose of 10 mg/kg are similar to the results from Study AVF2107g. Thus, we consider that a clinically significant effect is obtained at a dose of 5 mg/kg in first-line treatment and have no plan to investigate a dose of 10 mg/kg in first-line treatment. In second-line treatment, since there has been no report suggesting the usefulness of 5 mg/kg, we have no plan to investigate a dose of 5 mg/kg in second-line treatment.

PMDA understands that there is no plan to investigate the doses of bevacizumab in patients with colorectal cancer in a clinical study initiated by the applicant, but considers that it is necessary to grasp chemotherapy regimens combined with bevacizumab in the post-marketing experience in Japan and discuss the matters to be investigated in a future clinical study.

(2) Post-marketing surveillance

A post-marketing surveillance basic plan proposed by the applicant is as follows:

All-case investigation by a central registration system will be conducted. The objectives of surveillance are (a) to identify the occurrence of gastrointestinal perforation and haemorrhage (tumor-associated haemorrhage, mucosal haemorrhage) and explore the factors affecting the development of these events, (b) to perform a subgroup analysis between patients treated with bevacizumab 5 mg/kg and 10 mg/kg and identify the occurrence of adverse drug reactions at

each dose level, (c) to concentrate on collecting information on gastrointestinal perforation, haemorrhage (tumor-associated haemorrhage, mucosal haemorrhage), wound healing complications (wound dehiscence, post-operative haemorrhage, etc.), arterial thromboembolism, hypertension, proteinuria, and reversible posterior leukoencephalopathy syndrome (RPLS), which are important adverse drug reactions identified during the development and in foreign countries. The planned duration of surveillance is 18 months, the registration period lasts until 2,500 cases are accrued, and the observation period is 6 months. An interim analysis of patients registered in the early post-marketing phase is planned.

PMDA considers the above surveillance plan as follows:
With regard to the efficacy, as it is difficult to confirm the add-on effect of bevacizumab in a post-marketing surveillance, information should be collected so that the matters to be investigated in a future clinical study can be identified. For example, it is necessary to collect detailed information on the content of chemotherapy regimens combined with bevacizumab and to grasp drug utilization in Japan and information on the proper use. Also, as the details of the content of prior therapy were unknown in Foreign Study E3200 conducted by the ECOG, it is necessary to collect detailed information on patients who used bevacizumab as a second-line or subsequent treatment (previously treated patients), including prior chemotherapy. Taking into account these information, the necessity of a clinical study to further define the clinical positioning of bevacizumab should be examined.

As to the safety, since the submitted data includes a limited small number of Japanese patients, information should be collected via all-case investigation for a certain period of time after market launch. Although the items to be investigated, planned by the applicant, are mostly appropriate, it is necessary to not only put together safety information and perform an interim analysis, but also collect/analyze safety information obtained after market launch promptly and appropriately and swiftly disclose its content early after market launch.

The items to be investigated and the target number of cases and the registration period etc. will be finalized, taking account of a discussion on the efficacy, safety, clinical positioning, indications, and dosage regimen of bevacizumab with the expert advisors.

4.4 Adverse events etc. reported in clinical studies
The main adverse events other than deaths from clinical study safety data submitted were as follows.

1) Japanese phase I study of bevacizumab in combination with 5-FU/*l*-LV (Study Number JO18157)
All of the 18 patients enrolled into this study were included in the safety analysis and adverse events (MedDRA version 7.1) occurred in all of the 18 patients (273 events). Adverse events occurring in ≥ 30% (6/18 patients) of the patients were nausea in 12 patients (66.7%), diarrhoea in 11 patients (61.1%), anorexia in 10 patients (55.6%), neutrophil count decreased in 9 patients (50%), white blood cell count decreased in 9 patients (50%), stomatitis in 8 patients (44.4%),

vomiting in 8 patients (44.4%), lymphocyte count decreased in 7 patients (38.9%), weight decreased in 7 patients (38.9%), hypertension in 7 patients (38.9%), and epistaxis in 6 patients (33.3%) (data cutoff: [redacted] 20[redacted]).

Twenty two adverse events ≥Grade 3 were reported by 11 patients (61.1%) (lymphocyte count decreased, blood phosphorus decreased, neutrophil count decreased, diarrhoea, ileus, and hypertension, two cases each, blood cholesterol increased, nausea, colitis, abdominal pain, pyrexia, asthenia, infection, anorexia, dehydration, and palmar-plantar erythrodysaesthesia syndrome, one case each). Of which, neutrophil count decreased and hypertension (two cases each) and diarrhoea (one case) were those for which a causal relationship to the study drug, bevacizumab, could not be denied (adverse drug reactions) (data cutoff: [redacted] 20[redacted]).

2) Japanese safety confirmation study of bevacizumab in combination with FOLFOX4 (Study Number JO18158)

Safety analysis was performed for 14 patients excluding 1 untreated patient (Case No.X9) from the 15 enrolled patients at the time of interim data collection as of March 31, 2006. All of the 14 patients experienced adverse events (225 events) and those for which a causal relationship to the study drug (bevacizumab, 5-FU/*l*-LV and oxaliplatin) could not be denied (adverse drug reactions) were reported by all of the 14 patients (207 events). Adverse drug reactions occurring in ≥5 patients were neutrophil count decreased in 12 patients (85.7%), nausea in 11 patients (78.6%), white blood cell count decreased in 11 patients (78.6%), anorexia in 11 patients (78.6%), neurotoxicity in 10 patients (71.4%), diarrhoea in 7 patients (50.0%), epistaxis in 7 patients (50.0%), haematocrit decreased in 6 patients (42.9%), haemoglobin decreased in 6 patients (42.9%), platelet count decreased in 6 patients (42.9%), alopecia in 6 patients (42.9%), constipation in 5 patients (35.7%), vomiting in 5 patients (35.7%), lymphocyte count decreased in 5 patients (35.7%), blood bilirubin increased in 5 patients (35.7%), red blood cell count decreased in 5 patients (35.7%), and pigmentation disorder in 5 patients (35.7%). Hypertension occurred in 4 patients (28.6%) and protein urine present in 3 patients (21.4%) and all of these events were those for which a causal relationship to the study drug could not be denied.

Adverse events ≥Grade 3 were reported by 11 patients (78.6%) (15 events), which include neutrophil count decreased in 8 patients (of whom, 2 patients had Grade 4), white blood cell count decreased in 2 patients, diarrhoea in 1 patient, vomiting in 1 patient, periodontitis in 1 patient, gingivitis in 1 patient, and hyperglycaemia in 1 patient. All of these events except for hyperglycaemia in 1 patient, were classified as adverse drug reactions.

Between March 31, 2006 and [redacted], 7 serious adverse events were reported by 4 patients (Case No. X21, X22, X20, X23). Anorexia and fatigue in Case No. X21 and infectious gastroenteritis in Case No. X23 were all assessed as “probably related” to the study drug. Constipation and blood pressure increased in Case No. X21 were assessed as “possibly related” to the study drug. Gastrointestinal perforation in Case No. X20 was an event for which a causal relationship to the study drug “could not be denied” and cataract in Case No. X22 was assessed as “unrelated” to the study drug.

3) Foreign phase I, dose-escalation study (Study Number AVF0737g)
All of the 25 patients enrolled into this study were included in the safety analysis. Adverse events ≥Grade 3 were reported by 4 patients (16%), which include haemorrhage in 2 patients, dyspnoea in 1 patient, and anaemia in 1 patient. The events of haemorrhage include haemorrhage from a previously unrecognized brain metastasis in 1 patient and haemorrhage in epithelioid sarcoma of the right thigh in 1 patient and both of these events were assessed by the investigator as "possibly related" to bevacizumab. Increases in blood pressure >40 mmHg were noted in 1 patient each in the 3 mg/kg and 10 mg/kg groups.

Serious adverse events were reported by 2 patients in the 3 mg/kg group (Case No. X24, X25) (3 events), which include 2 events of haemorrhage and 1 event of anaemia, and 1 patient with haemorrhage from CNS metastasis was discontinued from the study. Both events of haemorrhage were assessed as "probably related" to bevacizumab and anaemia was assessed as "unrelated."

4) Foreign phase I, dose-escalation study (Study Number AVF0761g)
All of the 12 patients enrolled into this study were included in the safety analysis. Adverse events occurring in ≥2 patients were asthenia in 9 patients (75%), nausea in 8 patients (67%), anorexia in 5 patients (42%), alopecia, chest pain, pyrexia, and leukopenia (4 patients each [33%]), abdominal pain, back pain, depression, dyspepsia, glycosuria, mucous membrane disorder, and vomiting (3 patients each [25%]), anaemia, diarrhoea, dyspnoea, epistaxis, hypokalaemia, hypomagnesaemia, infection, rash maculo-papular, paraesthesia**,** rash, and thrombocytopenia (2 patients each [17%]).

Adverse events ≥Grade 3 were leukopenia in 4 patients (33%), thrombocytopenia in 2 patients (17%), nausea in 1 patient (8%), diarrhoea in 1 patient (8%), and hypotension in 1 patient (8%). Haemorrhage occurred in 3 patients (all in the CBDCA/PTX group): haematuria/ecchymosis in 1 patient and epistaxis in 2 patients.

5) Foreign phase II study of bevacizumab in combination with 5-FU/LV (Study Number AVF0780g)
Among the 104 patients enrolled into this study, 102 patients (35 patients in the 5-FU/LV group, 35 patients in the 5-FU/LV+bevacizumab 5 mg/kg group, 32 patients in the 5-FU/LV+bevacizumab 10 mg/kg group) excluding 2 enrolled patients who did not receive the study drug (5-FU/LV or bevacizumab) due to progressive disease were included in the safety analysis. All of the 102 patients had at least one adverse event. Commonly reported adverse events in patients treated with bevacizumab in combination with 5-FU/LV were epistaxis, vomiting, rhinitis, rash, pyrexia, and headache, etc.

Adverse events with a difference in incidence of ≥10% between the 5-FU/LV+bevacizumab group and the control group are as follows.

	5-FU/LV N=35 n, (%)		5-FU/LV+bevacizumab 5 mg N= 35 n, (%)		5-FU/LV+bevacizumab 10 mg N= 32 n, (%)	
	All Grades	Grade 3/4	All Grades	Grade 3/4	All Grades	Grade 3/4
Chills	1 (3%)	0 (0%)	5 (14%)	0 (0%)	5 (16%)	0 (0%)
Pyrexia	4 (11%)	0 (0%)	13 (37%)	0 (0%)	11 (34%)	1 (3%)
Headache	5 (14%)	0 (0%)	11 (31%)	0 (0%)	12 (38%)	1 (3%)
Infection	7 (20%)	0 (0%)	14 (40%)	0 (0%)	8 (25%)	1 (3%)
Mucous membrane disorder	9 (26%)	1 (3%)	6 (17%)	0 (0%)	4 (12%)	0 (0%)
Deep thrombophlebitis	0 (0%)	0 (0%)	4 (11%)	3 (9%)	0 (0%)	0 (0%)
Hypertension	1 (3%)	0 (0%)	4 (11%)	3 (9%)	9 (28%)	8 (25%)
Constipation	6 (17%)	0 (0%)	9 (26%)	1 (3%)	10 (31%)	1 (3%)
Gastrointestinal disorder	4 (11%)	1 (3%)	2 (6%)	0 (0%)	0 (0%)	0 (0%)
Gastrointestinal haemorrhage	0 (0%)	0 (0%)	2 (6%)	0 (0%)	5 (16%)	3 (9%)
Mouth ulceration	0 (0%)	0 (0%)	3 (9%)	0 (0%)	5 (16%)	1 (3%)
Vomiting	12 (34%)	2 (6%)	16 (46%)	2 (6%)	14 (44%)	2 (6%)
Rectal haemorrhage	1 (3%)	0 (0%)	6 (17%)	0 (0%)	4 (12%)	0 (0%)
Cough increased	5 (14%)	0 (0%)	9 (26%)	0 (0%)	8 (25%)	0 (0%)
Dyspnoea	3 (9%)	1 (3%)	7 (20%)	0 (0%)	5 (16%)	0 (0%)
Epistaxis	4 (11%)	0 (0%)	16 (46%)	0 (0%)	17 (53%)	0 (0%)
Pharyngitis	2 (6%)	0 (0%)	6 (17%)	0 (0%)	5 (16%)	0 (0%)
Rhinitis	9 (26%)	0 (0%)	17 (49%)	0 (0%)	12 (38%)	0 (0%)
Hyperglycaemia	2 (6%)	0 (0%)	6 (17%)	1 (3%)	0 (0%)	0 (0%)
Hypokalaemia	2 (6%)	1 (3%)	4 (11%)	1 (3%)	5 (16%)	3 (9%)
Healing abnormal	0 (0%)	0 (0%)	4 (11%)	1 (3%)	1 (3%)	0 (0%)
Weight decreased	8 (23%)	0 (0%)	5 (14%)	1 (3%)	3 (9%)	0 (0%)
Insomnia	9 (26%)	0 (0%)	5 (14%)	0 (0%)	6 (19%)	0 (0%)
Dry skin	7 (20%)	0 (0%)	4 (11%)	0 (0%)	2 (6%)	0 (0%)
Pruritus	1 (3%)	0 (0%)	6 (17%)	0 (0%)	6 (19%)	0 (0%)
Rash	7 (20%)	0 (0%)	16 (46%)	1 (3%)	11 (34%)	0 (0%)
Lacrimation disorder	3 (9%)	0 (0%)	4 (11%)	0 (0%)	6 (19%)	1 (3%)

Adverse events ≥Grade 3 were reported by 19/35 patients (54%) in the 5-FU/LV group, 26/35 patients (74%) in the 5-FU/LV+bevacizumab 5 mg/kg group, and 25/32 patients (78%) in the 5-FU/LV+bevacizumab 10 mg/kg group and the incidences of deep thrombophlebitis and hypertension etc. were higher in the 5-FU/LV+bevacizumab group compared to the 5-FU/LV group.

Serious adverse events occurring in ≥2 patients treated with 5-FU/LV+bevacizumab in this study are as follows.

	5-FU/LV N= 35 n, (%)		5-FU/LV+bevacizumab 5 mg N= 35 n, (%)		5-FU/LV+ bevacizumab 10 mg N= 32 n, (%)	
	Grade 3/4	All Grades	Grade 3/4	All Grades	Grade 3/4	All Grades
Total	8 (23%)	11 (31%)	14 (40%)	16 (46%)	14 (44%)	15 (47%)
Abdominal pain	0 (0%)	0 (0%)	0 (0%)	0 (0%)	2 (6%)	2 (6%)
Pyrexia	0 (0%)	0 (0%)	0 (0%)	1 (3%)	1 (3%)	3 (9%)
Deep thrombophlebitis	0 (0%)	0 (0%)	2 (6%)	2 (6%)	0 (0%)	0 (0%)
Hypertension	0 (0%)	0 (0%)	1 (3%)	1 (3%)	1 (3%)	1 (3%)
Colitis	0 (0%)	0 (0%)	0 (0%)	0 (0%)	2 (6%)	2 (6%)
Diarrhoea	4 (11%)	4 (11%)	4 (11%)	4 (11%)	3 (9%)	3 (9%)
Gastrointestinal haemorrhage	0 (0%)	0 (0%)	0 (0%)	0 (0%)	3 (9%)	3 (9%)
Intestinal obstruction	0 (0%)	0 (0%)	0 (0%)	2 (6%)	1 (3%)	1 (3%)
Vomiting	0 (0%)	0 (0%)	1 (3%)	1 (3%)	2 (6%)	2 (6%)
Leukopenia	1 (3%)	1 (3%)	2 (6%)	3 (9%)	0 (0%)	0 (0%)

6) Foreign phase II study of bevacizumab in combination with 5-FU/LV (Study Number AVF2192g)

Among the 214 patients enrolled into this study, 204 patients (104 patients in the 5-FU/LV group, 100 patients in the 5-FU/LV+bevacizumab group) excluding 5 patients with violations such as false reporting and 5 patients who received no study drug were included in the safety analysis.

Adverse events were reported by 102 patients (98.1%) in the 5-FU/LV group and 100 patients (100%) in the 5-FU/LV+bevacizumab group and adverse events occurring at a ≥10% higher incidence in the 5-FU/LV+bevacizumab group were asthenia (63 patients [60.6%] in the 5-FU/LV group and 76 patients [76.0%] in the 5-FU/LV+bevacizumab group), pain (21 patients [20.2%] and 33 patients [33.0%], respectively), pyrexia (11 patients [10.6%] and 24 patients [24.0%], respectively), hypertension (5 patients [4.8%] and 32 patients [32.0%], respectively), stomatitis (13 patients [12.5%] and 25 patients [25.0%], respectively), and proteinuria (20 patients [19.2%] and 38 patients [38.0%], respectively). Adverse events ≥Grade 3 were reported by 74 patients (71.2%) and 87 patients (87.0%), respectively, and of which, those with a difference in incidence of ≥2% between the groups are as shown below. Those occurring at a ≥5% higher incidence in the 5-FU/LV+bevacizumab group were asthenia, anaemia, hypertension, intestinal obstruction, and dyspnoea.

	5-FU/LV N=104 n, (%)	5-FU/LV+bevacizumab N=100 n, (%)
Asthenia	12 (11.5%)	17 (17.0%)
Pain	2 (1.9%)	6 (6.0%)
Sepsis	4 (3.8%)	8 (8.0%)
Abscess	1 (1.0%)	3 (3.0%)
Accidental injury	1 (1.0%)	3 (3.0%)
Hypertension	2 (1.9%)	15 (15.0%)
Deep thrombophlebitis	9 (8.7%)	6 (6.0%)
Syncope	2 (1.9%)	4 (4.0%)

Congestive heart failure	0 (0%)	2 (2.0%)
Supraventricular tachycardia	0 (0%)	2 (2.0%)
Cerebrovascular disorder	1 (1.0%)	3 (3.0%)
Nausea	12 (11.5%)	4 (4.0%)
Gastrointestinal disorder	0 (0%)	4 (4.0%)
Gastroenteritis	0 (0%)	2 (2.0%)
Intestinal obstruction	3 (2.9%)	9 (9.0%)
Bowel perforation	0 (0%)	2 (2.0%)
Ileus	1 (1.0%)	4 (4.0%)
Anaemia	0 (0%)	5 (5.0%)
Thrombocytopenia	0 (0%)	2 (2.0%)
Prothrombin decreased	1 (1.0%)	3 (3.0%)
Hypokalaemia	3 (2.9%)	5 (5.0%)
Sleepiness	0 (0%)	2 (2.0%)
Dyspnoea	2 (1.9%)	7 (7.0%)
Hypoxia	0 (0%)	2 (2.0%)
Urinary tract infection	0 (0%)	2 (2.0%)

7) Foreign phase III study of bevacizumab in combination with IFL (Study Number AVF2107g)

Adverse events (NCI-CTC) were reported for 309 patients (98 patients in the IFL group, 102 patients in the IFL+bevacizumab group, 109 patients in the 5-FU/LV+ bevacizumab group) for whom the reporting of all adverse events was required by the protocol, among 923 patients (Arm 1 [IFL]: 411 patients, Arm 2 [IFL+bevacizumab]: 402 patients, Arm 3 [5-FU/LV+bevacizumab]: 110 patients) excluding 2 patients with violations such as false reporting (Case No. X13, X14) from the 925 enrolled patients.

As of July 20, 2004, adverse events with a difference in incidence of $\geq$10% between the IFL group and the IFL+bevacizumab group or the 5-FU/LV+bevacizumab group are as shown below. Compared to the IFL group, adverse events occurring at a higher incidence in the IFL+bevacizumab group and the 5-FU/LV+bevacizumab group were pain (35.7%, 51.0%, 39.4%), hypertension (14.3%, 23.5%, 33.9%), anorexia (29.6%, 44.1%, 33.9%), headache (18.4%, 26.5%, 28.4%), and proteinuria (25.5%, 36.3%, 35.8%) etc.

	IFL N= 98 n, (%)	IFL+bevacizumab N=102 n, (%)	5FU/LV+ bevacizumab N=109 n, (%)
Total	98 (100%)	102 (100%)	109 (100%)
Pain	35 (35.7%)	52 (51.0%)	43 (39.4%)
Headache	18 (18.4%)	27 (26.5%)	31 (28.4%)
Hypertension	14 (14.3%)	24 (23.5%)	37 (33.9%)
Anorexia	29 (29.6%)	45 (44.1%)	37 (33.9%)
Constipation	28 (28.6%)	42 (41.2%)	33 (30.3%)
Stomatitis	13 (13.3%)	24 (23.5%)	19 (17.4%)
Rectal haemorrhage	2 (2.0%)	18 (17.6%)	9 (8.3%)
Leukopenia	53 (54.1%)	58 (56.9%)	12 (11.0%)
Epistaxis	10 (10.2%)	36 (35.3%)	35 (32.1%)
Dyspnoea	15 (15.3%)	26 (25.5%)	27 (24.8%)
Rhinitis	12 (12.2%)	26 (25.5%)	23 (21.1%)

Alopecia	25 (25.5%)	33 (32.4%)	6 (5.5%)
Dry skin	7 (7.1%)	7 (6.9%)	22 (20.2%)
Exfoliative dermatitis	3 (3.1%)	3 (2.9%)	21 (19.3%)
Skin discolouration	3 (3.1%)	2 (2.0%)	17 (15.6%)
Taste perversion	8 (8.2%)	12 (11.8%)	21 (19.3%)
Lacrimation disorder	2 (2.0%)	6 (5.9%)	20 (18.3%)
Proteinuria	25 (25.5%)	37 (36.3%)	39 (35.8%)

Adverse events ≥Grade 3 were reported by 296/397 patients (74.6%) in the IFL group and 339/392 patients (86.5%) in the IFL+bevacizumab group, and of which, those with a difference in incidence of ≥2% between the groups are as shown below. Those reported more frequently in the IFL+bevacizumab group as compared to the IFL group were deep thrombophlebitis, hypertension, diarrhoea, leukopenia, asthenia, abdominal pain, and pain.

	IFL N=397 n, (%)	IFL+bevacizumab N=392 n, (%)
Total	296 (74.6%)	339 (86.5%)
Asthenia	28 (7.1%)	38 (9.7%)
Abdominal pain	20 (5.0%)	35 (8.9%)
Pain	12 (3.0%)	21 (5.4%)
Deep thrombophlebitis	27 (6.8%)	35 (8.9%)
Hypertension	10 (2.5%)	49 (12.5%)
Diarrhoea	99 (24.9%)	135 (34.4%)
Vomiting	42 (10.6%)	31 (7.9%)
Nausea	38 (9.6%)	27 (6.9%)
Leukopenia	123 (31.0%)	147 (37.5%)

Other significant adverse events during first-line therapy among 397 patients in the IFL group and 392 patients in the IFL+bevacizumab group were haemorrhage ≥Grade 3 (10 patients [2.5%] in the IFL group and 13 patients [3.3%] in the IFL+bevacizumab group), hypertension (34 patients [8.6%] and 96 patients [24.5%], respectively), proteinuria (89 patients [22.4%] and 113 patients [28.8%], respectively), heart failure (4 patients [1.0%] and 5 patients [1.3%], respectively), diarrhoea ≥Grade 3 (99 patients [24.9%] and 135 patients [34.4%], respectively), and thromboembolism (67 patients [16.9%] and 79 patients [20.2%], respectively, including deep thrombophlebitis (27 patients [6.8%] and 36 pateints [9.2%], respectively).

8) Foreign phase III study of bevacizumab in combination with FOLFOX4 or XELOX (Study Number NO16966)

This study was initiated as an open-label comparative study of XELOX vs. FOLFOX4 (Study NO16966A). Then, combining XELOX or FOLFOX4 with bevacizumab or placebo, a randomized, double-blind comparative study evaluating the safety and efficacy of bevacizumab (Study NO16966C) was added.

In Study NO16966C, 10 patients in the two placebo groups received at least one dose of

bevacizumab due to drug-administration errors and these patients were included in the bevacizumab groups for analysis.

As of [redacted] 20[redacted], the safety was compared among the FOLFOX4+placebo group, the FOLFOX4+bevacizumab group, the XELOX+placebo group, and the XELOX+bevacizumab group. As a result, the incidence of adverse events ≥Grade 3, the rate of discontinuations due to adverse events, and the incidence of deaths within 28 days after the last dose were higher in the bevacizumab containing groups.

	FOLFOX4+placebo N= 336 n, (%)	FOLFOX4+bevacizumab N=341 n, (%)	XELOX+placebo N=339 n, (%)	XELOX+bevacizumab N=353 n, (%)
All adverse events	335 (99.7%)	339 (99.4%)	336 (99.1%)	351 (99.4%)
Adverse events ≥Grade 3	268 (79.8%)	288 (84.5%)	237 (69.9%)	267 (75.6%)
Discontinuations due to adverse events	69 (20.5%)	104 (30.5%)	71 (20.9%)	109 (30.9%)

Adverse events ≥ Grade 3 for which a causal relationship to the study drug could not be denied with a difference in incidence of ≥1% between the placebo and bevacizumab groups are as shown below. The incidences of gastrointestinal adverse events, palmar-plantar erythrodysaesthesia syndrome, venous thrombotic events, and hypertension were higher in the bevacizumab containing groups.

	FOLFOX4+placebo N=336 n, (%)	FOLFOX4 +bevacizumab N=341 n, (%)	XELOX+placebo N=339 n, (%)	XELOX +bevacizumab N=353 n, (%)
Neutropenia	148 (44%)	137 (40%)	26 (8%)	25 (7%)
Thrombocytopenia	11 (3%)	10 (3%)	16 (5%)	9 (3%)
Febrile neutropenia	16 (5%)	15 (4%)	1 (<1%)	4 (1%)
Leukopenia	5 (1%)	6 (2%)	1 (<1%)	2 (<1%)
Anaemia	4 (1%)	4 (1%)	3 (<1%)	2 (<1%)
Diarrhoea	31 (9%)	41 (12%)	67 (20%)	75 (21%)
Vomiting	6 (2%)	19 (6%)	16 (5%)	18 (5%)
Nausea	7 (2%)	11 (3%)	13 (4%)	22 (6%)
Stomatitis	6 (2%)	12 (4%)	6 (2%)	7 (2%)
Abdominal pain	4 (1%)	3 (<1%)	10 (3%)	10 (3%)
Paraesthesia	20 (6%)	21 (6%)	19 (6%)	18 (5%)
Peripheral sensory neuropathy	11 (3%)	16 (5%)	13 (4%)	8 (2%)
Peripheral neuropathies	10 (3%)	9 (3%)	10 (3%)	17 (5%)
Neuropathy	11 (3%)	10 (3%)	7 (2%)	6 (2%)
Dysaesthesia	8 (2%)	4 (1%)	5 (1%)	9 (3%)
Lethargy	2 (<1%)	5 (1%)	5 (1%)	2 (<1%)
Fatigue	22 (7%)	20 (6%)	16 (5%)	24 (7%)
Asthenia	15 (4%)	15 (4%)	18 (5%)	26 (7%)
Hypokalaemia	8 (2%)	7 (2%)	18 (5%)	10 (3%)
Anorexia	7 (2%)	8 (2%)	8 (2%)	11 (3%)
Dehydration	1 (<1%)	5 (1%)	8 (2%)	9 (3%)

Palmar-plantar erythrodysaesthesia syndrome	4 (1%)	7 (2%)	19 (6%)	42 (12%)
Hypertension	2 (<1%)	9 (3%)	4 (1%)	10 (3%)
Deep vein thrombosis	7 (2%)	10 (3%)	2 (<1%)	5 (1%)
Thrombosis	3 (<1%)	5 (1%)	2 (<1%)	2 (<1%)
Pulmonary embolism	2 (<1%)	7 (2%)	3 (<1%)	9 (3%)
Dyspnoea	6 (2%)	1 (<1%)	5 (1%)	6 (2%)
Dysaesthesia pharynx	1 (<1%)	0 (0%)	9 (3%)	4 (1%)
Allergic reaction	7 (2%)	6 (2%)	2 (<1%)	6 (2%)
Hyperbilirubinaemia	0 (0%)	1 (<1%)	5 (1%)	3 (<1%)

9) Foreign phase II study of bevacizumab in combination with IFL (Study Number E2200)

Among the 92 patients enrolled into this study, 87 patients excluding 5 untreated patients were included in the safety analysis. Adverse events with an incidence ≥20% were fatigue in 70 patients (80.5%), abdominal pain in 44 patients (50.6%), headache in 26 patients (29.9%), diarrhoea in 86 patients (98.9%), nausea in 67 patients (77.0%), vomiting in 43 patients (49.4%), anorexia in 33 patients (37.9%), constipation in 27 patients (31.0%), stomatitis in 26 patients (29.9%), white blood cell decreased in 71 patients (81.6%), neutropenia in 68 patients (78.2%), platelet count decreased in 29 patients (33.3%), cough in 26 patients (29.9%), epistaxis in 24 patients (27.6%), weight decreased in 19 patients (21.8%), alopecia in 34 patients (39.1%), rash/desquamation in 19 patients (21.8%), AST increased in 32 patients (36.8%), PTT prolonged in 26 patients (29.9%), and creatinine increased in 19 patients (21.8%).

Grade 3 adverse events were reported by 41 patients (47%) and adverse events ≥Grade 4 by 26 patients (30%). Among the adverse events with an incidence ≥20%, those ≥Grade 3 were fatigue in 9 patients, abdominal pain in 7 patients, headache in 1 patient, diarrhoea in 15 patients, nausea in 9 patients, vomiting in 10 patients, anorexia in 7 patients, constipation in 3 patients, white blood cell decreased in 10 patients, neutropenia in 31 patients, platelet count decreased in 1 patient, cough in 1 patient, epistaxis in 1 patient, PTT prolonged in 8 patients, and creatinine increased in 5 patients.

10) Foreign phase III study of bevacizumab in combination with FOLFOX4 (Study Number E3200)

Among the 829 patients enrolled into this study, 806 patients treated with the study drug were included in the safety analysis, and treatment-related, hematological adverse events ≥Grade 4 and non-hematological adverse events ≥Grade 3 were collected. Of which, those with a difference in incidence of ≥5% between the FOLFOX4 group and the FOLFOX4+bevacizumab group were diarrhoea (12.6% in the FOLFOX4 group and 17.8% in the FOLFOX4+bevacizumab group), nausea (4.2% and 10.8%, respectively), vomiting (3.2% and 10.1%, respectively), sensory neuropathy (9.1% and 16.4%, respectively), and fatigue (13.0% and 18.5%, respectively). Those with a difference in incidence of ≥ 2% are as shown below. The numbers of bevacizumab-treated patients who required dose reduction (10 mg/kg→5 mg/kg) or were discontinued due to toxicities were 97/241 patients (40.2%) in the

FOLFOX4+bevacizumab group and 54/202 patients (26.7%) in the bevacizumab alone group.

	FOLFOX4 N= 285 n, (%)	FOLFOX4+bevacizumab N= 287 n, (%)	Bevacizumab alone N=234 n, (%)
No. of patients with at least one adverse event	171 (60.0%)	219 (76.3%)	87 (37.2%)
Diarrhoea	36 (12.6%)	51 (17.8%)	4 (1.7%)
Nausea	12 (4.2%)	31 (10.8%)	7 (3.0%)
Vomiting	9 (3.2%)	29 (10.1%)	10 (4.3%)
Dehydration	14 (4.9%)	25 (8.7%)	8 (3.4%)
Ileus	1 (0.4%)	8 (2.8%)	5 (2.1%)
Neuropathy - sensory	26 (9.1%)	47 (16.4%)	2 (0.9%)
Neuropathy - other	8 (2.8%)	15 (5.2%)	3 (1.3%)
Fatigue	37 (13.0%)	53 (18.5%)	10 (4.3%)
Abdominal pain	10 (3.5%)	17 (5.9%)	12 (5.1%)
Headache	0 (0%)	8 (2.8%)	3 (1.3%)
Hypertension	5 (1.8%)	18 (6.3%)	17 (7.3%)
Dyspnoea	11 (3.9%)	17 (5.9%)	3 (1.3%)
Haemorrhage - other	0 (0%)	6 (2.1%)	1 (0.4%)

11) Foreign extension study (Study Number AVF2540g)

Among the 105 patients enrolled into this study, 104 patients treated with the study drug were included in the safety analysis. The most common adverse event ≥Grade 3 was hypertension, which was controllable in most patients. Two patients experienced arterial thrombotic events, but neither of them was discontinued from the study and administration of bevacizumab was resumed after thrombosis was treated. Although Grade 3 proteinuria was observed in 3 patients, no patients presented with nephrotic syndrome. None of the 13 patients who had at least one surgical operation during the study period experienced wound healing complications. Grade 4 gastrointestinal perforation was reported as perforation of gastric ulcer by 1 patient. Congestive heart failure was not noted in this study.

12) Foreign extension study (Study Number AVF0778g)

Fifty-six patients enrolled into this study were included in the analysis. The most common adverse event ≥ Grade 3 was hypertension. Venous thrombosis occurred in 5 patients, whereas there was no patient with pulmonary embolism. Among the 5 patients with venous thrombosis, 2 patients were discontinued from the study and 3 patients resumed bevacizumab after thrombosis was treated. Grade 3 proteinuria was noted in 1 patient, but there was no patient with nephrotic syndrome.

13) Foreign phase III clinical study of bevacizumab in combination with capecitabine in patients with previously treated metastatic breast cancer (Study Number AVF2119g, *J Clin Oncol* 2005; 23: 792-799, Study Period: November 2000 to [redacted] 2002)

Adverse events with an incidence ≥15% reported in this study are as follows.

	Capecitabine N=215 n, (%)		Capecitabine+bevacizumab N=229 n, (%)	
	≥Grade 3	All Grades	≥Grade 3	All Grades
All adverse events	123 (57.2%)	211 (98.1%)	156 (68.1%)	229 (100%)
Asthenia	14 (6.5%)	102 (47.4%)	17 (7.4%)	131 (57.2%)
Headache	1 (0.5%)	28 (13.0%)	4 (1.7%)	76 (33.2%)
Pain	4 (1.9%)	53 (24.7%)	7 (3.1%)	71 (31.0%)
Infection	1 (0.5%)	39 (18.1%)	2 (0.9%)	48 (21.0%)
Abdominal pain	1 (0.5%)	45 (20.9%)	4 (1.7%)	45 (19.7%)
Chest pain	5 (2.3%)	29 (13.5%)	3 (1.3%)	37 (16.2%)
Mucous membrane disorder	1 (0.5%)	28 (13.0%)	0 (0%)	35 (15.3%)
Hypertension	1 (0.5%)	5 (2.3%)	41 (17.9%)	54 (23.6%)
Diarrhoea	23 (10.7%)	113 (52.6%)	27 (11.8%)	129 (56.3%)
Nausea	4 (1.9%)	106 (49.3%)	6 (2.6%)	107 (46.7%)
Vomiting	9 (4.2%)	58 (27.0%)	6 (2.6%)	70 (30.6%)
Anorexia	5 (2.3%)	51 (23.7%)	2 (0.9%)	60 (26.2%)
Stomatitis	1 (0.5%)	41 (19.1%)	4 (1.7%)	58 (25.3%)
Constipation	0 (0%)	32 (14.9%)	1 (0.4%)	37 (16.2%)
Dyspnoea	11 (5.1%)	39 (18.1%)	17 (7.4%)	61 (26.6%)
Epistaxis	0 (0%)	3 (1.4%)	0 (0%)	36 (15.7%)
Exfoliative dermatitis	52 (24.2%)	162 (75.3%)	63 (27.5%)	193 (84.3%)
Proteinuria	0 (0%)	16 (7.4%)	2 (0.9%)	51 (22.3%)

14) Foreign phase II clinical study of bevacizumab in combination with CBDCA/PTX in patients with advanced non-small cell lung cancer (Study Number AVF0757g)

Among the 99 patients enrolled into this study, 98 patients excluding 1 patient who did not receive study drug because a brain metastasis was found before study treatment were included in the safety analysis. Adverse events occurring at a ≥10% higher incidence in the CBDCA/PTX+bevacizumab group compared to the CBDCA/PTX group or adverse events of which the number of occurrences in the CBDCA/PTX+bevacizumab group was at least twice that in the CBDCA/PTX group are as follows.

	CBDCA/PTX N= 32 n, (%)	CBDCA/PTX+bevacizumab 7.5 mg/kg N= 32 n, (%)	CBDCA/PTX+bevacizumab 15 mg/kg N= 34 n, (%)
Headache	3 (9.4%)	10 (31.3%)	16 (47.1%)
Infection	8 (25.0%)	10 (31.3%)	12 (35.3%)
Pyrexia	4 (12.5%)	11 (34.4%)	11 (32.4%)
Abdominal pain	3 (9.4%)	4 (12.5%)	8 (23.5%)
Back pain	2 (6.3%)	5 (15.6%)	4 (11.8%)
Hypertension	1 (3.1%)	5 (15.6%)	6 (17.6%)

Haemorrhage	0 (0%)	4 (12.5%)	0 (0%)
Hypotension	1 (3.1%)	4 (12.5%)	3 (8.8%)
Diarrhoea	6 (18.8%)	9 (28.1%)	14 (41.2%)
Anorexia	8 (25.0%)	9 (28.1%)	14 (41.2%)
Stomatitis	3 (9.4%)	5 (15.6%)	8 (23.5%)
Leukopenia	10 (31.3%)	15 (46.9%)	19 (55.9%)
Ecchymosis	0 (0%)	0 (0%)	4 (11.8%)
Hyperglycaemia	3 (9.4%)	4 (12.5%)	7 (20.6%)
Weight decreased	0 (0%)	2 (6.3%)	6 (17.6%)
ALP increased	1 (3.1%)	0 (0%)	3 (8.8%)
Myalgia	16 (50.0%)	9 (28.1%)	9 (26.5%)
Arthritis	2 (6.3%)	4 (12.5%)	0 (0%)
Paraesthesia	7 (21.9%)	9 (28.1%)	12 (35.3%)
Insomnia	14 (43.8%)	8 (25.0%)	5 (14.7%)
Depression	2 (6.3%)	5 (15.6%)	8 (23.5%)
Confusion	0 (0%)	2 (6.3%)	5 (14.7%)
Neuropathy	9 (28.1%)	4 (12.5%)	5 (14.7%)
Sleepiness	1 (3.1%)	0 (0%)	4 (11.8%)
Cough increased	8 (25.0%)	12 (37.5%)	17 (50.0%)
Epistaxis	2 (6.3%)	10 (31.3%)	15 (44.1%)
Haemoptysis	2 (6.3%)	9 (28.1%)	4 (11.8%)
Pharyngitis	3 (9.4%)	5 (15.6%)	9 (26.5%)
Rhinitis	0 (0%)	8 (25.0%)	7 (20.6%)
Voice alteration	0 (0%)	5 (15.6%)	8 (23.5%)
Sinusitis	1 (3.1%)	3 (9.4%)	7 (20.6%)
Bronchitis	1 (3.1%)	3 (9.4%)	4 (11.8%)
Hiccups	1 (3.1%)	2 (6.3%)	2 (5.9%)
Alopecia	17 (53.1%)	20 (62.5%)	22 (64.7%)
Rash	3 (9.4%)	11 (34.4%)	8 (23.5%)
Pruritus	0 (0%)	5 (15.6%)	2 (5.9%)
Acne	1 (3.1%)	0 (0%)	4 (11.8%)
Taste perversion	1 (3.1%)	3 (9.4%)	2 (5.9%)
Tinnitus	1 (3.1%)	2 (6.3%)	1 (2.9%)
Urinary tract infection	0 (0%)	1 (3.1%)	5 (14.7%)
Peripheral neuritis	9 (28.1%)	8 (25.0%)	13 (38.2%)

Among the above adverse events, events ≥Grade 3 were leukopenia in 7 patients, myalgia in 2 patients, peripheral neuritis in 1 patient in the CBDCA/PTX group, leukopenia in 10 patients, diarrhoea in 3 patients, haemoptysis in 3 patients, pyrexia in 2 patients, haemorrhage in 2 patients, headache, back pain, hyperglycaemia, arthritis, depression, cough increased, sinusitis, bronchitis, and urinary tract infection (1 patient each) in the CBDCA/PTX+bevacizumab 7.5 mg/kg group, leukopenia in 13 patients, headache, infection, pyrexia, hypertension, hyperglycaemia, myalgia, peripheral neuritis, and confusion (2 patients each), back pain, diarrhoea, neuropathy, sleepiness, haemoptysis, pharyngitis, bronchitis, hiccups, and urinary tract infection (1 patient each) in the CBDCA/PTX+bevacizumab 15 mg/kg group.

15) Foreign phase II study in patients with relapsed metastatic breast cancer (Study Number AVF0776g)

All of the 75 patients enrolled into this study (18 patients in the 3 mg/kg group, 41 patients in the 10 mg/kg, 16 patients in the 20 mg/kg group) were included in the safety analysis.

Adverse events with an incidence ≥10% among the 75 patients were asthenia in 43 patients (57%), infection in 30 patients (40%), pain in 29 patients (39%), headache in 27 patients (36%), back pain in 14 patients (19%), chest pain in 14 patients (19%), abdominal pain in 12 patients (16%), pyrexia in 10 patients (13%), hypertension in 17 patients (23%), vasodilatation in 13 patients (17%), nausea in 38 patients (51%), diarrhoea in 28 patients (37%), vomiting in 28 patients (37%), anorexia in 19 patients (25%), constipation in 18 patients (24%), stomatitis in 14 patients (19%), dyspepsia in 10 patients (13%), weight decreased in 10 patients (13%), myalgia in 24 patients (32%), arthritis in 23 patients (31%), hyperaesthesia in 15 patients (20%), paraesthesia in 15 patients (20%), dizziness in 14 patients (19%), depression in 12 patients (16%), cough increased in 21 patients (28%), dyspnoea in 21 patients (28%), sinusitis in 15 patients (20%), rhinitis in 12 patients (16%), lung disorder in 10 patients (13%), pharyngitis in 8 patients (11%), pruritus in 17 patients (23%), and urinary tract infection in 8 patients (11%).

Adverse events ≥Grade 3 were reported by 44 patients (59%) and of which, Grade 4 events were hypercalcaemia in 3 patients, asthenia, oedema, dyspnoea, and nephrosis (2 patients each). Among the above-mentioned adverse events with an incidence ≥10%, events ≥Grade 3 were hypertension in 14 patients, dyspnoea in 8 patients, asthenia in 7 patients, headache in 5 patients, pain in 4 patients, chest pain in 4 patients, abdominal pain in 4 patients, constipation in 3 patients, myalgia in 3 patients, arthritis in 3 patients, back pain in 2 patients, depression in 2 patients, vasodilatation in 1 patient, diarrhoea in 1 patient, anorexia in 1 patient, and cough increased in 1 patient.

16) Foreign phase II clinical study in patients with prostate cancer unresponsive to endocrine therapy (Study Number AVF0775g)

All of the 15 patients enrolled into this study were included in the safety analysis. Adverse events with an incidence ≥20% were asthenia in 9 patients (60%), constipation in 8 patients (53%), pain in 6 patients (40%), epistaxis in 6 patients (40%), anorexia in 5 patients (33%), back pain in 4 patients (27%), chest pain in 4 patients (27%), weight decreased in 4 patients (27%), cough increased in 4 patients (27%), infection in 3 patients (20%), dizziness in 3 patients (20%), and voice alteration in 3 patients (20%).

Serious adverse events reported were hyponatraemia in 2 patients (Case No. X26, X27) and abdominal pain in 1 patient (Case No. X27), and hyponatraemia was assessed as “probably related” to bevacizumab for both patients and abdominal pain was assessed as “unrelated.”

17) Antibody formation

Of the 1,032 patients included in a pooled analysis performed by Genentech (the US), 4 out of 837 patients who underwent anti-bevacizumab antibody test at baseline had positive results. On

the other hand, anti-bevacizumab antibodies were not detected in the specimens from 492 patients, collected after the start of administration of bevacizumab.

III. The Results of Compliance Review concerning the Documents Appended to the New Drug Application and Conclusion by the PMDA

1) PMDA's conclusion of the results of document compliance review

Document compliance review was conducted in accordance with the provisions of the Pharmaceutical Affairs Law for the documents appended to the new drug application. As a result, PMDA concluded that there should be no problem with conducting regulatory review based on the submitted documents.

2) PMDA's conclusion of the results of GCP on-site inspection

GCP on-site inspection took place in accordance with the provisions of the Pharmaceutical Affairs Law for the documents appended to the new drug application. As a result, PMDA concluded that there should be no problem with conducting regulatory review based on the submitted documents.

IV. Overall Evaluation

As a result of the above review, PMDA has judged that the efficacy and safety of bevacizumab have been demonstrated and that the product is approvable. PMDA will have a discussion with the expert advisors, focusing on the following points, and then make a final conclusion on the indications and the dosage regimen, based on the results of the Expert Discussion.

・The efficacy of bevacizumab in patients with advanced or recurrent colorectal cancer who are not candidates for curative resection

Previously untreated patients

Previously treated patients

・The safety of bevacizumab

・Indications

・Dosage and administration

・Post-marketing investigations

Review Report (2)

February 14, 2007

I. Overview of the Product

[Brand name]	Avastin 100 mg/4 mL Intravenous Infusion
	Avastin 400 mg/16 mL Intravenous Infusion
[Non-proprietary name]	Bevacizumab (Genetical Recombination)
[Applicant]	Chugai Pharmaceutical Co., Ltd.
[Date of application]	April 21, 2006

II. The Content of Review

The Pharmaceuticals and Medical Devices Agency (PMDA) sought the expert advisors' opinions based on the Review Report (1). Discussions with the expert advisors are outlined below.

1) The efficacy in patients with advanced or recurrent colorectal cancer who are not candidates for curative resection

(1) Previously untreated patients (first-line treatment)

PMDA considered that the key studies demonstrating the efficacy of bevacizumab in first-line treatment are Foreign Studies AVF2107g and NO16966 and judged that these study results have shown the add-on effect of bevacizumab in combination with fluoropyrimidine-based chemotherapy in first-line treatment. This judgment by the PMDA was supported by the expert advisors.

PMDA interpreted the results of subgroup analysis of Study NO16966 for PFS as follows:
(a) It has been suggested that the add-on effect of bevacizumab differs depending on the chemotherapeutic regimen combined with bevacizumab.
(b) The add-on effect of bevacizumab in combination with FOLFOX4 is uncertain at present and may be lower than the add-on effect of bevacizumab in combination with XELOX.

At the Expert Discussion, there was a comment from the expert advisors that the results of pre-planned subgroup analysis showing that the add-on effect of bevacizumab in combination with FOLFOX4 was not significant are important as an objective fact and PMDA's judgment was supported. The following PMDA's judgment was also supported: it is necessary to provide the results of pre-planned subgroup analysis (the add-on effect of bevacizumab in combination with FOLFOX4 was not significant) to the medical practice, in addition to the result that "the add-on effect of bevacizumab in combination with chemotherapy was demonstrated," as the efficacy results of Study NO16966.

On the other hand, the following comments were raised from the expert advisors:
Although we support the PMDA's judgment on the interpretation of the results of subgroup analysis, (a) This study is the only clinical study that evaluated the add-on effect of bevacizumab in combination with FOLFOX4 in first-line treatment and at present, it is impossible to draw a definitive conclusion on the relative relationship between the add-on effect of bevacizumab in combination with FOLFOX4 and the add-on effect of bevacizumab in combination with other chemotherapy regimens. (b) As Study NO16966 has confirmed the non-inferiority of XELOX over FOLFOX4 in terms of PFS, it is unclear how the results of secondary subgroup analysis that the add-on effect of bevacizumab in combination with FOLFOX4 was not significant, will be reflected when selecting medications/regimens for treatment, etc.

PMDA decided to establish "Indications," "Dosage and administration," and "Precautions," etc., taking account of the above comments from the expert advisors and the current situation that capecitabine, which is used for XELOX, is unapproved for colorectal cancer in Japan.

PMDA could not accept the use of the results of additional analyses of Study NO16966 performed by the applicant as a basis for drawing a conclusion on the preferred regimen [See the Review Report (1) "4.3 Clinical efficacy and safety, *Outline of review by the PMDA* 1) (1)(b) Study NO16966"]. This judgment by PMDA was supported at the Expert Discussion. The expert advisors pointed out as follows: Even if exploratory, these additional analyses were scientifically inappropriate as described below. If the applicant understands that "the add-on effect of bevacizumab in combination with FOLFOX4 can be expected" on the basis of the results of these analyses, there is a concern that information based on the results of inappropriate analyses will be provided.

Major problems

(a) "In Study NO16966, the proportion of subjects who had been continuously treated with bevacizumab also during one month prior to confirming PD was lower, which is one of the reasons why bevacizumab could not exert its full effect" is a meaningless inference since single agent bevacizumab does not provide adequate efficacy.

(b) "When the definition of event for PFS for Study NO16966 (first progression or death from the initiation of study treatment) was changed to the definition of event for Study AVF2107g (progressive disease or death within 28 days from the last dose of study drug), the hazard ratio was 0.63 ($p<0.0001$, log-rank test)." The maximum duration of treatment with bevacizumab was different between the two clinical studies (Study AVF2107g: 96 weeks, Study NO16966: 48 weeks) and comparison between the treatment groups is meaningless when deaths after 28 days from the last dose of study drug are censored in Study NO16966.

(c) "When analysis was performed excluding the patients with post-operative adjuvant chemotherapy, the hazard ratio was 0.72 ($p=0.0009$, log-rank test), demonstrating a significant add-on effect" is a claim that a significant difference was achieved by excluding a patient group with a good prognosis and it is extremely inappropriate

analysis.

PMDA asked for the applicant's view again on the appropriateness of the additional analyses performed by the applicant and the conclusion on the preferred regimen based on the results of these analyses.

The applicant responded as follows:
Although these additional analyses were performed as exploratory analyses, using them in the response to PMDA was a problem even for reference purposes. It is difficult to recommend FOLFOX4 as a chemotherapeutic regimen to be combined with bevacizumab based on the results of Study NO16966 alone.

(2) Factors affecting the efficacy of bevacizumab
At the Expert Discussion, the following comment was raised from the expert advisors:
It is necessary to examine the possibility that not only differences in the chemotherapy regimen combined, but also differences in the patient background affected the add-on effect of bevacizumab in combination with chemotherapy and to check (a) the results of a subgroup analysis by the differentiation grade of adenocarcinoma for Study AVF2107g, Study AVF2192g, and Study AVF0780g, and (b) the results of a subgroup analysis by the curability of patients with prior surgical therapy for colorectal cancer for Study NO16966.

PMDA asked the applicant to perform the above analyses, but the applicant responded that the differentiation grade or the curability of surgery as the patient background data had not been collected.

The applicant submitted the results of exploratory subgroup analysis by background factor relevant to surgery as shown below.

In Study NO16966, "curative" or "palliative" was documented for each surgical site. Using this data, patients were classified as "cases of curative surgery" if the intent of surgery was curative for all sites or as "cases of palliative surgery" if the intent of surgery was palliative for even one site. Then, a subgroup analysis was performed (FOLFOX4+bevacizumab/XELOX+ bevacizumab vs. FOLFOX4+placebo/XELOX+placebo) to assess the influence of the intent of surgery in patients with prior surgical therapy on the efficacy of bevacizumab (the add-on effect in combination with chemotherapy) as measured by PFS.

Subgroup	No. of cases	No. of events	Hazard ratio
Prior surgical therapy No	219	185	0.786 (97.5% CI, 0.563-1.097)
Yes	1181	875	0.828 (97.5% CI, 0.711-0.964)
Cases of curative surgery	562	400	0.943 (97.5% CI, 0.752-1.182)
Cases of palliative surgery	619	475	0.726 (97.5% CI, 0.591-0.893)

Although the hazard ratio was relatively high in "cases of curative surgery" than in "cases of palliative surgery," the point estimate was below 1 and it was considered that no qualitative interactions exist. Also, even when patients were classified as "cases of palliative surgery" if the intent of surgery was palliative for all sites or as "cases of curative surgery" if the intent of surgery was curative for even one site, similar results were obtained from the analysis. Therefore, although there was a difference in the hazard ratio between the subgroups, the definitions used for this analysis do not necessarily reflect the degree of complete cure achieved by surgery and this is unlikely to be a factor affecting the efficacy of bevacizumab also from a clinical point of view. Therefore, compared to ECOG Performance Status (0 or 1), the number of metastatic sites (1 or $\geq$2), alkaline phosphatase (normal or abnormal), or the presence or absence of hepatic metastases, i.e. the prognostic factors for which adjustment was made at randomization, the influence of curative or palliative surgery on the efficacy of bevacizumab is considered small.

PMDA considers as follows:
(a) The intent of surgery, i.e. curative or palliative, was documented by site in this study and patients classified as "cases of curative surgery" are not necessarily "those who relapsed after curative surgery" only and the patient background is unclear (for example, if a patient relapsed after curative surgery and then underwent colostomy [palliative surgery], this patient should essentially be "a case of curative surgery [a patient with a history of curative surgery]" but was counted as "a case of palliative surgery" in the analysis of the above table).
(b) An interaction between history of surgery and the presence or absence of bevacizumab has not been assessed.
(c) The possibility that the difference in the hazard ratio between the subgroups may be an apparent one due to other patient background factors, etc. has not been examined.
Therefore, based on the subgroup analysis submitted by the applicant, the applicant's conclusion is premature and whether there is a true difference in the hazard ratio between patients with and without a history of curative surgery for colorectal cancer can not be determined. This analysis is exploratory and the results of this analysis do not affect the PMDA's judgment on the add-on effect of bevacizumab in previously untreated patients, but it is necessary to perform analysis, also including the final data from Study NO16966, which will become available, as part of an investigation of predictive factors for the efficacy of bevacizumab, and appropriately provide information to the medical practice.

(3) Previously treated patients

The major study that evaluated the efficacy of bevacizumab in previously treated patients (second-line or subsequent treatment) is Study E3200, which demonstrated the add-on effect of bevacizumab in combination with FOLFOX4. PMDA judged that although the details of prior therapy in patients enrolled into this study are unknown, the add-on effect of bevacizumab in combination with chemotherapy in second-line or subsequent treatment has been demonstrated. This judgment by the PMDA was supported by the expert advisors.

2) Safety

At the Expert Discussion, the PMDA's judgment that there are no safety problems affecting the approval of bevacizumab at present was supported. The content of a review after the completion of the Review Report (1) and discussions with the expert advisors are described below.

(1) Thromboembolism and haemorrhage

Concerning thromboembolism and haemorrhage, in order to meet the post-marketing study commitments agreed at approval in the US, the effectiveness of monitoring the international normalization ratio (INR) has been assessed within Study NO16966. Analyses that "characterize the clinical consequences of both full-dose and low-dose anticoagulation therapy" have also been conducted in this clinical study. At the time of the preparation of the Review Report (1), PMDA was asking about the details of the above. The applicant responded that no data was available at present because analyses were ongoing.

As to the overseas post-marketing commitments, PMDA considers that it is necessary to provide information promptly as soon as the results become available.

Then, PMDA asked the applicant to explain the handling of anticoagulation therapy during treatment with bevacizumab in the major clinical studies as anticoagulation therapy prevents the development of thromboembolism, but increases the risk of bleeding.

The applicant responded as follows:

In the clinical studies submitted, the following rules with respect to anticoagulation therapy were generally provided.

- Patient exclusion criteria: Use of full-dose anticoagulants within the 10 days prior to enrollment (except as required to maintain patency of preexisting, indwelling IV catheters) or thrombolytic agents.
- Patient exclusion criteria: Chronic, daily treatment with aspirin (≥325 mg/day) or non-steroidal anti-inflammatory medications.
- Patient exclusion criteria: Evidence of bleeding diathesis or coagulopathy
- The use of low-dose warfarin, heparin, aspirin <325 mg/day, and non-steroidal anti-inflammatory medications of the kind known not to inhibit platelet function, is permitted.
- If patients develop Grade 4 symptomatic thromboembolism during the study period, bevacizumab should be discontinued. If patients develop thromboembolism ≤Grade 3 or asymptomatic Grade 4 thromboembolism, the patients may initiate anticoagulation

therapy with cessation of bevacizumab.

・In the case where anticoagulation therapy is initiated newly, if all of the following three conditions are met: (a) For patients receiving warfarin, the INR is stable in the target range (normally, 2-3) before the restart of study drug. (b) Haemorrhage ≥Grade 3 has not occurred after the start of study drug. (c) CT image obtained before the start of the study showed no tumor invasion into the major blood vessels, restart of bevacizumab under anticoagulation therapy is permitted.

In Studies AVF2107g and AVF2192g, among the patients randomized into the bevacizumab-containing group, the percentage of patients receiving aspirin at baseline was 8.4% and 23.0%, respectively, and the percentage of patients receiving warfarin at baseline was 10.0% and 11.0%, respectively. The incidences of arterial thromboembolism in the bevacizumab-containing group and the control group were 3.6% and 1.3%, respectively (Study AVF2107g) and 10.0% and 4.8%, respectively (Study AVF2192g).

In the foreign post-marketing experience, usually, patients indicated to receive anticoagulation therapy continue anticoagulation therapy during treatment with bevacizumab, but there has been no report recommending the use of anticoagulation therapy for all patients treated with bevacizumab and it should be unnecessary.

PMDA considers that it is necessary to provide information on the rules with respect to anticoagulation therapy employed in clinical studies to the clinical practice, using materials like a proper use guide.

The following judgment by the PMDA was supported at the Expert Discussion: Although the mechanism of development of thromboembolism or haemorrhage associated with bevacizumab is not clear, as it is known that the incidences of adverse events of thromboembolism and haemorrhage may generally be different between Japanese and American/European patients, it is necessary to perform coagulation/fibrinolysis tests, e.g. the prothrombin time INR and D-dimer and investigate whether such test values before or during treatment with bevacizumab can predict thrombosis and haemorrhage in Japan after market launch.

PMDA instructed the applicant to investigate predictive factors for thromboembolism and haemorrhage via post-marketing surveillance and asked the applicant to explain about the laboratory parameters planned to be monitored.

The applicant responded as follows:
According to the Japanese Society of Laboratory Medicine's "Clinical laboratory test guideline by diagnosis procedure combination 2003" (http://www.jscp.org/booklet/guideline/), PT-INR, APTT, and fibrinogen as coagulation/fibrinolysis parameters are listed as screening tests for hemostasis/thrombosis, and if DIC is suspected, FDP in addition to these parameters are listed. Combinations of these tests can identify most of the causes of common diseases with suspected bleeding tendency. For deep vein thrombosis and pulmonary thromboembolism, the Japanese

guideline (*Circ J* 68 [Suppl.4]: 1079-134, 2004) and overseas literature (*Ann Fam Med* 5: 57-62, 2007) recommend D-dimer as an indicator for diagnosis and D-dimer may be a predictive factor for thrombosis. This guideline and the stroke guideline (edited by Shinohara, Y. et al; Joint Committee on Stroke Guideline. Japanese Guidelines for the Management of Stroke 2004. Kyowakikaku; 2004) also recommend APTT (when heparin is used) and PT-INR (when warfarin is used) as monitoring tests for drug therapy and prophylaxis and these parameters need to be included in a post-marketing surveillance study as well. It has also been reported that hyperfibrinogenaemia is a risk factor for myocardial infarction and stroke (*N Engl J Med* 311: 501-5, 1984). Therefore, PT-INR, APTT, fibrinogen and D-dimer will be measured at baseline for patients registered in all-case investigation after market launch in order to investigate whether these parameters can predict thrombosis or haemorrhage associated with bevacizumab. The physicians will be asked to obtain PT-INR, APTT, and fibrinogen each time bevacizumab is administered (about every 2 weeks) for patients receiving anticoagulation therapy and about once a month for other patients, during treatment with bevacizumab and to obtain D-dimer about once a month for all patients.

PMDA accepted the response.

(2) Hypertension and proteinuria

PMDA considers as follows:

Among adverse events typically associated with bevacizumab, hypertension and proteinuria can be managed in the early stage in routine clinical practice. For proteinuria, it is necessary to perform periodic urinalysis and assessments at outpatient visits.

Regarding hypertension, blood pressure monitoring at home was discussed at the Expert Discussion. The following judgment by the PMDA was supported at the Expert Discussion: As bevacizumab is expected to be used mainly in an outpatient setting, it is necessary to educate patients about the importance of blood pressure monitoring and have them monitor their blood pressure at home using a home-use blood pressure meter wherever possible.

PMDA instructed the applicant to recommend blood pressure self-monitoring and indicate in what conditions patients should contact their doctor in patient educational materials, and the applicant accepted it.

(3) Typical adverse events associated with bevacizumab and the results of reexamination of their risk factors (foreign clinical studies)

PMDA considered that typical safety problems of bevacizumab are gastrointestinal perforation, delayed healing of wound, hypertension, proteinuria, arterial/venous thromboembolism, cardiotoxicity (congestive heart failure), infusion reactions, reversible posterior leukoencephalopathy syndrome (RPLS), and haemorrhage. PMDA included the incidence of each event in each clinical study and the results of examination of risk factors submitted at the time of NDA filing in the Review Report (1) “4.3 Clinical efficacy and safety, *Outline of review by the PMDA* 2) Safety (1)-(9)”.

However, the applicant explored the risk factors, using different clinical studies' data or different analysis methods for the incidences among different adverse events. Moreover, as a multivariate logistic regression analysis was performed using pooled data from the bevacizumab-treated groups and the control groups of multiple clinical studies, it was difficult to determine in what patient population each adverse event occurs frequently.

Therefore, PMDA asked the applicant to combine only the bevacizumab-treated groups from 3 studies: Foreign Studies AVF2107g, AVF2192g, and AVF0780g, explore the risk factors for each adverse event using a multivariate logistic regression analysis, and provide a discussion, and the applicant submitted the following response.

Gastrointestinal perforation:
As the incidence of gastrointestinal perforation was low at 1.6% (11/690 patients), a multivariate logistic regression analysis was not performed. When stratification of data was performed, there were no apparent differences in the incidence for all factors (gender [male/female], age [≥65 years/<65 years], race [Caucasian/Non-Caucasian], PS [0/≥1], concomitant gastrointestinal disease [present/absent], radiation therapy prior to the study [present/absent]), and risk factors for gastrointestinal perforation could not be identified due to the small number of cases with the event.

Delayed healing of wound:
As the incidence of delayed healing of wound was low at 3.3% (23/690 patients), a multivariate logistic regression analysis was not performed. When stratification of data was performed, there were no apparent differences in the incidence for all factors (gender, age, race, PS, a history of diabetes [present/absent], a history of malnutrition [present/absent], the use of warfarin or aspirin at baseline [present/absent], baseline albumin value [continuous quantity]), and risk factors for delayed healing of wound could not be identified. However, the incidence of delayed healing of wound was higher in the patient group with a history of malnutrition than in other patient groups.

Hypertension:
A multivariate logistic regression analysis was performed using gender, age, race, PS, BMI (≥25/<25), a history of hypertension (present/absent), a history of atherosclerosis (present/absent), a history of diabetes, and prior/concurrent hypercholesterolaemia or hyperlipidaemia (present/absent) as covariates. As a result, although factors significantly associated with the occurrence of hypertension were not identified, it was suggested that a history of hypertension may be a risk factor (adjusted odds ratio, 1.34; 95% CI, 0.97-1.86).

Proteinuria:
A multivariate logistic regression analysis was performed using gender, age, race, PS, a history of hypertension, a history of diabetes, and renal insufficiency at baseline (present/absent) as covariates. As a result, a history of hypertension (adjusted odds ratio, 1.64; 95% CI, 1.18-2.27)

and a history of diabetes (adjusted odds ratio, 2.31; 95% CI, 1.49-3.59) were identified as risk factors for proteinuria. The incidence of proteinuria in the patients with a history of hypertension was 39.3% (126/321 patients), which was higher than 26.6% in the patients without a history of hypertension. The incidence in the patients with a history of diabetes was 51.5% (51/99 patients), which was higher than 29.3% in the patients without a history of diabetes. The above results suggested that a history of hypertension and a history of diabetes are important risk factors for the development of proteinuria.

Arterial thromboembolism:
A multivariate logistic regression analysis was performed using gender, age, race, PS, BMI, a history of hypertension, a history of venous thrombosis (present/absent), a history of atherosclerosis, a history of diabetes, and prior/concurrent hypercholesterolaemia or hyperlipidaemia as covariates. As a result, a history of hypertension (adjusted odds ratio, 1.58 [95% CI, 1.01-2.48]) and a history of atherosclerosis (adjusted odds ratio, 2.33 [95% CI, 1.35-3.94]) were identified as risk factors for arterial thromboembolism and it was suggested that a history of diabetes (adjusted odds ratio, 1.62 [95% CI, 0.92-2.75]) may be a risk factor. The incidence of arterial thromboembolism in the patients with a history of hypertension was 18.4% (59/321 patients), which was higher than 10.8% in the patients without a history of hypertension. The incidence in the patients with a history of atherosclerosis was 28.0% (26/93 patients), which was higher than 12.2% in the patients without a history of atherosclerosis. The above results suggested that a history of hypertension and a history of atherosclerosis are important risk factors for the development of arterial thromboembolism.

Venous thromboembolism:
A multivariate logistic regression analysis was performed using gender, age, race, PS, BMI, a history of hypertension, a history of venous thrombosis, a history of atherosclerosis, a history of diabetes, and prior/concurrent hypercholesterolaemia or hyperlipidaemia as covariates. As a result, a history of venous thrombosis (adjusted odds ratio, 3.91; 95% CI, 1.62-9.08) was identified as a risk factor for venous thromboembolism, and it was suggested that a history of hypertension (adjusted odds ratio, 1.55; 95% CI, 0.98-2.46), gender (adjusted odds ratio, 0.71; 95% CI, 0.45-1.12), BMI (adjusted odds ratio, 1.55; 95% CI, 0.96-2.55), and prior/concurrent hypercholesterolaemia or hyperlipidaemia (adjusted odds ratio, 0.48; 95% CI, 0.21-0.95) may be risk factors. The incidence of venous thromboembolism in the patients with a history of venous thromboembolism was 42.3% (11/26 patients), which was higher than 12.7% in the patients without a history of venous thromboembolism. The above results suggested that a history of venous thrombosis is an important risk factor for the development of venous thromboembolism.

The risk factors for thromboembolism are different between arterial and venous events and the risk factor for arterial thromboembolism is a history of atherosclerosis and the risk factor for venous thromboembolism is a history of venous thrombosis. In addition, caution against both adverse events is needed for patients with a history of hypertension. More data will be collected and analyzed via post-marketing surveillance.

Heart failure:
As the incidence of heart failure was low at 1.9% (13/690 patients), a multivariate logistic regression analysis was not performed. Stratification of data was performed. As a result, among all factors (gender, age, race, PS, BMI, a history of hypertension, a history of atherosclerosis, a history of diabetes, prior/concurrent hypercholesterolaemia or hyperlipidaemia), ≥65 years, the presence of a history of atherosclerosis, and the presence of a history of diabetes were associated with higher incidences of heart failure and especially, the incidence in the patients with a history of atherosclerosis was high at 5.4%. Due to the small number of cases with heart failure, this analysis can not identify risk factors, but caution is required for elderly patients, atherosclerosis, and diabetes. Risk factors for heart failure will be identified via post-marketing surveillance.

Infusion reactions:
A multivariate logistic regression analysis was performed using gender, age, race, PS, and prior/concurrent drug hypersensitivity (present/absent) as covariates. As a result, although risk factors for the development of infusion reactions could not be identified, as allergic disease may be a risk factor, it is considered necessary to collect and analyze data via post-marketing surveillance.

Reversible posterior leukoencephalopathy syndrome:
Since reversible posterior leukoencephalopathy syndrome was not reported in these studies, stratification of data was not performed.

Haemorrhage:
As to haemorrhage, in Study AVF2107g, only adverse events ≥Grade 3 were collected during a portion of the study period. Therefore, haemorrhages were counted and analyzed separately for ≥Grade 3 and ≤Grade 2.

As the incidence of haemorrhage ≥Grade 3 was low at 4.9% (34/690 patients), a multivariate logistic regression analysis was not performed. Data was stratified and as a result, among all factors (gender, age, race, PS, the use of warfarin or aspirin at baseline, prior/concurrent rectal haemorrhage [present/absent], prior/concurrent anaemia [present/absent]), the incidence of haemorrhage ≥Grade 3 in the patients ≥65 years was 6.7%, which was higher than 3.6% in the patients <65 years while there were no major differences for other factors. Due to a low incidence of haemorrhage ≥Grade 3, risk factors can not be identified, but caution is required for elderly patients ≥65 years and it is considered necessary to collect and analyze data via post-marketing surveillance.

For haemorrhage ≤Grade 2, a multivariate logistic regression analysis was performed using gender, age, race, PS, the use of warfarin or aspirin at baseline, prior/concurrent rectal haemorrhage, and prior/concurrent anaemia as covariates. As a result, age (adjusted odds ratio, 1.59; 95% CI, 1.14-2.22) was identified as a risk factor and it was suggested that the use of warfarin or aspirin at baseline (adjusted odds ratio, 0.65; 95% CI, 0.40-1.01) and

prior/concurrent anaemia (adjusted odds ratio, 0.72; 95% CI, 0.47-1.08) may be risk factors. The incidence of haemorrhage ≤Grade 2 in the patients ≥65 years was 34.7% (103/297 patients), which was higher than 26.2% in the patients <65 years. The above results suggested that ≥65 years of age is an important risk factor for haemorrhage ≤Grade 2.

Although it was difficult to identify risk factors for haemorrhage ≥Grade 3 due to its low incidence, the incidence was higher in the patients ≥65 years. Also for haemorrhage ≤Grade 2, ≥65 years of age was suggested as a risk factor. Therefore, caution is required for elderly patients ≥65 years. However, as other factors may also be associated with haemorrhage ≥Grade 3, data will be collected and analyzed via post-marketing surveillance.

PMDA considers as follows:
Although it is difficult to identify risk factors for each adverse event based on the results of exploratory analyses, these analysis results are important information for selecting the data items to be collected in all-case investigation after market launch. Based on the results of exploratory analyses of risk factors by the applicant, (a) a history of hypertension may be a risk factor for hypertension, (b) a history of hypertension and a history of diabetes may be risk factors for proteinuria, (c) a history of hypertension, a history of atherosclerosis, a history of diabetes, and diabetes may be risk factors for arterial thromboembolism, (d) a history of hypertension, a history of venous thrombosis, gender, BMI, and prior/concurrent hypercholesterolaemia or hyperlipidaemia may be risk factors for venous thromboembolism, (e) a history of hypertension, a history of venous thromboembolism, a history of atherosclerosis, and a history of diabetes may be risk factors for arterial + venous thromboembolism, (f) age, the use of warfarin or aspirin at baseline, and prior/concurrent anaemia may be risk factors for haemorrhage.

PMDA considers as follows:
These factors can be collected via post-marketing surveillance and are useful information for developing CRFs. However, a lower incidence of venous thrombosis with the presence of prior/concurrent hypercholesterolaemia or hyperlipidaemia and a lower incidence of haemorrhage with the use of warfarin or aspirin at baseline are the results that are difficult to interpret clinically. It is necessary to clarify their causes using domestic post-marketing information and safety information from Foreign Study NO16966.

(4) Time to onset of adverse events typically associated with bevacizumab
As to gastrointestinal perforation, delayed healing of wound, haemorrhage, thromboembolism, hypertension, proteinuria, and cardiotoxicity (congestive heart failure), PMDA asked the applicant to examine the association of each event to the duration of treatment with bevacizumab, the number of doses, and the cumulative dose, using Study AVF2107g, Study AVF2192g, Study AVF0780g (the combined data from AVF2107g, AVF2192g, and AVF0780g), Study E3200, Study JO18157, and PSUR (patients with colorectal cancer only).

The applicant did not have detailed data from overseas PSUR for analysis and submitted the

following response based on the clinical study data.

Gastrointestinal perforation, delayed healing of wound, and heart failure:
Due to the small number of cases with each event, the time to onset could not be characterized.

Haemorrhage:
In all studies, the rate of events occurring early after the start of treatment was relatively high and especially the rate of events occurring within 1 month of treatment was very high at 11-46%, except for Study E3200 conducted by the ECOG. Based on the pooled data from the foreign clinical studies excluding Study E3200 and the results of Japanese Study JO18157, this high risk period seemed to continue for at least about 5 months after the initiation of treatment or for up to 10 doses. Particularly in Study JO18157, the rate of events occurring at <2 months after the initiation of treatment tended to be high at about 30% and caution needs to be exercised. Also in Study E3200 reporting the lowest incidence of haemorrhage, 24 out of the 32 patients with haemorrhage developed the event during the first 5 months after the initiation of treatment or 25 out of the 32 patients developed the event after up to 10 doses, showing a similar trend. As to the association with the cumulative dose, the risk for the development of haemorrhage was relatively high at cumulative doses up to about 5,000 mg in all studies. In Study JO18157 and Study E3200, the number of patients who developed haemorrhage at ≥5 months after the initiation of treatment or at cumulative doses of ≥5,000 mg was small and the results were considered to lack reliability. Based on the combined data from the foreign clinical studies excluding Study E3200, the upper 60% point of the distribution of cumulative dose was 5,060 mg and about 60% of the patient population to be treated with bevacizumab are very likely to receive a cumulative dose of ≥5,000 mg. Thus, many patients are likely to be exposed to the risk of haemorrhage and especially early in treatment (for about 5 months after the initiation of treatment, for up to about 10 doses, or at cumulative doses up to around 5,000 mg), strict control of haemorrhage risk is required.

Arterial thromboembolism:
In Study JO18157, only 1 patient had arterial thromboembolism and analysis of Japanese data was impossible. Based on the combined data from the foreign clinical studies excluding Study E3200, the rate of events occurring within 1 month after the initiation of treatment was high and then, the risk was distributed uniformly across time. In Study E3200, except for 1 patient who developed arterial thromboembolism at 17-18 months after the initiation of treatment, it was confirmed that the events are distributed uniformly throughout the treatment period. Likewise, in terms of the number of doses administered and the cumulative dose, the rate of events immediately after the initiation of treatment was high and then the events were distributed almost uniformly across time. Based on the above, the risk for the development of arterial thromboembolism is relatively high immediately after the start of treatment and after that, the risk exists regardless of the time from the initiation of treatment or the dose. Therefore, caution needs to be exercised continuously during treatment and especially early in treatment, even more caution is necessary.

Venous thromboembolism:
In Study JO18157 and Study E3200, venous thromboembolism did not occur. The combined data from the foreign clinical studies excluding Study E3200 suggested that the risk for the development of venous thromboembolism is high at around 6 months after the initiation of treatment, after 10-15 doses administered, or at cumulative doses of about 5,000 mg. This has not adequately been examined from a clinical point of view and a continued investigation is needed, but the possibility that the risk is high up to 6 months after the start of treatment can not be denied. Therefore, strict caution against venous thromboembolism is required continuously for at least about 6 months after the initiation of treatment, for up to 10-15 doses administered or at cumulative doses up to around 5,000 mg.

Hypertension:
The combined data from the foreign clinical studies excluding Study E3200 showed that hypertension developed over a long period of time after the initiation of treatment, regardless of the duration of treatment, the number of doses, or the cumulative dose. Thus, it was determined that there is no clear trend as to the association with the duration of treatment etc. Although the rate of events tends to rise with increasing cumulative dose, due to the small number of cases studied, we could not conclude that the risk of hypertension rises with increasing cumulative dose. Based on the above, long-term risk control is needed for hypertension.

Proteinuria:
Based on Study E3200 and the combined data from the foreign clinical studies excluding Study E3200, although the rate of events tended to rise with a longer duration of treatment or a higher number of doses administered, due to the small number of cases studied, its reliability is low. Although the rate of events tended to be increased with increasing cumulative dose, due to the small number of cases studied, we could not conclude that the risk of proteinuria is increased with increasing cumulative dose. In Study JO18157, the rate of events tended to be high early in treatment, but due to the smaller number of cases and shorter duration of observation as compared to the foreign clinical studies, no definitive conclusion could be drawn. Based on the above, the risk for the development of proteinuria is remotely related to the duration of treatment etc. and caution needs to be exercised continuously during treatment.

PMDA considers as follows:
Although the above analyses of the time to onset of adverse events typically associated with bevacizumab performed by the applicant are exploratory, the results may serve as a guide for the items to be investigated in a post-marketing surveillance program or the duration of monitoring in medical practice. It is necessary to collect information on the time to onset of adverse events typically associated with bevacizumab (or the cumulative doses) in Japanese patients after market launch, analyze/discuss the data obtained and disclose the results.

(5) Use in patients with brain metastases

As serious cerebral haemorrhage occurred in a hepatocellular carcinoma patient with a brain metastasis in a foreign phase I study (AVF0737g), patients with brain metastases were excluded from subsequent clinical studies. As a result, the safety information on patients with brain metastases is very limited. The European package insert states that bevacizumab is contraindicated in patients with "untreated CNS metastases" and the applicant clarified that this means "brain metastases have not been treated, e.g. with radiotherapy." On the other hand, the US labeling includes a caution statement about patients with CNS metastases in the WARNINGS section, but bevacizumab is not contraindicated in these patients.

Generally, patients with brain metastases are often excluded from clinical trials of anti-cancer agents because such patients have a poor prognosis and affect the efficacy and safety evaluation. Consequently, there is insufficient safety information on patients with brain metastases at the time of NDA filing, as with bevacizumab. However, in the case of bevacizumab, patients with brain metastases were excluded from subsequent clinical trials due to the development of cerebral haemorrhage in a clinical trial. Therefore, PMDA judged that the use of bevacizumab in patients with brain metastases needs to be handled very carefully from a safety point of view. However, considering the seriousness of the disease, if bevacizumab is contraindicated in patients with brain metastases, they might be excluded from receiving necessary therapy. Thus, PMDA judged that brain metastases should be listed as "Relative Contraindications (As a general rule, Avastin is contraindicated in the following patients. If the use of Avastin is considered essential, it should be administered with care)" and the use of bevacizumab in patients with brain metastases should not entirely be excluded.

At the Expert Discussion, the following comments were raised from the expert advisors: Bevacizumab should be contraindicated in patients with brain metastases; In the clinical practice of colorectal cancer treatment, due to a low frequency of brain metastases [Note by the PMDA: *Cancer* 94: 2698-705, 2002 has reported that the 5-year cumulative incidence is 1.2%], the presence or absence of brain metastases is not assessed until symptoms suggestive of brain metastases are noted. When a diagnosis of brain metastases is made, treatment of brain metastases, such as irradiation, takes precedence. Therefore, also in patients who have completed treatment of brain metastases, bevacizumab should not be "contraindicated" and a therapeutic opportunity of chemotherapy with bevacizumab should not be restricted, and brain metastases should be listed as "Relative Contraindications."; In routine clinical practice, since not all patients are assessed for brain metastases by imaging at the start of treatment, chemotherapy with bevacizumab might be initiated in patients with asymptomatic brain metastases at the start of treatment. A practical safety measure for such patients is to carefully monitor them for a possible occurrence of symptoms suggestive of brain metastases after the start of treatment.

Taking into account the above, PMDA instructed the applicant to list "patients with brain metastases" as "Relative Contraindications" and include the following statements in "Important Precautions": "Serious cerebral haemorrhage in a patient with a brain metastasis has been

reported in a clinical trial. Avastin should not be used in patients with untreated brain metastases." and "Even if no symptoms suggestive of brain metastases are noted and cancer chemotherapy with Avastin has been initiated, patients should be carefully monitored. If neurological abnormalities are suspected, appropriate measures, such as discontinuing Avastin, should be taken, in view of the possibility of brain metastases and cerebral haemorrhage." The applicant accepted it.

PMDA asked the applicant to explain the association between brain metastases and cerebral haemorrhage in patients with colorectal cancer based on foreign post-marketing information.

The applicant explained as follows:
In an observational study that is ongoing in Europe, as of [redacted] 20[redacted], among the 1,914 patients with colorectal cancer exposed to bevacizumab, no patient had symptomatic brain metastases at baseline or after the start of treatment with bevacizumab and 16 patients were found to have asymptomatic brain metastases (incidentally detected) at baseline or after the start of treatment with bevacizumab. None of these 16 patients developed cerebral haemorrhage.

(6) Pathologic fractures following treatment with bevacizumab
At the Expert Discussion, the following comment was raised from the expert advisors: Since bevacizumab has an angiogenesis inhibitory activity, it is necessary to investigate the potential for an increased risk of pathologic fractures at the site of bone metastases.

PMDA asked the applicant to explain the risk of pathologic fractures associated with bevacizumab and the applicant responded as follows:
Among the patients enrolled into the Japanese clinical studies, only 2 patients in Study JO18158 (1 patient each for the 5 mg group and 10 mg group) had bone metastases at baseline and pathologic fractures have not been reported by either patient. In foreign clinical studies, only 1 patient in Study AVF2107g and 2 patients in Study NO16966 had bone metastases at baseline (the bevacizumab-treated group) and all of these patients had pathologic fractures after the start of treatment. As the sites of bone metastases at baseline are unknown for these patients, whether the fractures occurred at the sites of bone metastases or not is unknown. However, the fact that fractures have been reported by all of the 3 patients with bone metastases at baseline in foreign clinical studies is noteworthy and we will collect and review data concerning the occurrence of adverse events of pathologic fractures in patients with bone metastases in all-case investigation after market launch in Japan.

PMDA accepted the response.

(7) Treatment as an inpatient
At the Expert Discussion, PMDA discussed whether bevacizumab should be used in an inpatient or outpatient setting.

The following comments were raised from the expert advisors: Under the current situation,

patients receive FOLFOX in an outpatient setting and it is not preferable to hospitalize patients for the use of bevacizumab; Although bevacizumab is associated with typical adverse events, these can be managed by careful monitoring in an outpatient setting.

PMDA judged that it is unnecessary to hospitalize patients for the use of bevacizumab.

(8) Safety information obtained from the Japanese clinical study

After the preparation of the Review Report (1), the applicant reported the results of the assessment of the initial safety of FOLFOX4+bevacizumab 10 mg/kg in second-line or subsequent treatment in an ongoing Japanese safety confirmation study (Study JO18158) as follows.

The safety confirmation study was conducted with the following two-step design:
When the first 12 patients were enrolled into each of the 5 mg/kg group and the 10 mg/kg group (step 1), the initial enrollment was suspended. After the initial safety in the 12 patients up to the 2nd cycle was confirmed, dose-limiting toxicity was evaluated and if the criteria for moving into the next step were met, enrollment was to be resumed to include further 38 patients.

On [redacted] 20[redacted], the last patient for step 1 was enrolled into the 5 mg/kg group and the initial safety in the 12 patients was evaluated by the members of the Data and Safety Monitoring Board between [redacted] and [redacted] 20[redacted] and whether to move into the next step was reviewed. All of the 12 patients were evaluable for the initial safety, no dose-limiting toxicity was observed, and it was determined that moving into the next step was acceptable.

On [redacted] 20[redacted], the last patient for step 1 was enrolled into the 10 mg/kg group and the initial safety in the 12 patients was evaluated by the members of the Data and Safety Monitoring Board between [redacted] and [redacted] 20[redacted] and whether to move into the next step was reviewed.

Of the 11 patients included in the assessment of the initial safety excluding 1 patient who was discontinued from the study due to consent withdrawal (Case No. X28), 2 patients (Case No. X29 and X30) developed Grade 4 neutropenia, which was defined as a dose-limiting toxicity. Case No. X29 experienced Grade 3 hypertension, but it could be controlled with anti-hypertensives and was considered as a clinically insignificant event. Although the start of the next cycle was postponed in many patients (the start of the second cycle and the third cycle was postponed in 5 patients and 6 patients, respectively), it was determined that moving into the next step was acceptable.

As of [redacted] 20[redacted], for step 1 and step 2, a total of 36 patients were enrolled into the FOLFOX4+bevacizumab 5 mg/kg group for first-line treatment and a total of 20 patients were enrolled into the FOLFOX4+bevacizumab 10 mg/kg group for second-line or subsequent treatment.

PMDA asked the applicant to present the number of cases with delayed healing of wound,

haemorrhage, thromboembolism, hypertension, proteinuria, or cardiotoxicity (congestive heart failure), i.e. adverse events typically associated with bevacizumab, in the Japanese clinical study.

The applicant presented the following data on a total of 46 patients (FOLFOX4+bevacizumab 5 mg/kg group: 33 patients, FOLFOX4+bevacizumab 10 mg/kg group: 13 patients) for whom data had been collected and entered as of [redacted] 20[redacted].

Adverse events typicallyassociated with bevacizumab reported in the Japanese clinical study

	FOLFOX4+bevacizumab 5 mg/kg N=33 n, (%)	FOLFOX4+bevacizumab 10 mg/kg N=13 n, (%)	Total N=46 n, (%)
Delayed healing of wound			
Grade 1	1 (3.0%)	0 (0%)	1 (2.2%)
Haemorrhage			
Grade 1	16 (48.5%)	6 (46.2%)	22 (47.8%)
Thromboembolism	0 (0%)	0 (0%)	0 (0%)
Hypertension			
Grade 1	15 (45.5%)	2 (15.4%)	17 (37.0%)
Grade 2	2 (6.1%)	0 (0%)	2 (4.3%)
Grade 3	0 (0%)	2 (15.4%)	2 (4.3%)
Subtotal	17 (51.5%)	4 (30.8%)	21 (45.7%)
Proteinuria			
Grade 1	4 (12.1%)	4 (30.8%)	8 (17.4%)
Grade 2	4 (12.1%)	0 (0%)	4 (8.7%)
Subtotal	8 (24.2%)	4 (30.8%)	12 (26.1%)
Cardiotoxicity (Congestive heart failure)	0 (0%)	0 (0%)	0 (0%)

Types of haemorrhages reported in the Japanese clinical study

	FOLFOX4+bevacizumab 5 mg/kg N=16 n, (%)	FOLFOX4+bevacizumab 10 mg/kg N=6 n, (%)	Total N=22 n, (%)
Epistaxis	13 (81.3%)	4 (66.7%)	17 (77.3%)
Anal haemorrhage	2 (12.5%)	0 (0%)	2 (9.1%)
Urinary occult blood positive	1 (6.3%)	1 (16.7%)	2 (9.1%)
Gingival bleeding	1 (6.3%)	0 (0%)	1 (4.5%)
Conjunctival haemorrhage	1 (6.3%)	0 (0%)	1 (4.5%)
Haemorrhage at the drain insertion site	1 (6.3%)	0 (0%)	1 (4.5%)
Implant site haemorrhage	0 (0%)	1 (16.7%)	1 (4.5%)

Myelosuppression ≥Grade 3 reported in the Japanese clinical study

	FOLFOX4+bevacizumab 5 mg/kg N=33 n, (%)	FOLFOX4+bevacizumab 10 mg/kg N=13 n, (%)	Total N=46 n, (%)
White blood cell count decreased			
Grade 3	7 (21.2%)	5 (38.5%)	12 (26.1%)
Neutrophil count decreased			

Grade 3	13 (39.4%)	7 (53.8%)	20 (43.5%)
Grade 4	10 (30.3%)	4 (30.8%)	14 (30.4%)
Subtotal	23 (69.7%)	11 (84.6%)	34 (73.9%)
Lymphocyte count decreased			
Grade 3	4 (12.1%)	1 (7.7%)	5 (10.9%)
Grade 4	1 (3.0%)	0 (0%)	1 (2.2%)
Subtotal	5 (15.2%)	1 (7.7%)	6 (13.0%)
Red blood cell count decreased	0 (0%)	0 (0%)	0 (0%)
Haemoglobin decreased			
Grade 3	2 (6.1%)	0 (0%)	2 (4.3%)
Haematocrit decreased	0 (0%)	0 (0%)	0 (0%)
Platelet count decreased			
Grade 3	1 (3.0%)	0 (0%)	1 (2.2%)

PMDA confirmed that (a) As of [redacted] 20[redacted], there has been no reversible posterior leukoencephalopathy syndrome and gastrointestinal perforation has been reported by 1 patient (Case No. X20) and (b) As of the preparation of the Review Report (1), there was no venous thrombosis in the Japanese clinical studies, but 1 patient in the FOLFOX4+bevacizumab 5 mg/kg group in Study JO18158 developed venous thrombosis after the completion of the study. Although this venous thrombosis was detected by CT scan obtained after the start of a further treatment, its causal relationship to the study drug could not be ruled out.

PMDA reviewed all adverse events collected up to [redacted] 20[redacted] including the above-mentioned events. As a result, PMDA judged that there are no safety problems that are clearly different from those in foreign clinical studies. However, the number of Japanese patients studied is limited and at present, it is difficult to determine whether there are any adverse events with a higher incidence in Japanese as compared to foreigners.

PMDA considers as follows:
Taking account of very limited safety data on bevacizumab from Japanese patients at the time of regulatory submission, the information obtained from the ongoing Japanese safety confirmation study is important and it is necessary to publish the final results of this study promptly and appropriately as soon as they become available.

Therefore, as “Instructions”, PMDA instructed the applicant to “summarize the data from the safety confirmation study promptly and publish the results as soon as the final results of the study become available.”

(9) Other matters relating to the safety
Haemoptysis with a fatal outcome has been reported in a foreign clinical study in patients with non-small cell lung cancer, and 5 out of the 44 patients developed gastrointestinal perforation in a foreign clinical study in patients with ovarian cancer or primary peritoneal carcinoma, leading to study termination. These data are from patients with types of cancer other than colorectal cancer: the claimed indication, but are important information on the safety of bevacizumab.

Therefore, PMDA instructed the applicant to include a caution statement in the package insert.

At the Expert Discussion, the following comment was raised: In Foreign Study AVF0757g in patients with non-small cell lung cancer, the incidence of an adverse event of depression was higher with bevacizumab compared to placebo and a dose response was also observed (CBDCA/PTX+placebo group, 6.3%; CBDCA/PTX+bevacizumab 7.5 mg/kg group, 15.6%; CBDCA/PTX+bevacizumab 15 mg/kg, 23.5%). Thus, it is necessary to check the details for patients with colorectal cancer as well.

PMDA asked the applicant about the details of the occurrence of neurological symptoms associated with bevacizumab in patients with colorectal cancer. The applicant analyzed the data from patients with colorectal cancer and submitted the following response.

Concerning adverse events relevant to nervous system disorders associated with bevacizumab in foreign clinical studies, the incidence of each event was checked, but there was no influence of bevacizumab on the occurrence and no association between bevacizumab and neurologic symptoms was suggested.

Incidence of neurologic symptoms in foreign clinical studies

	AVF2107/2192/0780 combined		NO16966		E3200	
Chemotherapy			FOLFOX4 or XELOX		FOLFOX4	
Study drug	Placebo	Bevacizumab	Placebo	Bevacizumab	Placebo	Bevacizumab
No. of cases	536	668	675	694	285	287
SOC Nervous system disorders	111 (20.7%)	151 (22.6%)	142 (21.0%)	125 (18.0%)	Not counted	Not counted
Depression	35 (6.5%)	52 (7.8%)	36 (5.3%)	24 (3.5%)	1 (0.4%)	0 (0%)

PMDA accepted the applicant's response that bevacizumab is not associated with increase in depression in patients with colorectal cancer at present. However, as to a dose-dependent increase in the incidence of depression in Foreign Study AVF0757g, the background is different from that of patients with colorectal cancer and a further investigation is needed.

3) Indications

As described in the Review Report (2) "1) The efficacy in patients with advanced or recurrent colorectal cancer who are not candidates for curative resection," PMDA judged as follows:

The efficacy of bevacizumab (the add-on effect in combination with fluoropyrimidine-based chemotherapy) in both previously untreated and treated patients has been demonstrated. As the use of bevacizumab in a post-operative adjuvant chemotherapy setting has not been evaluated at present, the indications should be "unresectable advanced or recurrent colorectal cancer" and it should be noted in the "Precautions for Indications" section that the efficacy and safety of bevacizumab in a post-operative adjuvant chemotherapy setting are unknown.

The above judgment by the PMDA was supported by the expert advisors at the Expert Discussion.

At the Expert Discussion, PMDA discussed the clinical positioning of bevacizumab in Japan as follows, taking into account the results of subgroup analysis of Foreign Study NO16966.

The efficacy of bevacizumab in previously untreated patients has been demonstrated in Studies AVF2107g and NO16966. However, under the current medical environment in Japan, (a) The XELOX regimen evaluated in Study NO16966 is not consistent with the approved package inserts of capecitabine and oxaliplatin in Japan, (b) Although Study AVF2107g has confirmed the add-on effect of bevacizumab in combination with IFL, there is a trend towards fewer opportunities to use IFL as a first-line therapy both in Japan and abroad and especially in Japan, IFL is not used frequently due to safety concerns, (c) FOLFOX is the mainstream as a first-line therapy in Japan. Therefore, the results of subgroup analysis of Study NO16966 as to the add-on effect of bevacizumab in combination with FOLFOX4 are extremely important information for the medical practice in Japan.
The above judgment by the PMDA was supported by the expert advisors.

The primary objective of Study NO16966 using PFS as the primary endpoint was not to detect a difference in PFS between the FOLFOX4+placebo group and the FOLFOX+bevacizumab group, but a subgroup analysis showed no difference between the groups (hazard ratio 0.89 [97.5% CI, 0.73-1.08]; log-rank test; p=0.1871). It was also suggested that the add-on effect itself of bevacizumab in combination with chemotherapy may be smaller with FOLFOX4 than with XELOX and PMDA considered that before using bevacizumab with FOLFOX4, physicians who are familiar with this information should fully explain the results of the clinical study to the patients and select a therapy according to individual patients' conditions. Therefore, PMDA judged that it is highly necessary to present the details of the clinical study, including the result that the add-on effect of bevacizumab in combination with FOLFOX4 was not definite, etc. in the package insert. This judgment by the PMDA was supported by the expert advisors at the Expert Discussion.

Based on the above, PMDA instructed the applicant to clearly indicate not only the results of primary analysis for superiority comparison (chemotherapy+placebo vs. chemotherapy +bevacizumab), but also the results of subgroup analysis for superiority comparison for Study NO16966 in the CLINICAL STUDIES section of the package insert, state that "there was no significant difference in PFS between FOLFOX4 in combination with bevacizumab and FOLFOX4 (in combination with placebo)," and provide information to the medical practice, and the applicant accepted it.

4) Dosage and administration

(1) Dosage regimen and precautions

PMDA judged that the appropriate dose of bevacizumab is 5 mg/kg for first-line treatment and 10 mg/kg for second-line or subsequent treatment for the reasons stated in the Review Report (1) "4.3 Clinical efficacy and safety, *Outline of review by the PMDA* 5) Dosage and administraion**,** 6) Post-marketing investigations (1) Dose". PMDA also judged that it is

necessary to advise that single agent bevacizumab treatment is not recommended and to provide information on the content and scope of currently available evidence from clinical trials. These judgments by the PMDA were supported by the expert advisors at the Expert Discussion. In Study NO16966, 7.5 mg/kg of bevacizumab was administered every 3 weeks in the XELOX+bevacizumab group, but the XELOX regimen in this study is not consistent with the approved package inserts of capecitabine and oxaliplatin, which are used in XELOX, in Japan and the above dosage regimen has not been proposed for approval at present. Therefore, PMDA concluded that it is unnecessary to establish the dosage regimen of bevacizumab when combined with XELOX.

The following comment was raised from the expert advisors:
As there is no conclusion on the order of priority of chemotherapeutic regimens to be combined with bevacizumab from the data obtained so far, it is also necessary to provide detailed information on the target patients enrolled into individual clinical studies and the dosage regimens of bevacizumab and concurrent chemotherapy.

Taking into account the above, PMDA instructed the applicant to indicate “The usual adult dosage is 5 mg/kg (body weight) or 10 mg/kg (body weight) of bevacizumab given as an intravenous infusion in combination with chemotherapy. The dosing interval should be ≥2 weeks” in the “DOSAGE AND ADMINISTRATION” section and include the following statements in the “Precautions for Dosage and Administration” section: “Avastin should be used in combination with fluoropyrimidine-based chemotherapy,” “The efficacy and safety of single agent Avastin have not been established,” and “The dose of Avastin should be chosen according to the patient's history of cancer chemotherapy with a full knowledge of the contents of the CLINICAL STUDIES section.” PMDA also instructed the applicant to clearly indicate the study population, the dosage regimens of chemotherapies studied, and the results of efficacy evaluation for the four major foreign studies (Study NO16966, Study E3200, Study AVF2107g, Study AVF2192g) and the two Japanese studies (Study JO18157, Study JO18158) in the CLINICAL STUDIES section.

In addition, PMDA instructed the applicant to provide detailed information on the criteria for delaying or discontinuing treatment with bevacizumab employed in the major clinical studies, via the package insert or the proper-use guide, etc., and the applicant accepted it.

Study	JO18157	JO18158
Dose of bevacizumab	3, 5, 10 mg/kg	5, 10 mg/kg
Concurrent chemotherapy	5-FU/*l*-LV	FOLFOX
Dose reduction criteria	None	5 mg/kg group: None 10 mg/kg ・Hemorrhage: Grade 2 ・Proteinuria: Grade 2, 3 ・Hypertension: Grade 1, 2 ・Hepatic dysfunction ≥ Grade 3 associated with bevacizumab If any of the above events occurs, reduce the dose to 5 mg/kg.
Criteria for delaying treatment	・Hematologic toxicities ≥Grade 3 or non-hematologic toxicities≥Grade 2, associated with bevacizumab ・Proteinuria: Grade 2	・Hemorrhage: Grade 2 ・Proteinuria: In the event of Grade 2 or 3, withhold until it decreased to ≤2g/day ・Hepatic dysfunction ≥Grade 3 associated with bevacizumab
Criteria for discontinuing treatment	・Thromboembolism (venous): ≥Grade 3 ・Thromboembolism (arterial): ≥Grade 1 ・Hemorrhage: ≥Grade 3 ・Haemoptysis: ≥Grade 1 ・Hypertension: ≥Grade 3 not controlled with medication ・Symptoms or image findings suggesting central nervous system disorder ・In the event of gastrointestinal perforation, wound dehiscence, or reversible posterior leukoencephalopathy syndrome ・Grade 4 hypersensitivity	・Thromboembolism (venous): ≥Grade 3 ・Thromboembolism (arterial): ≥Grade 1 ・Hemorrhage: ≥Grade 3 ・In the event of reversible posterior leukoencephalopathy syndrome ・Haemoptysis: ≥Grade 1 ・Proteinuria: Grade 4 ・Hypertension: ≥Grade 3 not controlled with medication ・In the event of gastrointestinal perforation or wound dehiscence ・Hypersensitivity ≥Grade 3 associated with bevacizumab ・Symptoms or image findings suggesting central nervous system disorder

Study	E3200	AVF2107g	NO16966
Dose of bevacizumab	10 mg/kg	5 mg/kg	5 mg/kg
Concurrent chemotherapy	FOLFOX4	IFL	FOLFOX4
Dose reduction criteria	・Hemorrhage: Grade 2 ・Proteinuria: worsening by ≥ Grade 1 from baseline and ≥500 mg/day ・Hypertension: Grade 1, 2 ・Hepatic dysfunction: ≥Grade 3 ・Coagulation disorder: Grade 2 If any of the above events occurs, reduce the dose to 5 mg/kg.	None	None
Criteria for delaying treatment	・Hemorrhage: Grade 2 ・Proteinuria: In the event of worsening ≥ Grade 1 from baseline, withhold until it decreased to ≤2 g/day ・Hepatic dysfunction : ≥Grade 3	・Thromboembolism: Grade 3 ・Proteinuria: >2g/day ・Hemorrhage: Grade 3	・Thromboembolism: Grade 3 ・Proteinuria: >2g/day ・Hepatic dysfunction ≥Grade 3 associated with bevacizumab

Criteria for discontinuing treatment	・Thromboembolism (arterial): ≥Grade 3 ・Hemorrhage: ≥Grade 3 ・Hypertension: ≥Grade 3 ・Coagulation disorder: ≥Grade 3	・Thromboembolism: Grade 4 ・Hemorrhage: Grade 4 ・Hypertension: ≥Grade 3 not controlled with medication	・Thromboembolism: Grade 4 ・Hemorrhage: ≥Grade 3 ・Proteinuria: Grade 4 ・Hypersensitivity ≥Grade 3 associated with bevacizumab ・In the event of gastrointestinal perforation or wound dehiscence ・Hypertension: ≥Grade 3 not controlled with medication

(2) Discussions on dosage and administration

At the Expert Discussion, there was a comment that whether the use of bevacizumab for second-line or subsequent treatment of patients previously treated with bevacizumab (the use of bevacizumab beyond first progression) is recommended is unclear based on the currently available data.

PMDA asked the applicant whether the use of bevacizumab beyond first progression is recommended and the applicant responded as follows:

As there has been no data from a confirmatory clinical study assessing the efficacy and safety of bevacizumab in combination with chemotherapy for second-line or subsequent treatment in patients who have received bevacizumab in combination with chemotherapy for first-line treatment, the use of bevacizumab beyond first progression can not be recommended at present. Genentech (the US) plans to analyze the efficacy of bevacizumab in combination with a second-line or subsequent chemotherapy in patients with PD after a first-line chemotherapy with bevacizumab, based on the interim results from a post-marketing, large observational study of patients with previously untreated colorectal cancer (Study BRiTE). Also, studies evaluating the efficacy, safety, and dosage regimen of bevacizumab in the same setting are ongoing by clinical research groups (Study AIO KRK 0504, etc.). Thus, taking account of the results of these studies, we will consider the necessity of investigating the efficacy and safety of bevacizumab beyond first progression.

PMDA largely accepted the applicant's response that the use of bevacizumab beyond first progression is not recommended until evidence for the efficacy and safety of bevacizumab in this setting is obtained. PMDA considers that it is necessary to advise that the efficacy and safety of Bevacizumab beyond first progression have not been established, via the proper-use guide etc. after market launch. PMDA also considers that the applicant should provide relevant information and determine the necessity of a further investigation promptly when the ongoing analysis of a post-marketing, large observational study in the US (Study BRiTE) is completed and the analysis results become available.

The following comment was raised from the expert advisors:

The applicant explained that there is no plan to assess the optimum dosage regimen of bevacizumab for second-line treatment [See the Review Report (1) "4.3 Clinical efficacy and safety, *Outline of review by the PMDA* 6)(1) Dose"]. Foreign Study AVF0780g that investigated the dose response of bevacizumab in patients with colorectal cancer showed no dose response

between 5 mg/kg and 10 mg/kg and the benefits of increasing the dose from 5 mg/kg to 10 mg/kg are unclear. The rationale for choosing a dose of 10 mg/kg of bevacizumab for Study E3200, a phase III study in second-line treatment, is unclear. Therefore, it is questionable whether the optimum dose for second-line treatment is 10 mg/kg.

PMDA asked for the applicant's view again on the necessity of assessing the dosage regimen of bevacizumab for second-line treatment after market launch.

The applicant responded as follows:
There has been no report suggesting the efficacy of 5 mg/kg of bevacizumab in second-line treatment and we predict that it will be hard to enroll patients into a comparative study at 5 mg/kg vs. 10 mg/kg. Thus, it is difficult to conduct a study.

PMDA considers as follows:
Although the PMDA's judgment that the dose of bevacizumab for second-line treatment may be determined based on the results of Study E3200, remains unchanged, the rationale for choosing the dose for Study E3200 is unclear as pointed out also at the Expert Discussion. The optimum dose of bevacizumab for second-line treatment is an issue for future investigation.

5) Post-marketing investigations
PMDA judged that it is necessary to conduct all-case investigation after market launch, identify the details of bevacizumab and concurrent chemotherapy (regimen), and collect safety data from Japanese patients since safety data obtained so far in Japan is very limited.

At the Expert Discussion, this judgment by the PMDA was supported by the expect advisors.

The following comments were raised from the expert advisors: (a) All-case investigation should be a prospective survey intended to determine the risk factors for serious adverse events (predictive factors), etc., instead of an unfocused survey. (b) Since Study E3200 is the only confirmatory study in previously treated patients, in order to study such population, it is necessary to collect detailed information on prior therapy and examine its association with the safety after market launch. (c) The number of patients treated with bevacizumab in Japan to date is limited and it is necessary to swiftly feedback (provide information) safety information from all-case investigation to the medical practice through the website etc.

Taking account of these comments from the expert advisors and the content of a review as described in the Review Report (2) "2) Safety," PMDA discussed as follows.

(1) Estimated number of candidate patients to receive bevacizumab and the target number of cases for all-case investigation after market launch
PMDA asked the applicant to explain with specific data the estimated number of candidate patients to receive bevacizumab and the number of medical institutions where bevacizumab will be used after market launch, and the number of cases that can be collected during the first year

of marketing.

The applicant responded as follows:
The number of new patients diagnosed with colorectal cancer each year is 123,040 (Future Estimation of Cancer Morbidity in Japan. *Cancer: Statistics White Paper －Incidence/Death/Prognosis －2004.* Shinohara Shuppan), 16.5% of whom have advanced colorectal caner (Stage IV) (Japanese Society for Cancer of the Colon and Rectum. Multi-institutional registry of large bowel cancer, Cases in 1995-1998. *Guideline for Treatment of Colorectal Cancer - For physicians 2005.* Kanehara & Co., Ltd), and assuming that 80% of these patients are not candidates for curative resection, the number of patients with advanced colorectal cancer who are not candidates for curative resection is 16,241. The number of patients with recurrent colorectal cancer is 18,792 (Japanese Society for Cancer of the Colon and Rectum. Research Project, Cases in 1991-1996. *Guideline for Treatment of Colorectal Cancer - For physicians 2005.* Kanehara & Co., Ltd), and assuming that 80.0% of these patients are not candidates for curative resection, the number of patients with recurrent colorectal cancer who are not candidates for curative resection is 15,033. Assuming that 80% of the total 31,275 patients who are not candidates for curative resection receive a first-line therapy and [redacted] of them will be prescribed bevacizumab, the number of patients prescribed bevacizumab as a first-line therapy is [redacted]. Assuming that [redacted]% of the patients who have received a first-line therapy will require second-line chemotherapy and [redacted]% of them will be prescribed bevacizumab, the number of patients prescribed bevacizumab as a second-line therapy is [redacted]. Based on the above, the number of patients prescribed bevacizumab during the first year of marketing is estimated at about 14,000.

The number of medical institutions registered for all-case investigation for an oxaliplatin preparation (brand name: Elplat for Injection 100 mg) approved for the indication of “unresectable advanced or recurrent colorectal cancer” is 1,127 (the number of medical institutions contracted is 1,205) (http://www.yakult.co.jp/ph/medical/product01/elplat/02.html). Thus, it seems that the number of medical institutions staffed by physicians experienced in chemotherapy, mainly FOLFOX, is over 1,000. However, as Avastin will be delivered to only the medical institutions capable of responding to emergencies involving adverse events associated with bevacizumab, we predict that the use of bevacizumab will be limited to around 800-900 institutions, although the details are yet to be determined.

PMDA asked the applicant to determine the target number of cases for all-case investigation based on its objectives.

The applicant responded as follows:
The primary objective of the all-case investigation is to identify the occurrence of gastrointestinal perforation and haemorrhage etc., which are unique to bevacizumab and have been reported as serious in some cases, under routine drug uses in Japan. The incidences of gastrointestinal perforation and haemorrhage (tumor-associated haemorrhage) $\geq$Grade 3 occurring during treatment with bevacizumab in foreign clinical studies were 8/392 patients

(2.0%) and 13/392 patients (3.3%), respectively, in Study AVF2107g and 2/100 patients (2.0%) and 5/100 patients (5.0%), receptively, in Study AVF2192g. Based on these results, assuming that gastrointestinal perforation occurs at an incidence of 2.0% and haemorrhage ≥Grade 3 occurs at an incidence of 5.0%, 1,124 cases and 2,181 cases, respectively, are required to assure with 95% power that the incidences of these adverse events are ≤3.0% and ≤6.0%, respectively. Based on the above, the target number of cases for all-case investigation has been determined to be 2,500.

The secondary objective of the investigation is to perform a subgroup analysis between patients treated with bevacizumab 5 mg/kg and 10 mg/kg and identify the occurrence of adverse drug reactions at each dose level. Since the patient background at baseline is different between patients treated with 5 mg/kg and those treated with 10 mg/kg, a rigorous comparison can not be made in this investigation, but we will examine whether there are any differences in the nature, incidence, and the time to onset of adverse drug reactions between the dose groups and also compare the data with the results from foreign clinical studies.

With 2,500 patients included in the investigation, according to the results of market research by the applicant, the numbers of patients treated with bevacizumab 5 mg/kg and those treated with 10 mg/kg are estimated at about 1,600-1,800 and about 700-900, respectively. For assessing whether the dose of bevacizumab is a risk factor for the development of adverse drug reactions or not, with 1,600 patients treated with bevacizumab 5 mg/kg and 900 patients treated with 10 mg/kg being investigated, if the incidence of adverse drug reactions with 5 mg/kg is 1-20% and the ratio of the incidence of adverse drug reactions with 5 mg/kg vs. 10 mg/kg is around 1.2-2.5, a statistical difference in the incidence of adverse drug reactions between the different doses can be detected. If analysis results suggest a dose-response relationship for safety, we will consider the conduct of another post-marketing surveillance study to further determine the dose response.

We will also perform subgroup analyses in terms of the patient background and the status of administration etc., taking into account the results of assessment of the risk factors for adverse reactions typicallyassociated with bevacizumab, such as gastrointestinal perforation, haemorrhage, arterial thrombosis, hypertension, and proteinuria. A multivariate logistic analysis will also be performed as appropriate.

PMDA largely accepted the applicant’s response.
However, instead of considering the conduct of a post-marketing surveillance study only when analysis results suggest a dose response for safety, it is necessary to consider the conduct of a further investigation also when (a) Any laboratory parameter or background factor predicting haemorrhage etc. has been suggested, and (b) Any adverse drug reaction with a possible difference in incidence between Japanese and foreigners has been identified. It is also necessary to evaluate the obtained results when 2,500 cases, i.e. the target number of cases for the all-case investigation, have been collected and consider whether to continue the all-case investigation and the necessity of changes to the methodology/content of the investigation, and then determine the termination of the all-case investigation carefully. The all-case investigation

should be continued while the data from 2,500 patients are being evaluated.

At a face-to-face consultation held before the NDA filing, PMDA had given the following advice to the applicant, in anticipation of a very limited number of subjects treated in Japanese clinical trials: (a) Consider the details of post-marketing surveillance prior to the NDA filing, and (b) Be fully prepared at the NDA filing so that post-marketing surveillance can be conducted smoothly upon approval of bevacizumab. However, judging from the draft post-marketing surveillance plan submitted by the applicant and their responses to a PMDA's question relating to post-marketing surveillance, it appears that no specific or detailed consideration has adequately been given to a post-marketing surveillance plan or the preparation of the company's internal system etc. and there is a concern about delay in preparation. The applicant should build a system for conducting the post-marketing surveillance as quickly as possible.

(2) The items to be investigated via post-marketing surveillance

As stated in the Review Report (2) "2) Safety," PMDA considers that it is necessary to perform coagulation/fibrinolysis tests, e.g. PT-INR and D-dimer and investigate whether such tests are useful for predicting haemorrhage and thromboembolism in Japanese patients. It is also necessary to find out the prior therapy as a patient background factor and examine its association with the safety, and collect information on the presumed risk factors (predictive factors) for adverse events typically associated with bevacizumab based on foreign clinical study data (age, a history of venous thrombosis, etc.) via the all-case investigation. In addition, the duration of observation and the test schedule should be established, taking into account the results of the examination of the time to onset of adverse events typically associated with bevacizumab.

PMDA instructed the applicant to develop a CRF to collect necessary information efficiently, in view of the above, and the applicant accepted it.

(3) Providing information on the results of all-case investigation

PMDA considered that it is necessary to collect/analyze post-marketing safety information promptly and appropriately and swiftly disclose its content on the applicant's website etc. early after market launch, and asked for the applicant's view on this matter.

The applicant responded as follows:

For a certain period of time after market launch, bevacizumab will be delivered to only the medical institutions that fulfill the conditions, e.g. those willing to cooperate with all-case investigation. Patients will be registered in advance. The status of use of bevacizumab (the number of registered cases, etc.) and the occurrence of adverse drug reactions obtained via the investigation will be published on our website and updated about once a month.

The plan presented by the applicant is largely acceptable. However, as to providing information on the results of all-case investigation through the applicant's website, PMDA instructed the

applicant to prepare the internal system so as to present the latest information on the status of registration of cases, the status of collection of CRFs, and the occurrence of adverse drug reactions, and to ensure that the development division and the post-marketing safety division will work in close cooperation so that necessary information will be disclosed without delay. It is necessary to update the information on the website precisely and feedback the status of progress of investigation (the number of registered cases for all-case investigation, the status of collection of CRFs, and adverse drug reaction information, etc.) appropriately.

(4) Material for promoting the proper use

The applicant presented a plan to prepare a "proper use guide" summarizing evidence obtained to date, notes of caution about the safety, and the points to note in diagnosis and treatment, etc., in order to provide information after market launch.

PMDA considers that preparing a proper use guide is important. PMDA instructed the applicant to clearly indicate (a) Patient selection and notes of caution in line with the dosing schedule of bevacizumab and (b) The regimens (dosage regimen) of bevacizumab and concurrent chemotherapy used in clinical studies, in the "proper use guide." PMDA also instructed the applicant to never use the content of inappropriate additional analyses performed by the applicant to claim that "there is" an add-on effect of bevacizumab in combination with FOLFOX4 in Study NO16966 [See the Review Report (1) "4.3 Clinical efficacy and safety, *Outline of review by the PMDA* 1)(1)(b) Study NO16966" and the Review Report (2) "1) The efficacy in patients with advanced or recurrent colorectal cancer who are not candidates for curative resection"] in information materials etc. for marketing.

The applicant accepted the PMDA's instructions including the instruction not to use the content of the additional analyses performed by the applicant.

6) Pharmacokinetics

At the Expert Discussion, the following comments were raised from the expert advisors: (a) Concerning the pharmacokinetic assessment of bevacizumab, although we understand that it is difficult to accurately analyze the pharmacokinetics of a genetically recombinant antibody drug, the applicant's response and discussion on the non-clinical study data are just an excuse [See the Review Report (1) "3.2 Pharmacokinetic studies, *Outline of review by the PMDA* 1) CL in single-dose studies in mice and rats, 2) CL after multiple doses"] and in order to judge its credibility, further data collection and accurate analysis are required, (b) There seem to be no major differences in the pharmacokinetics of bevacizumab between Japanese and foreigners and pharmacokinetic interactions are also unlikely to occur at present. However, it is important to investigate possible interactions between irinotecan and bevacizumab at the molecular level and those between bevacizumab and concurrently used capecitabine, and such investigations should be undertaken in the future.

As described in the Review Report (1) "3.2 Pharmacokinetic studies, *Outline of review by the PMDA* 1), 2)", there has been no adequate evidence directly supporting the applicant's response

on the non-clinical study data and a further investigation is required. Thus, PMDA asked for the applicant's view.

The applicant responded that UGT1A1 polymorphism will be investigated at the molecular level, taking into account ethnic differences, when a clinical study including a regimen of bevacizumab in combination with irinotecan in Japanese patients is conducted. Since this investigation is considered significant, PMDA included the conduct of these pharmacokinetic studies in the "Instructions".

7) Resistance

PMDA asked the applicant to explain about the mechanism of acquiring resistance to bevacizumab.

The applicant responded as follows:

We, as the applicant, have not performed any specific study on the acquisition of resistance to bevacizumab and its mechanism in a clinical or non-clinical setting. However, based on papers/reviews on the mechanism of acquiring resistance to angiogenesis inhibitors, including VEGF inhibitors (*Drug Resist Updat* 6: 111-27, 2003, *Cancer Metastasis Rev* 20: 79-86, 2001, *Nat Rev Cancer* 6: 626-35, 2006), the following mechanisms of acquiring resistance to bevacizumab are suggested: (a) Resistance acquisition via angiogenesis factors other than VEGF, (b) Resistance acquisition via angiogenesis-independent tumor growth, (c) Resistance acquisition due to oncogenic mutations, (d) Resistance acquisition due to the formation of anti-bevacizumab antibodies. At present, it is unclear how much influence each of the above mechanisms has had on the clinical data on bevacizumab. We hope that it will be elucidated gradually with an accumulation of clinical findings.

PMDA considers that relevant findings and resistance information are required also for physicians to determine a treatment plan and it is necessary to provide relevant information appropriately.

8) Deficiencies in the application dossier

Bevacizumab was a product discussed at the Investigational Committee for Usage of Unapproved Drugs and interested parties including patients submitted a petition for early approval of bevacizumab to the Minister of Health, Labour and Welfare: Early approval of bevacizumab was desperately awaited. PMDA understands that the applicant bore an enormous burden of preparing documents after the regulatory submission, due to the submission of an interim analysis of some study data during the filing process, etc. However, the documents submitted at the NDA filing contained more than 100 clerical errors and mistakes in the figures/tables, and in the applicant's response to a set of questions from the PMDA, completely irrelevant or non-informative references were cited. Such problems became apparent during the regulatory review, and the application data and the response to a set of questions were amended repeatedly after the regulatory submission. Given such enormous changes to the application dossier, it is no exaggeration to say that the new drug application and response were submitted

without ensuring quality control/quality assurance of the application data. For the review of this application, the reviewers had to spend a vast amount of time and labor on repeated checking of these amendments, which disturbed an efficient review process. In future, the applicant should be more aware of the importance of quality control/quality assurance of an application dossier and put an appropriate system in place promptly.

III. Overall evaluation

Bevacizumab is a recombinant antibody against VEGF that is known to be involved in tumor progression. While no clinically significant results have been obtained with single agent bevacizumab, it is considered that bevacizumab characteristically exhibits clinical usefulness in patients with colorectal cancer when combined with fluoropyrimidine-based chemotherapy.

As a result of its review of the application submitted, PMDA has concluded that the product may be approved for the following indications and dosage and administration, subject to the conditions as described below, provided that appropriate caution statements are included in the package insert and proper-use information is provided appropriately after market launch and that the product is properly used at a medical institution with adequate facilities for the treatment of emergencies under the supervision of a physician with adequate knowledge and experience in cancer chemotherapy.

Since this application falls under the category of drugs containing new active ingredients, a re-examination period of 8 years should be appropriate. The drug substance and the drug product are both classified as a powerful drug. The product is also classified as a biological product.

[INDICATIONS]

Unresectable advanced or recurrent colorectal cancer

[DOSAGE AND ADMINISTRATION]

The usual adult dosage is 5 mg/kg (body weight) or 10 mg/kg (body weight) of bevacizumab given as an intravenous infusion in combination with chemotherapy. The dosing interval should be ≥2 weeks.

[Conditions for approval]

Because of the very limited number of subjects treated in the domestic clinical trials, conduct all-case investigation until the data from a certain number of patients are accumulated after market launch in order to identify the background of patients treated with Avastin and collect safety and efficacy data on Avastin early, and take necessary measures for the proper use of Avastin.

[Instructions]

1. Summarize the final data from the safety confirmation study promptly and publish the results.

2. Conduct studies with an appropriate design in order to further determine the pharmacokinetics of Avastin and publish the results.

[WARNINGS]
1. Avastin in combination with chemotherapy should be administered only to patients eligible for the treatment under the supervision of physicians with sufficient knowledge and experience in cancer chemotherapy at medical institutions that can provide adequate emergency medical care. Eligible patients must be carefully selected after reading the package inserts for Avastin and concomitant chemotherapeutic agents. Treatment should be started only after obtaining informed consent from patients or their families who are fully informed of the efficacy and risk of treatment.
2. Gastrointestinal perforations, including fatal cases, have been reported. Gastrointestinal perforation should be included in the differential diagnosis of patients presenting with abdominal pain on Avastin. Discontinue Avastin and take appropriate measures in patients given a diagnosis of gastrointestinal perforation. The patients should not be re-exposed to Avastin. (See “Careful Administration,” “Clinically significant adverse reactions,” and “Other Precautions”).
3. Wound healing complications (e.g., wound dehiscence, post-operative haemorrhage) may occur.
(1) Examine the surgical wound in postoperative patients to determine whether they can receive Avastin. Do not administer Avastin to patients with unhealed wound after major surgery, except in situations where the therapeutic benefits are expected to outweigh the risk of wound healing complications. In clinical studies, patients were not allowed to receive Avastin until at least 28 days had elapsed following major surgery (See “Careful Administration”).
(2) In patients experiencing wound healing complications during treatment, Avastin should be interrupted until the wound is fully healed and appropriate measures should be taken (See “Clinically significant adverse reactions”).
(3) An adequate interval should be allowed between the termination of Avastin and subsequent elective surgery. The appropriate interval required to avoid the risks of delayed healing of wound has not been determined (See “Important Precautions” and “Clinically significant adverse reactions”).
4. Avastin may increase the risk of tumor-associated haemorrhage. Avastin therapy may result in cerebral haemorrhage in patients with brain metastases. The occurrence of cerebral haemorrhage in a patient with a brain metastasis has been reported in a foreign clinical trial. Discontinue Avastin and take appropriate measures in patients experiencing severe hemorrhage during treatment. The patients should not be re-exposed to Avastin. (See “RELATIVE CONTRAINDICATIONS”, “Important Precautions,” “Clinically significant adverse reactions,” and “Other Precautions”).
5. Arterial thromboembolic events (e.g., cerebrovascular accident, transient ischemic attack, myocardial infarction, angina pectoris, cerebral ischemia, and cerebral infarction), including fatal cases, have been reported. Patients should be carefully monitored for these events. Discontinue Avastin or take other appropriate measures in patients presenting with any abnormal findings. Patients who experience arterial thromboembolic events should not be

re-exposed to Avastin. (See “Careful Administration” and “Clinically significant adverse reactions”).
6. Hypertensive encephalopathy or hypertensive crisis, including fatal cases, has been reported. Discontinue Avastin in patients experiencing these events. The patients should not be re-exposed to Avastin. Blood pressure should be monitored periodically during Avastin therapy. (See “Important Precautions” and “Clinically significant adverse reactions”).
7. Reversible posterior leukoencephalopathy syndrome may occur rarely. Discontinue Avastin and take appropriate measures in patients suspected to have reversible posterior leukoencephalopathy syndrome. (See “Clinically significant adverse reactions”).

[CONTRAINDICATIONS]
Patients with a history of hypersensitivity to any of the ingredients of Avastin

[RELATIVE CONTRAINDICATIONS]
Patients with brain metastases (See “WARNINGS” and “Important Precautions”)

[Precautions for indications]
(1) The efficacy and safety of Avastin in combination with adjuvant chemotherapy have not been established.
(2) Avastin should be used with a full understanding of the information in “CLINICAL STUDIES.”

[Precautions for dosage and administration]
(1) Avastin should be used in combination with fluoropyrimidine-based chemotherapy. The chemotherapeutic agents used in combination with Avastin must be selected based on a full understanding of the information in “CLINICAL STUDIES.”
(2) Read carefully the package inserts for concomitant chemotherapeutic agents.
(3) The efficacy and safety of Avastin monotherapy have not been established.
(4) The dosage of Avastin should be selected according to prior chemotherapy regimens, based on a full understanding of the information in “CLINICAL STUDIES.”
(5) Preparation of infusion solution and infusion duration
1) Withdraw the necessary volume of Avastin with a syringe and dilute in a total volume of approximately 100 mL isotonic sodium chloride solution (JP). The initial dose should be administered over 90 minutes as an intravenous infusion.
2) If the first infusion is well tolerated, the second infusion may be administered over 60 minutes. If the 60-minute infusion is well tolerated, all subsequent infusions may be administered over 30 minutes.

IV. Additions to the Review Report (1)

The responses to the questions that were being asked to the applicant at the time of preparation of the Review Report (1) are described below.

2. Quality Data

Outline of review by the PMDA

1) Manufacturing process

(1) Timing of checking the stability of the WCB

Although the stability of the working cell bank (WCB) is to be checked when a new WCB is prepared, it was forecasted at the time of regulatory submission that an additional WCB would be prepared every [redacted] years. Thus, the PMDA asked the applicant to check the stability more frequently in order to provide an assurance during the period until a new WCB is prepared.

The applicant responded as follows:

Due to the change in the demand forecast after the regulatory submission, a new WCB is expected to be prepared every [redacted] years. Therefore, we have planned to check the stability only when preparing a new WCB. However, taking also account of the possibility of a temporary decrease in the production volume of bevacizumab in future, we will check the stability when preparing a new WCB or every [redacted] years, whichever comes first.

PMDA accepted the response as the stability will be checked at least every [redacted] years.

(2) The basis for allowing refiltration

Refiltration may be repeated up to [redacted] times either before the storage of the drug substance or before sterile filtration in the formulation step. PMDA asked the applicant to explain the reason for setting this criterion, showing the data that serves as a basis.

The applicant responded as follows:

[redacted] mL of the drug substance produced at a commercial scale (12,000 L) (Lot No. U) was taken and refiltrated [redacted] times and the results of specification tests for the drug substance were compared between the refiltrated and not refiltrated drug substances. As a result, their test results were comparable. Also, long-term testing ([redacted]℃, [redacted] months) and accelerated testing ([redacted]℃, [redacted] months) of the drug product (Lot No. G) produced from the drug substance manufactured at a commercial scale and refiltrated [redacted] times (Lot No. P) were conducted. As a result, the drug product produced from the drug substance refiltrated [redacted] times was also stable at [redacted]℃ for [redacted] months, like the drug product produced from the drug substance not refiltrated. Thus, it has been determined that refiltration of the drug substance may be repeated up to [redacted] times [For the results of stability studies of the drug product (Lot No. G), see the Review Report (1) ”2. Data relating to quality, *Summary of the submitted data* 3)(4) Stability of the drug product”].

PMDA accepted the above response.

2) Characterization

(1) Capillary isoelectric focusing (cIEF) and isoelectric focusing (IEF)

PMDA instructed as follows:

Regarding the results of cIEF and IEF, as bevacizumab is heterogeneous with respect to the carbohydrate chain and primary structure etc., multiple bands in addition to [redacted] main bands are detected. Thus, to present the results of a characterization study, the range of the pI value as well as the detection of [redacted] main bands should be mentioned.

The applicant responded as follows:

The relevant part will be changed as follows: IEF showed [redacted] main bands and the range of the pI value was [redacted].

Based on the values obtained from cIEF and IEF, the main species of bevacizumab (having Gly^{452} on both the heavy chains C-terminal ends and G0) has an estimated pI of [redacted].

PMDA accepted the response.

(2) Differently charged related substances

The applicant explained that since differently charged related substances (variants with unprocessed C-terminal Lys residues on the heavy chains) are regulated by in-process controls and its content is kept low at [redacted]% for the drug substance derived from the current G7 cell line, it is unnecessary to set a specification for them. However, PMDA asked the applicant to sort out clinical study data etc. obtained to date for assessing the impact of variations in the heavy chain C-terminal Lys content on the safety and then show their view on the necessity of setting a specification again.

The applicant responded as follows:

Since multiple lots of the drug product in which a molecular weight component of unprocessed heavy chain C-terminal Lys residues is [redacted]% (Lot No. V and W derived from the 107 cell line, Lot No. X and Y derived from the G7 cell line) were administered to each patient in clinical trials, no direct finding on the association between the safety and the heavy chain C-terminal Lys content could be obtained. However, (a) The content of variants with unprocessed heavy chain C-terminal Lys residues is kept under [redacted]% for the lots derived from the G7 cell line and the constancy is assured. (b) The heavy chain C-terminal Lys content does not affect the *in vitro* biological activity. (c) It has been confirmed that the pharmacokinetics of Lot No. Z derived from the 107N cell line (H-chain C terminal Lys:[redacted]%) is comparable to that of Lot No. X derived from the G7 cell line (H-chain C-terminal Lys: [redacted]%) in rats [Note by the PMDA: It has been suggested that C-terminal Lys residues are removed promptly by the active form of carboxypeptidase in blood after administration (*Trends Pharmacol Sci* 9: 299-304, 1988)]. Therefore, although there were differences in heavy chain C-terminal Lys content between the drug products derived from the 107N and G7 cell lines, we considered that these differences have no effects on the safety. Based on the above, no direct control with defined parameters in the form of in-process tests is implemented and setting a specification is also unnecessary.

PMDA accepted the decision not to include the heavy chain C-terminal Lys content in the specification for the following reasons:
With respect to variants having Lys residue variations at the C-terminal of their heavy chains, (a) Based on the data from 5 lots, the H-chain C-terminal of the drug produced from the current cell line at a commercial scale are processed at ≥[redacted]% and these have comparable biological activities. (b) It has been demonstrated that the pharmacokinetics is comparable between Lot No. Z derived from the 107N cell line and Lot No. X derived from the G7 cell line. (c) No safety concern has so far been reported even though the drug products derived from different cell lines were administered in clinical trials.

3) Control of the drug substance

(1) The necessity of control of the drug substance with respect to carbohydrates

PMDA asked the applicant to explain the impact of the presence or absence of carbohydrates on the pharmacokinetics and safety although even unglycosylated bevacizumab retains biological activity. PMDA also asked the applicant to include testing of carbohydrates in the specification in terms of assuring the quality.

The applicant evaluated (a) the impact of carbohydrate chains on clearance, (b) the immunogenicity of carbohydrate chains, and (c) the carbohydrate structure of bevacizumab, and then showed their view on the necessity of setting a specification test as follows.

(a) The impact of carbohydrate chains on clearance:
Carbohydrate chains may affect the clearance of the antibody by its clearance via binding of the non-reducing terminal sugar to the galactose receptor or the mannose receptor and its non-specific clearance via the neonatal Fc receptor (FcRn).

An IgG carbohydrate chain is in the Fc domain and it is predicted that its direct binding to the mannose receptor or the galactose receptor does not occur. When omalizumab and efalizumab, which have an identical amino acid sequence of the Fc region to that of bevacizumab were administered to mice and patients and serum glycopeptide was measured by MALDI-TOF-MS, there were no differences in carbohydrate distribution in the process of elimination, and it has also been reported that the carbohydrate structure does not affect clearance.

Although it is known that non-specific clearance of human antibodies is mediated by FcRn, it has been reported that unglycosylated antibody generated by site-directed mutagenesis is recognized by FcRn, like wild-type glycosylated antibody, and is eliminated from the circulation of mice or neonatal rats (*J Immunol* 143: 2595-601, 1989, *Mol Immunol* 29: 949-56, 1992), suggesting that the recognition of Fc by FcRn is not affected by the presence or absence of carbohydrate. Moreover, the results were also consistent in the above-mentioned study with efalizumab and it has been indicated that FcRn-mediated clearance in humans is unaffected by G0 or G1.

There are lot-to-lot variations in G0 content, and Lot No. AA with a low value (G0: [redacted]%) and

Lot No. AB with a high value (G0: [redacted]%) were administered as a single dose (10 mg/kg) to rats. As a result, the geometric mean ratio of AUC_{0-11} was 0.98 (90% CI, 0.91-1.06), which met the bioequivalence criteria and it was confirmed that the clearance is comparable.

(b) The immunogenicity of carbohydrate chains:
The predominant glycoform of bevacizumab is identical to carbohydrate chains on IgG molecule derived from human plasma and it is considered that bevacizumab in which the Fc region is glycosylated is safe due to immunotolerance. Since it has also been reported that the Fc structure is maintained with the presence of GlcNAc at the branch point of the two chains(1-6) (*J Mol Biol* 325: 979-89, 2003), bevacizumab having the predominant G0 glycoform, is considered to maintain the Fc structure and immunogenicity would not be elicited by conformational change. Based on the above, the carbohydrate structure of bevacizumab has little effects on the immunogenicity.

(c) The carbohydrate distribution of evacizumab:
Among the carbohydrate structures of bevacizumab observed, even the relative abundance of predominant glycoform, G0, is within the range of around [redacted]%, assuring that the carbohydrate distribution is constant.

Based on the results from the above studies with bevacizumab or other antibody drugs which have an identical amino acid sequence of the Fc region to bevacizumab, etc., the carbohydrates of bevacizumab do not affect the pharmacokinetics. Although no direct data on the impact of the presence or absence of the carbohydrates on the safety is available, there have been no literature or clinical findings suggesting the effects of various glycoforms of bevacizumab on the safety and at present, there should be no safety concerns. In addition, the manufacturing process for bevacizumab is well-controlled and the variation range of the carbohydrate distribution is narrow and the consistency is assured. Therefore, release specifications and limits for the carbohydrate of bevacizumab are not considered necessary.

PMDA accepted the decision not to set a carbohydrate structure specification for the drug substance and the drug product, because the variation range of the carbohydrate structure is small and there should be no clinically relevant effects of variations in the carbohydrate structure of bevacizumab on the disposition kinetics and safety.

(2) IEC
IEC samples were treated with carboxypeptidase B (CpB). PMDA asked the applicant to explain the necessity of establishing a specification for detecting also isoforms with unprocessed heavy chain C-terminal Lys residues, without CpB treatment.

The applicant responded as follows:
The purpose of CpB treatment is to remove a component of unprocessed heavy chain C-terminal Lys residues for simplifying the chromatogram pattern of [redacted] and facilitating the detection of other [redacted] component and new [redacted] component. Therefore, change to the

specification is unnecessary.

PMDA accepted the response.

(3) Peptide mapping

PMDA asked the applicant to explain the necessity of specifying the acceptance criteria as follows: “Typical peaks having the same retention time and height or area as the reference material are detected.”

The applicant responded as follows:

Setting the acceptance criteria as proposed by the PMDA is based on the premise that extremely high precision is assured with respect to the retention time and peak height or area among the elution patterns of different samples (for example, [redacted] and [redacted]) for peptide mapping. However, in reality, some variability is unavoidable due to complicated procedures unique to peptide mapping, which include (a) The complexity of the structure of a monoclonal antibody, (b) Multiple chemical or biochemical manipulations such as reduction, [redacted] cleavage and enzymatic digestion for the preparation of sample, (c) Chromatographic analysis of sample containing the end product of large quantities of peptides with an extensive molecular weight distribution. Therefore, it is difficult to specify the acceptance criteria as proposed by the PMDA for routine lot release, from a technical and operational standpoint.

On the other hand, according to our test procedures, [redacted] ([redacted]), [redacted], [redacted] of [redacted] and [redacted] ([redacted]) and [redacted] ([redacted]) are analyzed [redacted] for each sample. The acceptance criteria are as follows: [redacted] of [redacted] and [redacted] is [redacted] [redacted] of [redacted] at all analysis steps and [redacted] must be [redacted] for [redacted] and [redacted] must also be [redacted]. Even a subtle change in the molecular structure can be detected by [redacted] and [redacted] [redacted] for the following reasons.

・If the retention time or resolution of peaks differs significantly, [redacted], [redacted], and [redacted], and the acceptance criteria for identity are not met.
・Analysis of [redacted] makes it possible not only to offset inter-test variability but also to detect a subtle difference in the retention time between the reference material and sample.
・When [redacted] for [redacted] of [redacted] and [redacted], changes in the peak height for the sample solution are indirectly checked, which can detect a subtle change in the molecular structure of the sample.

Although the peptide map specification as indicated in General Information of the Japanese Pharmacopoeia is not directly employed, judging that even the specifications established by the applicant can assure the quality, PMDA accepted the response.

4) Viral inactivation/removal

PMDA asked the applicant to explain the appropriateness of the followings: (a) The ability of the affinity chromatography step and the cation exchange chromatography step to inactivate and remove retroviruses was evaluated by different methods (infectivity assay, quantitative PCR) and these reduction factors were summed up. (b) Virus reduction factors determined by quantitative PCR were used for process evaluation for adventitious viruses.

The applicant responded as follows:

(a) Adding up of reduction factors for determining the ability to inactivate/remove viruses:

Non-infectious, retrovirus-like particles are generally present in CHO cells. The ability of the purification process to clear these retrovirus-like particles was evaluated using X-MuLV, which is recommended by the ICH Guideline Q5A (PMSB/ELD Notification No. 329 dated February 22, 2000 "Viral Safety Evaluation of Biotechnology Products Derived From Cell Lines of Human or Animal Origin"). Namely, X-MuLV was added to a scaled-down version of the purification process and the virus clearance capacity was evaluated by cell-based infectivity assay and quantitative PCR [See the Review Report (1) "2. Data relating to quality 1) (5) Safety evaluation of adventitious infectious agents"].

"[redacted] treatment step and affinity chromatography step" and "[redacted] treatment step and cation exchange chromatography step" of the purification process were evaluated as a series of steps, but infectivity assay was applied to the steps that are expected to inactivate viruses ("[redacted] treatment" and "[redacted] treatment") and quantitative PCR was applied to the steps that are expected to remove viruses ("affinity chromatography," "anion exchange chromatography," and "cation exchange chromatography"). Although quantitative PCR used to evaluate the ability to remove viruses is an assay for virus particle numbers and can not directly assess the presence or absence of infectivity, since it has been reported that the number of X-MuLV particles is correlated with the infectivity when the specific activity of the virus is constant (*Biotechnol Bioeng* 87: 884-96, 2004) and it is anticipated that the specific activity of virus is constant or declines in the purification process, we consider that the results of assessment by quantitative PCR are an indicator of a reduction in the infectivity risk. For "the affinity chromatography step" and "the cation exchange chromatography step," only the ability to remove viral particles was evaluated by quantitative PCR, excluding the ability to inactivate the virus by buffers. Thus, overall virus reduction factors are not overestimated.

(b) Virus reduction factors determined by quantitative PCR:

Infectivity assay for adventitious viruses was carried out on the cell banks and pre-harvest cell culture fluids, which confirmed the absence of viruses [See the Review Report (1) "2. Data relating to quality 1) (5) Safety evaluation of adventitious infectious agents"]. In the manufacturing process, assuming adventitious introduction of virus, the ability of the purification process to clear adventitious viruses was evaluated using two model viruses that display a significant resistance to physical/chemical treatment (MMV and SV40). As these viruses can not be inactivated by "the low pH treatment step" or "the urea treatment step," the ability of the chromatography steps to remove viruses was evaluated by quantitative PCR [See

the Review Report (1) “2. Data relating to quality 1) (5) Safety evaluation of adventitious infectious agents”]. Since quantitative PCR can not assess the ability to inactivate viruses, the clearance capacity may be underestimated. However, as the sum of the reduction factors of the two steps is $\geq$6, the process is considered to have adequate ability to remove viruses. Therefore, we think that evaluating the viral clearance capacity by only quantitative PCR in this study was appropriate.

As to (a), PMDA accepted the adding up of the reduction factors determined by quantitative PCR and infectivity assay. As to (b), since the ability to remove adventitious viruses was evaluated by quantitative PCR only and the virus reduction factors obtained were by no means high, PMDA sought opinions from the expert advisors. As a result, the following comment was raised: Since *in vitro* assay has been performed on pre-harvest cell culture fluids and there is a certain assurance about the viral safety of the raw materials, taking also account of the necessity of bevacizumab in clinical practice, the process has a virus reduction factor of $\geq$6 for non-enveloped viruses and there should be no concerns.

PMDA accepted the response.

3.1 Pharmacology studies

The following responses to the questions that were being asked to the applicant at the time of preparation of the Review Report (1) were submitted.

PMDA asked the applicant to explain the binding activity of bevacizumab to the VEGF family that share domains highly homologous to VEGF isoforms such as $VEGF_{165}$.

The applicant responded as follows:

A review article indicated that bevacizumab does not exhibit neutralizing activity against the VEGF family molecules other than VEGF (*Nat Rev Drug Discov* 3: 391-400, 2004). However, as the original articles etc. upon which the review article is based, have not been published, data from a direct investigation of the binding activity of bevacizumab to the VEGF family other than VEGF is unknown. The amino acid residues within a VEGF molecule that seem particularly important for binding to bevacizumab are Met^{81}, Arg^{82}, Ile^{83}, Gly^{88}, Gln^{89}, and Gly^{92}, and to what extent they exist in the VEGF family molecules and in the platelet-derived growth factor molecule with a similar steric structure to that of VEGF was investigated. As a result, in human VEGF-B, in which the largest number out of the 6 amino acid residues were found among the VEGF family molecules, 3 of the 6 amino acid residues were different. Given that bevacizumab does not bind to mouse VEGF in which one out of the above-mentioned 6 amino acid residues is different, bevacizumab is very unlikely to bind to the human VEGF family other than VEGF.

Then, PMDA asked the applicant to explain the binding activity of VEGF to the VEGF family receptors other than VEGF receptors and the effects of bevacizumab on the VEGF family receptor signal transduction system.

The applicant responded as follows:
An isoform of VEGF, $VEGF_{165}$ binds to neuropilin-1 and -2, coreceptors, besides VEGFR-1 and -2. It is thought that neuropilin-1 and -2 bind to sites different from the binding sites of VEGFR-1 and -2 within the VEGF molecule, strengthening the binding between VEGFR-2 and $VEGF_{165}$ (*Mol Cancer Ther* 5: 1099-107, 2006), but whether the binding of $VEGF_{165}$ to neuropilin-1 and -2 is inhibited by bevacizumab is unknown.

PMDA considers that it is necessary to continue to collect information including a literature search, with respect to the physiological function of VEGF signal transduction and examine the effects of bevacizumab on the biofunction and the mechanism of development of adverse drug reactions.

4.2 Clinical pharmacology
The applicant submitted the following response concerning a future plan to investigate the effects of anti-bevacizumab antibodies on the pharmacokinetics of bevacizumab.

Reevaluation of the ECLA for anti-bevacizumab antibodies by Genentech has been completed and analysis of 1,884 samples collected from Study NO16966 has been started using the new assay method after reevaluation. After confirming the details of reevaluation, the samples collected from Japanese Study JO18157 and 4 ongoing Japanese clinical studies will be analyzed promptly for anti-bevacizumab antibodies. Although the details are not obtained, we have heard that the new ECLA after reevaluation can not detect anti-bevacizumab antibodies in the presence of bevacizumab at $\geq$100 μg/mL coexisting in the sample due to the interference of bevacizumab with this assay system. Thus, blood sampling timepoints for antibody assay in an ongoing Japanese clinical study will be reconsidered. It is expected that the effects of anti-bevacizumab antibodies on the pharmacokinetics of bevacizumab will be determined in future, by comparing the pharmacokinetics between subjects with positive antibody test results and those with negative results.

The PMDA confirmed that the effects of anti-bevacizumab antibodies on the pharmacokinetics, efficacy, and safety of Bevacizumab will be investigated in an ongoing Japanese clinical study. If a new finding on the relationship between anti-bevacizumab antibodies and the pharmacokinetics of Bevacizumab from Japanese or foreign clinical studies or published articles etc. is obtained, it is necessary to reconsider the necessity of a further investigation, taking account of the finding as well. It is also necessary to appropriately provide information on the results of such investigation to the medical practice.

4.3 Clinical efficacy and safety
The following responses were submitted to the questions that were being checked with or asked to the applicant at the time of preparation of the Review Report (1) [See the Review Report (2) “2) Safety” for the outline of review by the PMDA].

The detailed information on the incidences of delayed healing of wound (including post-operative haemorrhage) in major clinical studies overseas is shown below.

Study Number	Treatment group	No. of cases with delayed healing of wound			
		Cases of surgery during the study n, (%)		Cases of surgery before the study* n, (%)	
		All adverse events	≥Grade 3	All adverse events	≥Grade 3
AVF2107g **	IFL+bevacizumab	NA	5/60 (8.3%)	NA	5/180 (2.8%)
	IFL+placebo	NA	1/44 (2.3%)	NA	3/173 (1.7%)
AVF2192g ***	5FU/LV+ bevacizumab	NA	5/15 (33.3%)	NA	0/43 (0%)
	5FU/LV+ placebo	NA	0/3 (0%)	NA	0/39 (0%)
E3200	FOLFOX4+ bevacizumab	NA	NA	NA	NA
	FOLFOX4 alone	NA	NA	NA	NA
	Bevacizumab alone	NA	NA	NA	NA
NO16966	FOLFOX4+ bevacizumab	3/46 (6.5%)	0/46 (0%)	3/111 (2.7%)	0/111 (0%)
	FOLFOX4+ placebo	1/49 (2.0%)	1/49 (2.0%)	2/114 (1.8%)	2/114 (1.8%)
	XELOX+ bevacizumab	0/56 (0%)	0/56 (0%)	3/126 (2.4%)	1/126 (0.8%)
	XELOX+ placebo	0/41 (0%)	0/41 (0%)	2/111 (1.8%)	0/111 (0%)

*: Patients who initiated treatment with bevacizumab between the 28th and 60th postoperative days,
: Grade 3/4 haemorrhage, *: Grade 3/4 wound healing complications (haemorrhage, etc.),
NA: not applicable

Concerning delayed healing of wound, analysis was performed for the patients with diabetes, who are likely to experience delayed healing of wound, based on the data from Study AVF2107g, Study AVF2192g, and Study AVF0780g, and the following response was submitted.

Of the analysis population, 159/535 patients in the control group and 160/527 patients in the bevacizumab-containing group had some risk factor for delayed healing of wound, e.g. diabetes, at baseline. The percentage of patients who experienced delayed healing of wound as an adverse event was 1% (8/535 patients) in the control group and 2% (10/527 patients) in the bevacizumab-containing group. In the patient subgroup with any risk factor for delayed healing of wound at baseline, 4 patients in the control group and 5 patients in the bevacizumab-containing group had delayed healing of wound as an adverse event, which was similar to the results of the overall patient population (8 patients and 10 patients, respectively). Cox regression analysis was performed including treatment group, baseline risk factors associated with wound healing, and an interaction term between treatment group and baseline risk factors associated with wound healing. As a result, none of these factors, including an interaction between treatment group and baseline risk factors associated with wound healing, was statistically significant (p=0.99, Wald test).

Using the data from Foreign Study NO16966, an investigation of whether INR measurements can predict thromboembolism and haemorrhage following treatment with bevacizumab is under way (planned to be completed in [redacted] 20[redacted]), and no data has become available. With regard to the status of use of anticoagulant therapy in this study, 124/675 patients (18.4%) in the chemotherapy group (FOLFOX+placebo group and XELOX+placebo group) and 161/694 patients (23.2%) in the chemotherapy+bevacizumab group (FOLFOX+bevacizumab group and XELOX+bevacizumab group) received some anticoagulant therapy during study treatment

based on their CRF data. The commonly used anticoagulant therapy was warfarin and the rate of use of warfarin therapy was 17% in the FOLFOX+placebo group, 14% in the XELOX+placebo group, 25% in the FOLFOX+bevacizumab group, and 17% in the XELOX+bevacizumab group.

There was no adverse event reported as an infusion reaction in the Japanese clinical studies (as of [redacted] 20[redacted]). Then, the events translated into pyrexia, chills, rash, headache, nausea, vomiting, wheezing, anaphylactic reaction, anaphylactic shock, anaphylactoid reaction, henoch-schonlein purpura, and anaphylactoid shock on MedDRA (ver8.1) PT and severe respiratory system disorders classified as the SOC "Respiratory, thoracic and mediastinal disorders" ≥Grade 3 were defined as "infusion reactions" for analysis. As a result, in Study JO18157, events that meet the above definition occurred in 8/18 patients (44.4%). The events observed in the 8 patients were nauseas and vomiting and none of them were ≥Grade 3 (as of [redacted] 20[redacted]). In Study JO18158, adverse events that meet the above definition occurred in a total of 15 patients (32.6%): 13/33 patients (39.4%) in the 5 mg/kg group and 2/13 patients (15.4%) in the 10 mg/kg group. None of them were ≥Grade 3 and the common event was nausea (13 patients) (as of [redacted] 20[redacted] [including reports before the data lock]).

As of [redacted] 20[redacted], a total of 56 patients including 36 patients in the 5 mg/kg group (including 1 untreated patient) and 20 patients in the 10 mg/kg group have been enrolled into Study JO18158 that is ongoing in Japan. As of [redacted] 20[redacted] before the data lock (CRF has not been collected for patients who have just been enrolled), adverse events ≥Grade 3 reported in the FOLFOX4+bevacizumab 5 mg group were neutrophil count decreased in 23 patients, white blood cell count decreased in 7 patients, lymphocyte count decreased in 5 patients, diarrhoea in 5 patients, anorexia in 4 patients, haemoglobin decreased in 2 patients, gastrointestinal perforation, abdominal pain, CRP increased, febrile neutropenia, dehydration, platelet count decreased, hyperglycaemia, vomiting, nausea, ALP increased, ALT increased, asthenia, sodium low, cataract, infection (periodontitis), and gingivitis (one case each), and those reported in the FOLFOX4+bevacizumab 10 mg group were neutrophil count decreased in 11 patients, white blood cell count decreased in 5 patients, hypertension in 2 patients, lymphocyte count decreased, AST increased, ALT increased, and ALP increased (one case each).

As comparison/discussion of safety data between Japanese and foreigners, including data from an ongoing Japanese study JO18158, the following response was submitted.

A comparison was made between 18 patients in Japanese Study JO18157 (data cutoff: [redacted] 20[redacted]) and 276 patients from Foreign Studies AVF0780g, AVF2107g, and AVF2192g combined. The incidences of adverse events occurring at a ≥10% higher incidence in the Japanese clinical study compared to the foreign clinical studies combined are shown below. Concerning the difference in the incidence of protein urine present (Japan: 38.9%, Overseas: 0.7%), proteinuria (32.2%) and protein urine (0.4%) were reported separately apart from protein urine present in the foreign studies, and when a comparison was made including these two events, there was no ≥10% difference between Japan and overseas.

Adverse event	Japanese study	Foreign studies	Adverse event	Japanese study	Foreign studies
	5-FU/*l*-LV	5-FU/LV		5-FU/*l*-LV	5-FU/LV
Stomatitis	50.0%	17.8%	White blood cell count decreased *5	55.6%	3.7%
Cheilitis *1	22.2%	7.7%	Haematocrit decreased	44.4%	0.4%
Epistaxis	50.0%	34.1%	Blood ALP increased	16.7%	2.2%
Palmar-plantar erythrodysaesthesia syndrome	33.3%	8.7%	White blood cell count increased	44.4%	0.4%
Alopecia	27.8%	8.3%	Lymphocyte count decreased *6	44.4%	0.4%
Skin exfoliation	22.2%	5.4%	Platelet count decreased *7	38.9%	5.1%
Pigmentation disorder	44.4%	2.5%	PT prolonged	22.2%	0.7%
Nail disorder	11.1%	1.1%	ALT increased	16.7%	0.7%
Dermatitis contact	11.1%	0.4%	Blood phosphorus decreased *8	22.2%	0.8%
Anorexia *2	66.7%	43.5%	Protein total decreased	16.7%	0.7%
Upper respiratory tract infection	22.2%	10.9%	Blood albumin decreased *9	22.2%	1.1%
Nasopharyngitis	33.3%	10.1%	Blood Cl decreased *10	16.7%	0.8%
Infection	11.1%	1.1%	γ-GTP increased	11.1%	0.4%
Dental caries	11.1%	1.1%	Blood Na decreased *11	16.7%	3.6%
Weight decreased	44.4%	16.3%	Blood cholesterol increased *12	11.1%	0.7%
Neutrophil count decreased *3	55.6%	14.5%	Eye discharge	11.1%	1.1%
Blood bilirubin increased *4	33.3%	4.3%	Post procedural haemorrhage	16.7%	4.3%
Hb decreased	44.4%	1.1%			

*1 Including lip ulceration, chapped lips, lip blister, lip pain, and lip disorder, *2 Including decreased appetite and oral intake reduced, *3 Including neutropenia, *4 Including hyperbilirubinaemia, *5 Including leukopenia, *6 Including lymphopenia, *7 Including thrombocytopenia, *8 Including hypophosphataemia, *9 Including hypoalbuminaemia, *10 Including hypochloraemia, *11 Including hyponatraemia, *12 Including hypercholesterolaemia

Adverse events that were not reported in the foreign clinical studies combined and occurred at an incidence of ≥10% in Japanese Study JO18157 were neutrophil count increased (50.0%), red blood cell count decreased (38.9%), anal haemorrhage (16.7%), blood cholesterol decreased, blood uric acid decreased, blood urea decreased, and blood urea increased (11.1% each).

Among the above adverse events, events ≥Grade 3 occurring at a ≥5% higher incidence in Japanese Study JO18157 compared to the foreign studies combined were blood cholesterol increased (Japan: 5.6%, Overseas: 0%), blood phosphorus decreased (11.1%, 0%), lymphocyte count decreased (11.1%, 0%), and infection (5.6%, 0.4%).

Concerning the FOLFOX4+bevacizumab 5 mg/kg group, comparison was made between 33 patients in the FOLFOX4+bevacizumab 5 mg/kg group from Japanese Study JO18158 (data cutoff: [redacted] 20[redacted] [including reports before the data lock]) and 341 patients in the FOLFOX4+bevacizumab group from Foreign Study NO16966. Adverse events occurring at a ≥10% higher incidence in Japanese Study JO18158 compared to Foreign Study NO16966 are as follows.

Adverse event	Japanese study	Foreign study	Adverse event	Japanese study	Foreign study

Abdominal pain	39.4%	24.9%	INR increased	15.2%	1.2%
ALT increased	39.4%	1.2%	Lymphocyte count decreased *8	51.5%	0.3%
Alopecia	45.5%	15.5%	Malaise	24.2%	1.5%
Anorexia *1	87.9%	33.5%	Nasopharyngitis	27.3%	11.4%
AST increased	36.4%	1.5%	Nausea	78.8%	63.6%
Blood albumin decreased *2	27.3%	0.6%	Neuropathy peripheral	33.3%	17.9%
Blood ALP increased	21.2%	0.3%	Neurotoxicity	57.6%	4.4%
Blood bilirubin increased *3	30.3%	2.4%	Neutrophil count decreased *9	87.9%	56.6%
Blood cholesterol increased *4	36.4%	0.6%	Palmar-plantar erythrodysaesthesia syndrome	24.2%	13.5%
Blood Na decreased *5	21.2%	0.9%	Pigmentation disorder	39.4%	2.1%
Cheilitis *6	21.2%	3.6%	Platelet count decreased *10	45.5%	13.2%
Dehydration	15.2%	4.4%	Protein urine present *11	30.3%	6.5%
Dysgeusia	33.3%	14.4%	Toothache	12.1%	2.1%
Erythema	15.2%	4.1%	Weight decreased	33.3%	9.4%
Hb decreased	42.4%	1.8%	Weight increased	12.1%	2.1%
Hiccups	30.3%	6.2%	White blood cell count decreased *12	72.7%	7.6%
Hypertension *7	51.5%	20.9%			

*1 Including decreased appetite, *2 Including hypoalbuminaemia, *3 Including hyperbilirubinaemia, *4 Including hypercholesterolaemia, *5 Including hyponatraemia, *6 Including chapped lips and lip pain, *7 Including blood pressure increased, *8 Including lymphopenia, *9 Including neutropenia, *10 Including thrombocytopenia, *11 Including proteinuria, *12 Including leukopenia

Adverse events that were not reported in the FOLFOX4+bevacizumab group from Foreign Study NO16966 and occurred at an incidence of ≥10% in the FOLFOX4+bevacizumab 5 mg/kg group from Japanese Study JO18158 were red blood cell count decreased (30.3%), blood lactate dehydrogenase increased, haematocrit decreased (24.2% each), protein total decreased (21.2%), and CRP increased (15.2%).

Among the above adverse events, events ≥Grade 3 occurring at a ≥5% higher incidence in the FOLFOX4+bevacizumab 5 mg/kg group from Japanese Study JO18158 compared to the FOLFOX4+bevacizumab group from Foreign Study NO16966 were anorexia (Japan: 12.1%, overseas: 2.3%), Hb decreased (6.1%, 0.3%), lymphocyte count decreased (15.1%, 0%), neutrophil count decreased (69.7%, 41.1%), and white blood cell count decreased (21.2%, 0%).

Concerning the FOLFOX4+bevacizumab 10 mg/kg group, comparison was made between 13 patients in the FOLFOX4+bevacizumab 10 mg/kg group from Japanese Study JO18158 (data cutoff: [redacted] 20[redacted] [including reports before the data lock]) and 287 patients in the FOLFOX4+bevacizumab 10 mg/kg group from Foreign Study E3200. Adverse events occurring at a ≥10% higher incidence in Japanese Study JO18158 compared to Foreign Study E3200 are as follows.

Adverse event	Japanese study	Foreign study	Adverse event	Japanese study	Foreign study
Abdominal pain	23.1%	11.1%	Insomnia	15.4%	0.7%
Anorexia	53.8%	6.3%	Nausea	53.8%	16.4%
AST increased *[1]	30.8%	1.7%	Neutrophil count decreased *[6]	92.3%	24.4%
Blood albumin decreased *[2]	23.1%	1.7%	Malaise *[7]	38.5%	24.0%
Blood ALP increased *[3]	23.1%	2.1%	Pigmentation disorder	15.4%	0.7%
Constipation	53.8%	4.5%	Platelet count decreased *[8]	76.9%	9.4%
Epistaxis	30.8%	1.7%	Protein urine present *[9]	30.8%	18.5%
Headache	15.4%	4.2%	Pyrexia	15.4%	3.1%
Hb decreased *[4]	46.2%	6.6%	Stomatitis	46.2%	4.9%
Hiccups	15.4%	1.0%	Rash*[10]	23.1%	1.0%
Hypertension *[5]	30.8%	15.7%	White blood cell count decreased *[11]	84.6%	7.0%

*[1] Including AST, *[2] Including hypoalbuminaemia, *[3] Including blood ALP, *[4] Including Hb, *[5] Including blood pressure increased, *[6] Including neutropenia, *[7] Including fatigue, *[8] Including thrombocytopenia, *[9] Including proteinuria, *[10] Including exfoliative rash, *[11] Including white blood cell count

Adverse events that were not reported in the FOLFOX4+bevacizumab 10 mg/kg group from Foreign Study E3200 and occurred at an incidence of ≥10% in the FOLFOX4+bevacizumab 10 mg/kg group from Japanese Study JO18158 were neurotoxicity (61.5%), red blood cell count decreased, lymphocyte count decreased, blood cholesterol increased (46.2% each), alopecia (38.5%), haematocrit decreased, nasopharyngitis (30.8% each), blood lactate dehydrogenase increased, gingivitis, hypoaesthesia, and neuropathy peripheral (15.4% each).

Among the above adverse events, the incidences of events ≥Grade 3 were compared. As a result, events occurring at a ≥5% higher incidence in Japanese Study JO18158 compared to Foreign Study E3200 were AST increased (Japan: 7.7%, overseas: 0%), blood alkaline phosphatase increased (7.7%, 0%), lymphocyte count decreased (7.7%, 0%), neutrophil count decreased (84.6%, 18.1%), and white blood cell count decreased (38.5%, 0%).

Due to the small number of subjects in the Japanese clinical studies, it is difficult to identify adverse events that occur at a higher incidence in Japanese patients compared to foreign patients or occur uniquely in Japanese patients. But according to the above comparisons, adverse events occuring at a ≥10% higher incidence in the Japanese study than the foreign studies in both 5-FU/LV+bevacizumab and FOLFOX4+bevacizumab groups, or occurring at a ≥10% incidence only in the Japanese study in 5-FU/LV+bevacizumab and/or FOLFOX4+bevacizumab group were alopecia, anorexia, pigmentation disorder, blood albumin decreased, blood ALP increased, blood cholesterol increased, Hb decreased, lymphocyte count decreased, nasopharyngitis, neutrophil count decreased, platelet count decreased, white blood cell count decreased, haematocrit decreased, and red blood cell count decreased. Among the adverse events occurring at a ≥10% higher incidence in the Japanese clinical study compared to the foreign clinical studies, events ≥Grade 3 with a ≥5% higher incidence in Japan in any comparison were blood cholesterol increased, blood phosphorus decreased, lymphocyte count decreased, neutrophil count decreased, infection, anorexia, Hb decreased, white blood cell count decreased, AST increased, and blood ALP increased. Of the adverse events reported as those typically

associated with bevacizumab, haemorrhage (anal haemorrhage, epistaxis, post procedural haemorrhage), hypertension, and protein urine occurred at a higher incidence in the Japanese clinical studies compared to the foreign clinical studies. These adverse events may occur at a higher frequency in Japanese patients compared to foreign patients and need to be brought to attention. These events including the incidences outside Japan are incorporated in the proposed package insert, and a caution will also be included in information materials after market launch.

In the Review Report (1) "4.4 Adverse events reported in clinical studies," adverse events as of the data cutoff date: [redacted] are presented for the Japanese phase I study of bevacizumab in combination with 5-FU/*l*-LV (Study Number JO18157) while the adverse events ≥Grade 3 presented are those as of the data cutoff date: [redacted]. Adverse events including those ≥ Grade 3 reported in this study as of the data cutoff date: [redacted] are as follows.

All of the 18 patients enrolled into this study were included in the safety analysis and adverse events (MedDRA version 7.1) were reported by all of the 18 patients (378 events). Adverse events occurring in ≥30% (6/18 patients) of the patients were nausea in 13 patients (72.2%), anorexia in 12 patients (66.7%), diarrhoea in 11 patients (61.1%), vomiting, neutrophil count decreased, white blood cell count decreased (10 cases each: 55.6%), stomatitis, neutrophil count increased, epistaxis (9 cases each: 50.0%), haematocrit decreased, haemoglobin decreased, lymphocyte count decreased, weight decreased, white blood cell count increased, pigmentation disorder (8 cases each: 44.4%), platelet count decreased, red blood cell count decreased, protein urine present, hypertension (7 cases each: 38.9%), blood bilirubin increased, palmar-plantar erythrodysaesthesia syndrome, nasopharyngitis, and fatigue (6 cases each: 33.3%).