15 November 2012
EMA/755420/2012
Committee for Medicinal Products for Human Use (CHMP)

Assessment report

Zaltrap

International non-proprietary name: **aflibercept**

Procedure No. **EMEA/H/C/002532**

Note

Assessment report as adopted by the CHMP with all information of a commercially confidential nature deleted.

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Executive Summary

Colorectal cancer (CRC) is one of the most common cancers in both men and women, and the second most common cause of cancer mortality in Europe. Significant advances in the treatment of metastatic CRC have been made during the last 25 years with the introduction of chemotherapy agents. Current therapies used in clinical practice for first and second line treatment of metastatic CRC include irinotecan or oxaliplatin, each in combination with bolus and infusional 5FU/ LV. Standard second-line treatments for metastatic CRC have also evolved to include the addition of targeted biologic therapies such as bevacizumab, cetuximab and panitumumab. Despite these advances, the prognosis of patients with metastatic CRC undergoing second-line treatment is poor and the expected median overall survival is only approximately one year.

In November 2012, the European Medicines Agency's Committee for Medicinal Products for Human Use (CHMP) recommended the authorisation of aflibercept (Zaltrap) in combination with irinotecan/5-fluorouracil/folinic acid (FOLFIRI) chemotherapy in the treatment of adults with metastatic colorectal cancer (MCRC) that is resistant to or has progressed after an oxaliplatin-containing regimen. The recommended dose of aflibercept, administered as an intravenous infusion over 1 hour, is 4 mg/kg of body weight, followed by the FOLFIRI regimen. This is considered as one treatment cycle. The treatment cycle is repeated every 2 weeks.

Aflibercept is a recombinant human fusion protein that acts as a high-affinity soluble decoy receptor that can block the VEGF pathway by preferentially binding to vascular endothelial growth factor A (VEGF-A), VEGF-B and placenta growth factor (PlGF) and preventing these factors from activating their endogenous receptors. By blocking this pathway, aflibercept is believed to exert direct anti-cancer activity and to potentiate the anti-cancer activity of chemotherapy agents by preventing new tumour vessel growth, regressing existing tumour vessels, normalising vasculature, affecting tumour cell function, offsetting of effects of chemotherapy induction of VEGF levels and potentially inhibiting VEGF repression of dendritic cell function.

The demonstration of clinical benefit for aflibercept was based on a single randomised, double-blind controlled trial of aflibercept versus placebo in MCRC patients being treated with FOLFIRI after failure of an oxaliplatin based regimen (EFC10262- VELOUR). The primary efficacy analysis showed a small but clinically significant difference of 1.44 months in median overall survival between the study groups. In this trial, the risk of death associated with aflibercept was reduced by 18% compared to that observed in the control group (stratified Hazard Ratio 0.817, Confidence Interval: 0.713 to 0.937, p=0.0032). The median overall survival was 13.5 months in the aflibercept arm compared to 12.1 months in the placebo arm. Secondary efficacy endpoints were consistent with the effect observed in terms of overall survival. Aflibercept was associated with an improvement of 2.23 months in duration of median progression-free survival and of 9% in objective response rate (19.8% versus 11.1% for aflibercept and placebo, respectively).

The trial also included a subgroup of patients whose disease had progressed after treatment with bevacizumab. Bevacizumab has a mechanism of action similar to that of aflibercept. Because of the risk of cross-resistance between the two agents, a subgroup analysis was conducted to assess if an effect of aflibercept could be observed also in patients pre-treated with bevacizumab. In this subgroup analysis, a trend towards a favourable effect on overall survival was observed for aflibercept, but no definitive conclusions could be drawn.

Treatment-emergent adverse events led to permanent discontinuation of treatment in 26.8% of patients in the aflibercept arm compared to 12.1% of patients in the placebo arm, clearly reflecting the toxic potential of the study drug when combined with FOLFIRI. Furthermore, substantially more dose

modifications and premature discontinuation of all study drugs as well as cycle delays were seen in the experimental arm. However, overall exposure to background chemotherapy (irinotecan and 5-fluorouracil) on study was similar between the two treatment groups. Aflibercept was associated with anti-VEGF class side effects including hypertension, haemorrhage and fistulae. Addition of aflibercept also increased the frequency of adverse events associated with irinotecan and 5-FU, including diarrhoea, neutropenia, and stomatitis. Severe treatment-emergent adverse events occurred in 83.5% of patients in the aflibercept arm compared to 62.5% in the placebo arm. Severe events with a frequency ≥2% higher in the aflibercept arm included diarrhoea, hypertension, asthenic conditions, stomatitis and ulceration, and dehydration. Serious treatment-emergent adverse events (defined as events that are life-threatening, result in death, require in patient hospitalisation or prolong hospitalisation, result in persistent or significant disability, are congenital anomalies/birth defects or require intervention to prevent permanent impairment or damage) were reported in 48.1% of patients in the aflibercept arm compared to 32.7% in the placebo arm. The most common serious adverse events were gastrointestinal disorders (20% vs 11%) followed by infection and infestations (11.3% vs 6.3%). In patients ≥65 years the incidence of specific AEs, such as diarrhoea, dizziness, asthenia, weight decrease and dehydration was ≥5% higher than in the younger population.

In terms of balance of benefits and risks, the overall toxicity of aflibercept in the studied combination regimen was considered significant, not always manageable, and in some patients ultimately leading to termination also of the chemotherapy. However, despite this toxicity, there was still a small but clinically relevant survival advantage of 1.44 months (median). Thus, the benefits associated with aflibercept were considered to outweigh the risks.

In order to optimise benefit–risk balance, it is essential to identify the proper target population for therapy. This might be possible to accomplish through the judicious use of biomarkers in all phases of clinical drug development. However, no validated predictive serum or plasma biomarkers have been identified during the development of aflibercept that correlate with treatment outcomes. Thus, the CHMP has requested to the applicant company to analyse plasma and tissue samples from the available trials, with the primary aim to identify biomarkers to allow better selection of the population likely to experience a beneficial effect following treatment with aflibercept.

Finally, individual patient decisions should be based on clinical judgement and also take into account patient preferences.

Product information

Name of the medicinal product:	Zaltrap
Applicant:	sanofi-aventis groupe 54, rue La Boétie 75008 Paris France
Active substance:	aflibercept
International Nonproprietary Name:	aflibercept
Pharmaco-therapeutic group (ATC Code):	Other antineoplastic agents (ATC Code not yet assigned)
Therapeutic indication:	Zaltrap in combination with irinotecan/ 5-fluorouracil/ folinic acid (FOLFIRI) chemotherapy is indicated in adults with metastatic colorectal cancer (MCRC) that is resistant to or has progressed after an oxaliplatin-containing regimen
Pharmaceutical form:	Concentrate for solution for infusion
Strength:	25 mg/ml
Route of administration:	Intravenous use
Packaging:	vial (glass)
Package sizes:	1 vial with 100mg/4ml 1 vial with 200mg/8ml 3 vials with 100mg/4ml

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List of abbreviations

5-FU:	5-fluorouracil
ADA:	Anti aflibercept antibodies
AE:	Adverse Event
ALT:	Alanine amino transferase
AST:	Aspartate amino transferase
ATE:	Arterial thromboembolic events
AUC	Area under the curve
BMI	Body Mass Index
CHMP	Committee for Medicinal Products for Human Use
CI:	Confidence Interval
ClCr:	Creatinine clearance
DBP:	Diastolic blood pressure
DLT	Dose Limiting toxicity
DMC:	Data Monitoring Committee
EC	European Commission
ECOG PS:	Eastern cooperative oncology group performance status
e-CRF:	electronic-Case Report Form
EGFR:	Epidermal growth factor receptor
ELISA:	enzyme-linked immunosorbent assay
EMA	European Medicines Agency
EU	European Union
FDA:	Food and Drug Administration
GCP:	Good Clinical Practice
HLT:	High level term
HR:	Hazard ratio
HU	Hydroxyurea
HUS:	Hemolytic uremic syndrome
HUVEC:	Human umbilical vein endothelial cell
IA:	interim analysis
ICH:	International Conference for Harmonization
IRC:	Independent Review Committee, Independent review committee
ITT:	Intent-to-Treat
IV:	Intravenous
IVRS:	Interactive Voice Recognition System
KD:	Dissociation constant
LOQ:	Limit of Quantitation
LV:	Leucovorin
LV5FU2:	IV bolus 5-FU, 22-hour continuous infusion and leucovorin on Day 1 and Day 2
MCRC:	Metastatic colorectal cancer
MTD	Maximum tolerated dose
MW:	Molecular weight
Nab:	Anti-aflibercept neutralizing antibodies
NCI:	National Cancer Institute
OS	Overall Survival
PD:	pharmacodynamics
PFS:	Progression-free Survival

PK	Pharmacokinetics
PlGF:	placenta growth factor
PPE:	Palmar-Plantar Erythrodysaesthesia
PR:	partial response
PT:	Preferred term
RDI:	Relative Dose Intensity
RECIST:	Response evaluation criteria in solid tumours
RP2D:	Recommended Phase 2 Dose
RPLS:	Reversible posterior leukoencephalopathy syndrome
SAE:	Serious Adverse Event
SBP:	Systolic blood pressure
SC:	Subcutaneous
SD:	stable disease
SOC:	System organ class
TEAE:	Treatment-emergent adverse event
TMA:	Thrombotic microangiopathy
TMDD:	Target-mediated drug disposition
TTP:	Thrombotic thrombocytopenic purpura
ULN:	Upper limit of normal
VEGF:	vascular endothelial growth factor
VTE:	Venous thromboembolic event
WBC	White Blood Cell

1. Background information on the procedure

1.1. Submission of the dossier

The applicant Sanofi-aventis submitted on 24 November 2011 an application for Marketing Authorisation to the European Medicines Agency (EMA) for Zaltrap, through the centralised procedure falling within the Article 3(1) and point 1 of Annex of Regulation (EC) No 726/2004.

The applicant applied for the following indication: Zaltrap in combination with irinotecan-fluoropyrimidine-based chemotherapy is indicated in adults with metastatic colorectal cancer (MCRC) previously treated with an oxaliplatin-containing regimen.

The legal basis for this application refers to:

Article 8.3 of Directive 2001/83/EC - complete and independent application

The application submitted is composed of administrative information, complete quality data, non-clinical and clinical data based on applicants' own tests and studies and/or bibliographic literature substituting/supporting certain test(s) or study(ies).

Information on Paediatric requirements

Pursuant to Article 7 of Regulation (EC) No 1901/2006, the application included an EMA Decision P/345/2012 on the granting of a class waiver.

Information relating to orphan market exclusivity

Similarity

Pursuant to Article 8 of Regulation (EC) No. 141/2000 and Article 3 of Commission Regulation (EC) No 847/2000, the applicant did not submit a critical report addressing the possible similarity with authorised orphan medicinal products because there is no authorised orphan medicinal product for a condition related to the proposed indication.

New active Substance status

The applicant requested the active substance aflibercept contained in the above medicinal product to be considered as a new active substance in itself.

Scientific Advice

The applicant did not seek scientific advice at the CHMP.

Licensing status

Zaltrap has been given a Marketing Authorisation in the USA on 3 August 2012.

1.2. Steps taken for the assessment of the product

The Rapporteur and Co-Rapporteur appointed by the CHMP and the evaluation teams were:

Rapporteur: Kristina Dunder Co-Rapporteur: Daniela Melchiorri

- The application was received by the EMA on 24 November 2011.
- The procedure started on 21 December 2011.
- The Rapporteur's first Assessment Report was circulated to all CHMP members on 12 March 2012. The Co-Rapporteur's first Assessment Report was circulated to all CHMP members on 09 March 2012.
- During the meeting on 19 April 2012, the CHMP agreed on the consolidated List of Questions to be sent to the applicant. The final consolidated List of Questions was sent to the applicant on 23 April 2012.
- The applicant submitted the responses to the CHMP consolidated List of Questions on 18 July 2012.
- The Rapporteurs circulated the Joint Assessment Report on the applicant’s responses to the List of Questions to all CHMP members on 4 September 2012.
- During the CHMP meeting on 20 September 2012, the CHMP agreed on a list of outstanding issues to be addressed in writing and in an oral explanation by the applicant.
- The applicant submitted the responses to the CHMP List of Outstanding Issues on 12 October 2012.
- The Rapporteurs circulated the Joint Assessment Report on the applicant’s responses to the List of Outstanding Issues to all CHMP members on 30 October 2012.
- During the meeting on 12-15 November 2012, the CHMP, in the light of the overall data submitted and the scientific discussion within the Committee, issued a positive opinion for granting a Marketing Authorisation to Zaltrap on 15 November 2012.

2. Scientific discussion

2.1. Introduction

Colorectal cancer (CRC) is one of the most common cancers in both men and women, and the second most common cause of cancer mortality in Europe (Ferlay *et al,* 2010). In 2011 more than 1.2 million new cases, and more than 600.000 deaths was attributed to colorectal cancer worldwide (Jemal *et* al, 2011). In approximately 60% of CRC patients the initial diagnosis is carried out at late stages of disease which are characterised by a poor prognosis. In particular, the 5-year survival of metastatic colorectal cancer (MCRC) is around 12% (American Cancer Society, 2005).

Significant advances in the treatment of MCRC have been made in a step-wise way during the last 20-25 years, due to the introduction of active agents (5-FU, LV, irinotecan, oxaliplatin) and their use at different doses and schedule (ie, bolus and continuous infusion). Current therapies recognised in clinical practice as the standard of care for first and second line treatment of MCRC include irinotecan or oxaliplatin, each in combination with bolus and infusional 5FU/ LV (FOLFIRI and FOLFOX, respectively). No correlation between sequence of administration of the two regimens in first or second line and clinical efficacy have been reported (Tournigand *et* al, 2004). In current clinical practice, patients with MCRC who have received first-line oxaliplatin-based chemotherapy (ie, oxaliplatin/5-FU/leucovorin [LV]) typically receive second-line treatment with irinotecan-based chemotherapy.

Current standard second-line treatments for MCRC have also evolved to include the addition of targeted biologic therapies to the combination of 5-FU/LV with irinotecan. Targets for biologic therapies include VEGF and EGFR. MCRC is one of the first malignancies in which a clear benefit was demonstrated with an anti-VEGF treatment combined with chemotherapy in randomised clinical studies. Bevacizumab is a recombinant humanised monoclonal antibody that inhibits angiogenesis through binding to VEGF. Cetuximab and panitumumab are anti-epidermal growth factor receptor (EGFR) monoclonal antibodies approved for use in patients with MCRC whose tumours harbour wild-type KRAS.

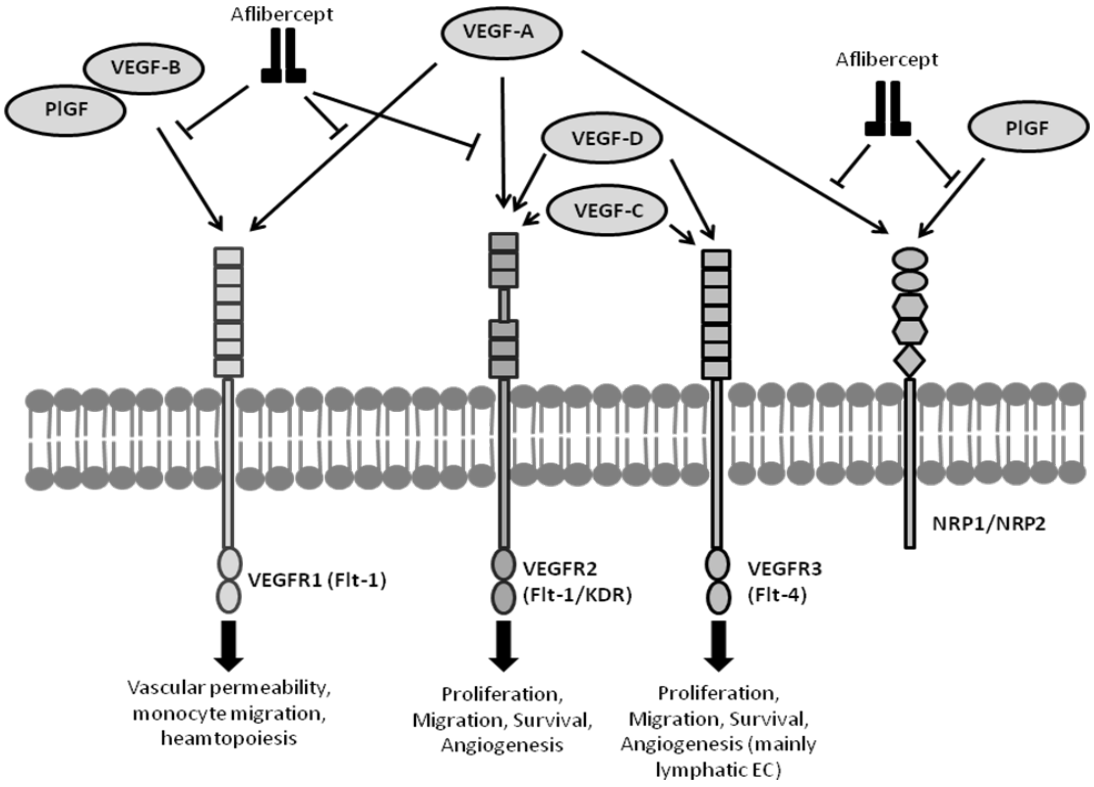
Aflibercept is a recombinant human fusion protein, composed of domain 2 from VEGFR-1 fused to domain 3 from VEGFR-2 attached to the hinge region of the Fc(a) domain of human immunoglobulin, acting as a high-affinity soluble decoy receptor that can block the VEGF pathway by preferentially binding to VEGF-A, VEGF-B and PlGF and preventing these factors from activating their endogenous receptors. It exhibits high binding affinity for the VEGF-A isoforms VEGF165 and VEGF121, VEGF-B, PlGF-1, and PIGF-2 but not for VEGF-C or VEGF-D.

VEGF-A is a major mediator of tumour angiogenesis through its effects on endothelial cell survival, migration and proliferation. Initially discovered as a vascular permeability factor, VEGF-A also decreases barrier function of the endothelium and may be a cause of the increased extravasation of macromolecules from tumour-associated vessels. Other members of the VEGF gene family include VEGF-B, VEGF-C, VEGF-D, VEGF-E and PlGF. Various VEGF family members are processed as different splice isoforms that differ in molecular weight and degree of binding to the extracellular matrix. For example, tumour-derived VEGFA generally occurs as VEGF121 and VEGF165 isoforms.

VEGF-A binds to two receptor tyrosine kinases, VEGFR-1 and VEGFR-2, to promote angiogenesis. The receptors VEGFR-1 and VEGFR-2 are found predominantly on the surface of vascular endothelial cells and, upon binding to VEGF-A, become phosphorylated and activate intracellular signals to promote cell survival, migration and proliferation. VEGFR-1 is also expressed by some leukocytes. PlGF binds only to VEGFR-1 and NRP1/NRP2, and may stimulate vessel formation directly by acting on endothelial cells,

or indirectly by recruiting leukocytes and endothelial progenitor cells. A third receptor VEGFR-3, which binds VEGF-C and VEGF-D, is mainly involved in the regulation of lymphatic vessels (Figure 1).

Figure 1: The VEGF pathway



As a VEGFR binding antagonist, aflibercept is believed to exert direct anti-cancer activity and to potentiate the anti-cancer activity of chemotherapy agents through a variety of modes of actions. These include prevention of new tumour vessel growth, regression of existing tumour vessels, vascular normalisation, direct effects on tumour cell function, offsetting of effects of chemotherapy induction of VEGF levels, and inhibition of VEGF repression of dendritic cell function.

The applicant applied for the following indication: Zaltrap in combination with irinotecan-fluoropyrimidine-based chemotherapy is indicated in adults with metastatic colorectal cancer (MCRC) previously treated with an oxaliplatin-containing regimen.

The finally approved indication is: Zaltrap in combination with irinotecan/5-fluorouracil/folinic acid (FOLFIRI) chemotherapy is indicated in adults with metastatic colorectal cancer (MCRC) that is resistant to or has progressed after an oxaliplatin-containing regimen.

The recommended dose of Zaltrap, administered as an intravenous infusion over 1 hour, is 4 mg/kg of body weight, followed by the FOLFIRI regimen. This is considered as one treatment cycle. The treatment cycle is repeated every 2 weeks.

2.2. Quality aspects

2.2.1. Introduction

Aflibercept (VEGF Trap) is a fusion protein synthetised by Chinese Hamster Ovary (CHO) cells as a dimeric, secreted, soluble glycoprotein. Aflibercept has a protein molecular weight of 97 kDa and contains glycosylation, constituting an additional 15% of the total molecular mass, resulting in a total molecular weight of 115 kDa. Aflibercept is composed of domain 2 from VEGFR-1 (VEGF Receptor 1)

(Flt-1) fused to domain 3 from VEGFR-2 (VEGF Receptor 2) (KDR, Flk-1), which is then fused to the hinge region of the Fc (a) domain of human immunoglobulin G1.

Aflibercept drug product is packaged as a concentrate for solution for infusion at 25 mg/mL. Aflibercept drug product is a sterile, clear, colourless to pale yellow, non-pyrogenic, preservative free solution, and is supplied as 100 mg and 200 mg single-use vials delivering 4 mL and 8 mL of 25 mg/mL aflibercept solution, respectively.

Aflibercept drug product has been formulated specifically for intravenous (IV) administration.

2.2.2. Active Substance

Zaltrap is formulated from aflibercept drug substance.

The production process is typical for a recombinant-Fc fusion protein. The upstream process includes expansion of the CHO host cells and expression of recombinant aflibercept. The downstream process involves clarification and purification of the protein from the culture medium.

Aflibercept drug substance is a recombinant human soluble fusion protein designed to provide pharmacological blockade of the vascular endothelial growth factor (VEGF) pathway through high affinity, specific binding to the VEGF ligand family members, VEGF-A, placenta growth factor (PlGF), and VEGF-B.

Aflibercept is a dimeric glycoprotein with a protein molecular weight of 97 kDa and contains glycosylation, constituting an additional 15% of the total molecular mass, resulting in a total molecular weight of 115 kDa.

Manufacture

Manufacturer

The drug substance is manufactured and released by Regeneron Pharmaceuticals Inc., New-York, USA.

Description of manufacturing process and process controls

The manufacturing process is initiated with the thawing and inoculation of one working cell bank (WCB) vial. The cell culture is expanded until reaching sufficient density for inoculation into the production bioreactor.

The downstream process consists of several chromatography steps (protein A affinity chromatography, Cation exchange chromatography, Anion exchange chromatography and Hydrophobic Interaction chromatography), and includes viral inactivation and filtration steps to clear potential adventitious viral agents. After processing through the step of concentration/diafiltration, the drug substance is filled into containers and stored frozen.

No reprocessing is claimed for the standard manufacturing of the product.

The drug substance manufacturing process was generally well described. In general, the in process control system is appropriate, including adequate tests to control consistency in product as well as to prevent or reduce bioburden, mycoplasma and adventitious viruses. The limits set for control of process parameters were satisfactorily justified.

The construction of the expression vector and the establishment of the production cell line were appropriately described. The characterisation of the MCB, WCBs and EPCBs were performed in accordance with relevant guidelines. The specifications for control of new WCBs were considered

acceptable. Limits are in place to control the maximum time/population doubling level in the production of new WCBs.

Process Validation

The studies reported from the validation of production in the commercial scale were considered acceptable, supporting consistent production.

Manufacturing process development

The manufacturing process evolved over the course of development through three process changes where the main objectives of the process modifications were to increase the scale and improve productivity and process yields. Comparability of product derived from the different production processes used in development has been demonstrated.

Specification

Characterisation

Aflibercept has been extensively characterised for elucidation of the primary, secondary and tertiary structure. Characterisation studies confirmed the homodimeric structure of the protein, its amino acid sequence, the C-terminal sequence, the molecular weight, the expected pattern of disulphide bonds, the carbohydrate profile including the content in sialic acid, the quantitative monosaccharide composition, the profiling of oligosaccharide structures and the presence of N-linked carbohydrates.

Adequate information was provided as regards product-related impurities (molecular variants of aflibercept resulting from various types of degradation). Process related impurities are reduced to an acceptable level during the manufacturing process.

Control of Drug Substance

The tests selected for control of drug substance specifications were overall considered appropriate.

Host cell proteins, DNA, Protein A, bioburden and endotoxins are routinely tested at release.

The purity is tested by SDS-PAGE and Size Exclusion HPLC, while charge heterogeneity is monitored by Isoelectric Focusing. Deamidation is also monitored on a routine basis by enzyme-linked detection of isoaspartate with reversed-phase HPLC.

A cell based bioassay and a binding assay were designed to evaluate potency.

The analytical procedures used to control the quality of the drug substance were appropriately validated.

Container closure system

Two container closure systems can alternatively be used for storage of the drug substance: polyethylene vinyl acetate bags and polycarbonate bottles.

Stability

Real-time stability data up to 24 months are available for three conformance batches showing that for most parameters the drug substance remains stable over the proposed storage time of 24 months and under the proposed conditions at -20°C protected from light.

2.2.3. Finished Medicinal Product

Aflibercept drug product is manufactured by formulation of aflibercept drug substance with sucrose and polysorbate 20 (stabilisers), sodium chloride, citrate buffer, and sodium phosphate buffer, adjusted to final pH. Aflibercept (VEGF Trap, AVE0005) Drug Product is supplied in two drug product presentations:

- a presentation at 100 mg / 4.0 mL (nominal concentration).

- a second presentation at 200 mg / 8.0 mL (nominal concentration).

Both presentations are manufactured from the same bulk sterile solution at 25 mg/mL of aflibercept.

Prior to infusion to the patient, the concentrate solution is diluted with 0.9% sodium chloride solution or 5% dextrose.

Manufacture of the product

Description of manufacturing process and process controls

The manufacturing process for the drug product is straightforward, including conventional steps for thawing, dissolving, mixing, pH-adjusting, sterile filtering and filling of product.

The manufacturing processes for each presentation are generally well described, appropriately controlled and acceptably validated, including the bioburden reduction filtration, hold times, aseptic filling, container closure integrity and shipping.

An acceptable review is provided of the studies conducted in development of drug product.

In general, the in process control system is appropriate, including adequate tests to control consistency in product.

Process Validation

Validation of the commercial process has been satisfactorily conducted. The proposed procedures for validation of reprocessing under the defined conditions are considered acceptable. Studies supporting shipping of product are acceptable.

Product specification

Control of drug product

The tests identified for control by drug product specifications were in general considered appropriate.

Testing for sterility and endotoxin content is routinely performed at release.

As for the drug substance, the purity is tested by SDS-PAGE and Size Exclusion HPLC, while charge heterogeneity and deamidation are monitored by Isoelectric Focusing and enzyme-linked detection of isoaspartate with reversed-phase HPLC, respectively.

The potency is determined using a cell-based bioassay and a binding assay.

The analytical procedures used to control the drug product quality were appropriately validated.

Container closure system

The drug product is filled into 5 mL or 10 mL, Type 1, clear borosilicate glass vial and capped with a flanged cap with tear-off lid and inserted rubber sealing disc, Flurotec® coated.

Stability of the product

The recommended storage conditions for aflibercept concentrate for solution for infusion at 100 mg/4 mL and 200 mg/8 mL are based on primary stability results on three batches of aflibercept concentrate at 100 mg/4 mL and three batches at 200 mg/8 mL.

These batches were manufactured at ¾ of the full scale with the final drug product process from full-scale batches of drug substance final process packed in EVA bags. Batches were packaged in the commercial packaging. 36-Month results under long-term conditions (5°C ± 3°C) are available on these batches.

Comprehensive data were reported and in general, the results support the product shelf-life of 36 months and storage conditions (2-8°C protected from light).

Adventitious agents

The overall viral safety of Zaltrap is considered satisfactory. The manufacturing process does not directly use any materials of biological origin. Cell banks were extensively controlled and no viral contaminants other than retroviral-like particles normally seen in CHO cells were observed.

Global reduction factors reported in viral clearance studies were satisfactory for both enveloped viruses as well as for non-enveloped viruses.

2.2.4. Discussion on chemical, pharmaceutical and biological aspects

No major objections were raised during the assessment of the quality part of the dossier.

The Applicant has responded satisfactorily to all of the other quality concerns and questions identified in the Day 120 List of Questions and in the Day 180 List of Outstanding Issues.

Concerning the specifications for the drug substance, the proposed limits were generally set too wide and, based on manufacturing history and clinical experience, most specifications were tightened by the Applicant. Likewise, the proposed limits for the drug product were generally considered too wide and were re-evaluated during the course of the procedure. Most acceptance limits were subsequently tightened.

The absence of test to control oxidation in the specifications for the drug substance and the choice of the ligand in the cell based bioassay were discussed and justified by the Applicant during the course of the evaluation.

The Western blot method, performed as identity test for the release of the drug substance and drug product, displayed a non specific background binding with the products tested other than Aflibercept. The Applicant committed to improve the assay by further reducing the non specific background.

A slightly deviant trend observed during stability studies between the drug substance stored in bags and that stored in bottles was discussed with the Applicant. Adequate justification to support storage in both types of containers was provided. Furthermore, satisfactory data was provided to support storage under the recommended conditions, showing that no trend can be identified for the drug substance stored at -20°C.

The Applicant confirmed during the procedure its manufacturing practice to aseptically fill the bulk sterile solution as soon as possible after sterile filtration and the maximum hold time between filtration and filling was justified. The Applicant committed to introduce a point-of-fill filtration in the production of the drug product.

In conclusion, information on development, manufacture and control of the drug substance and drug product has been presented in a satisfactory manner. The results of tests carried out indicate satisfactory consistency and uniformity of important product quality characteristics, and these in turn lead to the conclusion that the product should have a satisfactory and uniform performance in the clinic.

2.2.5. Conclusions on the chemical, pharmaceutical and biological aspects

Based on the review of the data on quality, the manufacture and control of the aflibercept drug substance and the Zaltrap drug product are considered acceptable.

The Quality of the product is considered to be acceptable when used in accordance with the conditions defined in the SPC. Physicochemical and biological aspects relevant to the uniform clinical performance of the product have been investigated and are controlled in satisfactory way.

Data has been presented to give reassurance on viral/TSE safety.

2.2.6. Recommendations for future quality development

In the context of the obligation of the MAHs to take due account of technical and scientific progress, the CHMP recommends the following points for investigation:

- *Drug Substance*: The Applicant is recommended to improve the method used for control of identity of the active substance, by further reducing the non-specific background of the assay.
- *Drug Product*: The Applicant is recommended to perform studies for introduction on a point-of-fill filtration in the production of finished product.

2.3. Non-clinical aspects

2.3.1. Introduction

In vivo pharmacodynamic studies were conducted in immunocompromised mice. Pharmacokinetic (PK) and toxicokinetic (TK) studies were conducted in mice, rats, and cynomolgus monkeys. Single-dose toxicity studies were conducted in rats and repeat-dose toxicity studies were conducted in mice, rats and cynomolgus monkeys. An embryofoetal development study and a local tolerance study were conducted in rabbits. Safety pharmacology was investigated as part of the general repeat-dose toxicology studies in mice, rats, rabbits and monkeys. With the exception of studies to evaluate the effects of aflibercept on cardiovascular and renal systems in mice, all safety pharmacology studies were claimed to have been conducted in compliance with Good Laboratory Practices (GLP). Repeat-dose toxicity studies in rats and monkeys, one of the two single-dose toxicity studies in rats, one rabbit embryofoetal development study, the rabbit local tolerance study and the human tissue cross reactivity study were also claimed to have been conducted in accordance with GLP.

2.3.2. Pharmacology

Primary pharmacodynamic studies

The equilibrium dissociation constants, KD for the interaction of aflibercept to nine VEGF family related ligands from human (monkey), mouse, rat and rabbit were determined by surface plasmon resonance technology (Table 1).

Table 1: Binding Parameters for the Interaction of VEGF Trap to VEGF Family Related Ligands (Study IVT0044)

Ligand	ka ($M^{-1}s^{-1}$)	kd (s^{-1})	K_D (pM)
Human VEGF-A_{165}	$4.05x10^{7}$	$2.01x10^{-5}$	0.497
Human VEGF-A_{121}	$3.75x10^{7}$	$1.35x10^{-5}$	0.360
Human VEGF-B_{108}	$3.52x10^{7}$	$6.74x10^{-5}$	1.92
Human PlGF-2	$1.75x10^{6}$	$6.81x10^{-5}$	38.8
Human PlGF-1	$6.73\ x10^{6}$	$2.64x10^{-3}$	392.0
Murine VEGF-A_{164}	$2.80x10^{7}$	$1.64x10^{-5}$	0.585
Murine VEGF-A_{120}	$2.15x10^{7}$	$1.23\ x10^{-5}$	0.571
Murine PlGF-2	$1.64x10^{7}$	$5.45x10^{-5}$	3.33
Rat VEGF-A_{164}	$3.67x10^{7}$	$1.73x10^{-5}$	0.471
Rabbit VEGF -A_{165}	$3.39x10^{7}$	$2.63\ x10^{-5}$	0.775
Human VEGF-C	NB	NB	NB
Human VEGF-D	NB	NB	NB

Abbreviations used: ka = Association rate constant, kd = Dissociation rate constant, K_D = Equilibrium dissociation constant, NB=No binding detected

Human umbilical vein endothelial cells (HUVEC), which express VEGF receptors can be cultured in vitro and their activation can be induced by exogenously added VEGF. The HUVECs were used to assess the ability of aflibercept to block VEGF-A165 dependent VEGFR-2 phosphorylation (Table 2).

Table 2: In vitro activities of aflibercept

Cellular assay	VEGF Trap activity
VEGF-A_{165} (1 nM) dependent VEGFR-2 phosphorylation (Human umbilical vein endothelial cells (HUVEC) used to assess the ability of VEGF Trap to block VEGF-A_{165} dependent VEGFR-2 phosphorylation), Study IVT0043 and HVT0136	Complete inhibition at an equimolar concentration of VEGF Trap (1 nM). In a separate experiment, both VEGF-A and VEGF-C stimulated the phosphorylation of VEGFR-2 but aflibercept effectively inhibited VEGFR-2 phosphorylation only when it was induced by VEGF-A (IC50 of 3.15 nM) and not when it was induced by VEGF-C.
$VEGF_{165}$ (50 pM) induced calcium mobilization, Study IVT0043	Inhibition IC_{50} = 1.2-1.7 nM
Suppression of VEGF-driven endothelial cell proliferation evaluated in primary human dermal microvascular endothelial cells, Study IVT 0042	Aflibercept potently inhibited VEGF-induced HDMEC proliferation, with an IC50 of 192 pM.
Inhibition of angiogenesis in ex vivo cultured rat aortas, Study 1104	Aflibercept inhibited the outgrowth of microvessels from rat aorta with an IC50 of 121 pM in presence of exogenous VEGF-A (10 ng/mL, 260 pM), and an IC50 of 42 pM in the absence of exogenous factors.

In vivo activity studies are summarised in the following Table 3.

Table 3: *In vivo* activity studies

Type of study, study number	Test system, dose schedule, no of animals per grp and gender	Noteworthy Findings
Effects on tumour blood vessel density, IVV0066	SCID mice, 25 mg/kg, sc, 3-10 animals per group, males	Reduction of vessel density in C6 glioma (80%), U87 glioblastoma (57%) and 786-0 renal cell carcinoma (60%)
Levels of VEGF-aflibercept complex, the levels of unbound aflibercept (free aflibercept) circulating in the blood and the effects of aflibercept on the tumour burden, IVV0064, VGT-NC-004, IVV0065	SCID mice, 0.5, 1, 2.5, 10, 25 mg/kg, administered a multiple occasions after tumour implantation, sc and iv administration, 3-6 animals per group, males	These studies showed that aflibercept efficiently captures and forms complexes with endogenous and tumour-derived VEGF. When aflibercept was administered at active doses that resulted in tumour

		growth inhibition, the concentration of free aflibercept greatly exceeded the concentrations of either the mouse or human VEGF/aflibercept complexes.
Human MKN-45 gastric adenocarcinomas subcutaneous xenografts (advanced stage), IVV0051	SCID mice, 2.5, 10 and 40 mg/kg sc twice a week for two weeks starting on day 8, 5-10 animals per group, females	Active from 2.5 to 40 mg/kg (1.1 to 2.8 log cell kill)
Human Hs746T gastric adenocarcinomas subcutaneous xenografts (advanced stage), IVV0051	SCID mice, 2.5, 10 and 40 mg/kg sc twice a week for two weeks starting on day 10, 5-10 animals per group, females	Active at 10 and 40 mg/kg (1.3 and 1.1 log cell kill) Inactive 2.5 mg/kg (0.5 log cell kill)
Human SNU-5 gastric adenocarcinomas subcutaneous xenografts (advanced stage), IVV0051	SCID mice, 2.5, 10 and 40 mg/kg sc twice a week for two weeks starting on day 10, 5-10 animals per group, females	Active from 2.5 to 40 mg/kg (1.4 to 2.5 log cell kill)
Murine C51 colon adenocarcinomas subcutaneous xenografts (early stage), IVV0051	SCID mice, 2.5, 10 and 40 mg/kg sc twice a week for three weeks starting on day 3, 5-10 animals per group, females	Active from 2.5 to 40 mg/kg (1.1 to 4.2 log cell kill)
Murine C51 colon adenocarcinomas subcutaneous xenografts (advanced stage), IVV0051	SCID mice, 2.5, 10, 25 and 40 mg/kg, sc on day 9 and 14, 5-10 animals per group, females	Active at 25 and 40 mg/kg (1.2 and 2.0 log cell kill). Inactive at 2.5 and 10 mg/kg (0.2 and 0.1 log cell kill)
Human HT-29 colon adenocarcinomas subcutaneous xenografts (advanced stage), IVV0051	SCID mice, 2.5, 10 and 40 mg/kg sc twice a week for three weeks starting on day 10, 5-10 animals per group, females	Active from 2.5 to 40 mg/kg (0.7 to 2.2 log cell kill)
Human COLO 205 colon carcinoma subcutaneous xenografts (advanced stage), IVV0080	ICR SCID mice, 10, 25 and 40 mg/kg, sc twice a week for two weeks starting on day 13, 8 mice per group, females	Active from 10 to 40 mg/kg (0.9 to 1.6 log cell kill)
Human HCT 116 colon adenocarcinomas subcutaneous xenografts (early stage), IVV0051	Swiss nude mice, 2.5, 10, 25 and 40 mg/kg, sc twice a week for two weeks starting on day 4, 5-10 mice per group, females	Active from 2.5 to 40 mg/kg (1.3 to 2.5 log cell kill)
Human HCT 116 colon adenocarcinomas subcutaneous xenografts (advanced stage)	ICR SCID mice, 10, 25 and 40 mg/kg, sc twice a week for three weeks starting on day 11, 8-10 mice per group, females	Active from 10 to 40 mg/kg (0.9 to 1.6 log cell kill)

log cell kill= tumour growth delay/3.32 x tumour doubling time, HDT: highest dose tested, HNTD: highest non-toxic dose

Secondary pharmacodynamic studies

No secondary pharmacology studies were submitted (see discussion on non-clinical aspects).

Safety pharmacology programme

No specific *in vivo* safety pharmacology studies were conducted but the potential undesirable pharmacodynamic effects of aflibercept on physiological functions were investigated as part of the general repeat-dose toxicology studies in mice, rats, rabbits or monkeys.

No significant direct treatment-related effects that could be related to an impairment of the central nervous system were observed in cynomolgus monkeys dosed subcutaneously at 1.5, 5, 15, and 30 mg/kg/adm twice a week for 3 months, intravenously at 2, 10, and 30 mg/kg/adm once a week for 1 month, intravenously at 3, 10, and 30 mg/kg/adm once a week for 3 months, or intravenously at 3, 10, and 30 mg/kg/adm once a week for 15 weeks and then once every other week up to Week 27.

No significant treatment-related effects on ECG parameters were observed in cynomolgus monkeys dosed SC at 1.5, 5, 15, and 30 mg/kg twice a week for 3 months, IV at 2, 10, and 30 mg/kg/ adm once a week for 1 month, IV at 3, 10, and 30 mg/kg once a week for 3 months, or IV at 3, 10, and 30 mg/kg once a week for 15 weeks and then once every other week up to Week 27.

SC administrations of aflibercept (12.5 or 25 mg/kg for 2 weeks) resulted in statistically significant reductions in microvessel density in the following normal tissues: liver, pancreatic islets and thyroid follicles at all doses and time points evaluated. Less consistent and less marked decreases in microvessel density were also noted in the anterior or posterior pituitary gland and adipose tissue. No decreases in microvessel density were detected in the adrenal gland (medulla and cortex), duodenum, exocrine pancreas or retina at any dose level or time point.

The potential effects of aflibercept on blood pressure were evaluated after a single subcutaneous administration at doses of 0, 2.5 and 25 mg/kg in telemetered C57BL/6 mice and at doses of 0, 0.05, 0.15, 0.5, 1, 2.5, 5, 10 and 25 mg/kg in telemetered Wistar-Kyoto rats (3 to 9 animals/group). Blood pressure and/or heart rate were recorded from at least 48 hours before treatment up to 3 to 4 weeks after treatment.

Aflibercept induced a moderate, sustained increase in blood pressure in rats and mice at doses (2.5 mg/kg and 0.5 mg/kg and above, respectively) lower than the active dose in pharmacological models. The duration of this increase in blood pressure was dose-related, and maximal changes in blood pressure were observed only at doses ≥10 mg/kg in rats, and ≥2.5 mg/kg in mice. The duration of blood pressure elevation was correlated with the presence of free aflibercept in the circulation, such that systolic and diastolic blood pressure remained elevated above pre-treatment baseline values until circulating aflibercept levels fell below approximately 1 µg/mL.

Multiple classes of anti-hypertensives were found to be effective in lowering blood pressure in rats treated with aflibercept. Angiotensin converting enzyme inhibition (Captopril, Ramipril), calcium modulators (Nifedipine and Hydralazine), alpha adrenergic receptor antagonism (Prazosin), and modulation of nitric oxide availability (Molsidomine) effectively reversed aflibercept-induced hypertension.

The potential effects of aflibercept on venous and arterial thrombus formation were evaluated in the New Zealand White rabbit electrolytic injury model. Intravenous administration of aflibercept at dose levels of 0.3, 3.0, and 30 mg/kg/adm did not affect venous and arterial thrombus formation as assessed by activated clotting times, haematology and coagulation parameters, blood pressure, heart rate, blood flows (both descending aorta and right jugular vein), time elapsed between electrical current initiation and thrombotic occlusion or vessel weights (with associated thrombus when present) in an electrolytic injury model in the rabbit.

The effects of aflibercept on the respiratory parameters were evaluated in the conscious, unrestrained Sprague Dawley rat (8 males/group), using whole body plethysmography, after a single 30-minute intravenous infusion at doses of 10, 50 and 150 mg/kg. Aflibercept did not induce any biologically relevant effects on the respiratory parameters regardless of the time of measurement on Day 1 after treatment and on Day 7.

The subcutaneous administration of aflibercept at 25 mg/kg/adm twice a week for 4 weeks to normal, adult C57BL/6 male mice (6 animals/group) did not induce any biologically relevant effects on renal function.

The effects of aflibercept on wound repair and healing were evaluated in a New Zealand White rabbit incisional wound healing model. Aflibercept was administered as a 30-minute intravenous infusion on Days -2, 3, 7 and 11; Day 1 corresponded to model induction. Wound tensile strength was reduced on Days 4, 8 and 12 at all doses, relative to a negative control group. The effects of aflibercept on wound

repair and healing were also evaluated in a New Zealand White rabbit excisional wound healing model. Aflibercept was administered as a 30-minute intravenous infusion on Days -2, 5, 11 and 17; Day 1 corresponded to model induction. Administration of aflibercept at 0.3, 3 and 30 mg/kg produced dose related effects on excisional wound healing and related parameters. At 0.3 mg/kg, impairment of wound repair and healing was noted mainly on day 8 characterised by a reduced fibrous response and neovascularisation relative to controls. In contrast, at the 3 and 30 mg/kg doses, neovascularisation was nearly undetectable at all time points evaluated (days 8, 14 and 20), and fibrous responses and epidermal hyperplasia were also markedly reduced. Consequently, IV administration of aflibercept at 3 and 30 mg/kg/adm resulted in larger wound areas on days 14 and 20, compared to controls, and an increased incidence of open wounds on day 20.

Finally, in order to examine any role that Fc-mediated effector function may play in aflibercept activity, several ex vivo cell-based assays were developed to measure complement-dependent cytotoxicity (CDC) and antibody-dependent cell-mediated cytotoxicity (ADCC). Aflibercept was not able to mediate ADCC activity in either primary human umbilical vein endothelial cells or tumour cell lines. Similarly, aflibercept was unable to mediate CDC activity in either primary HUVEC or tumour cell lines in contrast to the positive control in the assay.

Pharmacodynamic drug interactions

Pharmacodynamic interaction studies with irinotecan and 5-FU were submitted together with primary pharmacology studies and these are summarised in the following Table 4.

Table 4: Pharmacodynamic interaction studies

Type of study, study number	Test system, dose schedule, no of animals per grp and gender	Noteworthy Findings
Combination treatment of aflibercept and irinotecan in mice bearing subcutaneous colon carcinoma HCT 116, IVV0043	NCR nude mice, 2.5 to 40 mg/kg, sc, on days 12, 15 and 18, and/or irinotecan, iv, from 12.5 to 52.4 mg/kg, 8-10 mice per group, females	Synergistic combination (3.0 log cell kill for the HNTD of the combination versus 1.8 log cell kill for the HNTD of irinotecan and 1.7 log cell kill for the HDT of aflibercept)
Combination treatment of aflibercept and 5-fluorouracil in mice bearing subcutaneous mammary adenocarcinomas MA13/C, 1119	Swiss nude mice, 2.5-40 mg/kg, sc, twice a week for three weeks and/or 5-FU, iv, from 34.6 to 145 mg/kg, once a week for three weeks, 5-10 mice per group, females	Synergistic combination (2.7 log cell kill for the HDT of the combination versus 1.4 log cell kill for the HDT of aflibercept and 1.3 log cell kill for the HNTD of 5-FU)

2.3.3. Pharmacokinetics

The pharmacokinetic (PK) profiles of free aflibercept were determined following single dose IV and SC administration to mice, rats and cynomolgus monkeys, to support regimens used in non clinical toxicology and efficacy studies as well as the proposed clinical route of administration (IV). Aflibercept exposures determined in the pivotal toxicology studies are also described.

In mice following IV administration of 1 mg/kg, free aflibercept displayed a multicompartmental serum PK profile. The clearance was slow, t1/2 was prolonged and distribution was slightly larger than the central compartment. Free aflibercept serum concentrations were still detectable at 7 days after dosing. Bound aflibercept exposure (Cmax and AUC) increased in a linear manner with dose between 0.5 and 2.5 mg/kg and then rapidly approached a plateau with further increases in dose. Free aflibercept exposures increased in a nonlinear manner with doses between 0.5 and 2.5 mg/kg, becoming approximately linear with doses greater than 2.5 mg/kg.

In rat after IV administration of 1 mg/kg, free aflibercept displayed a multi-compartmental serum profile. The clearance was slow, t1/2 was prolonged and the steady state volume of distribution (Vss)

was slightly larger than the central compartment. Free aflibercept serum concentrations were still detectable at 7 days after dosing. The mean free aflibercept t1/2 was similar for IV and SC administration and bioavailability following SC dosing was 33%.

In monkey after a single 5 mg/kg IV administration, aflibercept displayed a multicompartmental serum profile. Clearance was slow, the t1/2 was prolonged, and distribution was slightly larger than the central compartment. Free aflibercept serum concentrations from all dose groups were still detectable in all monkeys 14 days after dosing.

Following a single 1 mg/kg IV dose of [125I]-aflibercept to female rats, approximately 75% of the total dose of radioactivity was found in the serum at 5 minutes post-dosing; at 24 hours post-dosing, this amount had declined to 12.3% of the dose and by 168 hours post-dosing, only 0.76% of the total radioactive dose remained in the serum. The highest tissue levels of radioactivity were found in the organs of clearance and other highly perfused tissues (11.4%, 1.33%, 0.42%, 0.34%, and 0.19% of the total dose of radioactivity was localised in the liver, kidney, spleen, lung and heart, respectively).

No formal metabolism studies were submitted (see discussion on non-clinical aspects). Following a single SC dose in mice, rats or cynomolgus monkeys, free aflibercept displayed nonlinear pharmacokinetics. Free aflibercept clearance ranged between 0.5 and 3.5 mL/h/kg. Elimination half-life ranged from 2 days in the mouse to 5 days in the monkey. At higher concentrations, in the absence of free aflibercept in circulation, the elimination half-life of bound aflibercept was about 7 days in mice, i.e., much longer than that of free aflibercept (around 1 to 2 days). In monkey repeat dose toxicology studies, bound aflibercept was still present in plasma 3 months after the last dose. By comparing bound aflibercept concentrations at steady-state and 3 months following the last dose in the 13-week toxicity study in monkeys, the elimination half-life of bound aflibercept was roughly estimated to be approximately 20 days.

To determine the potential for renal excretion of aflibercept, single 1 mg/kg IV doses were administered to functionally-nephrectomised and sham-operated female rats. Comparison of the key indices of free aflibercept exposure (Cmax, Tmax, AUC, and t1/2) revealed no apparent differences between the nephrectomised and sham-operated control animals.

No animal drug-drug interaction studies were submitted (see discussion on non-clinical aspects).

In a study aimed at investigating the potential effect of aflibercept sialylation on its PK parameters, different lots of aflibercept with different degrees of sialylation but with similar purity and potency, were administered to rats and PK parameters were determined. Above a certain threshold of sialylation, no difference was detected in PK parameters. Below this level, aflibercept exposure (AUC and MRT) was directly correlated with the degree of sialylation, with exposure decreasing (and clearance increasing) with decreasing degree of sialylation. The terminal volume of distribution (Vz) was inversely correlated with the degree of sialylation (data not shown).

2.3.4. Toxicology

The toxicology studies are summarised in the following Table 5.

Table 5: Aflibercept toxicology programme

Species (Strain)	Route and Regimen of Administration	Duration of Dosing	Doses (mg/kg/adm)	GLP Compliance	Study Number
Single-dose					
Rat (SD)	Intravenous 30-minute infusion	Single administration	0, 150, 500	No	TXP0166
Rat (SD)	Intravenous 30-minute infusion	Single administration	0, 50, 150, 500	Yes, Pivotal	TXA1004

Repeat-dose					
Mouse (CD-1)	Subcutaneous	3 times per week 4 weeks	0, 10, 15	No	PK01017
Mouse (SCID)	Subcutaneous	Twice a week 4 or 8 weeks	0, 2.5, 25	No	VGT3
Rat (SD)	Subcutaneous	3 times per week 4 weeks	0, 10, 15	No	PK01027
Rat (SD)	Subcutaneous	3 times per week 4 weeks	0, 2, 5	No	PK01034
Rat (SD)	Subcutaneous	3 times per week 4 weeks	0, 0.5, 1	No	PK01042
Rat (Nude)	Subcutaneous	Twice a week 4 or 8 weeks	0, 25	No	PK01032
Rat (SD)	Subcutaneous	3 times per week 13 weeks	0, 0.1, 0.5, 1, 2	Yes, Pivotal	0470RR20-001
Monkey (cynomolgus)	Intravenous	Once a week 4 weeks	0, 2, 10, 30	Yes, Pivotal	SNBL223-11
Monkey (cynomolgus)	Intravenous	Once a week 13 weeks	0, 3, 10, 30	Yes, Pivotal	SNBL223-18
Monkey (cynomolgus)	Intravenous	Weekly for the first 15 weeks and then once every two weeks 27 weeks	0, 3, 10, 30	Yes, Pivotal	670145
Monkey (cynomolgus)	Subcutaneous	3 times per week 4 weeks	0, 1.5, 5, 15	Yes, Pivotal	SNBL223-4
Monkey (cynomolgus)	Subcutaneous	Twice a week 13 weeks	0, 1.5, 5, 15, 30	Yes, Pivotal	SNBL223-09
Fertility					
Monkey (cynomolgus)	Intravenous	Weekly for the first 15 weeks and then once every two weeks 27 weeks	0, 3, 10, 30	Yes, Pivotal	670145
Embryo-foetal development					
Rabbit (NZW)	Intravenous	2 weeks	0, 15, 30, 45	No	DSE 2005-0569 - DIV0953
Rabbit (NZW)	Intravenous	GD6, 9, 12, 15, 18	0, 3, 15, 45	No	TEP0184
Rabbit (NZW)	Intravenous	GD6, 9, 12, 15, 18	0, 3, 15, 60	Yes, Pivotal	TER0506
Study in juvenile animals					
Monkey (cynomolgus)	Intravenous	Once a week 13 weeks	0, 0.5, 3, 30	Yes, Pivotal	670144
Local tolerance					
Rabbit (NZW)	Intravenous, intramuscular, subcutaneous	-	24.4, 25, 100 mg/mL	Yes, Pivotal	DSE 2005-0387 (TOL1079)
Human tissue cross reactivity					
Human tissue	In vitro	-	5.0, 25.0 µg/mL	Yes, Pivotal	SPS-01-141
Haemolysis and flocculation studies					
Monkey blood	In vitro	-	0.69, 2.09, 4.17 mg/mL	No	HEM-No1
Human blood	In vitro	-	0.5, 2.0, 4.0 mg/mL	No	HEM-No3
Human blood	In vitro	-	8.0 mg/mL	No	HEM-No5

Single dose toxicity

The pivotal single-dose toxicity study is summarised in the following Table 6.

Table 6: Pivotal single dose toxicity study

Study ID	Species/Sex/ Number/Group	Dose (mg/kg)/Route	Approx. lethal dose / observed max non-lethal dose (mg/kg)
TXA1004, C04008M630 B11 (DP)	Rat (SD)/5M, 5F per dose	0, 50, 150, 500/30-minute intravenous Infusion	>500/500
Major findings **Clinical signs:** 50 mg/kg; Lesions and/or redness at the injection site in 2F/5. 500 mg/kg; Lesions, redness, swelling and/or scabs at the injection site in 1M/5 and 1F/5. **Body weight:** ≥50 mg/kg; ↓body weight gain in M from Day 1 to 8 (-38, -49 and –65% as compared to control at 50, 150 and 500 mg/kg, respectively). **Food consumption:** ≥50 mg/kg ↓ in M from Day 3 to 8 (-16, -20 and –27% as compared to control at 50, 150 and 500 mg/kg, respectively). **Necropsy** [a] No-compound related macroscopic findings			

[a]: After a 2-week observation period

Repeat dose toxicity

Pivotal repeat-dose toxicity studies are summarised in the following Table 7.

Table 7: Pivotal repeat dose toxicity studies

Study ID/ Species/Number/Sex/Group	Dose (mg/kg)/Route	Duration/ Administration/Recovery	NOEL/ NOAEL (mg/kg/day)
0470RR20-001, Rat, 6M, 6F/group were treated for 4 weeks and 10M, 10F/group were treated for 13 weeks. 5F, 5M in the control.	0.1, 0.5, 1.0, 2.0, subcutaneously	1 and 3 months/three times weekly, 1.0, and 2.0 mg/kg groups remained on the study for a 6-week treatment-free recovery period after the 4 or 13-week dosing period.	One-month treatment : 0.5 mg/kg/adm Three-month treatment : 0.1 mg/kg/adm
Findings **Clinical signs:** Aflibercept was clinically well tolerated at all dose levels. No treatment-related on electrocardiograms, blood pressure readings and body temperature. **Clinical chemistry:** 1 and 30 mg/kg/adm males; high urinary total protein, ↓ albumin, ↓ albumin/globulin ratios, ↑ cholesterol (1 male in 30 mg/kg/adm). Only the Albumin/globulin ratio was resolved following the 6-week recovery phase. **Haematology:** Marginally higher red blood cell mass (red blood cell count, haemoglobin and haematocrit) was noted in both sexes at all dose levels on Weeks 2 and 4. This change was completely resolved on Week 10. **Necropsy: Growth plate changes;** ↓ in metaphyseal capillary invasion, ↓ in primary bony trabeculae, degeneration of the cartilage matrix, disorganization of the chondrocyte columns, ↑ thickening of the physeal cartilage, and transverse subchondral bony plate. **Kidneys;** very slight or slight (and moderate in 1 monkey at 30 mg/kg/adm) increase in the mesangial matrix (1 male following dosing at 2 mg/kg/adm, 1 male following dosing at 10 mg/kg/adm and 3 males and 2 females following dosing at 30 mg/kg/adm). Three monkeys with kidney histopathological findings (one dosed at 10 and two dosed at 30 mg/kg/adm) also had increased urine protein and BUN levels and decreased serum albumin and/or serum total protein. Changes were not completely resolved after recovery. **Adrenal glands;** ↓ vacuolation of adrenal zona fasciculata cells with cytoplasmic eosinophilia was observed at all dose levels at the end of the dosing period only. **Ovaries;** Very slight or slight decreases in numbers of maturing follicles, granulosa and/or theca cells (10 and 30 mg/kg/adm). Resolved at the end of recovery. **Antibody formation;** 2 (30 mg/kg/adm) out of 36 animals developed aflibercept antibodies.			
Study ID/ Species/Number/Sex/Group	**Dose (mg/kg)/Route**	**Duration/ Administration/Recovery**	**NOEL/ NOAEL (mg/kg/day)**
SNBL223-18 Monkey (cynomolgus), 8M/8F in control and 20 and 30 mg/kg/adm groups, 4M/4F in 3 mg/kg/adm group.	0, 3, 10, 30 30-minute intravenous infusion	13 weeks, once a week, 13 weeks	<3 mg/kg/adm
Findings **Clinical signs:** Hunched posture and kyphosis at all dose level (increased at weeks 11, 12, and 13). Red nasal discharge; 1 male in 3 mg/kg/adm, 1 male and 2 female in 10 mg/kg/adm, 2 females (30 mg/kg/adm). No treatment-related changes on electrocardiograms, blood pressure readings and body temperature. **Clinical chemistry:** High urinary total protein in 5 animals (4 in 10 mg/kg/adm group and 1 in 30 mg/kg/adm group), high microalbumin in 9 animals (6 in 10 mg/kg/grp and 3 in 30 mg/kg/adm). Urinary total protein and microalbumin levels tended to return to pretest values at the end of the recovery period. ↑ triglyceride levels (2 animals) and ↓ serum phosphorus levels (1 animal) were noted in 30 mg/kg/adm group. A slight and transient increase in C-reactive protein was noted in 1 male treated at 10 mg/kg/adm and 1 male and 2 females treated at 30 mg/kg/adm. **Haematology:** Marginal increases in mean red blood cell mass in all dose groups. **Necropsy: Ovaries;** ↓ in ovary (all doses) and uterus (30 mg/kg/adm) weights. Resolved after recovery. ↓ number of granulose cells, theca cells and/or maturing follicles (all doses). No fully resolved after recovery. **Vertebral column;** slight curvature in 2			

monkeys treated at 10 mg/kg/adm and 1 monkey treated at 30 mg/kg/adm. No resolved after recovery. **Kidneys;** ↑ mesangial matrix (all doses). Ultrastructural changes in the kidney, evaluated by electron microscopy, were characterised by reduction or loss of filtration slit diaphragms between terminal foot processes of podocytes (epithelial cells) and hypertrophy and swelling of endothelial cells with irregularity of their cytoplasmic fenestrations. ↑ in immunohistochemical staining for IgG, IgM and C3 was apparent in all groups treated with aflibercept. No fully resolved after recovery. **Bones;** Disorganization of the chondrocyte columns, thickening of the growth plate cartilage and transverse subchondral bony plate (all doses). Not fully resolved after recovery. **Adrenal glands;** ↓ in vacuolation with ↑ eosinophilia (all doses). **Antibody formation;** 2 (at 10 and 30 mg/kg/adm) out of 40 animals developed aflibercept antibodies.

Study ID / Species / Number / Sex / Group	Dose (mg/kg) / Route	Duration / Administration / Recovery	NOEL / NOAEL (mg/kg/day)
670145 Monkey (cynomolgus), 6M/6F per group	0, 3, 10, 30 30-minute intravenous infusion	26 weeks, once a week for the initial 15 weeks then once every two weeks for the remaining 12 weeks, 22 weeks recovery	< 3 mg/kg/adm

Findings

Clinical signs: One male monkey dosed at 3 mg/kg/adm was euthanised prematurely on Day 182. This animal showed marked anaemia related to nasal bleeding, increased white blood cell, reticulocyte and neutrophil counts, decreased platelet counts, decreases in cholesterol, total protein, albumin, globulin and albumin/globulin ratio, increases in triglycerides and urea levels. Gross necropsy confirmed extensive macroscopic findings in the nasal cavities, including blood clots, bent nasal septum and absence of the right middle concha. Haematological evidence showed marked anaemia. These changes were regarded as compound-related. All aflibercept-treated groups showed sneezing, stained red fur, dry skin, swelling, scabbing or redness of the muzzle or lower jaw, hunched posture, reduced appetite, thinness and/or hypoactivity.↓ in group mean body weights were noted at all dose levels (0.4%, 7.8% and 20.4% for the 3, 10 and 30 mg/kg/adm groups, respectively). Initial weights were gained back after the recovery period. Aflibercept-treated females at all doses stopped exhibiting signs of regular menstrual bleeding during the dosing phase of the study. Not fully resolved during recovery. Induction of pronounced, but fully reversible, impaired sperm motility and abnormal sperm morphology was observed at all doses. No treatment-related changes on electrocardiograms, blood pressure readings and body temperature. **Clinical chemistry:** ↑ in cholesterol and significant elevations in C-reactive protein in all animals at all dose levels (weeks 4, 12 and 25 for cholesterol and weeks 4, 13 and 26 for C-reactive protein). Changes were fully reversible. ↑ in gamma glutamyl transferase (GGT), aspartate aminotransferase (AST), alkaline phosphatase (ALP) and/or alanine aminotransferase (ALT) were noted at all doses in individual males and females. Changes were not fully resolved during recovery. Mild increases in group mean urine microalbumin and urine protein levels in all doses. Aflibercept at ≥3 mg/kg/adm abrogated normal cyclical fluctuations of 17β-estradiol, progesterone, follicule stimulating hormone (FSH) and inhibin B. **Haematology:** Slight to mild increases in fibrinogen, red cell distribution width, mean platelet volume, platelet counts, red blood cell counts, hemoglobin, hematocrit and reticulocyte counts in males and/or females treated at doses of 3 mg/kg/adm and higher during dosing Weeks 4, 12 and 25. Partially or fully reversible after recovery. **Necropsy: Bone;** Femur bone mineral content (BMC) values at all treated animals were slightly lowered. Not fully reversible after recovery. Radiography showed an irreversible increased incidence of kyphosis, degenerative joint disease of vertebral articular facets, periosteal reaction of the femur and ilium. **Nasal cavity** (⩾3 mg/kg/adm); atrophy/loss of the septum and/or turbinates associated with necrotizing inflammation and various other epithelial, microvascular, cartilaginous and osseous were noted. **Bone** (≥ 3 mg/kg/adm); irreversible osteocartilaginous exostoses, exostoses in the arches of the thoracic and lumbar vertebrae. **Kidneys** (≥ 3 mg/kg/adm females); ↑ eosinophilic matrix in the glomerular tuft that stained positively with the periodic acid Schiff reaction. Glomerulopathy, often with tubulointerstitial inflammation and/or cast formation. Not fully reversible after recovery. **Ovaries** (≥ 3 mg/kg/adm); Compromised ovarian luteal development and follicular maturation, uterine endometrial and myometrial atrophy and vaginal epithelial atrophy. Effects were fully reversible after recovery. Vascular degeneration/proliferation was noted in duodenum and gallbladder at doses ⩾10 mg/kg/adm. **Adrenal glands** (≥3 mg/kg/adm); ↓ cytoplasmic vacuolation with increased cytoplasmic eosinophilia, which correlated with macroscopic dark discoloration. **Vascular proliferation** (≥ 10 mg/kg/adm); In several other tissues, including the heart. **CNS;** Macrophage infiltration in the choroid plexus. **Antibody formation:** Fourteen study animals developed anti-aflibercept antibodies (3, 4 and 7 animals out of 12 in the 3, 10 and 30 mg/kg/adm groups, respectively).

Study ID / Species / Number / Sex / Group	Dose (mg/kg) / Route	Duration / Administration / Recovery	NOEL / NOAEL (mg/kg/day)
SNBL223-4 Monkey (cynomolgus), 5M/5F in the control and high dose groups, 3M/3F in low and mid dose groups.	0, 1.5, 5.0, 15 Subcutaneous injection	4 weeks, three times weekly, 4 weeks	<1.5 mg/kg/adm

Findings

All treatment groups: ↑ in erythrocyte count, hemoglobin and hematocrit. Not fully resolved in the 15 mg/kg group. ↑ in glomerular mesangial matrix in the kidney, ↓ in serum total protein and albumin and ↑ in serum BUN and urine protein levels. Treatment related decreased vacuolation of adrenal zona fasciculata cells with cytoplasmic eosinophilia. **5 and 15 mg/kg group:** Degeneration and disorganization in the growth plate of the femur and decrease in numbers of maturing follicles, granulosa cells, and/or thecal cells. Moderate pericholangitis and slight focal hypertrophy of the bile duct epithelium in one female (15 mg/kg) (considered incidental in this study) and elevated GGT and C-reactive protein levels on Day 29.

Study ID / Species / Number / Sex / Group	Dose (mg/kg) / Route	Duration / Administration / Recovery	NOEL / NOAEL (mg/kg/day)
SNBL223-09 Monkey (cynomolgus), 5M/5F in the control and high dose groups, 3M/3F in low and mid dose groups	0, 1.5, 5.0, 15, 30 Subcutaneous injection	13 weeks, two times weekly, 6 weeks	<1.5 mg/kg/adm
Findings **All treatment groups:** Slight increase in mean hemoglobin, hematocrit and red blood cell numbers. Not reversible after recovery. Increase in blood pressure on dosing week 13 (2 females at 15 mg/kg and 2 females at 30 mg/kg). **15 and 30 mg/kg:** Decrease number of maturing follicles, granulose cells and/or thecal cells.			

Genotoxicity

No relevant studies were submitted (see discussion on non-clinical aspects).

Carcinogenicity

No relevant studies were submitted (see discussion on non-clinical aspects).

Reproduction Toxicity

The effects of aflibercept on fertility were investigated in the context of repeat-dose toxicity studies in sexually mature Cynomolgus monkeys by IV administration (see repeat dose toxicity in Table 7 above).

The pivotal embryofoetal development study is summarised in the following Table 8.

Table 8: Pivotal embryofoetal development study

Study ID / Species / Number / Sex / Group	Dose (mg/kg) / Route	Duration / Administration / Recovery	NOEL / NOAEL (mg/kg/day)
TER0506, Rabbit, 3/dose for PK, 22 to 26/dose in main study	0, 3, 15, 60 30-minute intravenous infusion	GD6, GD9, GD12, GD15, GD18 C-section GD29	Maternal 3 mg/kg/adm Foetus ≤ 3 mg/kg/adm
Findings **Clinical signs:** Decreased maternal body weight. Some food consumption effects in the 15 and 60 mg/kg/adm groups. **Necropsy:** Decrease in uterus weight in the high-dose group leading to a lower number of viable foetuses. Increase in mean number of post-implantation loss (early resorption) in the high-dose group. **Malformations:** Anasarca in 2 fetuses (including 1 polymalformed fetus) from the 3 mg/kg/adm group, ectrodactyly in 1 fetus from the 15 mg/kg/adm group and anasarca, gastroschisis, anal atresia and/or short tail in 9 fetuses (including 6 polymalformed fetus) from the 60 mg/kg/adm group. **Heart;** Ventricular septum defect, small or enlarged ventricular chamber and absence of the atrioventricular valve of fetuses from dams treated at 60 mg/kg/adm. **Great vessels and arteries;** Atresia of pulmonary trunk, narrowed pulmonary trunk, overriding aorta, reduced pulmonary artery branch, absent or narrowed ductus arteriosus, retroesophageal or dilated aortic arch, dilated aorta and retroesophageal subclavian artery of fetuses from dams treated at 60 mg/kg/adm. **Skeletal (including variations);** Fused caudal vertebrae, fused or supernumerary ribs, fused sternebrae, supernumerary arch and/or centrum of lumbar vertebrae and absence of arch and/or centrum of sacral vertebrae, increase in the incidence of absent or small interparietal skull bone, incomplete ossification of the hyoid, thoracic and lumbar vertebrae, sternebrae, talus and forepaw and hindpaw phalanxes in fetuses from the 60 mg/kg/adm group.		

Finally, a 13-week intravenous toxicity study (670144) was specifically conducted in sexually immature monkeys (2 to 2.5 years of age). This study showed similar safety signals as those seen in adult monkeys in the repeat-dose toxicity studies (data not shown).

Toxicokinetic data

In general, a no-observable adverse effect level (NOAEL) was not established following IV or SC administration of aflibercept in monkeys. Exposure ratios of free aflibercept, calculated at the lowest doses associated with findings in animals by comparing average Cmax and AUC (corrected to take into account the different schedules of administration) to exposures observed in cancer patients after a first

IV dose of 4 mg/kg (administered every 2 weeks) or 6 mg/kg (administered every 3 weeks), are presented in the following table 9.

Table 9: Exposure comparisons between animals and cancer patients

Study Type / Regimen of administration and Study No.	Dose	Ratio [a]	Dose	Ratio [a]
Humans				
Pharmacokinetics in cancer patients 1q2w at 4 mg/kg [Study TED6115/TED6116] 1q3w at 6 mg/kg [Study TCD6120]	**4 mg/kg**	NA	**6 mg/kg**	NA
C_{max} (µg/mL) [b]	97.4	NA	118	NA
$AUC_{0-\infty}$ (µg.day/mL) [b]	293	NA	464	NA
Cynomolgus Monkeys				
6-month IV Toxicity Once a week for the first 15 weeks and once every 2 weeks for the remaining 12 weeks [Study 670145,	**LOAEL[e]: 3 mg/kg**	NA	**LOAEL[e]: 3 mg/kg**	NA
C_{max} [c, d] (µg/mL)	94.2	**0.97**	94.2	**0.80**
AUC_{0-168h} [d] (µg.day/mL)	348 (174x2)	**1.19**	522 (174x3)	**1.13**
3-month IV Toxicity in Juveniles Once weekly for 3 months [Study 670144,	**LOAEL[e]: 0.5 mg/kg**	NA	**LOAEL[e]: 0.5 mg/kg**	NA
C_{max} [c, f] (µg/mL)	8.8	**0.09**	8.8	**0.07**
AUC_{0-168h} [f] (µg.day/mL)	33 (16.5x2)	**0.11**	50 (16.5x3)	**0.11**

Abbreviations: AUC: area under the concentration time curve, Cmax: maximal concentration, IV: intravenous, NA: not applicable, ND: not determined, NOAEL: no observable adverse effect level, LOAEL: lowest observable adverse effect level, Tmax: time of maximal concentration.

[a] Exposure ratio = Cmax or AUC values in the nonclinical toxicity studies divided by the Cmax or AUC0-∞ estimated in humans at doses and regimens indicated in the table.

[b] Cmax and AUC0-∞ values indicated in the table were calculated after the first administration in patients.

[c] Cmax was observed at Tmax of 5 minutes after completion of IV infusion.

[d] All Cmax values from monkeys obtained during the 27-week dosing duration were averaged; AUC0-168h values from monkeys were averaged over the 27-week dosing duration, taking into account the weekly regimen applied for the first 15 weeks and the 1q2w regimen applied during the 12 remaining weeks; the calculated AUC0-168h values were then multiplied by 2 or 3 to allow the calculation of exposure ratios in comparison with the 1q2w and 1q3w regimens applied in cancer patients dosed at 4 and 6 mg/kg, respectively.

[e] Dose expressed as a LOAEL because no NOAEL was defined in this study.

[f] Cmax and AUC0-168h values from monkeys obtained after 1, 5, 9 and 13 weekly administrations were averaged; the calculated AUC0-168h values were then multiplied by 2 or 3 to allow the calculation of exposure ratios in comparison with the 1q2w and 1q3w regimens applied in cancer patients dosed at 4 and 6 mg/kg, respectively.

Local Tolerance

No compound-related local reactions were noted at the injection sites following IV, IM, and SC injection of 24.4 mg/mL, 25 or 100 mg/mL of aflibercept to New Zealand White rabbits. There were no compound-related macroscopic and microscopic observations at the end of the 8-day observation period. The local tolerance of aflibercept was also assessed following intravenous administration in the 4-week, 13-week and 6-month toxicity studies conducted in cynomolgus monkeys. No compound-related microscopic findings were noted at the injection sites in monkeys after repeated intravenous infusions.

Other toxicity studies

In human tissue cross-reactivity studies aflibercept did not cross-react with any of the 33 human tissues tested (data not shown).

Serum levels of anti-aflibercept antibodies were measured during the repeat-dose toxicity studies and the effect of these antibodies on aflibercept clearance was determined. Repeated administration of aflibercept to rats and mice resulted in an anti-aflibercept antibody response (ADA) that resulted in increased aflibercept clearance and nephrotoxicity that was associated with mortality. This precluded the use of these species in sub-chronic or chronic toxicity studies.

In some pregnant female rabbits, a total of five IV administrations resulted in the presence of anti-aflibercept antibodies associated with decreased levels of free aflibercept concentrations. In no case was toxicity in rabbits or monkeys associated with the presence of an anti-aflibercept antibody response, nor was the overall safety profile modified by the presence of anti-aflibercept antibodies in these animals. In monkeys after IV dose, ADA responses increased in frequency with the duration of dosing, with up to 12% of animals being ADA positive in the 3-month study and 36% being ADA positive in the 6-month study. Two of the 14 animals that were ADA positive in the 6-month toxicity study demonstrated increased clearance of both free and bound aflibercept.

No haemolysis nor protein precipitate were observed when mixing whole heparinised blood, serum and plasma from monkeys and humans with aflibercept solutions at concentrations including the ones used in animal toxicity studies and in clinical practice (data not shown).

2.3.5. Ecotoxicity / environmental risk assessment

No Environmental Risk Assessment was submitted (see discussion on non-clinical aspects).

2.3.6. Discussion on non-clinical aspects

In vitro, aflibercept blocked VEGF-induced proliferation of endothelial cells and inhibited microvessel outgrowth from rat aorta in vitro. In vivo pharmacology studies have indicated that treatment with aflibercept inhibits tumour growth of a wide variety of murine, rat, and human tumour cell lines implanted in mice. Aflibercept treatment of several established tumours also resulted in a decrease in tumour vessel density.

Aflibercept was effective in combination with several widely-used chemotherapeutic agents. Combination of aflibercept with the antimetabolite, 5-FU, was synergistic in inhibiting the growth of early mammary MA13/C tumours. Combining aflibercept with the topoisomerase I inhibitor, irinotecan, was also synergistic over several dose levels in advanced colon HCT 116 tumours.

No secondary pharmacology studies were submitted. The lack of secondary pharmacology studies was considered acceptable by the CHMP based on the safety pharmacology studies conducted in mice, rats and rabbits using the intravenous (IV) and subcutaneous (SC) routes of administration to help identify possible sequelae of stringent systemic inhibition of VEGF. Moreover, in human tissue cross-reactivity studies aflibercept did not cross-react with any of the 33 human tissues tested.

No treatment related effects on ECG parameters were observed in any of the pivotal monkey repeat-dose toxicity studies.

Subcutaneous administrations of aflibercept resulted in statistically significant reductions in microvessel density within the liver, pancreatic islets and thyroid follicles at all doses and time points evaluated. Less consistent and less marked decreases in microvessel density were also noted in the anterior or posterior pituitary gland and adipose tissue. No decreases in microvessel density were detected in the adrenal gland (medulla and cortex), duodenum, exocrine pancreas or retina at any dose level or timepoint. It has been previously shown in the mouse that a subset of capillaries remains dependent on VEGF into adulthood, mainly in the pancreatic islets and thyroid (Kamba, et al. 2006). Several other tissues have also been identified, in which a subset of the capillaries remain VEGF dependent, including liver, pituitary and adipose tissue. The pharmacological inhibition of VEGF has been shown to increase endothelial cell thickness, and reduce the number of fenestrations in the capillaries of several endocrine organs (Kamba, T et al.). All classes of VEGF inhibitors evaluated to date, irrespective of their mechanism of action, produce these structural changes in susceptible subsets of capillaries showing that these are class effects attributable to inhibition of the VEGF/VEGF Receptor signaling pathway. Hypertension has been included as an identified risk in the RMP.

A single subcutaneous administration of aflibercept induced a moderate, sustained but reversible increase in blood pressure in rats and mice at doses (2.5 mg/kg and 0.5 mg/kg and above, respectively) lower than the active dose in pharmacological models. Multiple classes of anti-hypertensives were found to be effective in lowering blood pressure in rats treated with aflibercept. Aflibercept has also shown blood pressure effect in clinical trials (see clinical AR). Similar effects on blood pressure were also noted in rats receiving a single dose of cediranib, a VEGF receptor tyrosine kinase inhibitor (Curwen, et al. 2008). Hypertension was reported first in patients treated with bevacizumab and is the predominant and expected side effect of anti-VEGF therapies (Launary-Vacher, et al. 2009). Hypertension induced by this class of drugs is related to the role of the VEGF/VEGFR signaling pathway in blood pressure homeostasis (Roodhart, et al. 2008). Blocking VEGFR signaling can result in a decrease in the secretion of vasodilation factors (nitric oxide and prostacyclin) and/or an increase in the secretion of vasoconstriction factors (endothelin- 1) by the endothelial cells leading to an increase in peripheral vascular resistance and an increase in blood pressure (Roodhart et al, 2008), (Verheul, et al. 2007). Since VEGF can also induce hypotension through an endothelial baroreceptor signaling cascade, VEGF pathway inhibition could result in hypertension through the disturbance of the baroreceptor response (Verheul, et al. 2007). In addition, hypertension induced by angiogenesis inhibitors may be due to an inappropriate reduction in the density of capillaries and arterioles (Verheul, et al. 2007).

In rabbit, impairment of wound repair and healing was noted on day 8 at 0.3 mg/kg/adm, characterised by a reduced fibrous response and neovascularization relative to controls. Compromised wound healing was also evaluated in the clinical material, see clinical AR. The signal on compromised wound healing is included in section 5.3 of the SmPC and as wound healing impairment was included as an identified risk in the RMP.

No safety pharmacology signals on central nervous system effects were detected in any of the repeat-dose monkey studies, no effects on thrombus formation in exposed rabbits were detected, aflibercept treatment did not affect the respiratory function in conscious rats and no biologically relevant renal effects were detected in treated mice.

Following IV administration to mice, rats and monkeys, free aflibercept displayed a multicompartmental PK serum profile. Clearance was slow, t1/2 long and the Vss was slightly greater than the volume of the central compartment. In rats, mice and monkeys administered SC doses of aflibercept at multiple dose levels, a nonlinear PK profile was observed, indicative of target-mediated clearance. This PK profile was characterised by an increased free aflibercept t1/2 and a positive deviation of exposure from dose proportionality at highest aflibercept dose levels. Aflibercept SC bioavailability was 94% in mice, 85% in monkeys and 33% in rats. Aflibercept complex accounted for a significant percentage of the circulating form of the drug, with the relative percentage increasing as the aflibercept dose was reduced.

A study with radioactivity-labelled aflibercept showed that 5 minutes post-dosing, approximately 11.4%, 1.33%, 0.42%, 0.34%, and 0.19% of the total dose of radioactivity was localised in the liver, kidney, spleen, lung and heart respectively.

In summary, aflibercept has a long circulating t1/2, characteristic of an IgG1 fusion protein, and is cleared by multiple mechanisms, which include saturable binding to endogenous VEGF as well as proteolytic degradation. This also gives that the maximum pharmacological consequences of aflibercept binding to VEGF are reached, since all VEGF is bound, at relative low doses (2.5 mg/kg, single dose, in mice).

Weekly/every two weeks intravenous administration of aflibercept to cynomolgus monkeys for up to 6 months resulted in mean body weight decrease. Initial weights were gained back after the recovery period. Decreased body weight was also observed in male rats. More clinical signs of toxicity in the

longer monkey studies included: red nasal discharge, stained red fur, dry skin, swelling, scabbing or redness of the muzzle or lower jaw and hunched posture and kyphosis.

Changes in clinical chemistry included high urinary total protein, high microalbumin, increased triglyceride levels, increased cholesterol, elevated C-reative proteins, increase in liver enzymes (GGT, AST, ALP, ALT) and decrease in phosphorus levels. Aflibercept abrogated normal cyclical fluctuations of 17 β-estradiol, progesterone, follicule stimulating hormone (FSH) and inhibin B. Aflibercept-treated females at all doses stopped exhibiting signs of regular menstrual bleeding during the dosing phase of the study, which was not fully resolved during recovery. Some minor changes were also observed in fibrinogen, red cell distribution width, mean platelet volume, platelet counts, red blood cell counts, haemoglobin, haematocrit and reticulocyte counts.

Organ toxicity was noted at all doses tested and the target organs were:

- Bone; interference with growth plate maturation of long bones and osteocartilaginous exostoses of vertebrae. Similar changes in the growth plate have been reported in cynomolgus monkeys administered recombinant humanised monoclonal antibody to vascular endothelial cell growth factor.

- Kidney; frequently increased glomerular mesangial matrix, occasionally hyperplasia of parietal epithelium and periglomerular fibrosis at 30 mg/kg/adm. The renal findings were reversible when the recovery period was 13 weeks or longer. Kidney glomerular changes were apparently not immune mediated (lack of staining of glomeruli with antibody against monkey IgM, monkey IgG, or human C3 complement) and may be related to lack of circulating VEGF. No alterations in urinalysis parameters reflective of impaired renal function were observed in repeat dose monkey studies with a recombinant humanised monoclonal antibody to vascular endothelial cell growth factor.

- Testis; Induction of pronounced, but fully reversible, impaired sperm motility and abnormal sperm morphology was observed at all doses.

- Ovary; decreased number of maturing follicles, granulosa cells and/or theca cells. Similar changes have been reported in cynomolgus monkeys administered recombinant humanised monoclonal antibody to vascular endothelial cell growth factor.

- The respiratory and olfactory epithelium of nasal turbinates and adrenal gland.

- Skeletal changes: decreased metaphyseal capillary invasion, decreased primary bony trabeculae, increased thickness of physeal cartilage, disorganization of the chondrocyte columns, and degeneration of the cartilage matrix. These skeletal changes showed reversibility after shorter recovery periods. Osteocartilaginous exostoses and degenerative joint disease were also observed and were not reversible following a 5-month recovery period.

No studies evaluating carcinogenicity or mutagenicity of aflibercept were submitted in agreement with the ICH S6 and ICH S9 guidelines (EMA/CHMP/ICH/731268/1998 and EMEA/CHMP/ICH/646107/2008).

No specific studies with aflibercept have been conducted in animals to evaluate the effect on fertility. However, results from a repeat dose toxicity study suggest there is a potential for aflibercept to impair reproductive function and fertility. In sexually mature female cynomolgus monkeys inhibition of ovarian function and follicular development was evidenced. These animals also lost normal menstrual cycling. In sexually mature male cynomolgus monkeys a decrease in sperm motility and an increase in incidence of morphological abnormalities of spermatozoa were observed. There was no margin of exposure to patients in relation to these effects. These effects were fully reversible within 8-18 weeks after the last injection. This is described in section 5.3 of the SmPC.

Women of childbearing potential should be advised to avoid becoming pregnant while on Zaltrap, and should be informed of the potential hazard to the foetus. Women of childbearing potential and fertile

males should use effective contraception during and up to a minimum of 6 months after the last dose of treatment. This is described in section 4.6 of the SmPC.

Aflibercept has been shown to be embryotoxic and teratogenic when administered intravenously to pregnant rabbits every 3 days during the organogenesis period (gestation days 6 to 18) at doses approximately 1 to 15 times the human dose of 4 mg/kg every 2 weeks. Observed effects included decreases in maternal body weights, an increased number of foetal resorptions, and an increased incidence of external, visceral, and skeletal foetal malformations. This is described in section 5.3 of the SmPC.

The applicant has not calculated AUC-values in all presented studies, but relied on C_{max} in order to estimate exposure margins to human. Since the presented pharmacokinetic studies indicate a target saturation multicompartmental serum profile and a long half-life in both rat and monkey it is agreed that a C_{max} approach to estimate human exposure margins is acceptable. The data show no margins between human exposure and toxic exposure in animals.

In local tolerance studies, no compound-related local reactions were noted at the injection site after either way of administration. These findings are somewhat in contradiction with the finding of injection site lesions in the single dose toxicity study. From a clinical point of view, injection site lesions were not identified as a major side effect and thus the inconclusive non-clinical data were no longer considered.

In rat, most animals developed anti- aflibercept antibodies. In high-titer animals an increase in clearance was observed, indicating that this was antibody mediated. In low-titer animals no increase in clearance was observed. In the 27 week repeat-dose monkey study, some animals developed anti-drug antibodies (14 out of 36 animals). In two of these animals the antibodies gave rise to an increased clearance of aflibercept. In the rest of the animals the applicant argued that, due to the low antibody titer, that there was no reduction in free aflibercept levels. In the embryo-foetal study no toxicokinetic data were collected from the main study group and it is not known whether the presence of anti-aflibercept antibodies (found in 21 out of 71 animals) in the main study group influences aflibercept exposure levels in the dams. However, all dose groups showed maternal and embryo-foetal toxicity so that it is clear that the aflibercept-directed antibodies did not abolish exposure.

Finally, the absence of an ERA was considered acceptable by the CHMP as aflibercept is made up by naturally occurring peptides and therefore aflibercept is not expected to pose a risk to the environment, in accordance with the Guideline on the Environmental Risk Assessment of medicinal products for human use (EMA/CHMP/SWP/4447/00).

2.3.7. Conclusion on the non-clinical aspects

Non-clinical aspects were adequately addressed by the applicant and there are no outstanding issues.

2.4. Clinical aspects

2.4.1. Introduction

The applicant submitted 14 clinical pharmacokinetic studies, 3 clinical pharmacodynamic studies, 1 pivotal clinical efficacy and safety study and 2 supportive Phase III studies in support of this Marketing Authorisation application.

GCP

The Clinical trials were performed in accordance with GCP as claimed by the applicant.

The applicant has provided a statement to the effect that clinical trials conducted outside the community were carried out in accordance with the ethical standards of Directive 2001/20/EC.

- Tabular overview of clinical studies

Table 10: Clinical oncology studies with aflibercept

Study type	Study code	Number of patients treated
Pharmacokinetics in Phase 1 single agent studies		
Safety	TED6113	38
	TED6114 (extension study)	18
	TED6115	57
	TED6116 (extension study)	40
Pharmacokinetics and pharmacokinetics/pharmacodynamics in Phase 2 single agent studies		
Safety+efficacy	ARD6122	215
	ARD6123	96
	ARD6772	16
	EFC6125	58
Pharmacokinetics and pharmacokinetics/pharmacodynamics in Phase 1 combination studies		
Safety	TCD6117 (FOLFOX4)	32
	TCD6118 (irinotecan/LV5FU2)	65
	TCD6120	134
	TCD6119 (TCF)	44
	TCD6121)	61
	TCD10173	28
Pharmacodynamics in healthy subjects (Phase 1 studies)		
Pharmacodynamics	PDY6655	40
	PDY6656	48
Pharmacodynamics related to safety in patients with a solid malignancy		
Safety	TES10897+docetaxel	87
Efficacy, safety and pharmacokinetics in patients with colorectal cancer		
Efficacy, safety, pharmacokinetics	EFC10262 (VELOUR)	1226 (611 aflibercept treated)
Efficacy, safety and pharmacokinetics in patients with others tumour types		
Efficacy, safety, pharmacokinetics	EFC10547 (VANILLA)	541 (270 aflibercept treated)
	EFC10261 (VITAL)	905 (452 aflibercept treated)

2.4.2. Pharmacokinetics

In order to characterise the pharmacokinetics of aflibercept, two analytes were quantified in healthy subjects and in patients: free aflibercept (compound not complexed to VEGF) and bound aflibercept (VEGF: aflibercept complex in a ratio 1:1). A commercially available assay was also used to detect free endogenous VEGF. Finally, two methods were developed and validated to detect binding anti-aflibercept antibodies. A comparison study of both methods demonstrated that the assay used in phase 2 and 3 studies was more sensitive than the original assay used in phase 1 and early phase 2 studies. A method for detecting neutralizing anti-aflibercept antibodies was also developed and validated.

For the pharmacokinetic data analysis in healthy volunteers, a non-compartmental analysis was performed for free, bound and total aflibercept. Pharmacokinetic parameters were calculated using non-compartmental methods.

For the pharmacokinetic data analysis in cancer patients, pharmacokinetic properties of free and bound aflibercept were determined from non-compartmental PK analysis (NCA) in single agent (TED6115/TED6116) and 5 combination studies (TCD6117, TCD6118, TCD6119, TCD6120 and TCD6121). Then, PK was characterised by population approach from a pooled dataset using data from TED6115/TED6116, TCD6120-VT, TCD6118, ARD6122, ARD6123 and EFC6125. These population PK models for free and bound aflibercept were used to estimate PK parameters in the pivotal phase 3 study (POH0265 for EFC10262/VELOUR) as well as in phase 3 studies in other indications (POH0262 for EFC10547/VANILLA, POH0274 for EFC10261/VITAL) after implementation of their data in the pooled database.

For the population pharmacokinetic data analysis, a population pharmacokinetic (PK) model of aflibercept was first developed for free and bound aflibercept in 56 healthy subjects after single IV doses of 1 to 4 mg/kg in PDY6655 and PDY6656 Phase 1 clinical studies (POH0251). Then Phase 1 and 2 studies were conducted in patients in which aflibercept was administered intravenously every two weeks as either a single agent or in combination with various chemotherapy drugs at dose levels ranging from 2.0 to 6.0 mg/kg every two weeks or from 2.0 to 9.0 mg/kg every three weeks. Data from 433 patients were used in a population pharmacokinetic analysis of free aflibercept concentrations (POH0253). In this analysis, the influence of demographic factors (age, gender weight, race such as Caucasian, Black and Asian), laboratory determinations (albumin, serum alkaline phosphatase, total bilirubin, aspartate amino transferase, alanine amino transferase, total protein and creatinine clearance) and concomitantly used chemotherapy agent on the pharmacokinetics of free aflibercept was investigated. Later on, a population pharmacokinetic analysis was performed in 416 patients with both free and bound aflibercept concentrations (POH0263) using the structural model that was developed in POH0251. The pharmacokinetics of free (including covariates assessment) and bound aflibercept was further investigated with the successive addition of 204 patients from EFC10547/VANILLA study (POH0262), 370 patients from EFC10261/VITAL study (POH0274) and 500 patients from EFC10262/VELOUR study (POH0265 & POH0265- Amendment01).

Absorption

Aflibercept is to be administered by intravenous infusion; therefore no relevant studies were submitted. A single formulation of aflibercept was used during preclinical and clinical development and the same formulation will be used for marketing purposes.

Distribution

Aflibercept is distributed via target-mediated drug disposition (TMDD), as it is bound with high affinity to its pharmacologic target such that the interaction is reflected in the pharmacokinetic properties of the drug.

According to the population PK model, free aflibercept volume of distribution (Vss) was 7.8, slightly greater than blood compartment (POH0265), at doses greater than 2 mg/kg. PK parameters for free aflibercept, as determined in the POH0265 final population PK analysis, are presented in the following Table 11.

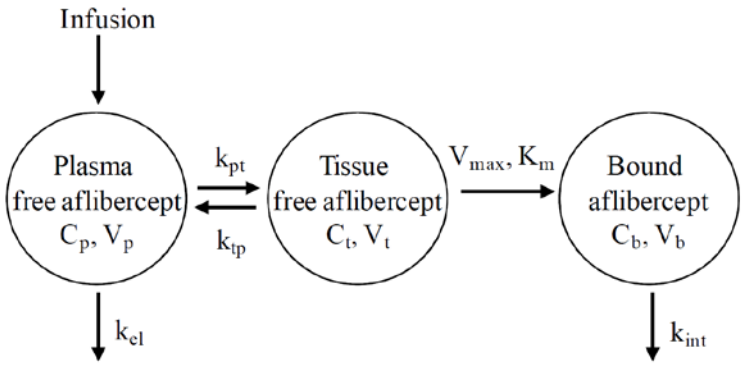
Table 11: PK parameters for free aflibercept in a typical patient, from the POH0265 final population PK model

	CL (L/day)	V_{ss} (L)	$t_{½z}$ (day)	Cycle 1			Steady State		
				Cmax (µg/mL)	Ctrough (µg/mL)	$AUC_{0-\tau}$ (µg*day/mL)	$Cmax_{SS}$ (µg/mL)	$Ctrough_{ss}$ (µg/mL)	AUC_{SS} (µg*day/mL)
4 mg/kg q2w	1.02	7.77	5.79	59.1	5.33	218	65.6	6.55	263
6 mg/kg q3w				88.6	3.45	365	92.4	3.76	394

Typical patient : male 67 kg; normalized ALB 0.769; normalized ALK 0.816; normalized TP 0.866;CLCR 75.9= mL/min. τ = 336h for 4 mg/kg q2 weeks and 504 h for 6 mg/kg q3 weeks

In the same analysis (POH0265), the final structural model, shown in Figure XXX below, involved two compartments for free aflibercept and one for bound aflibercept, with a Michaelis-Menten type binding of free aflibercept to VEGF from the peripheral compartment. Free aflibercept in plasma distributes to tissues then binds to VEGF to form the complex. The bound aflibercept is assumed to be directly eliminated through internalization and not to any appreciable extent through reversible dissociation to re-generate free aflibercept and free VEGF. Typical clearance and central volume of distribution for a male subject were 0.04 L/h (1.0 L/day) and 4.5 L, respectively. The terminal half-life was 140 hours (about 6 days).

Figure 2: Structural model for free and bound aflibercept



The Applicant hypothesised that maintaining a free/bound aflibercept ratio above 1 throughout the dosing interval would maximise binding of available endogenous VEGF. This ratio, measured in all studies, showed that free was consistently in excess of bound aflibercept throughout the treatment period at dose levels greater than 2 mg/kg every 2 weeks or greater than 4 mg/kg every three weeks (data not shown).

Elimination

According to the population PK model, free aflibercept clearance is around 1.0 L/day with a terminal half-life of around 6 days, at doses greater than 2 mg/kg. Elimination of VEGF-bound aflibercept is slower with an apparent clearance of 0.182 L/day and a half-life of 15 days. At dose levels of 2 mg/kg and above, plasma clearance values were in the same order of magnitude across studies.

Dose proportionality and time dependencies

The dose proportionality of aflibercept administered as single agent has been evaluated between 0.3 to 7 mg/kg in study TED6115/TED6116 (single agent) and between 2 and 9 mg/kg in combination with

docetaxel in study TCD6120 and POH0253. Since docetaxel probably does not impact the pharmacokinetics of aflibercept, dose proportionality of free aflibercept was evaluated up to 9 mg/kg.

Aflibercept exhibits a non linear PK with higher clearance at lowest dose levels. Then, pharmacokinetics of free aflibercept is linear over the 2-9 mg/kg dose range. In cancer patients, mean free aflibercept clearance decreased from 1.95 L/day at the dose of 0.3 mg/kg to 1.13 L/day at the dose of 2 mg/kg and then, remained stable over the 2-9 mg/kg range. Consistently, terminal half-life increased over the 0.3-2 mg/kg range and then was stable. This could be related to saturable high-affinity binding of aflibercept to endogenous VEGF at higher doses, limited by VEGF availability.

Based on population PK analysis (POH0265), at 4 mg/kg every 2 weeks and 6 mg/kg every 3 weeks, the accumulation ratio for free aflibercept (AUCss/AUC0-336 and AUCss/AUC0-504, respectively) was 1.2 and 1.1, respectively. After 4 mg/kg every 2 weeks, time to steady state estimated was 10 weeks, steady state is as good as reached at the 4th dose (by 6 weeks), and 81% of $C_{trough,ss}$ reached at the end of the first dose.

Special populations

No specific studies to assess the effect of gender, race, weight, age, renal or hepatic function were submitted. These effects were evaluated from the population PK analysis.

Among the 1507 patients included in the population PK analysis (POH0265 and POH0265 - Amendment 01), 549 (36%) patients were identified with mild renal impairment (50 ml/min ≤CLCR ≤80 ml/min), 96 (6%) patients with moderate renal impairment (30 ml/min ≤CLCR <50 ml/min), and 5 (<1%) patients with severe renal impairment (CLCR <30 ml/min). Clearance decreased by 6.48% for a CLCR of 47.8 ml/min and increases by 10.2% for a CLCR of 148 ml/min (compared to a CLCR of 75.9). Mean free aflibercept clearance (based on individual post hoc estimates) was 0.65 l/day in patients with severe renal impairment (n=5), 0.80 l/day in moderately impaired patients (n=96), and 0.91 l/day in the mildly impaired patients (n=549) compared to 1.1 l/day in normal patients (n=857).

Among the 1507 patients included in the population PK analysis (POH0265 and POH0265 - Amendment 01), 63 were classified with mild hepatic impairment and 5 with moderate hepatic impairment. Patients with low serum albumin concentrations (≤0.568×upper limit of normal [ULN]) or high concentration of alkaline phosphatase (≥3.24×ULN) had a 18.7% and 12.9% increase in clearance respectively compared with a typical patient. No effect of total bilirubin, aspartate amino transferase and alanine amino transferase was identified.

Gender was the most significant covariate for explaining the inter-individual variability of free aflibercept with a 15.5% higher clearance and a 20.6% higher volume of distribution in males than in females. However, no difference in AUC was noted between males and females.

No effect of race was identified in the population analysis, where 92% of the included patients were Caucasians.

Weight has a minor effect on aflibercept clearance with an 8.47% increase in clearance for extreme body weight (≥ 99.8 kg) and a higher effect on volume of 16.9%. The effect of weight on free aflibercept clearance and volume of distribution, combined with the weight-based dosing, resulted in a higher exposure in patients with higher body weight. Area under the curve (AUC) was 29% higher in the > 100 kg patient category compared to the 50-100 kg category.

No effect of age was identified in the population analysis, where 31% of the patients were 65-75 years old and 5% of the patients were 75 years or older.

Pharmacokinetic interaction studies

No specific drug-drug interaction studies with aflibercept were submitted. However, PK data from five combination Phase I studies were used to assess PK interactions between aflibercept and various other anti-cancer agents based on inter-study comparisons. In addition, the effect of combinations on the PK of aflibercept was also investigated via population PK analysis (data not shown, see discussion on clinical pharmacology).

Pharmacokinetics using human biomaterials

Human tissue cross-reactivity studies are described under non-clinical aspects.

2.4.3. Pharmacodynamics

Mechanism of action

No studies addressing the mechanism of action were submitted.

Primary and Secondary pharmacology

In the phase I study TED6115 the aflibercept dose of 4 mg/kg administered every 2 weeks as a 1-hour IV infusion was established as the recommended dose for further studies based on dose limiting toxicities observation (rectal ulcer and proteinuria observed at 7 mg/kg), analysis of the overall safety profile and free/bound level results. The mean trough free/bound aflibercept ratio was greater than 1 at dose levels greater than 2 mg/kg. The dose of 4 mg/kg every 2 weeks was further confirmed when aflibercept was administered in combination with standard doses of the irinotecan and 5-fluorouracil/leuvovorin regimen in the TCD 6118 Phase 1 study in patients with solid tumours. This phase 1 study included a dose escalation and an extension phase, which allowed confirmation of the aflibercept dose as well as assessment of the safety profile and preliminary anti-tumour activity of the combination at the aflibercept recommended dose of 4 mg/kg every 2 weeks. The trough free/bound aflibercept ratio exceeded 1 throughout the dosing interval for most of the patients treated at 4 mg/kg. In addition, this study showed promising antitumour activity in heavily pre-treated MCRC patients.

Studies TED6115 and TCD6118 are described in more detail under dose-response studies.

The potential aflibercept liability for QT prolongation was assessed in study TES10897. This was a prospective, multicentre, international, randomised, double-blind, placebo-controlled, parallel-group study to assess the effect on QTcF interval (QTc Fridericia) of aflibercept versus placebo in cancer patients. Aflibercept was administered at 6 mg/kg q3w in combination with docetaxel (every 3 weeks dose <75mg/m2). 88 patients were randomised, of whom 87 were treated and evaluated for PK, while 84 patients were evaluated for PD. Free aflibercept and VEGF-bound aflibercept plasma concentrations and Cmax were measured. The PK samples for aflibercept were collected predose, at Cycle 1 and Cycle 3 (different time points), and at the final Day 60 follow-up visit. ECG intervals (QTcF, QTcB, QTcN, HR, PR, QRS) were measured and the exposure-QT relationship (between baseline adjusted QTcF changes and log free aflibercept concentration plasma) was assessed.

In TES10897, after infusion of 6 mg/Kg of aflibercept, the upper bound of the two sided 90% CI for the baseline-adjusted QTcF change was below 20 msec at both cycle 1 and cycle 3. The QT-exposure and relationship with free aflibercept were calculated at Cycle 1 and Cycle 3. At Cycle 1, the estimated slope of the relationship (95%CI) was - 0.013 (-0.044;0.019) versus + 0.048 (0.013;0.082) at Cycle 3 showing a PK/PD relationship between QTcF change from baseline and free aflibercept concentrations; every increase in 100 µg/mL of free aflibercept being associated with null or small (5 msec) increase in

QTcF. In the safety population, analysis showed no QTcF >500 ms but one transient QTcF change from baseline ≥ 60 ms (in a patient with a past medical history of myocardial infarction).

The effect of aflibercept on 24-hour ambulatory systolic and diastolic blood pressures (SBP/DBP) and allied physical and laboratory tests was evaluated in 2 single administration Phase 1 healthy male subjects studies (PDY6655, PDY6656). Aflibercept significantly increased 24-hour mean SBP, with noticeably greater effects at 4 mg/kg when compared to 1 and 2 mg/kg. The increase in 24-hour mean SBP observed in subjects receiving aflibercept 4 mg/kg reached a peak of +14.54 mm Hg (placebo-corrected mean difference) at week 2, and remained elevated, at +5.47 mmHg 6 weeks after the administration. DBP and night time blood pressure were also affected but not pulse pressure.

Analysis of endothelium dysfunction markers suggested no major alteration of endothelium by aflibercept. In single dose Phase 1 studies, aflibercept did not induce proteinuria or microalbuminuria and did not modify electrolyte excretion or ClCr. Plasma active renin and aldosterone were decreased in aflibercept-treated subjects.

Relationship between plasma concentration and effect

In the pivotal VELOUR study (EFC10262), overall survival was significantly correlated with free aflibercept clearance (p=0.0147), as well as Cmax (p=0.0005), AUC extrapolated (p<0.0001), AUC at first cycle (p<0.0001) and average AUCcumOS (cumulative AUC up to last administration + 90 days + date of death or cutoff date whichever comes first) (p<0.0001). A significant relationship was also shown with bound aflibercept clearance (p<0.0001). For average AUCcumOS, an increase of 1000 µg.h/mL was associated with a 13% increase in the survival hazard rate.

Similar results to those observed with OS were observed for PFS: decreased free aflibercept clearance (p<0.0001), increased AUC extrapolated (p<0.0001), increased average AUCcumPFS (cumulative AUC up to last administration + 90 days or date of progression or cutoff date whichever comes first) (p=0.0001) and AUC of first cycle (p<0.0001) were significantly correlated with a higher PFS. Bound aflibercept clearance was also correlated with a higher PFS (p=0.0048). For OS and PFS, the results of multivariate analyses were consistent with those of the univariate analyses. When adding endogenous VEGF at baseline in the multivariate model (356 patients) the relationship between PK parameters and efficacy endpoints also remained significant, with a hazard ratio estimate in the same range as without VEGF in the model.

With regard to safety and in the VELOUR study, the occurrence of any hypertension during cycles 1 and 2 was significantly correlated with free aflibercept Cmax, AUC of first cycle, AUC extrapolated and cumulative AUC at cycles 1 and 2. The occurrence of haemorrhage at cycles 1 and 2 was significantly correlated with free aflibercept AUC extrapolated and cumulative AUC at cycles 1 and 2 (respective p-values: 0.0577 and 0351). For proteinuria, a significant relationship was found with only one PK parameter: AUCcum at cycles 1 and 2, but with an opposite trend to the one observed for hypertension: an increase of 2000 µg.h/mL corresponded to a 51% decrease (p=0.0006 in the univariate analysis) in the odds of experiencing proteinuria.

In the VANILLA study (metastatic pancreatic cancer), the occurrence of hypertension during cycles 1 and 2 was found to be significantly associated with a decrease in free aflibercept clearance. In the VITAL study (advanced NSCLC), for the occurrence of hypertension during cycles 1 and 2, a significant relationship was shown with free aflibercept Cmax, AUC of first cycle and clearance. Increased free aflibercept Cmax was the only PK parameter significantly associated with the occurrence of any proteinuria grade ≥ 2 during cycles 1 and 2. A trend was observed towards higher incidence of venous thromboembolic event for lower free aflibercept clearance.

2.4.4. Discussion on clinical pharmacology

The analytical assays used to measure free and VEGF-bound aflibercept were considered adequately validated.

Being a high molecular weight protein, aflibercept is expected to be eliminated to a large extent in a predictable manner and no specific studies have been performed to evaluate the aflibercept metabolism, excretion, as well as drug-drug interactions. This was considered acceptable by the CHMP.

The pharmacokinetic properties of aflibercept and the effect of impaired organ functions, gender, race, weight or age on aflibercept pharmacokinetics have to a large extent been derived from a population pharmacokinetic analysis with data from 1507 patients with various types of advanced malignancies.

In preclinical tumour models, biologically active doses of aflibercept correlated with those necessary to produce circulating concentrations of free aflibercept in excess of VEGF bound aflibercept. Circulating concentrations of VEGF bound aflibercept increase with the aflibercept dose until most available VEGF is bound. Further increases in the aflibercept dose resulted in dose-related increases in circulating free aflibercept concentrations but only small further increases in the VEGF bound aflibercept concentration.

In patients, Zaltrap is administered at the dose of 4 mg/kg intravenously every two weeks for which there is an excess of circulating free aflibercept compared to VEGF bound aflibercept.

At the recommended dose regimen of 4 mg/kg every two weeks, concentration of free aflibercept were near steady state levels by the second cycle of treatment with essentially no accumulation (accumulation ratio of 1.2 at steady-state compared to the first administration).

The volume of distribution of free aflibercept at steady state is approximately 8 litres.

No metabolism studies have been conducted with aflibercept since it is a protein. Aflibercept is expected to degrade to small peptides and individual amino acids. Free aflibercept is primarily cleared by binding to endogenous VEGF to form a stable, inactive complex. As with other large proteins, both free and bound aflibercept are expected to be cleared, more slowly, by other biological mechanisms, such as proteolytic catabolism. At doses greater than 2 mg/kg, free aflibercept clearance was approximately 1.0 L/day with a terminal half life of 6 days. High molecular weight proteins are not cleared by the renal route, therefore renal elimination of aflibercept is expected to be minimal.

Consistent with target mediated drug disposition, free aflibercept exhibits a faster (non linear) clearance at doses below 2 mg/kg, likely due to the high affinity binding of aflibercept to endogenous VEGF. Linear clearance observed in the dose range of 2 to 9 mg/kg is likely due to non saturable biological mechanisms of elimination such as protein catabolism.

There was no effect of age on the pharmacokinetics of free aflibercept and no effect of race was identified in the population analysis, but these observations are based on a small number of patients. (see also discussion on clinical safety).

Weight had an effect on free aflibercept clearance and volume of distribution resulting in a 29% increase in aflibercept exposure in patients weighing ≥100 kg. This variability was not considered clinically relevant.

There have been no formal studies with Zaltrap in patients with hepatic impairment. In a population pharmacokinetic analysis with data from 1507 patients with various types of advanced malignancies receiving Zaltrap with or without chemotherapy, 63 patients with mild hepatic impairment (total bilirubin >1.0 x – 1.5 x ULN and any AST) and 5 patients with moderate hepatic impairment (total bilirubin >1.5 x – 3 x ULN and any AST) were treated with Zaltrap. In these mild and moderate hepatic impairment patients, there was no effect on clearance of aflibercept. There are no data available for

patients with severe hepatic impairment (total bilirubin >3 x ULN and any AST). This information is included in section 5.2 of the SmPC (see also discussion on clinical safety).

There have been no formal studies with Zaltrap in patients with renal impairment. A population pharmacokinetic analysis was conducted with data from 1507 patients with various types of advanced malignancies receiving Zaltrap with or without chemotherapy. This population included; 549 patients with mild renal impairment (CLCR between 50 80 ml/min), 96 patients with moderate renal impairment (CLCR between 30 50 ml/min), and 5 patients with severe renal impairment (CLCR <30 ml/min). This population pharmacokinetic analysis revealed no clinically meaningful differences in clearance or systemic exposure (AUC) of free aflibercept in patients with moderate and mild renal impairment at the 4 mg/kg dose of Zaltrap as compared to the overall population studied. No conclusion can be drawn for patients with severe renal impairment due to very limited data available. In the few patients with severe renal impairment, drug exposure was similar to that observed in patients with normal renal function. This information is included in section 5.2 of the SmPC (see also discussion on clinical safety).

Safety in terms of hypertension and venous thrombo-embolic event as wells as efficacy in terms of overall and progression free survival were significantly positively correlated with exposure to free aflibercept. A significant impact of the exposure level of free aflibercept on treatment outcome in terms of OS and PFS is shown with a decrease of the hazard rate by 70.1 % and 66.1 % respectively comparing 5^{th} and 95^{th} percentiles of exposure. This information is included in section 5.2 of the SmPC.

No pharmacokinetic interaction studies were submitted. Population pharmacokinetics analysis and inter study comparisons did not reveal any pharmacokinetic drug drug interaction between aflibercept and the FOLFIRI regimen. This is described in section 4.5 of the SmPC. Considering that aflibercept is a protein and eliminated via protein catabolism, clinically relevant interactions are not expected, and the lack of proper drug-drug interaction studies is acceptable.

2.4.5. Conclusions on clinical pharmacology

The clinical pharmacology of aflibercept in healthy volunteers and patients with metastatic colorectal cancer is considered to be sufficiently characterised. A relationship between free aflibercept exposure and treatment outcome (OS and PFS) as well as AEs (hypertension and venous thrombo-embolic events) was established.

2.5. Clinical efficacy

The efficacy of aflibercept in its proposed indication is based on a single pivotal phase 3 trial (EFC10262- VELOUR) of aflibercept versus placebo in MCRC patients being treated with FOLFIRI after failure of an oxaliplatin based regimen. No formal phase II studies, preceding in development the pivotal trial, were submitted.

2.5.1. Dose response studies

A series of dose finding phase I studies of aflibercept single agent (TED6115 and its extension part TED6116 study) and combined with various standard chemotherapy regimens (TCD6117, TCD6118, TCD6119, TCD6120, TCD6121, TCD10173), including irinotecan plus LV5FU2 (Douillard regimen, TCD6118), were submitted.

TED6115: An open-label, sequential-cohort, dose-escalation, safety, tolerability, and pharmacokinetic study of VEGF Trap (aflibercept) administered intravenously in patients with advanced solid tumours or lymphoma

Evaluated for safety: 47 patients in the IV treatment group.

IV administration: at the following order of dose levels: 0.3, 1.0, 2.0, 4.0, 3.0, 5.0, and 7.0 mg/kg.

Duration of treatment: 1 dose on Day 1 and 1 dose on Day 15 (2 treatment cycles).

Safety results

A total of 47 patients were treated with IV aflibercept ranging from 0.3 to 7.0 mg/kg per a 2-week interval schedule. Aflibercept overall was well tolerated up to 7.0 mg/kg IV. Proteinuria and hypertension are TEAEs of particular interest, as they are known to be a common effect of this class of drugs, ie VEGF blockade. Both of these TEAEs increased in frequency and severity at the 4.0 mg/kg IV dose level and above. Across all IV dose levels, the overall frequency of hypertension was reported in 18 (38.3%) patients and proteinuria in 5 (10.6%) patients.

A total of 6 dose limiting toxicities were reported in 5 IV patients:

- 1.0 mg/kg (N = 7): 1 Grade 3 arthralgia and 1 Grade 3 dysphonia (in the same patient)
- 2.0 mg/kg (N = 6): 1 Grade 3 dyspnea
- 4.0 mg/kg (N = 7): 1 Grade 3 hypertension
- 7.0 mg/kg (N = 13): 1 Grade 2 rectal ulcer and 1 Grade 3 proteinuria

The MTD of IV aflibercept was not formally determined after dosing at the 7.0 mg/kg level.

TCD6118: A Phase 1, dose-escalation, sequential-cohort study of the safety, tolerability, and pharmacokinetics of intravenous AVE0005A (VEGF Trap) in combination with intravenous irinotecan/5-fluorouracil/leucovorin administered every 2 weeks in patients with advanced solid malignancies.

Number of patients: Study Part 1: Enrolled: 48 Treated: 38. Study Part 2: Randomised: 28 Treated: 27. Total treated: 65

Aflibercept dose: 2.0 mg/kg during the initial dosing and escalated to either 3.0 or 4.0 mg/kg, then escalated by 1.0 or 2.0 mg/kg increments until the RPTD was determined. In Part 2 aflibercept was administered at the defined RPTD dose.

Combination therapy: Patients were given aflibercept followed by LV5FU2-CPT11 every 2 weeks in the absence of study withdrawal criteria.

Safety results

Part 1: Overall, 38 patients received 585 cycles of aflibercept across the 4 doses levels: 2, 4, 5 and 6 mg/kg. Out of these, 60.5% had primary colorectal localisation.

There were 2 patients who experienced a DLT (stomatitis, esophagitis) at the 5 mg/kg dose level and 1 patient with 1 DLT (febrile neutropenia) reported at the 6 mg/kg dose level.

Part 2: The 4 mg/kg aflibercept dose was selected for Part 2 of the study. Overall, 27 patients were treated whereof 70.4% with primary colorectal localisation.

All 27 patients experienced at least 1 TEAE while on study treatment. The most frequent clinical TEAEs were asthenia/fatigue, diarrhoea, dysphonia, nausea, anorexia, alopecia, hypertension, stomatitis, vomiting, dyspnoea, epistaxis, and dry skin. Grade 3/4 clinical TEAEs reported most commonly were hypertension, asthenia/fatigue, dyspnea, and anorexia. Proteinuria was observed in 85% of patients

with 34.6% ≥ Grade 2. Hematuria concomitant with proteinuria was observed in 6 patients (23.1%). There was 1 patient with a Grade 3 event of left ventricular dysfunction reported at Cycle 31, and another patient with a recto-vaginal fistula. Five patients were taken off study treatment due to AEs. One death was caused by drug-related cerebral haemorrhage and 1 by cardiac insufficiency.

Efficacy results

Seven patients out of 42 with MCRC showed objective PRs and 27 had an SD as best response category (ie, 81% of disease control).

2.5.2. Main study

EFC10262 (VELOUR)

Methods

This was a randomised, double-blind study, comparing the efficacy of aflibercept versus placebo in patients with metastatic Colorectal Cancer (MCRC) treated with the irinotecan/5-fluorouracil/folinic acid combination (FOLFIRI) after failure of an oxaliplatin based regimen.

The study was conducted in 176 centres in 28 countries; participating sites were from Western and Eastern Europe, North and South America, Australia, South Africa and in South Korea.

Study Participants

Inclusion criteria

Patients meeting all of the following criteria were to be considered for enrolment into the study:

- Histologically or cytologically proven adenocarcinoma of the colon or rectum
- Metastatic disease that was not amenable to potentially curative treatment (ie, inoperable)
- Measurable or non-measurable disease (as per RECIST).
- One and only one prior chemotherapeutic regimen for metastatic disease. This prior chemotherapy had to be an oxaliplatin containing regimen. Patients had to have progressed during or following the last administration of the oxaliplatin based chemotherapy. Patients who relapsed within 6 month of completion of oxaliplatin-based adjuvant chemotherapy were eligible.

Key exclusion criteria

- Prior therapy with irinotecan.
- Less than 28 days elapsed from prior radiotherapy, from prior surgery and prior chemotherapy to the time of randomization.
- Age <18 years.
- ECOG PS >2.
- History of brain metastases, uncontrolled spinal cord compression, or carcinomatous meningitis or new evidence of brain or leptomeningeal disease.
- Presence of anti-VEGF class related events: Proteinuria, uncontrolled hypertension, uncontrolled thromboembolic events within 3 months, deep vein thrombosis within 4 weeks, coagulopathy, non-healing wounds.

- Conditions contraindicating FOLFIRI treatment: Dihydropyrimidine dehydrogenase deficiency, uncontrolled small bowel or colonic disorders, enteropathy, chronic diarrhoea, bowel obstruction, known Gilbert's syndrome.
- Inadequate bone marrow function: ANC <1.5 x 10⁹/L, platelet count <100 x 10⁹/L, hemoglobin <9.0 g/dL
- Serum creatinine >1.5 x upper limit of normal (ULN). If creatinine 1.0-1.5 x ULN, creatinine clearance, calculated according to Cockroft-Gault formula, <60 ml/min excluded the patient.
- Inadequate liver function tests: Total bilirubin >1.5 x ULN, transaminases >3 x ULN (unless liver metastasis present, 5 x ULN in that case). Alkaline phosphatase >3 x ULN (unless liver metastasis present, 5 x ULN in that case).

Treatments

Study treatment administration had to start within 3 days of randomisation.

- Arm A, aflibercept: 4 mg/kg administered IV over 1 hour on Day 1, every 2 weeks.
- Arm B, placebo: 4 mg/kg administered IV over 1 hour on Day 1, every 2 weeks

Immediately after aflibercept/placebo administration, all the patients were to receive the FOLFIRI regimen, administered as follows:

- Irinotecan 180 mg/m2 IV infusion in 500 mL D5W over 90 minutes and dl leucovorin 400 mg/m² IV infusion over 2 hours (leucovorin expressed in dl racemic, when the l-isomer form is used the dose should be divided by 2, ie, 200 mg/m²), at the same time, in bags using a Y-line, followed by:
- 5-FU 400 mg/m² IV bolus given over 2-4 minutes, followed by:
- 5-FU 2400 mg/m² continuous IV infusion in 500 mL D5W (recommended) over 46-hours.

In case BSA >2.0 m², the actual doses of irinotecan and 5-FU were to be adjusted to a maximum BSA of 2.0 m², for safety reasons.

Objectives

The primary objective of the study was to demonstrate improvement in OS with aflibercept by comparison to placebo in patients with colorectal cancer treated with FOLFIRI as second line treatment for metastatic disease.

Secondary objectives included: to compare PFS in the two treatment arms; to evaluate overall RR, as per response evaluation criteria in solid tumours (RECIST), in the two treatment arms; to evaluate the safety profile in the two treatment arms; to assess immunogenicity of intravenous aflibercept; to assess pharmacokinetics of IV aflibercept and perform population PK evaluation.

Outcomes/endpoints

In terms of efficacy, the primary endpoint was Overall Survival (OS). Progression-free Survival (PFS) as assessed by an Independent Review Committee (IRC) was a secondary endpoint and it was to be considered as co-primary endpoint, if a statistically significant difference for OS failed to be observed (see statistical methods). Since IRC review was not in place at the start of the study, investigator assessment was used for patients who died prior to the implementation of Amendment 2 of the study protocol (see Conduct of the study below) and for those patients who did not consent to third party

review. If death or progression was not observed, the patient was censored at the date of last valid tumour assessment without evidence of progression or at the study cut-off date, whichever came first, regardless of initiation of further anti-tumour therapies.

Response Rate (RR) based on IRC assessment was to be testedonly if OS or PFS tested positive.

Sample size

For the primary endpoint of OS, the expected median survival time in the control arm (FOLFIRI + placebo) was 11 months. A 20% risk reduction in aflibercept + FOLFIRI arm compared to FOLFIRI + placebo arm was expected (HR of 0.80, corresponding to a median OS improvement from 11 months in the control arm to 13.75 months in the test arm). Assuming that survival times would be exponentially distributed in both treatment arms, a total of 863 deaths was required to detect with 90% power a 20% risk reduction in the aflibercept arm relative to the placebo arm, using a two sided log-rank test at a significance level of 0.0499.

Based on an anticipated accrual period of 30 months followed by 9 months of follow-up after the randomisation of the last patient, a total of 1200 patients (600 in each arm) were required to achieve the targeted number of events.

Randomisation

Eligible patients were randomly assigned to either the control arm or the experimental arm in a 1:1 ratio. Randomisation was stratified according to baseline Eastern cooperative oncology group performance status ([ECOG PS] 0 versus 1 versus 2) and prior bevacizumab (yes versus no). Patients who, at the time of randomisation, were on the follow-up phase of a double-blind controlled study with bevacizumab (either bevacizumab versus placebo or bevacizumab versus another biologic agent) while that study was still blinded, could still be randomised in the present study. In such cases, stratification for prior bevacizumab was to be ‘yes’.

Blinding (masking)

This was a double-blind study.

Statistical methods

As per initial protocol, one formal interim analysis was planned when 561 death events (65% information fraction) had occurred, using a two-sided nominal significance level of 0.0107 based on an O’Brien-Fleming alpha spending function. An early stopping of the study for efficacy was to be considered if the O’Brien Fleming efficacy boundary was met. The final PFS analysis was performed at the time of that interim OS analysis.

Upon request of the Data Monitoring Committee (DMC), an additional interim analysis of OS was performed to provide an early evaluation of the benefit-risk ratio, when 315 death events (36.5% information fraction) had occurred. A futility boundary was planned for that analysis, and in order to maintain the integrity of the trial (penalty for type-I error), a stopping boundary for possible overwhelming efficacy was also planned using the O’Brien-Fleming alpha spending function. The two-sided nominal significance level for efficacy at this interim analysis was 0.00042.

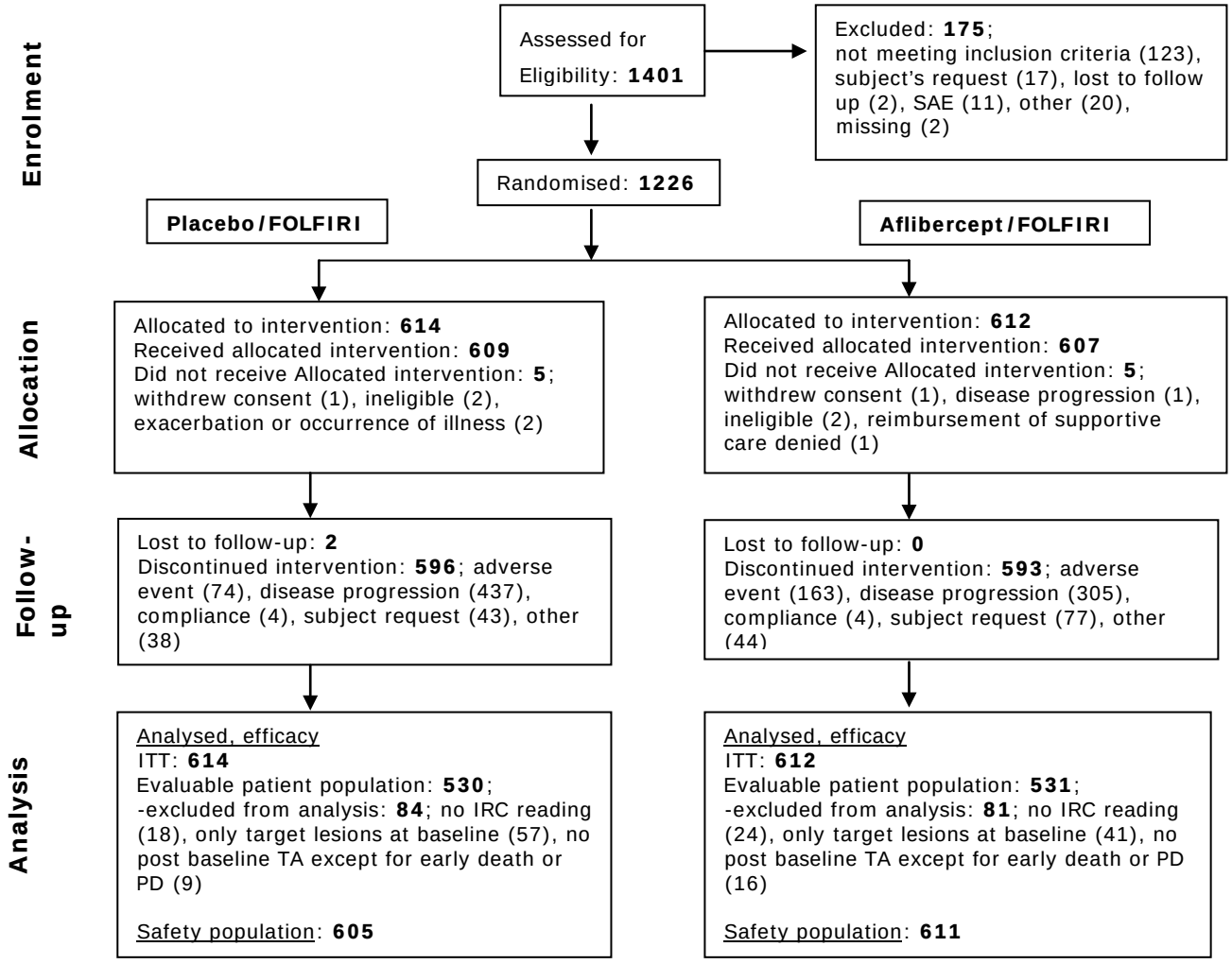
If the O'Brien-Fleming boundary was crossed by OS at the second interim (ie, p-value of OS was <0.0107), the study could be stopped; otherwise, the PFS would be used as a co-primary endpoint while the study would continue for OS. In that context, the alpha level was split between OS and PFS to adequately control the overall type I error rate. PFS was tested at a 2-sided 0.0001 level and the

overall alpha level for OS was at a 2-sided 0.0499 level. Response rate was to be tested only after either OS or PFS was tested positive.

Using a group sequential approach with an O'Brien Fleming Alpha-spending function and an overall two-sided α level of 0.0499, the two-sided nominal significance level to be used at the final analysis of OS was 0.0466.

Results

Participant flow



Recruitment

The first patient was enrolled in the study on 19 November 2007 and the last patient was enrolled on 16 March 2010. The date for the data cut-off was 7 February 2011, upon occurrence of the 863rd patient's dearth on study and final planned OS data analysis.

Conduct of the study

The original protocol was subject to 5 amendments whereof the first was dated before start of enrolment. In the first amendment, in order to balance the number of patients with ECOG performance status equal to 0, 1 and 2, across treatment arms, stratification at randomisation for PS was changed

from 0-1 versus 2 to 0 versus 1 versus 2. Moreover, response rate (RR) was added as secondary efficacy endpoint. With the 2nd amendment, patients that were on the follow up phase of double blind trials with bevacizumab in first line metastatic colorectal cancer were allowed to participate in the study while the previous study was still blinded. An independent imaging third party review was set up. In the 3rd amendment, a supplemental analysis for an early evaluation of the benefit to risk ratio requested by the DMC was introduced, including an analysis of OS and a descriptive analysis of the secondary endpoint, PFS, as assessed by the investigators, to be performed when 315 death events had occurred. Furthermore, a change in the protocol was implemented, in which the disease progression event in the primary analysis of PFS was to be based on assessment of radiological tumour progression by the IRC. With amendment 4, use of G-CSF as necessary was recommended following DMC review of unblinded data and with amendment 5 the duration of study participation was extended for approximately 9 months beyond the cut-off data for the primary OS analysis.

Overall, 16 patients had one important selection criteria deviation: 10 patients (1.6%) in the placebo arm and 6 patients (1.0%) in the aflibercept arm. These patients were not excluded from the ITT population or the evaluable population (EP). The most common reason was relapse more than 6 months after completion of adjuvant chemotherapy, 5 patients in the placebo arm and 2 patients in the aflibercept arm.

Seven patients received the wrong study medication: Four patients who were randomised to the placebo arm received at least one infusion of aflibercept and were therefore included in the aflibercept arm for the analysis of safety, and three patients who were randomised to the aflibercept arm received at least one infusion of placebo without impact on their treatment group for safety analysis.

Baseline data

Baseline demographic and disease characteristics are presented in the following Tables 12-16.

Table 12: Patient demographics and patient characteristics at baseline, ITT population

	Placebo/Folfiri (N=614)	Aflibercept/Folfiri (N=612)	All (N=1226)
Gender [n(%)]			
Number	614	612	1226
Male	353 (57.5%)	365 (59.6%)	718 (58.6%)
Female	261 (42.5%)	247 (40.4%)	508 (41.4%)
Age (Years)			
Number	614	612	1226
Median	61.0	61.0	61.0
Mean (SD)	60.2 (10.8)	59.5 (10.5)	59.8 (10.7)
Min : Max	19 : 86	21 : 82	19 : 86
Age class [n(%)]			
Number	614	612	1226
<65	376 (61.2%)	407 (66.5%)	783 (63.9%)
≥65 but <75	199 (32.4%)	172 (28.1%)	371 (30.3%)
≥75	39 (6.4%)	33 (5.4%)	72 (5.9%)
Race [n(%)]			
Number	614	612	1226
Caucasian/White	523 (85.2%)	548 (89.5%)	1071 (87.4%)
Black	27 (4.4%)	16 (2.6%)	43 (3.5%)
Asian/Oriental	51 (8.3%)	35 (5.7%)	86 (7.0%)
Other	13 (2.1%)	13 (2.1%)	26 (2.1%)
Region			
Number	614	612	1226
Western Europe	217 (35.3%)	208 (34.0%)	425 (34.7%)
Eastern Europe	136 (22.1%)	161 (26.3%)	297 (24.2%)
North America	75 (12.2%)	63 (10.3%)	138 (11.3%)
South America	56 (9.1%)	62 (10.1%)	118 (9.6%)
Other countries	130 (21.2%)	118 (19.3%)	248 (20.2%)

Note: Other countries = Australia, New Zeland, South Africa and Korea

Table 13: Summary of patients randomised by level of stratification factor (as per eCRF), ITT population

	Placebo/Folfiri (N=614)	Aflibercept/Folfiri (N=612)	All (N=1226)
ECOG PS [n(%)]			
0	354 (57.7%)	350 (57.2%)	704 (57.4%)
1	248 (40.4%)	249 (40.7%)	497 (40.5%)
2	12 (2.0%)	13 (2.1%)	25 (2.0%)
Prior Bevacizumab [n(%)]			
Yes	177 (28.8%)	169 (27.6%)	346 (28.2%)
No	437 (71.2%)	443 (72.4%)	880 (71.8%)

Table 14: Disease characteristics at initial diagnosis, ITT population

	Placebo/Folfiri (N=614)	Aflibercept/Folfiri (N=612)	All (N=1226)
Primary site [n(%)]			
Number	614	612	1226
Colon	302 (49.2%)	289 (47.2%)	591 (48.2%)
Recto sigmoid	136 (22.1%)	123 (20.1%)	259 (21.1%)
Rectum	174 (28.3%)	197 (32.2%)	371 (30.3%)
Other	2 (0.3%)	3 (0.5%)	5 (0.4%)
- cea & ck20 postive - presumed colorectal primary	1 (0.2%)	0	1 (<0.1%)
- Appendix	0	1 (0.2%)	1 (<0.1%)
- Colon plus appendix	0	1 (0.2%)	1 (<0.1%)
- Presumed colorectal,cea positive and history of colon cancer>20 years ago	0	1 (0.2%)	1 (<0.1%)
- Synchronous primary, cecum and rectum	1 (0.2%)	0	1 (<0.1%)
Histology type [n(%)]			
Number	614	612	1226
Adenocarcinoma	614 (100%)	612 (100%)	1226 (100%)
Time from 1st diagnosis to randomization (months) [n(%)]*			
Number	614	611	1225
Mean (SD)	20.88 (21.10)	20.98 (24.08)	20.93 (22.62)
Median	13.67	14.62	14.26
Min : Max	2.4 : 214.7	2.1 : 325.1	2.1 : 325.1

*If the day of initial date of diagnosis is missing, it is considered as the first day of the month

Table 15: Prior chemotherapies, ITT population

	Placebo/Folfiri (N=614)	Aflibercept/Folfiri (N=612)	All (N=1226)
Prior chemotherapy [n(%)]			
Number	614	612	1226
Yes	614 (100%)	612 (100%)	1226 (100%)
Neoadjuvant/Adjuvant only	64 (10.4%)	60 (9.8%)	124 (10.1%)
Advanced only	442 (72.0%)	450 (73.5%)	892 (72.8%)
Neoadjuvant/Adjuvant + Advanced	108 (17.6%)	102 (16.7%)	210 (17.1%)
Patients entering the study directly from neoadjuvant/adjuvant [a]	64	60	124
With Bevacizumab	9 (14.1%)	8 (13.3%)	17 (13.7%)
Patients with advanced chemotherapy only [a]	442	450	892
With Bevacizumab	141 (31.9%)	145 (32.2%)	286 (32.1%)
Patients with neoadjuvant/adjuvant followed by advanced chemotherapy [a]	108	102	210
With Bevacizumab	26 (24.1%)	15 (14.7%)	41 (19.5%)

Table 16: Prior anti-hypertensive medications for patients with history of hypertension, Safety Population

	Placebo / Folfiri (N=605)	Aflibercept / Folfiri (N=611)	All (N=1216)
Patients with history of hypertension	265 (43.8%)	262 (42.9%)	527 (43.3%)
Patients with prior anti-hypertensive medication	240/265 (90.6%)	240/262 (91.6%)	480/527 (91.1%)

Numbers analysed

The number of patients in each arm included in the efficacy analyses is reported in Table 17.

Table 17: Analysis populations

	Placebo/Folfiri	Aflibercept/Folfiri	All
Randomized population	614 (100%)	612 (100%)	1226 (100%)
Efficacy populations			
Intent-to Treat (ITT)	614 (100%)	612 (100%)	1226 (100%)
Evaluable Patient population[a]	530 (86.3%)	531 (86.8%)	1061 (86.5%)
Safety population	605	611	1216

[a]: For response rate only

Note: For the safety population, patients are tabulated according to treatment actually received. Patients who received at least one dose of [illegible] are tabulated in the [illegible] group. For efficacy populations, patients are tabulated according to their randomized treatment.

The ITT population was the primary population for analysis of efficacy parameters, with the exception of response rate for which the EP population was used.

Outcomes and estimation

Primary endpoint

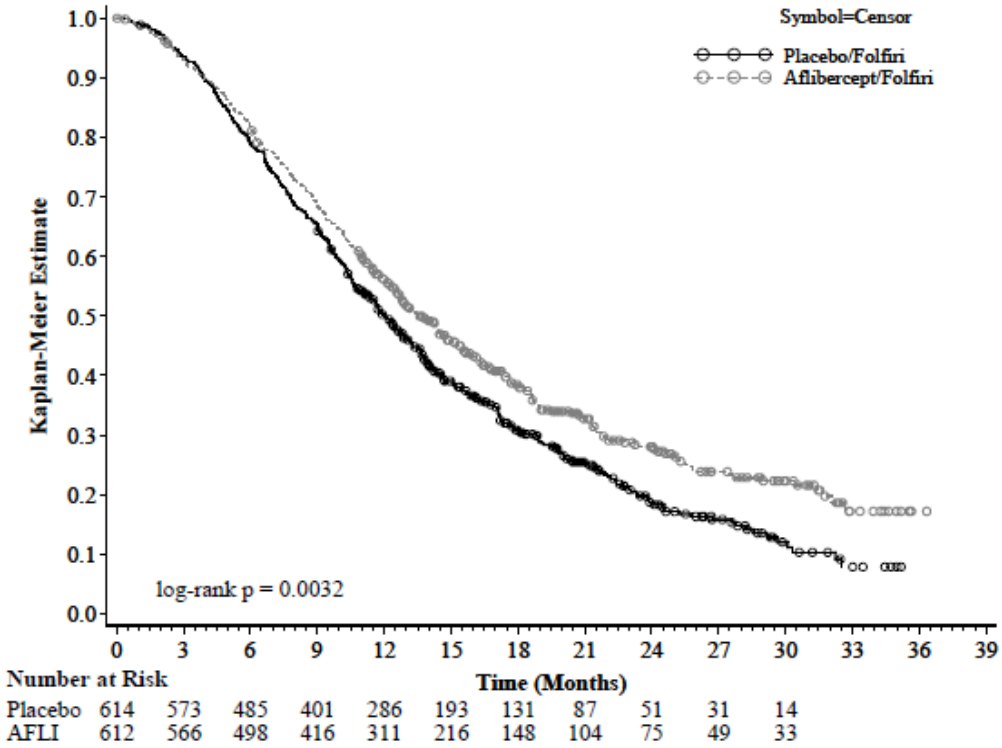
Data on the primary endpoint of OS at the cut-off date for the final OS analysis (7 February 2011) are summarised in the following Table 18 and Figure 3.

Table 18: KM survival estimates by treatment group, stratified according to stratification factors at randomisation (IVRS), ITT population

Time to Event or Censoring	Placebo/Folfiri (N=614)	Aflibercept/Folfiri (N=612)
Overall		
Number of death events, n/N(%)	460/614 (74.9%)	403/612 (65.8%)
25% quantile overall survival (95.34% CI) (months)	6.83 (6.144 to 7.589)	7.62 (6.571 to 8.476)
Median overall survival (95.34% CI) (months)	**12.06 (11.072 to 13.109)**	**13.50 (12.517 to 14.949)**
75% quantile overall survival (95.34% CI) (months)	21.03 (18.924 to 22.801)	25.59 (22.012 to 31.704)
Survival probability (95.34% CI)		
6 months	0.791 (0.759 to 0.824)	0.819 (0.788 to 0.850)
12 months	0.503 (0.462 to 0.543)	0.561 (0.521 to 0.602)
24 months	0.187 (0.149 to 0.225)	0.280 (0.237 to 0.324)
Stratified Log-Rank test p-value[a]		
vs Placebo/Folfiri	-	**0.0032**
Stratified Hazard ratio (95.34% CI)[a]		
vs Placebo/Folfiri	-	**0.817 (0.713 to 0.937)**

[a]: Stratified on ECOG Performance Status (0 vs 1 vs 2) and Prior Bevacizumab (yes vs no) according to IVRS . Significance threshold is set to 0.0466 using the O'Brien-Fleming alpha spending function.

Figure 3: KM curves by treatment group, ITT population



The unadjusted HR for the comparison of median survival times by unstratified logrank test was 0.809 (95.34% CI: 0.706 to 0.927), p = 0.0019.

Information on patients without an event at the time of the data cut-off is presented in Table 19.

Table 19: Summary of patients censored, ITT population

	Placebo/Folfiri (N=614)	Aflibercept/Folfiri (N=612)
Number of patients without event [n(%)]	154 (25.1%)	209 (34.2%)
Reason of censoring		
Alive at the cut off date	149 (24.3%)	201 (32.8%)
Alive at the last contact before cut off date	0	6 (1.0%)
Lost to follow-up	5 (0.8%)	2 (0.3%)
Time from last contact to cutoff date (months)		
Number	5	8
Median	8.44	17.28
Mean (SD)	10.51 (7.35)	17.10 (7.38)
Min : Max	3.8 : 22.1	5.4 : 26.8
Time from last contact to cutoff date (class)		
Number	5	8
> 2 months	5 (100%)	8 (100%)

Secondary endpoints

For 42 patients (26 in the aflibercept arm, 16 in the placebo arm) who died prior to the implementation of IRC review or who refused consent for this review, the investigator's tumour assessment was used. The final analysis of PFS was performed at the time of the second interim analysis of OS (cut-off date:

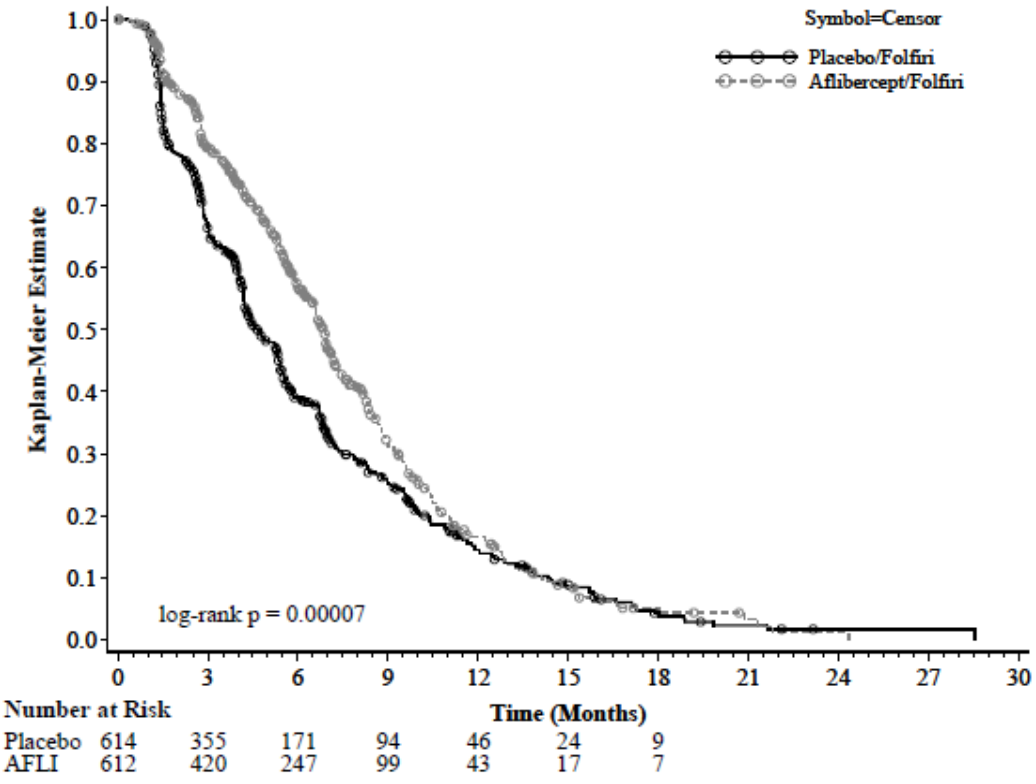
6 May 2010) and was conducted in the ITT population. Results are presented in the following Table 20 and Figure 4.

Table 20: PFS according to IRC (months) stratified according to IVRS, ITT population

Time to Event or Censoring	Placebo/Folfiri (N=614)	Aflibercept/Folfiri (N=612)
Overall		
Number of events, n/N(%)	454/614 (73.9%)	393/612 (64.2%)
Median PFS (99.99% CI) (months)	4.67 (4.074 to 5.552)	6.90 (5.881 to 7.852)
Type of event		
Documented disease progression	371 (81.7%)	292 (74.3%)
Death without disease progression	83 (18.3%)	101 (25.7%)
Time from last TA* to death date (months)		
Number	83	101
Median	3.35	2.04
Probability of surviving (99.99% CI)		
6 months	0.390 (0.306 to 0.475)	0.573 (0.488 to 0.659)
12 months	0.146 (0.076 to 0.216)	0.166 (0.085 to 0.246)
18 months	0.043 (0.000 to 0.091)	0.051 (0.000 to 0.108)
Stratified Log-Rank test p-value[a]		
vs Placebo/Folfiri	-	**0.00007**
Stratified Hazard ratio (99.99% CI)[a]		
vs Placebo/Folfiri	-	**0.758 (0.578 to 0.995)**

[a]: Stratified on ECOG Performance Status (0 vs 1 vs 2) and Prior Bevacizumab (yes vs no) according to IVRS
Significance threshold is set to 0.0001.

Figure 4: PFS according to IRC (months) – ITT population



Evaluation of response rate was conducted in the evaluable patient population and based on assessment by IRC. Overall, 165 patients were excluded from the EP population.

Table 21: Reasons for exclusion from evaluable population for RR, ITT population

	Placebo/Folfiri (N=614)	Aflibercept/Folfiri (N=612)
Any reason	84 (13.7%)	81 (13.2%)
Reason for exclusion from evaluable population		
No IRC reading	18 (2.9%)	24 (3.9%)
Only non target lesions at baseline*	57 (9.3%)	41 (6.7%)
No post baseline TA except for early death or PD*	9 (1.5%)	16 (2.6%)

*among patients read by the IRC

RR results are presented in the following Table 22.

Table 22: Overall objective response rate, evaluable patient population for response rate

	Placebo/Folfiri (N=530)	Aflibercept/Folfiri (N=531)
Best Overall Response [n(%)]		
Complete response	2 (0.4%)	0
Partial response	57 (10.8%)	105 (19.8%)
Stable disease	344 (64.9%)	350 (65.9%)
Progressive disease	114 (21.5%)	55 (10.4%)
Not evaluable	13 (2.5%)	21 (4.0%)
Overall Response		
Responders (Complete response or Partial response)	59 (11.1%)	105 (19.8%)
95% CI[a]	8.5% to 13.8%	16.4% to 23.2%
Stratified Cochran-Mantel-Haenszel test p-value[b]		
Vs Placebo/Folfiri	-	0.0001

Ancillary analyses

Subgroup OS analyses by stratification factors as 'per IVRS' are presented in the following Figures 5 and 6.

Figure 5: Subgroup analyses by stratification factors as per IVRS, ITT population

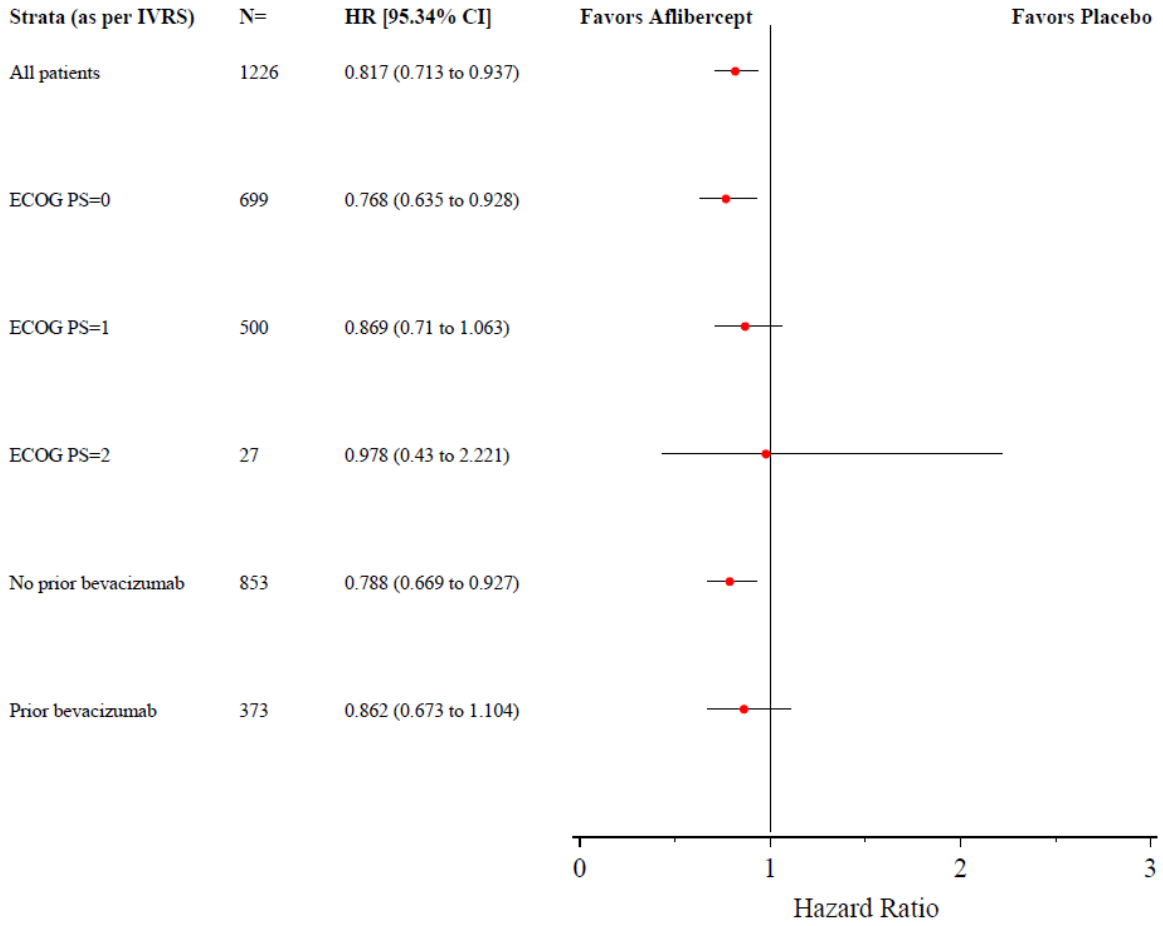
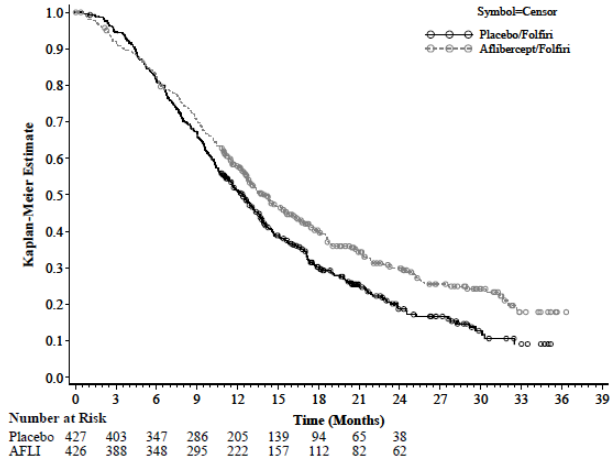
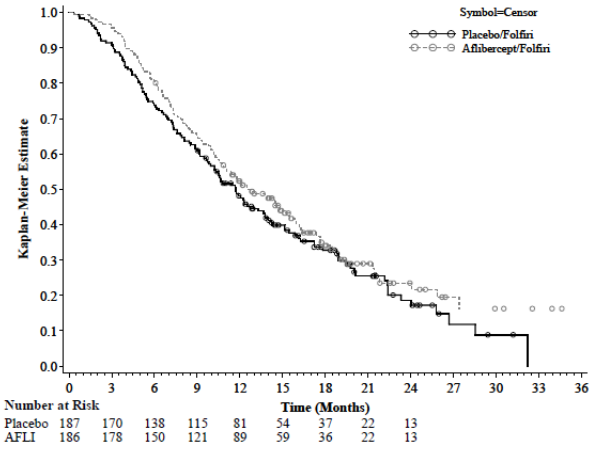


Figure 6: Subgroup analyses by prior bevacizumab treatment as per IVRS, ITT population

No prior bevacizumab



Prior bevacizumab



Post-hoc analyses excluding patients who progressed during or within 6 months of adjuvant therapy for patients with or without prior bevacizumab treatment are summarised in Table 23.

Table 23: Post-hoc analyses excluding adjuvant patients[a,b]

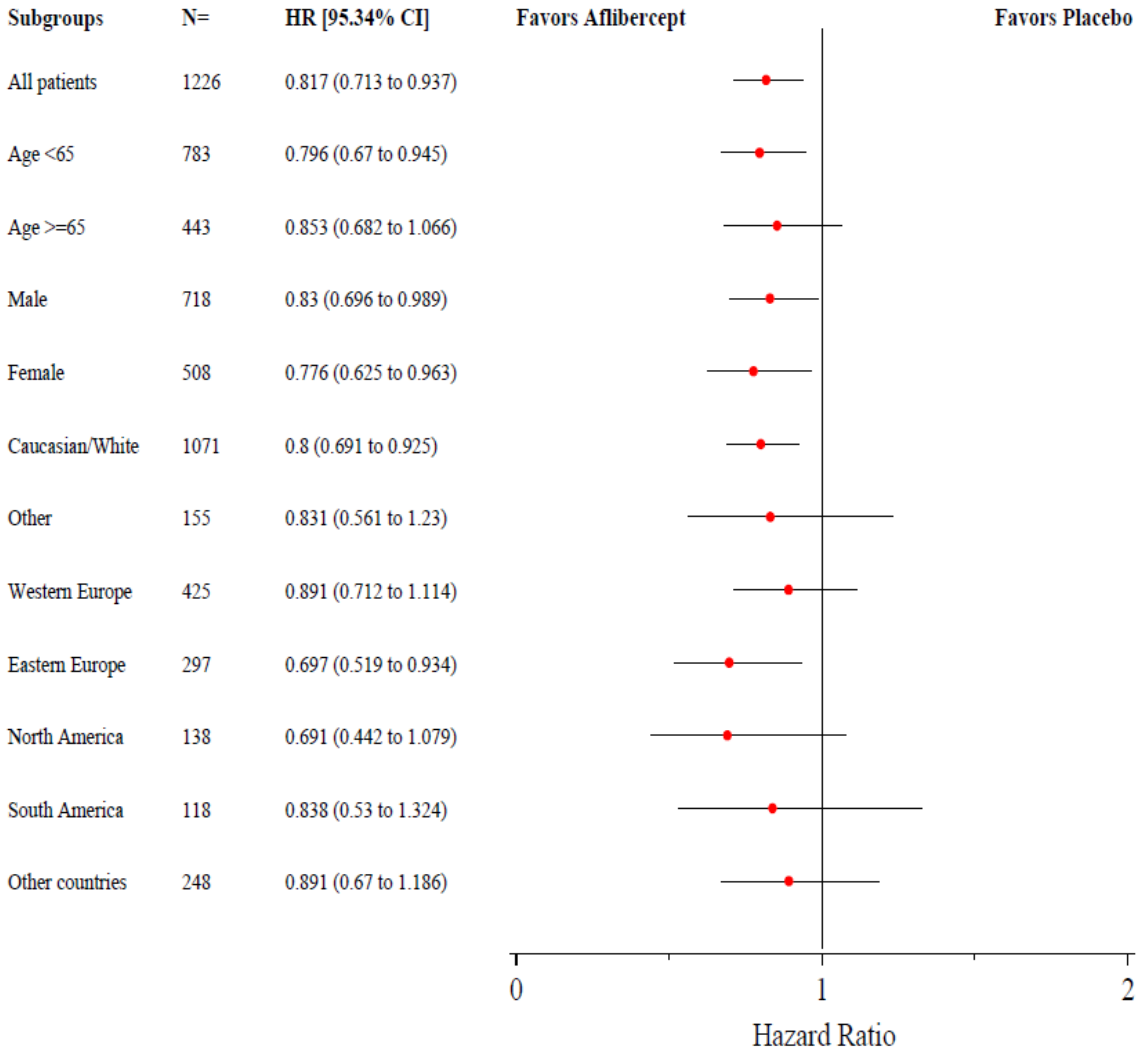
	Placebo/FOLFIRI (N=550)	Zaltrap/FOLFIRI (N=552)
Patients with prior bevacizumab excluding adjuvant only (n (%))	179 (32.5%)	177 (32.1%)
Median overall survival (95% CI) (months)	11.7 (9.66 to 13.27)	13.8 (11.01 to 15.87)
Hazard ratio (95% CI)	0.812 (0.634 to 1.042)	
Median PFS (95% CI) (months)	3.9 (3.02 to 4.30)	6.7 (5.72 to 8.21)
Hazard ratio (95% CI)	0.645 (0.498 to 0.835)	
Patients with no prior bevacizumab excluding adjuvant only (n (%))	371 (67.5%)	375 (67.9%)
Median overall survival (95% CI) (months)	12.4 (11.17 to 13.54)	13.7 (12.71 to 16.03)
Hazard ratio (95% CI)	0.766 (0.645 to 0.908)	
Median PFS (95% CI) (months)	5.3 (4.50 to 5.55)	6.9 (6.24 to 7.20)
Hazard ratio (95% CI)	0.777 (0.655 to 0.921)	

[a] As determined per IVRS

[b] Overall survival in ITT population excluding patients who progressed during or within 6 months of adjuvant therapy demonstrated an HR (95% CI) of 0.78 (0.68 to 0.90) [median OS (95% CI) with Placebo/FOLFIRI 11.9 months (10.88 to 13.01) and with Zaltrap/FOLFIRI 13.8 months (12.68 to 15.44)]

Subgroup analyses by demographic factors are presented in the following Figure 7.

Figure 7: Subgroup analyses by demographic factors, ITT population



Subgroup analyses by prior hypertension are presented in the following Figures 8 and 9.

Figure 8: Subgroup analyses by prior hypertension, ITT population

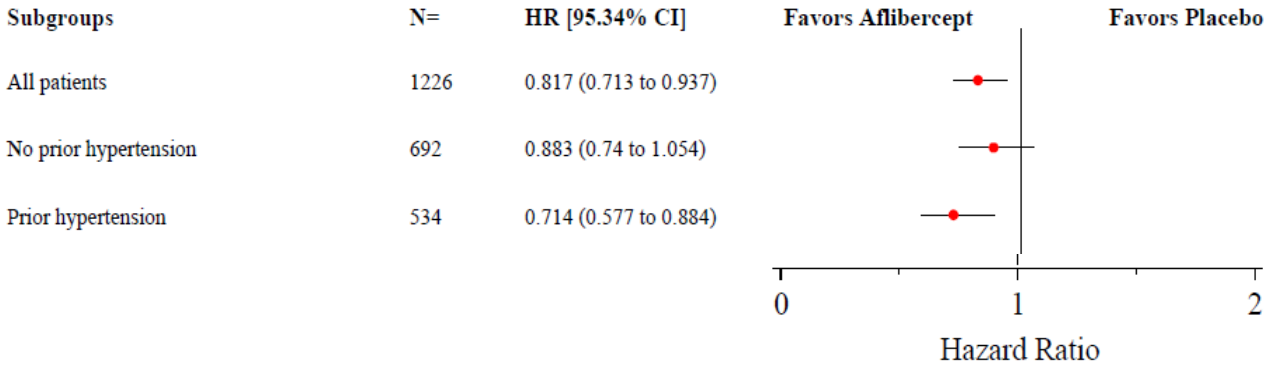
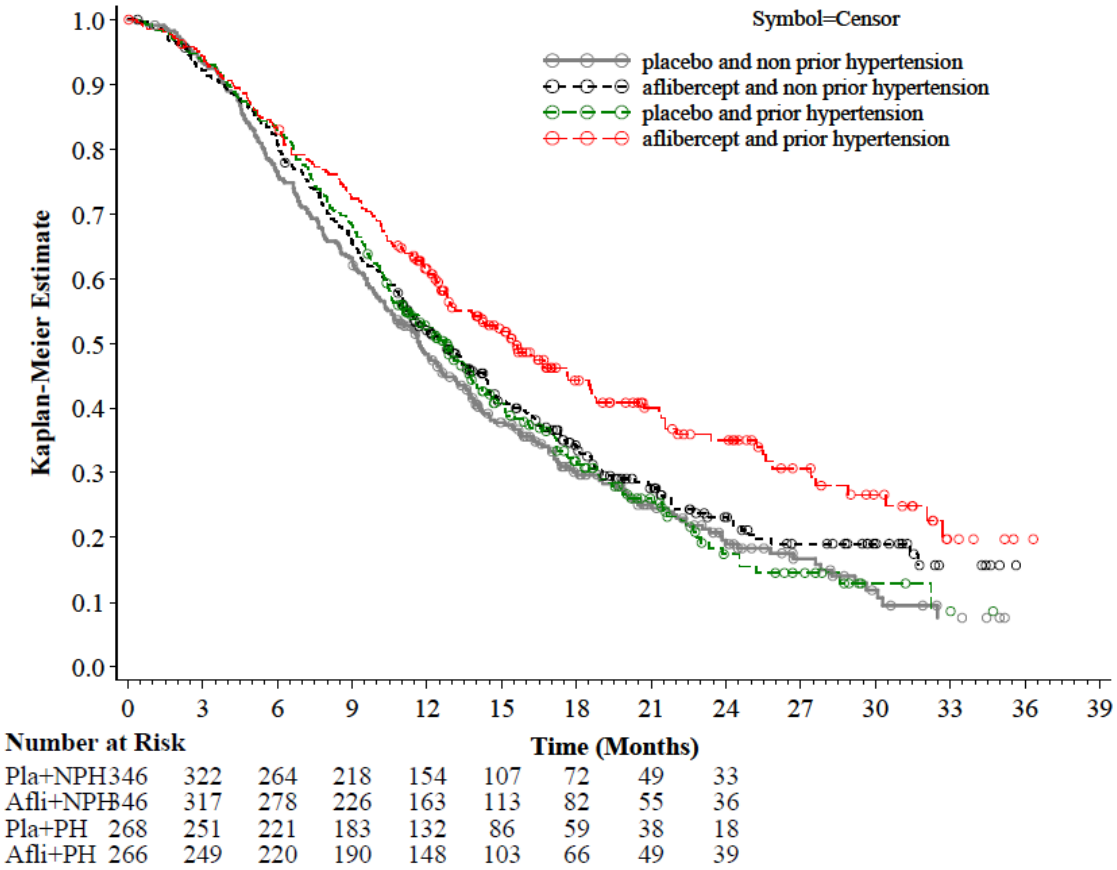


Figure 9: OS by hypertension at baseline – ITT population



Summary of main study

The following table summarises the efficacy results from the main studies supporting the present application. These summaries should be read in conjunction with the discussion on clinical efficacy as well as the benefit risk assessment (see later sections).

Table 24: Summary of Efficacy for trial EFC10262-VELOUR

Title: A Multinational, Randomised, Double-blind Study, Comparing the Efficacy of Aflibercept Once Every 2 Weeks versus Placebo in Patients with Metastatic Colorectal Cancer (MCRC) Treated with Irinotecan/5-FU Combination (FOLFIRI) after Failure of an oxaliplatin based regimen			
Study identifier	EFC10262		
Design	Prospective, multinational, phase 3 randomised, double-blind, placebo controlled study		
	Duration of main phase: Duration of Run-in phase: Duration of Extension phase:		until PD, unacceptable toxicity, patient's refusal, or investigator's decision not applicable not applicable
Hypothesis	Superiority		
Treatments groups	Placebo/FOLFIRI		matching placebo + dl-LV 400 mg/m² over 2 hours IV infusion and irinotecan 180 mg/m² over 90-minute infusion via a Y-connector followed by bolus 5-FU 400 mg/m² and 5-FU C.I. 2400 mg/m² over 46 hours infusion, every 2 weeks: 614 patients randomised
	Aflibercept/FOLFIRI		Aflibercept 4 mg/kg + dl-LV 400 mg/m² over 2 hours IV infusion and irinotecan 180 mg/m² over 90-minute infusion via a Y-connector followed by bolus 5-FU 400 mg/m² and 5-FU C.I. 2400 mg/m² over 46 hours infusion, every 2 weeks: 612 patients randomised
Endpoints and definitions	Primary endpoint	OS	Time interval from the date of randomization to the date of death, due to any cause
	Secondary endpoint	PFS	Time interval from the date of randomization to the date of first observation of disease progression or the date of death (due to any cause)
	Secondary endpoint	ORR	Percent of patients achieving a confirmed CR or confirmed PR according to RECIST (EP population)
Database lock	Cutoff date 7 February 2011		

Results and Analysis

Analysis description	**Primary Analysis**		
Analysis population and time point description	Intent to treat (863rd patient death)		
Descriptive statistics and estimate variability	Treatment group	Placebo/FOLFIRI	Aflibercept/FOLFIRI
	Number of subjects	614	612
	OS (median, in months)	12.1	13.5
	95.34% CI	11.07-13.11	12.52-14.95
	PFS (median, in months)	4.67	6.90
	99.99% CI	4.07-5.55	5.88-7.85

	ORR (by RECIST) [number of patients (%)]	59 (11.1)	105 (19.8)
	95% CI	8.5%-13.8%	16.4%- 23.2%
Effect estimate per comparison	Primary endpoint (OS)	Comparison groups	aflibercept vs placebo
		Stratified Hazard Ratio	0.817
		95.34% CI	0.713-0.937
		Stratified p-value (Log-Rank Test)	0.0032
	Secondary endpoint (PFS)	Comparison groups	aflibercept vs placebo
		Stratified Hazard Ratio	0.758
		99.99% CI	0.578-0.995
		Stratified p-value (Log-Rank Test)	0.00007
	Secondary Endpoint (ORR)	Comparison groups	aflibercept vs placebo
		Stratified p-value (Cochran-Mantel-Haenszel test)	0.0001

Analysis performed across trials (pooled analyses and meta-analysis)

Not applicable

Clinical studies in special populations

No studies in special populations were submitted, but subgroup analyses in the pivotal study focused on efficacy according to gender or patient age (see Figure 7). Few non-caucasian patients and limited patients with moderate or severe renal or hepatic impairment were included in the pivotal trial, but no relevant differences in efficacy were noted.

Supportive studies

Not applicable

2.5.3. Discussion on clinical efficacy

Design and conduct of clinical studies

Data from a single placebo-controlled phase III pivotal study, EFC10262 or VELOUR, are presented. In 2007, when the pivotal study was being designed, the utility of the anti-VEGF agent, bevacizumab, had already been demonstrated in first-line therapy, with significant prolongation of survival observed when bevacizumab was added to irinotecan-based treatment (IFL). In patients with previously treated MCRC, a survival benefit of bevacizumab had been demonstrated with oxaliplatin-based treatment (FOLFOX). However, at that time, no large, randomised, controlled study had demonstrated a survival benefit with the use of an antiangiogenic agent, or with other targeted agents in combination with an irinotecan-based regimen, which had evolved to be the backbone chemotherapy for the second-line setting.

The rationale for choice of dose and concomitant therapies is accepted. The results are considered valid for the EU. No protocol amendments or violations are identified that would substantially impact

on the efficacy analyses and conclusions. Thus, no major concerns regarding the conduct of the study are noted.

Efficacy data and additional analyses

For the primary efficacy endpoint, OS, the analysis was based on reasonably mature data and performed after a median follow-up time of 22.3 months. A statistically significant difference was observed between treatment groups in terms of the primary efficacy endpoint OS. The difference in median OS was 1.44 months in favour of the aflibercept arm. The sensitivity analyses exploring the effect in relevant subgroups were in support of the primary analysis.

No baseline patient or disease characteristics significantly predictive of response to the addition of aflibercept to FOLFIRI have been identified so far. A numerically less beneficial effect on OS within the aflibercept arm was seen for patients previously treated with bevacizumab. However, no significant treatment interaction at the 10% level was noted in the subgroup analyses. Moreover, the study was not powered to demonstrate superiority of aflibercept over placebo in any particular subgroup so that any apparent lower OS benefit, e.g. for patients who received prior bevacizumab, could be due to random variation. Relevant information has been included in section 5.1 of the SmPC.

PFS according to independent review was a secondary endpoint, performed at the second interim analysis. Median PFS was 2.23 months significantly longer in the aflibercept arm than in the placebo arm, HR 0.758. The sensitivity analyses performed are considered to be in support of the primary analysis, although the difference between study arms was not formally statistically significant (p=0.0017) in the investigators' assessment-based analysis.

Subgroup analyses for overall survival and progression free survival according to ECOG PS, age (<65; ≥65), gender, presence of liver metastasis only (data not shown), history of prior hypertension and number of organs involved (data not shown), showed a treatment effect favouring the Zaltrap/FOLFIRI regimen over the placebo/FOLFIRI regimen.

Overall objective response rate, performed in the evaluable population, was a secondary endpoint and showed a significantly better response rate in the aflibercept arm, 19.8% vs 11.1% in the placebo arm, stratified p=0.0001.

2.5.4. Conclusions on the clinical efficacy

The application is supported by a well-conducted controlled study. Superiority of aflibercept combined with FOLFIRI over placebo combined with FOLFIRI in terms of OS was shown convincingly although the effect was small. The outcome of the primary efficacy analysis is supported by the outcome of the secondary endpoints PFS and OR. The external validity of the study is not questioned.

No validated predictive serum or plasma biomarkers have been identified that correlate with treatment outcomes to aflibercept. The Applicant is planning to retrospectively analyse plasma and tissue samples from the EFC10262, EFC10668 and EFC11338 trials, with the primary aim to determine prognostic or predictive biomarkers correlating with OS. This information is considered key to the benefit-risk balance of the product, as it promises to help better define the target population.

2.6. Clinical safety

Introduction

The core safety data in support of the safety of aflibercept in the proposed indication were derived from the pivotal ECF10262/VELOUR study in MCRC patients.

Eleven additional studies in which aflibercept was administered as single agent (6 Phase 1 or Phase 2 studies) and in combination with other agents (5 combination Phase I studies) have been taken into account for the safety analysis, as well as data coming from two further completed Phase 3 studies (VANILLA/ VITAL).

All together safety data from the three Phase 3 studies, single-agent Phase 1 and Phase 2 studies, and combination Phase 1 study have been reported in a integrate safety database (ISD) with a cut-off date of 07 February 2011. Overall, 2073 patients were exposed to aflibercept.

Furthermore, in order to explore the relative risk of specific adverse events, a meta-analysis was performed on the 3 Phase 3 studies (ECF 10262/VELOUR, EFC10547/VANILLA and EFC10261/VITAL).

Results from a Phase 1 QT interval prolongation study (TES10897), not included in the integrate safety database, have been also reported together with a summary of SAEs from ongoing trials sponsored by the Applicant and NCI.

The source of safety data are summarised in the following Table 25.

Table 25: Summary of integrated safety database

Integrated Safety Database	**Completed studies not included in the ISD***	**Ongoing studies°**	**NCI sponsored studies°**
Pivotal study EFC10262/VELOUR Single agent-Phase 1 -TED6115 -TED6116 Single agent-Phase 2 -ARD6122 (advanced epithelial ovarian cancer) -ARD6772 (advanced ovarian cancer) -EFC6125 (advanced ovarian cancer) -ARD6123 (NSCLA) Combination Phase 1 -TCD6117 -TCD6118 -TCD6119 -TCD6120 -TCD6121 (solid tumours) Other Phase 3 : -ECF10547/VANILLA (metastatic pancreatic cancer) -ECF 10261/VITAL (advanced NSCLC)	TED6113/6114 (SC administration) PDY6655, PDY6656 (healthy subjects) TCD10173 (Non Hodgkin Lymphoma phase I combination) TES10897 (QTc prolongation study)	EFC6546/VENICE EFC10668/AFFIRM TCD10767 TCD10089, TCD10091, TCD10794 (Japanese Phase 1 studies) TCD11382 (Chinese Phase 1 study)	ARD5537 ARD5538 ARD6836 ARD6839 ARD6842 ARD6843 ARD6844 ARD10676 (ADVL0714) ARD6124 LOI S-0802 NABTC-07-01 TED5540 TED5541 TED5542

*Summary of safety and class event AEs from clinical database, °Summary of SAEs from GPE database

Patient exposure

Patient exposure information in the pivotal study VELOUR (EFC10262) is summarised in the following Tables 26 and 27.

Table 26: Summary of overall study treatment exposure, EFC10262, safety population

	Placebo/Folfiri (N=605)	Aflibercept/Folfiri (N=611)
Number of cycles received by patient		
Sum	6127	6362
Mean (SD)	10.1 (8.1)	10.4 (7.6)
Median	8.0	9.0
Duration of exposure (weeks)		
Number	605	611
Mean (SD)	22.6 (17.9)	24.2 (17.4)
Median	18.1	21.4

Table 27: Exposure to aflibercept / placebo, irinotecan, 5-FU, EFC10262, safety population

	Placebo / Folfiri (N=605)	Aflibercept / Folfiri (N=611)
Aflibercept / placebo		
Number of infusions received by patient		
Sum	6035.0	5632.0
Median	8.0	7.0
Duration of exposure to aflibercept/placebo (weeks)		
Median	18.0	17.9
Total cumulative dose received (mg/kg)		
Median	32.00	28.00
Actual dose intensity (mg/kg/week)		
Median	1.84	1.66
< 1.5	57 (9.4%)	205 (33.6%)
>= 1.5 and < 2.5	548 (90.6%)	406 (66.4%)
Relative dose intensity		
Median	0.92	0.83
Irinotecan		
Number of infusions received by patient		
Sum	5992.0	6157.0
Median	8.0	9.0
Duration of exposure to irinotecan (weeks)		
Number	604	610
Median	18.1	21.0
Total cumulative dose received (mg/m²)		
Number	605	611
Median	1440.00	1472.50
Actual dose intensity (mg/m²/week)		
Number	605	611
Median	82.08	75.60
Relative dose intensity		
Number	605	611
Median	0.91	0.84

	Placebo / Folfiri (N=605)	Aflibercept / Folfiri (N=611)
5-FU		
Number of infusions received by patient		
Sum	6033.0	6161.0
Median	8.0	9.0
Duration of exposure to 5-FU (weeks)		
Number	603	611
Median	18.1	21.0
Total cumulative dose received (mg/m²)		
Number	605	611
Median	22400.00	22702.44
Actual dose intensity (mg/m²/week)		
Number	605	611
Median	1276.38	1165.56
Relative dose intensity		
Number	605	611
Median	0.91	0.83

Adverse events

An overview of adverse events in the pivotal study is presented in the following Table 28.

Table 28: Patients with at least one TEAE, EFC10262, safety population

n(%)	Placebo / Folfiri (N=605)	Aflibercept / Folfiri (N=611)
Patients with any TEAE	592 (97.9%)	606 (99.2%)
Patients with any grade 3-4 TEAE	378 (62.5%)	510 (83.5%)
Patients with any grade 3-4 related TEAE	284 (46.9%)	451 (73.8%)
Patients with any serious TEAE	198 (32.7%)	294 (48.1%)
Patients with any serious related TEAE	93 (15.4%)	194 (31.8%)
Patients with any TEAE with a fatal outcome[a]	29 (4.8%)	37 (6.1%)
Any patient who permanently discontinued due to TEAE	73 (12.1%)	164 (26.8%)

[a] the number (%) of events based on the start date of the AEs includes all TEAEs leading to death whatever the date and cause of death

Adverse drug reactions reported with aflibercept are summarised in the following Table 29.

Table 29: Adverse Drug Reactions (ADRs) reported with aflibercept presented by frequency and grade

System Organ Class / Frequency Category	Adverse Reaction	
	All grades	**Grades ≥3**
Infections and infestations		
Very common	Infection (1)	Infection (1)
Common	Neutropenic infection/sepsis (1) Urinary tract infection Nasopharyngitis	Neutropenic infection/sepsis (1)
Uncommon		Urinary tract infection

System Organ Class Frequency Category	Adverse Reaction	
	All grades	**Grades ≥3**
Blood and lymphatic system disorders		
Very common	Leucopenia (2) Neutropenia (1),(2) Thrombocytopenia (2)	Leucopenia (2) Neutropenia (2)
Common	Febrile neutropenia	Febrile neutropenia Thrombocytopenia (2)
Immune system disorders		
Common	Hypersensitivity (1)	
Uncommon		Hypersensitivity (1)
Metabolism and nutrition disorders		
Very common	Decreased appetite Weight loss	
Common	Dehydration (1)	Dehydration (1) Decreased appetite Weight loss
Nervous system disorders		
Very common	Headache	
Common		Headache
Uncommon	PRES (1),(4)	PRES (1),(4)
Vascular disorders		
Very common	Hypertension (1) Haemorrhage (1)	Hypertension
Common	Arterial thromboembolism (1) Venous thromboembolism (1)	Arterial thromboembolism (1) Venous thromboembolism (1) Haemorrhage (1)
Respiratory, thoracic and mediastinal disorders		
Very common	Dyspnoea Epistaxis Dysphonia	
Common	Oropharyngeal pain Rhinorrhoea	
Uncommon		Dyspnoea Epistaxis Dysphonia Oropharyngeal pain
Gastrointestinal disorders		
Very common	Diarrhoea (1) Stomatitis Abdominal pain Abdominal pain upper	Diarrhoea (1) Stomatitis
Common	Rectal haemorrhage Fistula (1) Aphthous stomatitis Haemorrhoids Proctalgia Toothache	Abdominal pain Abdominal pain upper

System Organ Class Frequency Category	Adverse Reaction	
	All grades	**Grades ≥3**
Uncommon	GI perforation (1)	GI perforation (1) Rectal haemorrhage Fistula (1) Aphthous stomatitis Proctalgia
Hepatobiliary disorders		
Very common	Increased AST (2) Increased ALT (2)	
Common		Increased AST (2) Increased ALT (2)
Skin and subcutaneous tissue disorders		
Very common	Palmar-Plantar Erythrodysaesthesia syndrome	
Common	Skin hyperpigmentation	Palmar-Plantar Erythrodysaesthesia syndrome
Uncommon	Compromised wound healing (1)	Compromised wound healing (1)
Renal and urinary disorders		
Very common	Proteinuria (1),(3) Increased serum creatinine	
Common		Proteinuria (1),(3)
Uncommon	Nephrotic syndrome (1) Thrombotic microangiopathy (1)	Nephrotic syndrome (1) Thrombotic microangiopathy (1)
General disorders and administration site conditions		
Very common	Asthenic conditions	Asthenic conditions
Note: Adverse reactions are reported using MedDRA version MEDDRA13.1 and graded using NCI CTC version 3.0 (1) See “Description of selected adverse reactions” in this section (2) Based on laboratory values (percentages done on patients with laboratory assessments) (3) Compilation of clinical and laboratory data (4) Not reported in MCRC study; however, PRES was reported in patients from other studies treated with aflibercept as monotherapy and in combination with chemotherapies other than FOLFIRI		

The most frequently reported TEAEs were gastrointestinal disorders (placebo arm: 86.1%; aflibercept arm: 93.5%) and general disorders and administration site conditions (placebo arm: 62.6%; aflibercept arm: 71.5%).

The most frequently reported gastrointestinal disorders (all grades, placebo arm versus aflibercept arm) were diarrhoea (56.5% versus 69.2%), nausea (54.0% versus 53.4%), stomatitis and ulceration (HLT, 34.9% versus 54.8%) and vomiting (33.4% versus 32.9%).

Among general disorders, the most frequently reported TEAEs (all grades, placebo arm versus aflibercept arm) were asthenic conditions (HLT, 50.2% versus 60.4%).

Events with an excess in incidence (all grades) in the aflibercept arm of more than 10% over the placebo arm were: hypertension (41.4% vs 10.7%), dysphonia (25.4% vs 3.3%), proteinuria (62.2% vs 40.7%), epistaxis (27.7% vs 7.4%), stomatitis and ulceration (54.8 vs 34.9%), weight decrease (31.9% vs 14.4%), thrombocytopenia (47.4% vs 33.8%), headache (22.3% vs 8.8%), infections and infestations (46.2% vs 32.7%), diarrhoea (69.2% vs 56.5%), neutropenia (67.8% vs 56.3%), asthenic conditions (60.4% vs 50.2%), ALT increase (47.3% vs 37.1%).

Grade 3 or 4 events were reported in 378 patients (62.5%) in the placebo arm and 510 patients (83.5%) in the aflibercept arm. A frequency ≥2% higher in the aflibercept arm in the incidence of grade 3 or 4 events was reported for hypertension (1.5% of patients in the placebo arm versus 19.1% of patients in the aflibercept arm), diarrhoea (7.8% versus 19.3%), stomatitis and ulceration (HLT; 5.0% versus 13.7%), asthenic conditions (HLT; 10.6% versus 16.9%), GI and abdominal pains (HLT; 3.3% versus 5.4%), dehydration (1.3% versus 4.3%), and palmar-plantar erythrodysesthesia syndrome (0.5% versus 2.8%).

The addition of aflibercept to FOLFIRI resulted in increased frequency of certain adverse events that are characteristic of irinotecan and 5-FU administration. The frequencies of all grades and of grade 3-4 of both diarrhoea and neutropenia were increased in the aflibercept arm compared to the placebo arm. Similarly, the characteristic 5-FU toxicities of stomatitis and PPE syndrome both occurred more frequently under aflibercept treatment compared to placebo (all grades and grade 3 or 4).

Potential anti-VEGF class events were analysed according to groups of TEAE terms, with clusters based upon the safety profile of aflibercept as observed in Phase 1 and Phase 2 studies as well as upon the known risks associated with other agents targeting the VEGF pathway. These specific group terms included: hypertension, haemorrhage, cardiac dysfunction, arterial and venous thromboembolic events, fistula, gastrointestinal perforation, compromised wound healing, osteonecrosis, reversible posterior leukoencephalopathy syndrome (RPLS), thrombotic microangiopathy (TMA), haemolytic uremic syndrome (HUS) and thrombotic thrombocytopenic purpura (TTP).

A summary of grouped events (all grades and grade≥3) in VELOUR by risk ratio is provided in Tables 30 and 31 below. Additionally, to asses the relative risk associated with aflibercept versus placebo, a meta-analysis of the 3 phase 3 studies completed was performed to explore the impact of the following selected events of interest.

Table 30: Summary of grouped AEs by risk ratio, all grades, EFC10262, Safety Population

Grouped terms	Placebo/Folfiri (N=605)	Aflibercept/Folfiri (N=611)	RR (95% CI)
Acute drug reaction	26 (4.3%)	26 (4.3%)	0.99 (0.58 to 1.69)
Arterial thromboembolic event	9 (1.5%)	16 (2.6%)	1.76 (0.78 to 3.95)
Cardiac dysfunction	0	2 (0.3%)	NC (NC)
Fistula from gastrointestinal origin	2 (0.3%)	7 (1.1%)	3.47 (0.72 to 16.62)
Fistula from other origin than gastrointestinal	1 (0.2%)	2 (0.3%)	1.98 (0.18 to 21.78)
Gastrointestinal perforation	3 (0.5%)	3 (0.5%)	0.99 (0.20 to 4.89)
Haemorrhage	115 (19.0%)	231 (37.8%)	1.99 (1.64 to 2.41)
Hypertension	65 (10.7%)	253 (41.4%)	3.85 (3.01 to 4.94)
Osteonecrosis	0	2 (0.3%)	NC (NC)
Venous thromboembolic event	44 (7.3%)	57 (9.3%)	1.28 (0.88 to 1.87)
Wound healing	5 (0.8%)	3 (0.5%)	0.59 (0.14 to 2.47)

NC: not calculated

Table 31: Summary of grouped AEs by risk ratio, Grade ≥3, EFC10262, Safety Population

Grouped terms	Placebo/Folfiri (N=605)	Aflibercept/Folfiri (N=611)	RR (95% CI)
Acute drug reaction	3 (0.5%)	3 (0.5%)	0.99 (0.20 to 4.89)
Arterial thromboembolic event	3 (0.5%)	11 (1.8%)	3.63 (1.02 to 12.95)
Cardiac dysfunction	0	1 (0.2%)	NC (NC)
Fistula from gastrointestinal origin	1 (0.2%)	2 (0.3%)	1.98 (0.18 to 21.78)
Gastrointestinal perforation	2 (0.3%)	3 (0.5%)	1.49 (0.25 to 8.86)
Haemorrhage	10 (1.7%)	18 (2.9%)	1.78 (0.83 to 3.83)
Hypertension	9 (1.5%)	118 (19.3%)	12.98 (6.65 to 25.33)
Venous thromboembolic event	38 (6.3%)	48 (7.9%)	1.25 (0.83 to 1.89)
Wound healing	0	2 (0.3%)	NC (NC)

NC: not calculated

Hypertension: In EFC10262, the number of patients with all grade hypertension was greater in the aflibercept arm (41.4%) than in the placebo arm (10.7%). In particular, the proportion of patients with grade 3 hypertension (requiring more than one drug or more intensive anti hypertensive therapy than previously) was higher in the aflibercept arm than in the placebo arm (19.1% versus 1.5%). One patient in the aflibercept arm developed a grade 4 event with no end-organ damaged reported.

In both treatment arms, more than half of the patients who experienced hypertension had the first occurrence during the first two cycles of treatment with a median time to onset similar between both groups.

Meta-analysis: The summary incidence of all grade hypertension in patients treated with aflibercept was 33.5% versus 7.9% in placebo-treated patients.For hypertension, treatment effects were consistent across Phase 3 studies (p-value for interaction: 0.32). The risk of occurrence of hypertension was consistently higher in the aflibercept arm compared to placebo whatever the background chemotherapy . The risk of developing hypertension was multiplied by 4.24-fold in aflibercept as compared to placebo (RR = 4.24, 95% CI: 3.48 to 5.18).

Haemorrhage: In EFC10262, the number of patients with at least one haemorrhagic event (all grades) was greater in the aflibercept arm (37.8%) than in the placebo arm (19.0%). The most frequently reported TEAE (all grades) in this category was epistaxis, in both the placebo and aflibercept arms, reported in 7.4% and 27.7% of patients, respectively. In addition there was more haemorrhage (all grades) from gastrointestinal origin in the aflibercept arm compared to placebo (10.0% versus 5.1%, respectively), and more haemoptysis (all grade 1 or 2 events).

The timing of first occurrence of haemorrhagic events was comparable between arms, with more than half of the patients in each treatment arm first experiencing such events during the first 3 treatment cycles.

One patient in the placebo arm and 12 patients in the aflibercept arm discontinued study treatment (premature or permanent discontinuation) due to haemorrhagic events.

One patient in the aflibercept arm experienced a fatal gastrointestinal haemorrhage consequent to duodenal haemorrhage.

Grade 3 or 4 haemorrhagic events were seen in other studies in solid tumour patients (total of 43/1462 including 16 fatal events), including cases of intracranial and pulmonary haemorrhage.

Meta-analysis: The summary incidence of all grade haemorrhage in aflibercept treated patients was 31.6% versus 14.6% among patients treated with placebo (the difference being mostly due to grade 1 or 2 epistaxis). For haemorrhage, treatment effects were consistent across Phase 3 studies (p-value for interaction: 0.41). The risk of developing haemorrhage was multiplied by 2.16-fold in aflibercept as compared to placebo (RR = 2.16, 95%CI: 1.86 to 2.52). When considering grade 3 or 4 events, the overall incidence was numerically increased with aflibercept compared to placebo (3.1% and 1.5%, respectively).

Cardiac dysfunction: Meta-analysis: The summary incidence of all grade cardiac dysfunction was 0.2% of placebo treated patients and 0.7% of aflibercept treated patients (0.3% in EFC10262, 1.9% in EFC10547, and 0.4% in EFC10261). The risk ratio of aflibercept versus placebo for all grades cardiac dysfunction was 2.99 (95% CI: 0.81 to 11.02).

The incidence reported in single-agent and combination Phase 1 and Phase 2 studies was 2% and 1%, respectively.

Arterial thromboembolic events: In study EFC10262 all grade ATEs were reported in 9 patients (1.5%) in the placebo arm and 16 patients (2.6%) in the aflibercept arm. These arterial events were mainly from cardiac ischemic origin. Grade 3 or 4 events were reported in 3 patients in the placebo arm and 11 patients in the aflibercept arm. No fatal ATEs were reported during the study.

Of the 16 patients in the aflibercept arm who experienced ATEs, 10 (62.5%) discontinued study treatment whilst in the placebo arm, 1 of the 9 patients (11.1%) with ATEs discontinued study treatment.

The incidences of ATEs were similar in single-agent (2.7%) and combination (1.8%) Phase 1 and Phase 2 studies.

Meta-analysis: ATEs occurred in 1.7% of placebo treated patients in the 3 Phase 3 studies and 2.3% in aflibercept treated patients. The risk ratio of aflibercept over placebo for all grade ATE was 1.36 (95% CI: 0.79 to 2.34).

Venous thromboembolic events: In study EFC10262, all grade VTEs were slightly more common in patients in the aflibercept arm (9.3%) than in the placebo arm (7.3%). The incidence of grade 3 or 4 VTEs was 7.9% in the aflibercept arm and 6.3% in the placebo arm with over half of these grade 3 or 4 events being pulmonary embolism events (aflibercept: 4.7%, placebo: 3.5%).

Discontinuation of study treatment due to VTE was reported for 45.6% of patients with VTE in the aflibercept arm as compared to 36.4% in the placebo arm.

One patient in the aflibercept arm experienced a fatal pulmonary embolism.

Meta-analysis: In study EFC10547, VTE occurred in 11.1% of patients receiving placebo versus 8.9% of patients receiving aflibercept. In EFC10261, VTE occurred in 4.6% of patients in the placebo arm versus 3.1% of patients receiving aflibercept. Overall, in the Phase 3 studies, the incidence of all grade VTE was 7.1% in both treatment groups (placebo and aflibercept). Aflibercept did not increase the risk of VTE compared to placebo (RR = 1.00 [95%CI: 0.76 to 1.31]).

Fistula: In study EFC10262, 3 patients in the placebo arm and 9 patients in the aflibercept arm had a fistula either from GI or non GI origin. In the aflibercept arm, fistula events were observed without specific pattern in the timing of occurrence. Most of the primary tumours were rectum or rectosigmoid (8 out of 9). Four fistulae occurred in patients who had local abscess or tumour necrosis. No other specific risk factors could be identified. Fistula prevented continuation of study treatment in 7 patients. No fistula was fatal.

Meta-analysis: Fistulae from GI and non GI origin were pooled for meta-analysis, due the low number of reported cases. Amongst the patients treated with aflibercept, the summary incidence of all grade fistulae was 1.1% versus 0.2% in the placebo arm. The overall risk of fistula was significantly increased with aflibercept, with respect to placebo (OR = 4.57, 95%CI: 1.42 to 20.01).

Gastrointestinal perforation: In study EFC10262, 3 patients in the placebo arm and 3 patients in the aflibercept arm had a GI perforation. In the integrated safety database 19 patients exposed to aflibercept (0.9%) had a gastrointestinal perforation (9 ovarian/cervix, 6 NSCL, 3 rectum/rectosigmoid, and 1 pancreas cancer). In the integrated safety database, 23 patients experienced GI perforation, 19 of them were treated with aflibercept (0.9%); there were 3 cases in rectum/rectosigmoid cancer patients. All but one were grade 3 or 4 and the events led to study treatment discontinuation in 15 patients. Overall, GI perforation events were fatal in 7 patients (3 with ovarian cancer, 3 with NSCLC and 1 rectosigmoid) despite corrective or palliative surgery, which was performed in 3 of them. Among the 7 fatal cases, 6 were diagnosed at cycle 1 or 2.

Meta-analysis: In the 3 Phase 3 studies, gastrointestinal perforation occurred in 0.5% of patients in each arm in study EFC10262 and at rates of 0.3% and 0.8% for placebo and aflibercept patients, respectively. The risk ratio of aflibercept over placebo for all grades GI perforation was 2.49 (95% CI: 0.78 to 7.93).

Compromised wound healing: In EFC10262, compromised wound healing was reported in 3 patients (0.5%) in the aflibercept arm and 5 patients (0.8%) in the placebo arm. Grade 3 compromised wound healing was reported in 2 patients treated with aflibercept (0.3%) and in none of the placebo-treated patients.

In the integrated safety database overall 9 patients (0.4%) had a compromised wound healing. Compromised wound healing led to aflibercept treatment discontinuation or cycle delay in 7 patients. The events resolved in 7 patients and were still present at the time of death for the 2 other patients. None of these events was fatal.

Meta-analysis: Overall, the incidence of all grade compromised wound healing was 0.5% in the aflibercept arm and 0.4% in the placebo arm, RR of aflibercept versus placebo was 1.40 (95%CI: 0.44 to 4.39).

Osteonecrosis: Two cases of osteonecrosis were reported in patients receiving aflibercept in EFC10262.

In the integrated safety database, a total of 7 cases of osteonecrosis have been reported, 6 of them in aflibercept treated patients (0.3%). No specific pattern was observed in the timing of occurrence. In 3 cases there was a history of jaw inflammation and in 3 other cases, there was a history of biphosphonate use. Treatment was discontinued in 2 out of 6 patients.

Meta-analysis: The RR of aflibercept over placebo for all grades osteonecrosis was 2.99 (95% CI: 0.31 to 28.72.

Reversible posterior leukoencephalopathy syndrome (RPLS): No RPLS was reported in study EFC10262.

In the integrated safety database, RPLS occurred in 4 patients treated with aflibercept, was grade 3-4 in 3 of them, and contributed to death in one of the patients (primary cause of death was disease progression) and treatment discontinuation in 2 others. Amongst the 4 RPLS, 2 occurred in patients receiving aflibercept as single-agent therapy (2/404, 0.5%).

Notably, the 0708 study, in which aflibercept was combined with pemetrexed and cisplatin for the treatment of advanced carcinoma, was closed due to the occurrence of RPLS in 3 patients out 62

enrolled (4.8%) compared to 8 patients out 2926 (0.27%) in all other trials sponsored by the Applicant.

Overall, there are 17 cases of RPLS reported:

• Study 0708 3 cases of RPLS in 62 patients (4.8%)

• Phase 1 to 2 single agent studies with 2 cases in 404 patients (0.5 %);

• Phase 1 to 2 studies with aflibercept in combination with cytotoxic chemotherapy with 4 cases in 577 patients (0.7%); and

• Phase 3 studies (non-squamous NSCLC, pancreatic cancer, colorectal cancer, and prostate cancer) with 2 cases in approximately 2069 patients (~ 0.10%)

• NCI studies with 6 in 683 patients exposed (0.9%)

Overall, there were 13 females and 4 males who developed the syndrome. Overall the mean age was 60.5 years (SD 12.5) with a median age of 59 years (range 34 to 76 years). The mean cycle at diagnosis was 4.8 (SD 5.3), mean day from last administration was 10.4 (SD 6.8). Twelve cases were reported as having recovered, (for 1 of the 12 cases the outcome was not specifically reported however the end date for the event was reported as 3 days following the event onset). The mean duration for these 12 events was 13.5 days (SD 11.2). The dosing regimen of 4mg/kg aflibercept administered every 2 weeks was background treatment in 11 of the 17 cases. Of these 11 cases, 8 were with single agent aflibercept and 3 were with aflibercept administered in combination with cytotoxic chemotherapy.

Eight of the patients had a reported past medical history of hypertension. Of the 9 patients with no past medical history of hypertension 5 developed increased blood pressure on treatment prior to the event. Thus, careful treatment of hypertension might be an important prophylactic measure.

Thrombotic microangiopathy (TMA), hemolytic uremic syndrome, and thrombotic thrombocytopenic purpura (TTP): In the integrated safety database, overall 9 patients (0.4%) were reported as having TMA including one patient in EFC10262. Most of the cases appeared mild in severity, however, 3 biopsy-confirmed TMA were observed with concomitant abnormalities supporting the diagnosis. All of these 3 cases led to treatment discontinuation, needed corrective treatment and resolved.

Two patients had a TTP and 1 patient a HUS. Two events led to treatment discontinuation and all resolved.

Acute drug reactions: In EFC10262, acute drug reactions were reported with the same frequency in both treatment arms (26 patients = 4.3% in each arm), with 3 patients in each arm (0.5%) reporting grade 3 events. Events lasted less than 1 day in both arms. Skin disorders were more common in the aflibercept arm. Two patients in each treatment arm discontinued study treatment due to acute drug reactions.

Meta-analysis: Among the patients treated with aflibercept, the summary incidence of all grade acute drug reaction was 4.4%, versus 3.5% in patients treated with placebo. The RR of aflibercept over placebo for all grades acute drug reactions was 1.26 (95% CI: 0.86 to 1.84).

Immunological events: Serum samples were evaluated for binding anti-aflibercept antibodies using a sandwich or a bridging immunoassay (anti-drug antibody (ADA) assay). Serum samples that were positive in this bridging ADA assay were further evaluated for neutralising activity in the neutralising antibody (NAb) assay. It is noted that presence of rheumatoid factor could generate a positive response in the bridging immunoassay. In all Phase 1 studies, the lower limit of quantitation (LOQ)

was 238.4 ng/mL, while in all Phase 2 and Phase 3 studies, the assay used a non-quantitative titre-based measurement.

Overall, among the 1671 patients treated with aflibercept and evaluable for immunogenicity, 63 patients (3.8%) had a positive response for anti-aflibercept antibodies and 17 patients (1.3%) had neutralising anti-aflibercept antibodies.

Table 32: Patients exposed with positive response in ADA assay, EFC10262, Safety Population

	Placebo/Folfiri (N=605)	Aflibercept/Folfiri (N=611)	Overall (N=1216)
Patients evaluable (tested in the assay at least once post-baseline)[1]	526 (86.9%)	521 (85.3%)	1047 (86.1%)
Patients with positive ADA (all time-points, baseline or post-baseline)[2, 3]	18 (3.4%)	9 (1.7%)	27 (2.6%)
Patients with positive ADA (at least once post-baseline)[2, 3]	18 (3.4%)	8 (1.5%)	26 (2.5%)
Patients with positive ADA post-baseline and negative (or missing) at baseline[3]	8 (1.5%)	4 (0.8%)	12 (1.1%)
Patients with positive neutralizing antibodies in patients with positive ADA post-baseline [3]	2 (0.4%)	1 (0.2%)	3 (0.3%)

1: among treated patients (safety population), 2: with positive screening and confirmation assays, 3: among evaluable patients

In the pivotal EFC10262 study 1.5% (8 patients) had a positive response for ADA and 0.2% (1 patient) for NAb in the aflibercept arm, and 3.4% (18 patients) had positive response for ADA at least once post baseline and 0.4% (2 patients) for NAb in the placebo arm. Among patients with ADA positive, 4 (50%) and 10 (56%) were positive at baseline in the aflibercept and placebo arms, respectively. The patient with NAb positive in aflibercept arm at cycle 3 received 10 cycles of treatment, had stable disease as best response and experienced disease progression after 189 days (6.2 months) on study.

Serious adverse event/deaths/other significant events

The total number of patients with SAEs was greater in the aflibercept arm (48.1%) than in the placebo arm (32.7%). Similarly, the incidence of grade 3-4 SAEs was more common in the aflibercept arm (41.6% of patients vs 28.8%).

At the SOC level, the most frequently reported SAEs were gastrointestinal disorders, with a higher incidence seen amongst patients in the aflibercept arm (20.3% versus 11.2%), infection and infestations (11.3% vs 6.3%), blood and lymphatic system disorders (6.5% versus 2.5%), metabolism and nutrition disorders (4.9% versus 1.8%) and respiratory, thoracic and mediastinal disorders (5.9% versus 3.0%).

Serious adverse events in the pivotal study are summarised in the following Table 33.

Table 33: Summary of serious TEAEs by SOC, HLGT, HLT, PT (worst grade by patient), EFC10262, Safety Population

PRIMARY SYSTEM ORGAN CLASS HLGT: High Level Group Term HLT: High Level Term Preferred Term n(%)	Placebo/Folfiri (N=605)		Aflibercept/Folfiri (N=611)	
	All grades	Grades ≥3	All grades	Grades ≥3
Any class	198 (32.7%)	174 (28.8%)	294 (48.1%)	254 (41.6%)
INFECTIONS AND INFESTATIONS	38 (6.3%)	32 (5.3%)	69 (11.3%)	60 (9.8%)
HLGT: Infections - pathogen unspecified	37 (6.1%)	32 (5.3%)	63 (10.3%)	55 (9.0%)
HLT: Lower respiratory tract and lung infections	14 (2.3%)	12 (2.0%)	14 (2.3%)	12 (2.0%)
Pneumonia	5 (0.8%)	3 (0.5%)	11 (1.8%)	10 (1.6%)
HLT: Sepsis, bacteraemia, viraemia and fungaemia NEC	5 (0.8%)	5 (0.8%)	15 (2.5%)	15 (2.5%)
Sepsis	5 (0.8%)	5 (0.8%)	8 (1.3%)	8 (1.3%)
HLT: Urinary tract infections	4 (0.7%)	2 (0.3%)	10 (1.6%)	4 (0.7%)
Urinary tract infection	3 (0.5%)	2 (0.3%)	8 (1.3%)	4 (0.7%)
NEOPLASMS BENIGN, MALIGNANT AND UNSPECIFIED (INCL CYSTS AND POLYPS)	6 (1.0%)	4 (0.7%)	8 (1.3%)	6 (1.0%)
HLGT: Neoplasm related morbidities	5 (0.8%)	4 (0.7%)	5 (0.8%)	4 (0.7%)
HLT: Oncologic complications and emergencies	5 (0.8%)	4 (0.7%)	5 (0.8%)	4 (0.7%)
BLOOD AND LYMPHATIC SYSTEM DISORDERS	15 (2.5%)	13 (2.1%)	40 (6.5%)	36 (5.9%)
HLGT: Anaemias nonhaemolytic and marrow depression	3 (0.5%)	2 (0.3%)	9 (1.5%)	6 (1.0%)
HLT: Anaemias NEC	3 (0.5%)	2 (0.3%)	7 (1.1%)	4 (0.7%)
Anaemia	3 (0.5%)	2 (0.3%)	7 (1.1%)	4 (0.7%)
HLGT: White blood cell disorders	10 (1.7%)	9 (1.5%)	30 (4.9%)	30 (4.9%)
HLT: Neutropenias	10 (1.7%)	9 (1.5%)	30 (4.9%)	30 (4.9%)
Febrile neutropenia	6 (1.0%)	6 (1.0%)	19 (3.1%)	19 (3.1%)
Neutropenia	4 (0.7%)	3 (0.5%)	11 (1.8%)	11 (1.8%)
METABOLISM AND NUTRITION DISORDERS	11 (1.8%)	8 (1.3%)	30 (4.9%)	24 (3.9%)
HLGT: Appetite and general nutritional disorders	3 (0.5%)	3 (0.5%)	3 (0.5%)	2 (0.3%)
HLGT: Electrolyte and fluid balance conditions	7 (1.2%)	5 (0.8%)	26 (4.3%)	20 (3.3%)
HLT: Total fluid volume decreased	7 (1.2%)	5 (0.8%)	24 (3.9%)	18 (2.9%)
Dehydration	7 (1.2%)	5 (0.8%)	24 (3.9%)	18 (2.9%)
PSYCHIATRIC DISORDERS	2 (0.3%)	1 (0.2%)	6 (1.0%)	4 (0.7%)
NERVOUS SYSTEM DISORDERS	9 (1.5%)	8 (1.3%)	12 (2.0%)	10 (1.6%)
CARDIAC DISORDERS	4 (0.7%)	3 (0.5%)	10 (1.6%)	8 (1.3%)
HLGT: Cardiac arrhythmias	2 (0.3%)	1 (0.2%)	4 (0.7%)	3 (0.5%)
HLT: Supraventricular arrhythmias	2 (0.3%)	1 (0.2%)	4 (0.7%)	3 (0.5%)

VASCULAR DISORDERS	17 (2.8%)	16 (2.6%)	25 (4.1%)	22 (3.6%)
HLGT: Embolism and thrombosis	16 (2.6%)	16 (2.6%)	10 (1.6%)	10 (1.6%)
HLT: Peripheral embolism and thrombosis	14 (2.3%)	14 (2.3%)	8 (1.3%)	8 (1.3%)
Deep vein thrombosis	7 (1.2%)	7 (1.2%)	7 (1.1%)	7 (1.1%)
HLGT: Vascular hypertensive disorders	0	0	10 (1.6%)	8 (1.3%)
HLT: Vascular hypertensive disorders NEC	0	0	10 (1.6%)	8 (1.3%)
Hypertension	0	0	10 (1.6%)	8 (1.3%)
RESPIRATORY, THORACIC AND MEDIASTINAL DISORDERS	18 (3.0%)	16 (2.6%)	36 (5.9%)	27 (4.4%)
HLGT: Lower respiratory tract disorders (excl obstruction and infection)	3 (0.5%)	2 (0.3%)	5 (0.8%)	3 (0.5%)
HLGT: Pulmonary vascular disorders	12 (2.0%)	12 (2.0%)	21 (3.4%)	21 (3.4%)
HLT: Pulmonary thrombotic and embolic conditions	12 (2.0%)	12 (2.0%)	20 (3.3%)	20 (3.3%)
Pulmonary embolism	12 (2.0%)	12 (2.0%)	19 (3.1%)	19 (3.1%)
HLGT: Respiratory disorders NEC	3 (0.5%)	2 (0.3%)	6 (1.0%)	3 (0.5%)
GASTROINTESTINAL DISORDERS	68 (11.2%)	61 (10.1%)	124 (20.3%)	98 (16.0%)
HLGT: Gastrointestinal haemorrhages NEC	7 (1.2%)	6 (1.0%)	12 (2.0%)	10 (1.6%)
HLT: Intestinal haemorrhages	5 (0.8%)	4 (0.7%)	7 (1.1%)	5 (0.8%)
Rectal haemorrhage	4 (0.7%)	3 (0.5%)	6 (1.0%)	4 (0.7%)
HLT: Non-site specific gastrointestinal haemorrhages	2 (0.3%)	2 (0.3%)	4 (0.7%)	4 (0.7%)
HLGT: Gastrointestinal inflammatory conditions	2 (0.3%)	2 (0.3%)	14 (2.3%)	12 (2.0%)
HLT: Colitis (excl infective)	1 (0.2%)	1 (0.2%)	6 (1.0%)	6 (1.0%)
HLT: Gastrointestinal inflammatory disorders NEC	1 (0.2%)	1 (0.2%)	7 (1.1%)	6 (1.0%)
HLGT: Gastrointestinal motility and defaecation conditions	19 (3.1%)	15 (2.5%)	49 (8.0%)	35 (5.7%)
HLT: Diarrhoea (excl infective)	14 (2.3%)	12 (2.0%)	44 (7.2%)	34 (5.6%)
Diarrhoea	14 (2.3%)	12 (2.0%)	44 (7.2%)	34 (5.6%)
HLGT: Gastrointestinal stenosis and obstruction	24 (4.0%)	23 (3.8%)	26 (4.3%)	20 (3.3%)
HLT: Duodenal and small intestinal stenosis and obstruction	3 (0.5%)	3 (0.5%)	5 (0.8%)	5 (0.8%)
HLT: Gastrointestinal stenosis and obstruction NEC	20 (3.3%)	19 (3.1%)	18 (2.9%)	14 (2.3%)
Ileus	5 (0.8%)	5 (0.8%)	4 (0.7%)	2 (0.3%)
Intestinal obstruction	11 (1.8%)	11 (1.8%)	10 (1.6%)	8 (1.3%)
HLGT: Gastrointestinal ulceration and perforation	3 (0.5%)	2 (0.3%)	4 (0.7%)	4 (0.7%)
HLGT: Oral soft tissue conditions	0	0	9 (1.5%)	9 (1.5%)
HLT: Stomatitis and ulceration	0	0	9 (1.5%)	9 (1.5%)
Stomatitis	0	0	8 (1.3%)	8 (1.3%)

HEPATOBILIARY DISORDERS	11 (1.8%)	10 (1.7%)	9 (1.5%)	6 (1.0%)
HLGT: Hepatic and hepatobiliary disorders	8 (1.3%)	7 (1.2%)	3 (0.5%)	2 (0.3%)
HLT: Cholestasis and jaundice	5 (0.8%)	5 (0.8%)	2 (0.3%)	2 (0.3%)
Hyperbilirubinaemia	4 (0.7%)	4 (0.7%)	2 (0.3%)	2 (0.3%)
MUSCULOSKELETAL AND CONNECTIVE TISSUE DISORDERS	9 (1.5%)	7 (1.2%)	6 (1.0%)	4 (0.7%)
HLGT: Musculoskeletal and connective tissue disorders NEC	6 (1.0%)	4 (0.7%)	4 (0.7%)	3 (0.5%)
HLT: Musculoskeletal and connective tissue pain and discomfort	6 (1.0%)	4 (0.7%)	3 (0.5%)	3 (0.5%)
Back pain	4 (0.7%)	4 (0.7%)	3 (0.5%)	3 (0.5%)
RENAL AND URINARY DISORDERS	10 (1.7%)	6 (1.0%)	16 (2.6%)	11 (1.8%)
HLGT: Renal disorders (excl nephropathies)	5 (0.8%)	4 (0.7%)	7 (1.1%)	6 (1.0%)
HLT: Renal failure and impairment	1 (0.2%)	1 (0.2%)	5 (0.8%)	5 (0.8%)
HLGT: Urinary tract signs and symptoms	4 (0.7%)	1 (0.2%)	6 (1.0%)	3 (0.5%)
HLT: Bladder and urethral symptoms	2 (0.3%)	0	4 (0.7%)	1 (0.2%)
GENERAL DISORDERS AND ADMINISTRATION SITE CONDITIONS	42 (6.9%)	28 (4.6%)	44 (7.2%)	31 (5.1%)
HLGT: Body temperature conditions	15 (2.5%)	3 (0.5%)	10 (1.6%)	2 (0.3%)
HLT: Febrile disorders	15 (2.5%)	3 (0.5%)	10 (1.6%)	2 (0.3%)
Pyrexia	15 (2.5%)	3 (0.5%)	10 (1.6%)	2 (0.3%)
HLGT: General system disorders NEC	27 (4.5%)	23 (3.8%)	29 (4.7%)	25 (4.1%)
HLT: Asthenic conditions	7 (1.2%)	5 (0.8%)	8 (1.3%)	5 (0.8%)
Asthenia	4 (0.7%)	2 (0.3%)	5 (0.8%)	3 (0.5%)
HLT: General signs and symptoms NEC	15 (2.5%)	15 (2.5%)	17 (2.8%)	17 (2.8%)
Disease progression	14 (2.3%)	14 (2.3%)	16 (2.6%)	16 (2.6%)
INVESTIGATIONS	4 (0.7%)	2 (0.3%)	6 (1.0%)	1 (0.2%)
INJURY, POISONING AND PROCEDURAL COMPLICATIONS	8 (1.3%)	6 (1.0%)	8 (1.3%)	6 (1.0%)
HLGT: Procedural related injuries and complications NEC	2 (0.3%)	2 (0.3%)	5 (0.8%)	5 (0.8%)

An overview of deaths in the pivotal study is presented in the following Table 34.

Table 34: Death and cause of death by period of occurrence, EFC10262, Safety Population

	Placebo/Folfiri (N=605)	Aflibercept/Folfiri (N=611)
Total number of deaths	452 (74.7%)	403 (66.0%)
Cause of death [n(%)]		
Adverse event	4 (0.7%)	14 (2.3%)
Disease progression	436 (72.1%)	369 (60.4%)
Other reason*	12 (2.0%)	20 (3.3%)
Number of deaths within 30 days from last dose [n(%)]	19 (3.1%)	30 (4.9%)
Cause of death [n(%)]		
Adverse event	4 (0.7%)	14 (2.3%)
Disease progression	13 (2.1%)	14 (2.3%)
Other reason* (including sudden death and unknown)	2 (0.3%)	2 (0.3%)
Deaths more than 30 days of last dose due to AE	0	0
Deaths within 60 days from first dose	16 (2.6%)	20 (3.3%)
Cause of death [n(%)]		
Adverse event	1 (0.2%)	4 (0.7%)
Disease progression	13 (2.1%)	13 (2.1%)
Other reason*	2 (0.3%)	3 (0.5%)

More than 50% of the deaths in context other than disease progression (10/16 aflibercept; and 3/6 placebo patients) were aged ≥65, while approximately one third of patients in the overall safety population were aged ≥65.

Death occurrence was observed after an average of 6 cycles of study treatment in both arms with an average day of occurrence between Day 9 (placebo group) and Day 12 (aflibercept group) of the last cycle. In the aflibercept group, 7/16 events and half of the fatal events in the placebo group (3/6 events) were assessed as related to study treatment.

Deaths in the context of disease progression and in context other than disease progression are presented in the following Tables 35 and 36.

Table 35: Fatal AEs* in the context of disease progression, EFC10262, Safety Population

Primary System Organ Class Preferred term	Placebo/Folfiri (N=605)	Aflibercept/Folfiri (N=611)
Any class	13 (2.1%)	14 (2.3%)
Gastrointestinal disorders	1 (0.2%)	2 (0.3%)
Intestinal obstruction	0	1 (0.2%)
Ileus	1 (0.2%)	0
Ileal perforation	0	1 (0.2%)
General disorders and administration site conditions	11 (1.8%)	12 (2.0%)
Disease progression	11 (1.8%)	12 (2.0%)
No fatal adverse event reported	1 (0.2%)	0
No fatal adverse event reported	1 (0.2%)	0

* AEs leading to death within 30 days of last dose of study medication

Table 36: Fatal AEs* in other context than disease progression, EFC10262, Saf Population

Primary System Organ Class Preferred term	Placebo/Folfiri (N=605)	Aflibercept/Folfiri (N=611)
Any class	6 (1.0%)	16 (2.6%)
Infections and infestations	3 (0.5%)	4 (0.7%)
Neutropenic infection	1 (0.2%)	0
Sepsis	1 (0.2%)	1 (0.2%)
Neutropenic sepsis	0	1 (0.2%)
Lobar pneumonia	1 (0.2%)	0
Septic shock	0	1 (0.2%)
Rectal abscess	0	1 (0.2%)
Metabolism and nutrition disorders	0	2 (0.3%)
Dehydration	0	2 (0.3%)
Nervous system disorders	0	1 (0.2%)
Metabolic encephalopathy	0	1 (0.2%)
Vascular disorders	0	1 (0.2%)
Hypovolaemic shock	0	1 (0.2%)
Respiratory, thoracic and mediastinal disorders	1 (0.2%)	3 (0.5%)
Pulmonary embolism	0	1 (0.2%)
Pneumonia aspiration	0	1 (0.2%)
Interstitial lung disease	1 (0.2%)	0
Acute respiratory failure	0	1 (0.2%)
Gastrointestinal disorders	0	3 (0.5%)
Gastrointestinal inflammation	0	1 (0.2%)
Duodenal ulcer haemorrhage	0	1 (0.2%)
Large intestinal obstruction	0	1 (0.2%)
General disorders and administration site conditions	2 (0.3%)	2 (0.3%)
Death	1 (0.2%)	2 (0.3%)
Sudden death	1 (0.2%)	0

* AEs leading to death within 30 days of last dose of study medication or more than 30 days of last dose and due to AE

The most frequent AEs leading to death within 30 days of last dose (other than disease progression) across both treatment groups was infection (4/16 in the aflibercept group and 3/6 in the placebo group). Two of the 4 fatal events in the aflibercept arm and 1 of the 3 in the placebo arm occurred in the context of neutropenia. The events of rectal abscess in the aflibercept group and the events of neutropenic infection and lobar pneumonia in the placebo group were assessed as related to study treatment.

In the aflibercept arm, hypovolaemia and dehydration were contributing factors to fatal events in 4 out of 16 deaths. The fatal events were dehydration (2 cases), hypovolemic shock (1 case) and metabolic encephalopathy (1 case). Grade 3 or 4 dehydration was observed in all cases, and was a consequence of either grade 3 vomiting (1 patient who died from metabolic encephalopathy) or grade 3 or 4 diarrhoea (3 patients). One of these cases was considered as related to study treatment (hypovolemic shock).

Laboratory findings

Haematology abnormalities in the pivotal study are summarised in the following Table 37.

Table 37: Haematology abnormalities, worst grade per patient, EFC10262, Saf Population

Laboratory parameter n/N1(%)	Placebo/Folfiri (N=605)	Aflibercept/Folfiri (N=611)
Neutropenia		
All grades	336/597 (56.3%)	409/603 (67.8%)
Grade 3	114/597 (19.1%)	139/603 (23.1%)
Grade 4	62/597 (10.4%)	82/603 (13.6%)
Anemia		
All grades	544/597 (91.1%)	496/603 (82.3%)
Grade 3	21/597 (3.5%)	20/603 (3.3%)
Grade 4	5/597 (0.8%)	3/603 (0.5%)
Thrombocytopenia		
All grades	202/597 (33.8%)	286/603 (47.4%)
Grade 3	5/597 (0.8%)	10/603 (1.7%)
Grade 4	5/597 (0.8%)	10/603 (1.7%)

Note: % calculated using the number of patients with at least one event (n) over the number of patients assessed for each parameter (N1) during the on-treatment period

An overview of liver and renal abnormalities as well as proteinuria events in the pivotal study is presented in the following Table 38.

Table 38: Liver and renal abnormalities and proteinuria events, EFC10262, Safety population

Laboratory parameter n/N (%)	Placebo/Folfiri (N=605)	Aflibercept/Folfiri (N=611)
ALT		
All grades	221/595 (37.1%)	284/600 (47.3%)
Grade 3	13/595 (2.2%)	15/600 (2.5%)
Grade 4	0/595	1/600 (0.2%)
AST		
All grades	296/590 (50.2%)	339/590 (57.5%)
Grade 3	9/590 (1.5%)	16/590 (2.7%)
Grade 4	1/590 (0.2%)	2/590 (0.3%)
Alkaline phosphatase		
All grades	411/594 (69.2%)	424/599 (70.8%)
Grade 3	38/594 (6.4%)	29/599 (4.8%)
Grade 4	0/594	0/599
Total bilirubin		
All grades	138/595 (23.2%)	137/600 (22.8%)
Grade 3	13/595 (2.2%)	8/600 (1.3%)
Grade 4	3/595 (0.5%)	2/600 (0.3%)
Creatinine		
All grades	108/596 (18.1%)	136/601 (22.6%)
Grade 3	2/596 (0.3%)	0/601
Grade 4	1/596 (0.2%)	0/601
Creatinine clearance		
<50 mL/mn	78/596 (13.1%)	92/601 (15.3%)
>=50 mL/mn and <=80 mL/mn	266/596 (44.6%)	281/601 (46.8%)
>80 mL/mn	252/596 (42.3%)	228/601 (37.9%)

Laboratory parameter n/N (%)	Placebo/Folfiri (N=605)	Aflibercept/Folfiri (N=611)
Number of patients with at least one proteinuria event [a]	246 (40.7%)	380 (62.2%)
Worst grade		
Grade 1	198 (32.7%)	218 (35.7%)
Grade 2	41 (6.8%)	114 (18.7%)
Grade 3	7 (1.2%)	46 (7.5%)
Grade 4 (Nephrotic syndrome)	0	2 (0.3%)
Outcome of grade > 1 events		
Recovered	31 (12.6%)	108 (28.4%)
Not recovered	17 (6.9%)	54 (14.2%)
Duration of grade > 1 events (day)		
Number	48	162
Median	16.0	27.0

[a] Includes grouped terms from AE page and proteinuria (morning spot and/or 24 hour urinalysis) from laboratory data

Safety in special populations

In the pivotal study, there was only one patient, who was in the placebo group, in the age category of ≥85 years. An overview of safety information according to age in the pivotal study is presented below.

Table 39: Number (%) of patients with ADRs by age, EFC10262, Safety population

	<65		65-74		75-84	
	Placebo Folfiri (N=372)	Aflibercept Folfiri (N=406)	Placebo Folfiri (N=196)	Aflibercept Folfiri (N=172)	Placebo Folfiri (N=36)	Aflibercept Folfiri (N=33)
Total ADRs	334(89.8%)	384(94.6%)	182(92.9%)	167(97.1%)	33(91.7%)	33(100%)
Serious ADRs - Total	51(13.7%)	101(24.9%)	35(17.9%)	75(43.6%)	7 (19.4%)	18(54.5%)
- Fatal	2 (0.5%)	1 (0.2%)	1 (0.5%)	4 (2.3%)	0	1 (3.0%)
- Hospitalisation/ prolongation of hospitalisation	47 (12.6%)	94 (23.2%)	35 (17.9%)	69 (40.1%)	7 (19.4%)	17 (51.5%)
- Life-threatening	5 (1.3%)	6 (1.5%)	1 (0.5%)	6 (3.5%)	0	2 (6.1%)
- Disability/incapacity	0	1 (0.2%)	1 (0.5%)	1 (0.6%)	0	0
- Other (medically important)	5(1.3%)	15(3.7%)	2 (1.0%)	7 (4.1%)	0	0
Drug withdrawal (SMQ)	0	0	0	0	0	0
Psychiatric disorders (SOC)	16 (4.3%)	9 (2.2%)	3 (1.5%)	5 (2.9%)	0	3 (9.1%)
Nervous system disorders (SOC)	91 (24.5%)	134(33.0%)	52 (26.5%)	53 (30.8%)	8 (22.2%)	13 (39.4%)
Accidents and Injuries (SMQ)	1 (0.3%)	4 (1.0%)	0	0	0	0
Cardiac disorders (SOC)	5 (1.3%)	7 (1.7%)	4 (2.0%)	1 (0.6%)	0	2 (6.1%)
Vascular disorders (SOC)	45 (12.1%)	169(41.6%)	34 (17.3%)	2 (41.9%)	7 (19.4%)	10 (30.3%)
Cerebrovascular disorders (SMQ)	0	2 (0.5%)	1 (0.5%)	1 (0.6%)	0	0
Infections and infestations (SOC)	23 (6.2%)	62 (15.3%)	21 (10.7%)	30 (17.4%)	2 (5.6%)	6 (18.2%)
Quality of life decreased (PT)	0	0	0	0	0	0

Note: Adverse Events are reported using MedDRA version MEDDRA13.1 and graded using NCI CTC Version 3.0.

Safety analyses by baseline creatinine clearance categories (<50, ≥50-80, >80 mL/min) were performed in all evaluable patients from the 3 placebo-controlled studies (n=2067). In patients receiving aflibercept, the adverse reactions in patients with mild renal impairment at baseline (n=352) were generally comparable with that of patients without renal impairment (n=642). A limited number of patients having moderate/severe renal impairment at baseline (n=49) were treated with aflibercept in these trials. In these patients, a >10% all grades higher incidence in dehydration was noted while other non-renal events were generally comparable to that of patients without renal impairment.

Safety related to drug-drug interactions and other interactions

No drug-drug interaction studies were submitted (see discussion on clinical pharmacology).

Discontinuation due to adverse events

An overview of adverse events leading to treatment discontinuation in the pivotal study is presented in the following Table 40.

Table 40: TEAEs ≥0.5% leading to permanent treatment discontinuation, EFC10262, Safety Population

	Placebo / Folfiri (N=605)		Aflibercept / Folfiri (N=611)	
Primary System Organ Class Preferred Term n(%)	**All grades**	**Grades ≥3**	**All grades**	**Grades ≥3**
Any class	73 (12.1%)	53 (8.8%)	164 (26.8%)	124 (20.3%)
Infections and infestations	10 (1.7%)	9 (1.5%)	21 (3.4%)	16 (2.6%)
Pneumonia	0	0	4 (0.7%)	3 (0.5%)
Blood and lymphatic system disorders	6 (1.0%)	3 (0.5%)	12 (2.0%)	8 (1.3%)
Neutropenia	4 (0.7%)	2 (0.3%)	7 (1.1%)	5 (0.8%)
Thrombocytopenia	1 (0.2%)	1 (0.2%)	4 (0.7%)	2 (0.3%)
Metabolism and nutrition disorders	1 (0.2%)	1 (0.2%)	9 (1.5%)	7 (1.1%)
Dehydration	1 (0.2%)	1 (0.2%)	6 (1.0%)	5 (0.8%)
Vascular disorders	4 (0.7%)	4 (0.7%)	23 (3.8%)	17 (2.8%)
Hypertension	0	0	14 (2.3%)	10 (1.6%)
Deep vein thrombosis	1 (0.2%)	1 (0.2%)	5 (0.8%)	5 (0.8%)
Respiratory, thoracic and mediastinal disorders	10 (1.7%)	10 (1.7%)	12 (2.0%)	9 (1.5%)
Pulmonary embolism	7 (1.2%)	7 (1.2%)	7 (1.1%)	7 (1.1%)
Gastrointestinal disorders	15 (2.5%)	9 (1.5%)	47 (7.7%)	36 (5.9%)
Diarrhoea	4 (0.7%)	2 (0.3%)	14 (2.3%)	11 (1.8%)
Stomatitis	1 (0.2%)	0	7 (1.1%)	3 (0.5%)
Renal and urinary disorders	3 (0.5%)	2 (0.3%)	15 (2.5%)	7 (1.1%)
Proteinuria	0	0	9 (1.5%)	2 (0.3%)
General disorders and administration site conditions	12 (2.0%)	7 (1.2%)	33 (5.4%)	24 (3.9%)
Fatigue	6 (1.0%)	4 (0.7%)	13 (2.1%)	11 (1.8%)
Asthenia	2 (0.3%)	1 (0.2%)	10 (1.6%)	7 (1.1%)

Post marketing experience

Not applicable

2.6.1. Discussion on clinical safety

In total 2073 patients treated with aflibercept and 1354 patients who received placebo and harbouring solid tumours were included in the integrated safety database. The pivotal safety population consists of all patients in the EFC10262 trial receiving at least one dose of study therapy, 605 in the placebo arm and 611 in the aflibercept arm (in total 1216 patients). A meta-analysis of certain selected side effects considered to be of special interest encompassing all 3 phase III studies was presented. The safety data base in mCRC is considered to be large enough for assessment of the benefit-risk balance.

The toxic potential of aflibercept when combined with FOLFIRI was clearly reflected by the higher treatment discontinuation rates, both premature (not all treatment components) and permanent (all treatment components), dose modifications of 5-FU and irinotecan, and cycle delays seen in the experimental arm (data not shown). Of note, treatment-emergent AEs led to permanent discontinuation of treatment in 26.8% of patients in the aflibercept arm compared to 12.1% of patients in the placebo arm. This means that not all aflibercept-associated side effects are clinically manageable.

Grade 3-4 TEAEs occurred in 83.5% of patients in the aflibercept arm compared to 62.5% in the placebo arm while serious TEAEs were reported in 48.1% of patients in the aflibercept arm compared to 32.7% in the placebo arm. Not unexpected, an increased frequency of typical anti-VEGF class side effects, including e.g. hypertension, proteinuria and haemorrhage, as well as of side effects associated with the chemotherapy backbone was seen in the aflibercept arm.

More patients in the aflibercept arm died from AE (2.3% vs 0.7%) while more patients in the placebo arm died from progressive disease (72% vs 60%). Fatal AEs in other context than disease progression in the safety database included infection (also in context of neutropenia), hypovolaemia/dehydration, and haemorrhage.

Zaltrap is contraindicated in case of hypersensitivity to aflibercept or to any of its excipients. Ophthalmic / intravitreal use is also contraindicated due to the hyperosmotic properties of Zaltrap. This information is included in section 4.3 of the SmPC.

The following warnings have been included in section 4.4 of the SmPC. As appropriate, relevant dosing recommendations have been included in section 4.2 of the SmPC and information has been included under the description of selected adverse reactions in section 4.8 of the SmPC.

- An increased risk of haemorrhage, including severe and sometimes fatal haemorrhagic events has been reported in patients treated with aflibercept. Patients should be monitored for signs and symptoms of gastrointestinal bleeding and other severe bleeding. Aflibercept should not be administered to patients with severe haemorrhage. Thrombocytopenia has been reported in patients treated with the Zaltrap/FOLFIRI regimen. Monitoring of complete blood count (CBC) with platelets is recommended at baseline, prior to initiation of each cycle of aflibercept, and as clinically necessary. Administration of the Zaltrap/FOLFIRI should be delayed until platelet count is ≥75 x 109/L.

- Gastrointestinal perforation including fatal GI perforation has been reported in patients treated with aflibercept. Patients should be monitored for signs and symptoms of GI perforation. Aflibercept treatment should be discontinued in patients who experience GI perforation.

- Fistula formation involving gastrointestinal and non-gastrointestinal sites has occurred in patients treated with aflibercept. Aflibercept treatment should be discontinued in patients who develop fistula.

- An increased risk of grade 3-4 hypertension (including hypertension and one case of essential hypertension) has been observed in patients treated with the Zaltrap/FOLFIRI regimen. Pre existing hypertension must be adequately controlled before starting aflibercept. If hypertension cannot be

adequately controlled, treatment with aflibercept should not be initiated. It is recommended to monitor blood pressure every two weeks, including before each administration or as clinically indicated during treatment with aflibercept. In the event of hypertension on aflibercept treatment, blood pressure should be controlled with appropriate anti hypertensive therapy and blood pressure should be monitored regularly. In case of severe hypertension, the treatment should be suspended until controlled and the aflibercept dose should be reduced to 2 mg/kg for subsequent cycles. Aflibercept should be permanently discontinued if hypertension cannot be adequately managed with appropriate anti hypertensive therapy, or if hypertensive crisis or hypertensive encephalopathy occurs.

- Hypertension may exacerbate underlying cardiovascular disease. Caution should be exercised when treating patients with history of clinically significant cardiovascular disease such as coronary artery disease, or congestive heart failure with Zaltrap. Patients with NYHA class III or IV congestive heart failure should not be treated with Zaltrap.

- Arterial thromboembolic events (ATE) (including transient ischaemic attack, cerebrovascular accident, angina pectoris, intracardiac thrombus, myocardial infarction, arterial embolism, and ischaemic colitis) have been observed in patients treated with aflibercept. Aflibercept treatment should be discontinued in patients who experience an ATE. Venous thromboembolic events (VTE) including deep vein thrombosis (DVT) and pulmonary embolism (infrequently fatal) have been reported in patients treated with aflibercept. Zaltrap should be discontinued in patients with life threatening (Grade 4) thromboembolic events (including pulmonary embolism). Patients with Grade 3 DVT should be treated with anticoagulation as clinically indicated, and aflibercept therapy should be continued. In the event of recurrence, despite appropriate anticoagulation, aflibercept treatment should be discontinued. Patients with thromboembolic events of Grade 3 or lower need to be closely monitored.

- Severe proteinuria, nephrotic syndrome, and thrombotic microangiopathy (TMA) have been observed in patients treated with aflibercept. Proteinuria should be monitored by urine dipstick analysis and urinary protein creatinine ratio (UPCR) for the development or worsening of proteinuria before each aflibercept administration. Patients with a UPCR >1 should undergo a 24 hour urine collection. Aflibercept administration should be suspended for ≥2 grams of proteinuria/24 hours and restarted when proteinuria is <2 grams/24 hours. If there is recurrence, the administration should be suspended until <2 grams/24 hours and then the dose reduced to 2 mg/kg. Aflibercept treatment should be discontinued in patients who develop nephrotic syndrome or TMA.

- A higher incidence of neutropenic complications (febrile neutropenia and neutropenic infection) has been observed in patients treated with the Zaltrap/FOLFIRI regimen. Monitoring of complete blood count (CBC) with differential count is recommended at baseline and prior to initiation of each cycle of aflibercept. Administration of Zaltrap/FOLFIRI should be delayed until neutrophil count is ≥1.5 x 109/L. Therapeutic use of G CSF at first occurrence of grade ≥3 neutropenia and secondary prophylaxis may be considered in patients who may be at increased risk for neutropenia complications.

- In the pivotal study of MCRC patients, diarrhoea and dehydration (all grade and grade 3-4) was observed more frequently in patients treated with Zaltrap compared to patients treated with placebo. Dose modification of FOLFIRI regimen, anti diarrhoeal medicinal products, and rehydration should be instituted as needed.

- In the pivotal study of MCRC patients, severe hypersensitivity reactions were reported in 0.3% of patients treated with Zaltrap and 0.5% of patients treated with placebo. In the event of a severe hypersensitivity reaction (including bronchospasm, dyspnoea, angioedema, and anaphylaxis), aflibercept should be discontinued and appropriate medical measures should be administered. In the event of a mild to moderate hypersensitivity reaction to Zaltrap (including flushing, rash, urticaria, and pruritus), aflibercept should be temporarily suspended until the reaction is resolved. Treatment with corticosteroids and/or antihistamines can be initiated as clinically indicated. Pre treatment with

corticosteroids and/or antihistamines may be considered in subsequent cycles (see section 4.2). Caution should be used when retreating patients with prior hypersensitivity reactions as recurrent hypersensitivity reactions have been observed in some patients despite prophylaxis, including corticosteroids.

- Aflibercept impaired wound healing in animal models (see discussion on non-clinical aspects). Potential for compromised wound healing (wound dehiscence, anastomotic leakage) has been reported with aflibercept. Aflibercept should be suspended for at least 4 weeks prior to elective surgery. It is recommended that aflibercept not be initiated for at least 4 weeks following major surgery and not until the surgical wound is fully healed. For minor surgery such as central venous access port placement, biopsy, and tooth extraction, aflibercept may be initiated/restarted once the surgical wound is fully healed. Aflibercept should be discontinued in patients with compromised wound healing requiring medical intervention.

- Reversible posterior (leuko)encephalopathy syndrome (RPLS/PRES), a serious but known anti-VEGF class effect was not reported in the pivotal phase III study of MCRC patients. In other studies, PRES was reported in patients treated with aflibercept as monotherapy and in combination with other chemotherapies. PRES may present with altered mental status, seizure, nausea, vomiting, headache, or visual disturbances. The diagnosis of PRES is confirmed by brain Magnetic Resonance Imaging (MRI). Aflibercept should be discontinued in patients that develop PRES.

- Elderly patients ≥65 years had an increased risk of diarrhoea, dizziness, asthenia, weight loss and dehydration. Careful monitoring is recommended in order to rapidly detect and treat signs and symptoms of diarrhoea and dehydration and to minimise potential risk. Safety in elderly patients is included as important missing information in the RMP. An observational cohort study will provide further safety information in this patient population.

- The safety of aflibercept in patients with severe liver or renal impairment is unknown, and information is limited for patients with moderate organ impairment. Patients with ECOG performance status ≥2 or having significant co morbidities may be at greater risk for a poor clinical outcome and should be carefully monitored for early clinical deterioration. There were no noteworthy differences between males and females in pivotal study. Analyses according to race were not preformed as the large majority (87.3%) of patients were Caucasians. Safety in patients with ECOG PS ≥2, non-caucasian patients or patients with renal or hepatic impairment is included as important missing information in the RMP. An observational cohort study will provide further safety information in non-caucasian patients or patients with renal or hepatic impairment.

From the safety database all the adverse reactions reported in clinical trials have been included in the Summary of Product Characteristics. With regard to selected adverse reactions:

- Infections occurred at a higher frequency in patients receiving Zaltrap than in patients receiving placebo, including urinary tract infection, nasopharyngitis, upper respiratory tract infection, pneumonia, catheter site infection and tooth infection.

- As with all therapeutic proteins, there is a potential for immunogenicity with Zaltrap. Overall across all clinical oncology studies, similar incidence of low titre anti drug antibody (ADA) responses (post baseline) in the ADA assay were observed in both patients treated with placebo and Zaltrap. High titre antibody responses to aflibercept were not detected in any patients. Some patients were also positive in the neutralising antibody assay. In the pivotal study of MCRC patients, positive responses in the ADA assay were observed at higher levels in patients treated with placebo than with Zaltrap. Positive results in the neutralising antibody assay in the MCRC pivotal study were also higher in patients treated with the placebo. There was no observed impact on the pharmacokinetic profile of aflibercept in patients who were positive in the immunogenicity assays. Given the similar ADA assay results in patients

treated with placebo or Zaltrap, the actual incidence of immunogenicity with Zaltrap based on these assays is likely to be overestimated.

Immunogenicity data are highly dependent on the sensitivity and specificity of the assay. Additionally, the observed incidence of antibody positivity in an assay may be influenced by several factors, including sample handling, timing of sample collection, concomitant medicinal products, and underlying disease. For these reasons, comparison of the incidence of antibodies to Zaltrap with the incidence of antibodies to other products may be misleading.

There are no data from the use of aflibercept in pregnant women. Studies in animals have shown reproductive toxicity (see discussion on non-clinical aspects). As angiogenesis is critical to foetal development, the inhibition of angiogenesis following administration of Zaltrap may result in adverse effects on pregnancy. Zaltrap should be used only if the potential benefit justifies the potential risk during pregnancy. If the patient becomes pregnant while taking Zaltrap, the patient should be informed of the potential hazard to the foetus. This information was included in section 4.6 of the SmPC.

No studies have been conducted to assess the impact of Zaltrap on milk production, its presence in breast milk or its effects on the breast fed child. It is unknown whether aflibercept is excreted in human milk. A risk to the newborns/infants cannot be excluded. A decision must be made whether to discontinue breast feeding or to discontinue/abstain from Zaltrap therapy taking into account the benefit of breast feeding for the child and the benefit of therapy for the woman. This information was included in section 4.6 of the SmPC.

Zaltrap has no or negligible influence on the ability to drive and use machines. If patients are experiencing symptoms that affect their vision or concentration, or their ability to react, they should be advised not to drive or use machines. This information was included in section 4.7 of the SmPC.

There is no information on the safety of aflibercept given at doses exceeding 7 mg/kg every 2 weeks or 9 mg/kg every 3 weeks. The most commonly observed adverse reactions at these doses were similar to those observed at the therapeutic dose. There is no specific antidote to Zaltrap overdose. Cases of overdose should be managed by appropriate supportive measures particularly with regards to monitoring and treatment of hypertension and proteinuria. The patient should remain under close medical supervision to monitor any adverse reactions. This information was included in section 4.9 of the SmPC.

2.6.2. Conclusions on the clinical safety

The size of the presented database is considered to be large enough for assessment of the benefit-risk balance. A substantially higher rate of treatment discontinuation, dose modifications of 5-FU/irinotecan, and cycle delays in the experimental arm all show an important toxic potential of aflibercept when combined with FOLFIRI. The frequency of TEAEs grade 3-4, serious TEAEs, and fatal AEs were all considerably higher in the experimental arm including typical anti-VEGF class side effects as well as those associated with the chemotherapy backbone. Patients ≥65 years constitute a vulnerable group. RPLS/PRES has been reported in 18 patients in the aflibercept experience. It is concluded that the addition of aflibercept to FOLFIRI is associated with significant toxicity that not always is manageable and for certain patients ultimately leads to termination also of the 5-FU/irinotecan backbone.

2.7. Pharmacovigilance

Detailed description of the pharmacovigilance system

The CHMP considered that the Pharmacovigilance system as described by the applicant fulfils the legislative requirements.

Risk Management Plan

The applicant submitted a risk management plan.

Table 41: Summary of the risk management plan

Safety concern	Proposed pharmacovigilance activities (routine and additional)	Proposed risk minimization activities (routine and additional)
Important identified risks		
Hypertension	Routine pharmacovigilance*, OCS	**Routine**: SPC section 4.2 and 4.4, PL "Pre existing hypertension must be adequately controlled before starting Zaltrap treatment. If hypertension cannot be adequately controlled, treatment with Zaltrap should not be initiated. It is recommended to monitor blood pressure every 2 weeks, including before each administration, or as clinically indicated, during treatment with aflibercept. In the event of hypertension, on aflibercept treatment, blood pressure should be controlled with appropriate anti-hypertensive therapy and blood pressure monitored regularly. In case of severe hypertension, aflibercept treatment should be suspended until controlled and the dose reduced to 2 mg/kg for subsequent cycles. Aflibercept should be permanently discontinued if blood pressure cannot be adequately managed with appropriate anti-hypertensive therapy, or if hypertensive crisis or hypertensive encephalopathy occurs. Hypertension may exacerbate underlying cardiovascular disease." In the SPC section 4.8, hypertension is listed as a very common adverse reaction.
Proteinuria/nephrotic syndrome	Routine pharmacovigilance*, OCS	**Routine**: SPC section 4.2 and 4.4, PL "Proteinuria should be monitored by urine dipstick analysis and urinary protein creatinine ratio (UPCR) for the development or worsening of proteinuria before each aflibercept administration. Patients with a UPCR >1 should undergo a 24-hour urine collection. Aflibercept administration should be suspended for ≥2 grams of proteinuria/24 hours and restarted when proteinuria is <2 grams/24 hours. If there is recurrence, the administration should be suspended until <2 grams/24 hours and then the dose reduced to 2 mg/kg. Aflibercept therapy should be discontinued in patients who develop nephrotic syndrome or TMA." In the SPC section 4.8, proteinuria is listed as a very common ADR, and nephrotic syndrome as uncommon ADR.

Safety concern	Proposed pharmacovigilance activities (routine and additional)	Proposed risk minimization activities (routine and additional)
Haemorrhage	Routine pharmacovigilance*, OCS	**Routine**: SPC section 4.2 and 4.4, PL "Patients should be monitored for signs and symptoms of GI bleeding and other severe bleeding. Aflibercept should not be administered to patients with severe haemorrhage", "and aflibercept be discontinued in case of severe haemorrhage". Haemorrhage and Epistaxis, and rectal haemorrhage are listed respectively as a very common and a common ADR in the SPC section 4.8.
Arterial thromboembolic events	Routine pharmacovigilance*, OCS	**Routine**: SPC section 4.2 and 4.4, PL "Aflibercept treatment should be discontinued in patients who experience ATEs." ATEs are listed as common ADR in SPC section 4.8.
Venous thromboembolic events	Routine pharmacovigilance*, OCS	**Routine**: SPC section 4.2 and 4.4, PL "VTE including deep vein thrombosis (DVT) and pulmonary embolism (infrequently fatal) have been reported in patients treated with aflibercept". Zaltrap should be discontinued in patients with life-threatening (Grade 4) thromboembolic events (including pulmonary embolism). Patients with Grade 3 DVT should be treated with anticoagulation as clinically indicated, and aflibercept therapy should be continued. In the event of recurrence despite appropriate anticoagulation, aflibercept treatment should be discontinued. Patients with thromboembolic events of Grade 3 or lower need to be closely monitored." VTE is listed as common ADR in SPC section 4.8.
Gastrointestinal perforation	Routine pharmacovigilance*, OCS	**Routine**: SPC section 4.2 and 4.4, PL "Patients should be monitored for signs and symptoms of GI perforation. Aflibercept treatment should be discontinued in patients who experience GI perforation." GI performation is listed as uncommon ADR in SPC section 4.8.
Fistula (from GI and non-GI origin)	Routine pharmacovigilance*, OCS	**Routine**: SPC section 4.2 and 4.4, PL "Aflibercept treatment should be discontinued in patients who develop fistula". Fistula is listed as common ADR in SPC section 4.8.
Posterior reversible encephalopathy syndrome (PRES)	Routine pharmacovigilance*, OCS	**Routine**: SPC section 4.2 and 4.4, PL "PRES may present with altered mental status, seizure, nausea, vomiting, headache, or visual disturbances. The diagnosis of PRES is confirmed by brain Magnetic Resonance Imaging (MRI). Aflibercept should be discontinued in patients that develop PRES." PRES is listed as uncommon ADR in SPC section 4.8.
Thrombotic microangiopathy (TMA)	Routine pharmacovigilance*, OCS	**Routine**: SPC section 4.2 and 4.4, PL "Aflibercept treatment should be discontinued in patients who develop nephrotic syndrome or TMA." In the SPC section 4.8, thrombotic microangiopathy is listed as uncommon ADR.

Safety concern	Proposed pharmacovigilance activities (routine and additional)	Proposed risk minimization activities (routine and additional)
Hypersensitivity reactions	Routine pharmacovigilance*, OCS	**Routine**: SPC section 4.2, 4.3 and 4.4, PL "Hypersensitivity to aflibercept or to any of the excipients" is listed in contraindications. "In the event of a severe hypersensitivity reaction (including bronchospasm, dyspnoea, angioedema, and anaphylaxis), aflibercept should be discontinued and appropriate medical measures should be administered." "In the event of a mild to moderate hypersensitivity reaction to Zaltrap (including flushing, rash, urticaria, and pruritus), aflibercept should be temporarily suspended until the reaction is resolved. Treatment with corticosteroids and/or antihistamines can be initiated as clinically indicated. Pre treatment with corticosteroids and/or antihistamines may be considered in subsequent cycles. Caution should be used when retreating patients with prior hypersensitivity reactions as recurrent hypersensitivity reactions have been observed in some patients despite prophylaxis, including corticosteroids." Hypersensitivity reaction is listed as common ADR in SPC section 4.8.
Wound healing complications	Routine pharmacovigilance*, OCS	**Routine**: SPC section 4.2 and 4.4, PL "Aflibercept should be suspended for at least 4 weeks prior to elective surgery. " "It is recommended that aflibercept not be initiated for at least 4 weeks following major surgery and not until the surgical wound is fully healed. For minor surgery such as central venous access port placement, biopsy, and tooth extraction, aflibercept may be initiated/restarted once the surgical wound is fully healed. Aflibercept should be discontinued in patients with compromised wound healing requiring medical intervention." Compromised wound healing is listed as uncommon ADR in SPC section 4.8.

Safety concern	Proposed pharmacovigilance activities (routine and additional)	Proposed risk minimization activities (routine and additional)
Increased chemotherapy-associated toxicity, affecting either hematopoiesis (including neutropenia and neutropenic complications, and thrombocytopenia), or GI tract (including diarrhoea and its dehydration complication), or skin and subcutaneous tissues (including stomatitis and palmar plantar erythrodysaesthesia syndrome)	Routine pharmacovigilance*, OCS	**Routine**: SPC section 4.2 and 4.4, PL "Monitoring of complete blood count (CBC) with differential count is recommended at baseline and prior to initiation of each cycle of aflibercept. Administration of Zaltrap/FOLFIRI should be delayed until neutrophil count is ≥1.5 x 109/l. Therapeutic use of G-CSF at first occurrence of grade ≥3 neutropenia and secondary prophylaxis may be considered in patients who may be at increased risk for neutropenia complications." "Monitoring of complete blood count (CBC) with platelets is recommended at baseline, prior to initiation of each cycle of aflibercept, and as clinically necessary. Administration of Zaltrap/FOLFIRI should be delayed until platelet count is ≥75 x 109/l." "Dose modification of FOLFIRI regimen, anti diarrhoeal medicinal products, and rehydration as needed should be instituted." "Elderly patients ≥65 years had an increased risk of diarrhoea, dizziness, asthenia, weight loss and dehydration. Careful monitoring is recommended in order to rapidly detect and treat signs and symptoms of diarrhoea and dehydration and to minimise potential risk." "Dose modification of FOLFIRI regimen is recommended for severe stomatitis and PPE syndrome". Neutropenia, leucopenia and thrombocytopenia are listed as very common and neutropenic infection/sepsis as common adverse reactions in SPC section 4.8. Stomatitis, Diarrhoea, PPE syndrome are also listed as very common, and Dehydration as common.
Important potential risks		
Off-label use (ie, intravitreous)	Routine pharmacovigilance*, DUS	**Routine**: SPC section 4.2 4.3 and 4.4, PL "Zaltrap should be administered under the supervision of a physician experienced in the use of antineoplastic medicinal products." "Zaltrap is to be administered only as an intravenous infusion and over 1 hour." "Zaltrap is contraindicated for "Ophthalmic / intravitreal use due to hyperosmotic properties of Zaltrap". "Zaltrap is a hyperosmotic solution, which is not formulated for compatibility with the intraocular environment. Zaltrap must not be administered as an intravitreal injection." In addition, an appropriate statement is written in the packaging (vial and carton) stating "For intravenous use only".
Reproductive and developmental toxicity	Routine pharmacovigilance*, Use of specific form to document pregnancy and birth outcomes	**Routine**: Specific labeling statements in SPC section 4.6 and PL "Women of childbearing potential should be advised to avoid becoming pregnant while on Zaltrap, and should be informed of the potential hazard to the foetus. Women of childbearing potential and fertile males should use effective contraception during and up to a minimum of 6 months after the last dose of treatment." "Zaltrap should be used only if the potential benefit justifies the potential risk during pregnancy. If the patient becomes pregnant while taking Zaltrap, she should be apprised of the potential hazard to the foetus."

Safety concern	Proposed pharmacovigilance activities (routine and additional)	Proposed risk minimization activities (routine and additional)
Cardiac dysfunction	Routine pharmacovigilance* OCS	**Routine**: SPC section 4.4, PL "Caution should be exercised when treating patients with history of clinically significant cardiovascular disease such as coronary artery disease, or congestive heart failure with Zaltrap. Patients with heart failure NYHA class III or IV should not be treated with Zaltrap."
Osteonecrosis	Routine pharmacovigilance* OCS	There is no specific recommendation about osteonecrosis.
Delayed fracture healing	Routine pharmacovigilance* OCS	There is no specific recommendation about delayed fracture healing.
Bone exostosis	Routine pharmacovigilance* OCS	There is no specific recommendation about bone exostosis.
Important missing information		
Safety in patients with renal impairment	Routine pharmacovigilance*, OCS	**Routine:** SPC section 4.2 states that "there have been no formal studies with aflibercept in patients with RI." "Clinical data suggest that no change in starting dose is required in patients with mild to moderate renal impairment. There are very limited data in patients with severe renal impairment; therefore, these patients should be treated with caution." The section 5.2 gives information on renal impairment.
Safety in patients with hepatic impairment	Routine pharmacovigilance*, OCS	**Routine:** SPC section 4.2 states that "there have been no formal studies with aflibercept in patients with hepatic impairment." "Clinical data suggest that no change in aflibercept dose is required in patients with mild to moderate hepatic impairment. There are no data regarding the administration of aflibercept in patients with severe hepatic impairment." The SPC section 5.2 gives information on hepatic impairment.
Safety in noncaucasian patients	Routine pharmacovigilance, OCS	**Routine:** SPC There is no specific recommendation about the use of aflibercept in noncaucasian patients in the SPC. The SPC section 5.2 states that there was no effect of ethnic groups/race on the pharmacokinetics of free aflibercept.
Safety in elderly patients (≥65 yo)	Routine pharmacovigilance*, OCS	**Routine**: SPC section 4.2 states that "No dose adjustments of Zaltrap is required in the elderly." In addition, there are specific recommendations in section 4.4, as follows: "elderly patients ≥65 years had an increased risk of diarrhoea, dizziness, asthenia, weight loss and dehydration. Careful monitoring is recommended in order to rapidly detect and treat signs and symptoms of diarrhoea and dehydration and to minimise potential risk."
Safety in children and adolescents	Routine pharmacovigilance*	**Routine:** SPC section 4.2 "There is no relevant use of Zaltrap in the paediatric population in the indication metastatic colorectal cancer".

Safety concern	Proposed pharmacovigilance activities (routine and additional)	Proposed risk minimization activities (routine and additional)
Safety in pregnant and lactating women, and fertile males	Routine pharmacovigilance*	**Routine:** SPC section 4.6, PL "Women of childbearing potential should be advised to avoid becoming pregnant while on Zaltrap, and should be informed of the potential hazard to the foetus. Women of childbearing potential and fertile males should use effective contraception during and up to a minimum of 6 months after the last dose of treatment." "Zaltrap should be used only if the potential benefit justifies the potential risk during pregnancy. If the patient becomes pregnant while taking Zaltrap, she should be apprised of the potential hazard to the foetus. " Moreover, "it is unknown whether aflibercept is excreted in human milk or not. A risk to the newborns/infants cannot be excluded. A decision must be made whether to discontinue breast-feeding or to discontinue/abstain from Zaltrap therapy taking into account the benefit of breast feeding for the child and the benefit of therapy for the woman."
Safety in long-term treatment use	Routine pharmacovigilance, OCS	There is no specific recommendation about long-term treatment use.
Safety in patients with ECOG ≥2	Routine pharmacovigilance	There is no specific recommendation about patients with ECOG ≥2.
Safety in patients with immune response to aflibercept	Routine pharmacovigilance	There is no specific recommendation about patients with positive aflibercept ADA assay.

* will include a cumulative review in each PSUR; DUS: drug utilization study; OCS: observational cohort study: of note: all safety of interest will not be systematically available in this study since dependent on their rate of occurrence

The CHMP, having considered the data submitted, was of the opinion that the below pharmacovigilance activities in addition to the use of routine pharmacovigilance are needed to investigate further some of the safety concerns:

Description	Due date
Submit report of an observational cohort study in order to provide further safety information on important identified and potential risks and in subpopulations such as elderly patients, patients with hepatic or renal impairment and non-caucasian patients. A study protocol will be submitted within 3 months of CHMP Opinion.	30/06/2018
Submit first status report of a Drug Utilisation Study to address potential for off-label use and particularly intravitreal off-label use. A study protocol will be submitted within 4 months of CHMP Opinion.	31/12/2013

No additional risk minimisation activities were required beyond those included in the product information.

2.8. User consultation

The results of the user consultation with target patient groups on the package leaflet submitted by the applicant show that the package leaflet meets the criteria for readability as set out in the *Guideline on the readability of the label and package leaflet of medicinal products for human use.*

3. Benefit-Risk Balance

Benefits

Beneficial effects

First line treatment of metastasing colorectal cancer (mCRC) in Europe commonly consists of a combination of 5-FU, leucovorin, and irinotecan (FOLFIRI) followed by second line treatment with a combination of 5-FU, leucovorin, and oxaliplatin (FOLFOX), or the reverse chronological order. There is no data to support superiority of any of the orders. The anti-VEGF compound bevacizumab is licensed for the treatment of mCRC but regional differences in its use are at hand, with a substantial higher fraction of patients in US being exposed to the drug in the first line setting compared to European patients.

Aflibercept is a novel anti-VEGF compound with a broader target range than bevacizumab. With the present application, licensure for aflibercept in combination with the FOLFIRI backbone in the 2nd line setting of mCRC after failure of an oxaliplatin-containing regimen is sought. Data from a single placebo-controlled phase III pivotal study, EFC10262, is presented.

The rationales for choice of dose and companion drugs are accepted. The results are considered valid for the EU. No major concerns regarding the conduct of the study are noted.

The primary efficacy endpoint, overall survival, was evaluated in the ITT population consisting of 612 patients in the aflibercept arm and 614 patients in the placebo arm. The analysis was based on reasonably mature data and performed after a median follow-up time of 22.3 months. A statistically significant difference in overall survival between the study arms was noted, stratified HR 0.817 (95.34% CI: 0.713 to 0.937), p=0.0032. The difference in median OS was 1.44 months in favour of the aflibercept arm, 13.50 months (95.34% CI:12.517 to 14.949) in the aflibercept arm compared to 12.06 months (11.072 to 13.109) in the placebo arm. Sensitivity analyses were in support of the primary analysis. A sustained positive effect of aflibercept was still present at 30 months with a survival probability two-fold higher than in the placebo arm (22.3% vs 12%).

Progression-free survival according to independent review was a secondary endpoint, performed at the second interim analysis and subject to a highly conservative split of alpha level with overall survival. Median PFS was 2.23 months significantly longer in the aflibercept arm (6.90 months) than in the placebo arm (4.67 months); stratified HR 0.758 (99.99% CI: 0.578-0.995), p=0.00007. The sensitivity analyses performed are considered to be in support of the primary analysis, although the difference between study arms was not formally statistically significant in the investigators' assessment-based analysis.

Overall objective response rate, performed in the evaluable population, was a secondary endpoint and showed a significantly better response rate in the aflibercept arm, 19.8% (95%CI: 16.4-23.2%) vs 11.1% (8.5-13.8%) in the placebo arm, stratified p=0.0001.

Thus, the results of the secondary analyses support the beneficial treatment effect seen with the addition of aflibercept to FOLFIRI in the primary analysis of overall survival.

Uncertainty in the knowledge about the beneficial effects

A numerically lower treatment effect on OS with the Zaltrap/FOLFIRI regimen was reported for patients with prior bevacizumab as compared to patients without prior bevacizumab exposure. Considering that this result was based on a subgroup analysis and that there was no evidence of heterogeneity in treatment effect (non significant interaction test), the CHMP considered that this did not raise concern.

A biomarker program encompassing the EFC10262, EFC10668 and EFC11338 studies has been initiated. The results of these studies may aid in the selection of patients for treatment with aflibercept and should be presented to the EMA as a post-authorisation commitment which is considered key to the benefit-risk balance.

Risks

Unfavourable effects

Treatment-emergent AEs led to permanent discontinuation of treatment in 26.8% of patients in the aflibercept arm compared to 12.1% of patients in the placebo arm, clearly reflecting the toxic potential of the study drug when combined with FOLFIRI. Furthermore, substantially more dose modifications and premature discontinuation of all study drugs as well as cycle delays were seen in the experimental arm.

Grade 3-4 TEAEs occurred in 83.5% of patients in the aflibercept arm compared to 62.5% in the placebo arm. Events with a frequency ≥2% higher in the aflibercept arm included diarrhoea, hypertension, asthenic conditions, stomatitis and ulceration, and dehydration.

Serious TEAEs were reported in 48.1% of patients in the aflibercept arm compared to 32.7% in the placebo arm. The most common SAE (SOC, all grades) and also with the largest difference versus placebo was gastrointestinal disorders (20% vs 11%) followed by infection and infestations (11.3% vs 6.3%).

While more patients in the placebo arm died from progressive disease (72% vs 60%) more patients in the aflibercept arm died from AE (all within 30 days from last dose, 2.3% vs 0.7%). Fatal AEs in other context than disease progression in the safety database include, but are not restricted to, infection (also in context of neutropenia), hypovolaemia and dehydration, and haemorrhage.

Aflibercept is associated anti-VEGF class side effects. Deduced from the meta-analysis including data from the 3 phase III studies, potential aflibercept-associated anti-VEGF class side effects considered to be of major clinical importance due to increased risk are: Hypertension (RR = 4.24 compared to placebo), haemorrhage (RR = 2.16), GI and non-GI fistulae (OR = 4.57).

Addition of aflibercept also increased the frequency of certain AEs associated with irinotecan and 5-FU, including diarrhoea, neutropenia, and stomatitis.

Reversible posterior leukoencephalopathy syndrome, a serious but known anti-VEGF class effect, has been reported in 18 patients (0.5%) in the aflibercept experience, including cases associated with single drug therapy. This is within the range reported for other anti-VEGF compounds.

Uncertainty in the knowledge about the unfavourable effects

In elderly patients ≥65 years the incidence of specific AEs, such as diarrhoea, dizziness, asthenia, weight decrease and dehydration was ≥5% higher than in the younger population. These data should not be underestimated, considering the CRC epidemiology. Further information on elderly patients is expected from an observational cohort study.

The safety of aflibercept in patients with severe liver or renal impairment is unknown and restricted information exists for patients with moderate organ impairment. This information is included in section 4.4 of the SmPC and organ impairment is included as important missing information in the RMP also to be addressed via an observational cohort study as additional pharmacovigilance activity.

Preclinical findings suggest a possible risk for decreased bone metabolism and it is not clear whether this translates into clinical relevance in terms of prolonged fracture healing. As a result, osteonecrosis, delayed fracture healing and bone exostosis are included as important potential risks in the RMP. These will also be addressed via an observational cohort study as additional pharmacovigilance activity.

Benefit-risk balance

Importance of favourable and unfavourable effects

In the 2nd line setting of mCRC a median OS of approximately 10-13 months is currently expected. An overall survival benefit of HR 0.817, albeit robust, is considered as a relatively modest clinical benefit.

The toxicity of aflibercept when combined with FOLFIRI is generally considered pronounced with a treatment discontinuation rate of 26.8% and a substantially larger fraction of patients experiencing all types of AEs than in the placebo arm.

Benefit-risk balance

In terms of balance of benefits and risks, the overall toxicity of aflibercept in the studied combination regimen was considered significant, not always manageable, and in some patients ultimately leading to termination also of the chemotherapy. However, despite this toxicity, there was still a small but clinically significant survival advantage. Thus, the benefits associated with aflibercept were considered to outweigh the risks.

Discussion on the benefit-risk balance

In order to optimise benefit–risk balance, it is essential to identify the proper target population for therapy. This might be possible to accomplish through the judicious use of biomarkers in all phases of clinical drug development. Regrettably, no validated predictive serum or plasma biomarkers have been identified during the development of aflibercept that correlate with treatment outcomes. Thus, the EMA has requested to the applicant company to analyse plasma and tissue samples from the available trials, with the primary aim to identify biomarkers to allow better selection of the population likely to experience a beneficial effect following treatment with aflibercept.

4. Recommendations

Outcome

Based on the CHMP review of data on quality, safety and efficacy, the CHMP considers by majority decision that the risk-benefit balance of Zaltrap in combination with irinotecan/5-fluorouracil/folinic acid (FOLFIRI) chemotherapy in the treatment of adults with metastatic colorectal cancer (MCRC) that is resistant to or has progressed after an oxaliplatin-containing regimen is favourable and therefore recommends the granting of the marketing authorisation subject to the following conditions:

Conditions or restrictions regarding supply and use

Medicinal product subject to restricted medical prescription (See Annex I: Summary of Product Characteristics, section 4.2).

Other conditions and requirements of the Marketing Authorisation

- **Periodic safety update reports**

The marketing authorisation holder shall submit the first periodic safety update report for this product within 6 months following authorisation. Subsequently, the marketing authorisation holder shall submit periodic safety update reports for this product in accordance with the requirements set out in the list of Union reference dates (EURD list) provided for under Article 107c(7) of Directive 2001/83/EC and published on the European medicines web-portal.

Conditions or restrictions with regard to the safe and effective use of the medicinal product

- **Risk Management Plan (RMP)**

The MAH shall perform the required pharmacovigilance activities and interventions detailed in the agreed RMP presented in Module 1.8.2 of the Marketing Authorisation and any agreed subsequent updates of the RMP.

An updated RMP shall be submitted annually until renewal.

When the submission of a PSUR and the update of a RMP coincide, they should be submitted at the same time.

In addition, an updated RMP should be submitted:

- At the request of the European Medicines Agency;
- Whenever the risk management system is modified, especially as the result of new information being received that may lead to a significant change to the benefit/risk profile or as the result of an important (pharmacovigilance or risk minimisation) milestone being reached.

- **Obligation to complete post-authorisation measures**

The MAH shall complete, within the stated timeframe, the following measures:

Description	Due date
Submission of the results of the biomarker programme encompassing the EFC10262, EFC10668 and EFC11338 studies	31/12/2016

Conditions or restrictions with regard to the safe and effective use of the medicinal product to be implemented by the Member States

Not applicable.

Divergent positions to the majority recommendation are appended to this report.

New Active Substance Status

Based on the CHMP review of data on the quality properties of the active substance, the CHMP considers that aflibercept is qualified as a new active substance.

DIVERGENT POSITION EXPRESSED BY CHMP MEMBERS

Although aflibercept exerts an anti angiogenesis activity, the favourable effects are considered modest. The toxicity is substantial. In the overall population, the observed improvement in OS by 1.44 month is associated with a 2.23 months gain in median PFS. This is considered of modest clinical relevance and not able to outweigh the substantial risks related to treatment with aflibercept.

In the sub-group of patients pre-treated with bevacizumab the even smaller activity reported (when compared with bevacizumab naïve patients) is a major concern, in particular since most patients with mCRC will be treated with bevacizumab as part of first line palliative treatment in line with the current EU treatment guidelines. This population has not been sufficiently addressed and no markers are defined to distinguish patients that may encounter clinically relevant results. This will probably never be further addressed after the marketing of aflibercept.

Crucial questions on the benefit-risk balance of this medicinal product will thus remain unanswered and all in all the benefit/risk is considered negative.

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Pierre Démolis

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Alar Irs

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Andrea Laslop

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Hubert Leufkens

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Emilia Mavrokordatou

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Jan Mazag

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Barbara van Zwieten-Boot

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