20 March 2014
EMA/CHMP/797690/2013
Committee for Medicinal Products for Human Use (CHMP)

Assessment report

Vynfinit

International non-proprietary name: vintafolide

Procedure No. EMEA/H/C/002571/0000

Note

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

This AR reflects the CHMP opinion on 20 March 2014, which originally recommended to approve this medicine. The recommendation was conditional to the results of the on-going confirmatory study EC-FV-06. Before the marketing authorisation was granted by the EC, the results of this study became available and did not support the initial recommendation. Subsequently, the company decided to withdraw the application and not to pursue any longer the authorisation for marketing this product. The current report does not include the latest results of this study as the withdrawal of the application did not allow for the CHMP to revise its opinion in light of the new data.

For further information please refer to the Q&A which followed the company's withdrawal of the application.

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List of abbreviations

^{99m}Tc-EC20, Technetium 99m-EC20	etarfolatide-technetium 99m complex
AE	adverse event
ALT	alanine aminotransferase
ANC	absolute neutrophil count
ASCO	American Society of Clinical Oncology
AST	aspartate aminotransferase
ATC	Anatomic Therapeutic Chemical
BSA	body surface area
BUN	blood urea nitrogen
CA-125	cancer antigen 125
CBC	complete blood cell
CI	confidence interval
CR	complete response
CRF	case report form
CT	computed tomography
CTCAE	Common Terminology Criteria for Adverse Events
DCR	disease control rate
DSMB	data safety monitoring board
EC20	folate-targeting imaging agent
EC145	folic acid-desacetylvinblastine hydrazide conjugate
ECOG	Eastern Cooperative Oncology Group
FDA	Food and Drug Administration
FIGO	International Federation of Gynaecology and Obstetrics
FR	folate receptor
GCP	Good Clinical Practice
G-CSF	granulocyte colony-stimulating factor
GGT	gamma–glutamyl transferase
GCIG	Gynaecologic Cancer Intergroup
GERD	gastroesophageal reflux disease
HR	hazard ratio
IA	interim analysis
IBW	ideal body weight
ICH	International Conference on Harmonisation
IE	insufficient evaluation
IEC	independent ethics committee
IM	intramuscular
IRB	institutional review board
IRF	independent review facility
ITT	intent to treat
IV	intravenous, intravenously
LD	longest diameter
LVEF	left ventricular ejection fraction
MedDRA	Medical Dictionary for Regulatory Activities
mITT	intent to treat population of all measurable patients
MRI	magnetic resonance imaging
MUGA	multiple gated acquisition
ORR	objective response rate
OS	overall survival
PD	progressive disease
PFS	progression-free survival
PLD	pegylated liposomal doxorubicin
PP	per-protocol
PROC	platinum resistant ovarian cancer
PR	partial response
RBC	red blood cell

RECIST	Response Evaluation Criteria in Solid Tumours
RFC	reduce folate carrier
SAE	serious adverse event
SAP	statistical analysis plan
SD	stable disease
SOC	system organ class
SPECT	single photon emission computed tomography
TEAE	treatment emergent adverse event
ULN	upper limit of normal
WBC	white blood cell
WHO	World Health Organization
AUC	area under curve
BIW	twice a week
Cl	clearance
Cmax	maximum concentration
CR	complete response
CYP	cytochrome P-450
DAVLBH	desacetylvinblastinehydrazide
DTPA	diethylenetriaminepentaacetic acid
EC119	Pteroyl-γ-Glu-Asp-Arg-Asp-Asp-Cys
EC17	folate-ethylenediamine-fluorescein
EC20	Pteroyl- β -Glu-β-Dap-Asp-Cys
FR	folate receptor
GLP	Good Laboratory Practice
IC50	concentration that causes 50% cell kill
K3EDTA	tripotassiumethylenediaminotetraacetic acid
LC-MS/MS	Liquid chromatography-mass spectrometry/mass spectrometry
LLOQ	lower limit of quantitation
LOEL	low observed effect level
MTD	maximum tolerated dose
PBS	phosphate buffered saline
PK	pharmacokinetic
PLD	pegylated liposomal doxorubicin
PROC	platinum-resistant ovarian cancer
RA	relative affinity
RFC	reduced folate carrier
TIW	3 times per week
Vc	volume of central compartmentalization
Vz	volume of distribution

1. Background information on the procedure

1.1. Submission of the dossier

The applicant Endocyte Europe, B.V. submitted on 26 October 2012 an application for Marketing Authorisation to the European Medicines Agency (EMA) for Vynfinit, through the centralised procedure falling within the Article 3(1) and point 4 of Annex of Regulation (EC) No 726/2004. The eligibility to the centralised procedure was agreed upon by the EMA/CHMP on 21 June 2012.

Vynfinit, was designated as an orphan medicinal product EU/3/12/959 on 9 February 2012. Vynfinit was designated as an orphan medicinal product in the following indication: Treatment of ovarian cancer.

The applicant applied for the following indication: Vynfinit, in combination with pegylated liposomal doxorubicin (PLD) is indicated for the treatment of adult patients with platinum resistant ovarian cancer (PROC) who express the folate receptor on all target lesions as assessed by Folcepri.

The legal basis for this application refers to:

Article 8.3 of Directive 2001/83/EC, as amended - complete and independent application. The applicant indicated that vintafolide was considered to be a new active substance.

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 tests or studies.

Information on Paediatric requirements

Pursuant to Article 7 of Regulation (EC) No 1901/2006, the application included an EMA Decision CW/1/2011 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 submit a critical report addressing the possible similarity with authorised orphan medicinal products.

Applicant's request(s) for consideration

Conditional Marketing Authorisation

The applicant requested consideration of its application for a Conditional Marketing Authorisation in accordance with Article 14(7) of the above mentioned Regulation based on the following claims:

- The risk-benefit balance of the medicinal product, as defined in Article 1(28a) of Directive 2001/83/EC, is positive.

In study EC-FV-04, a randomised, multicentre, open-label phase 2 study, treatment with vintafolide in combination with pegylated liposomal doxorubicin (PLD) resulted in a statistically significant reduction in the risk of progression or death and an associated clinically meaningful

difference in median PFS compared to the PLD alone arm. The efficacy was related to folate receptor (FR) expression, with the greatest benefit observed in the population with the worse prognosis, the population who express the folate receptor on all target lesions [FR(100%)] as assessed by ^{99m}Tc-etarfolatide imaging procedure. Balanced against the outlined benefit, the risk of vintafolide use in combination with PLD in the overall platinum resistant ovarian cancer (PROC) patient population was acceptable and manageable. While the addition of vintafolide to PLD added some toxicity to that associated with PLD alone, the safety profile of the combination was comparable to the safety profile of other agents used in the treatment of ovarian cancer.

- It is likely that the applicant will be in a position to provide comprehensive clinical data.

Additional comprehensive data are likely to be available from the ongoing phase 3 study EC-FV-06, a randomised double-blind phase 3 trial comparing vintafolide and PLD in combination versus PLD in patients with PROC. The study has been designed to confirm and support the benefit-risk balance in the 100% FR-positive PROC patient population. The primary analysis for Study EC-FV-06 will compare PFS (based on RECIST V 1.1 criteria) in patients with platinum-resistant ovarian cancer with all target lesions ^{99m}Tc-etarfolatide positive [FR(100%)] who receive combination therapy with vintafolide and PLD to patients with platinum-resistant ovarian cancer who receive PLD and placebo. Additional analyses will evaluate the lower FR positive levels. A total of up to approximately 600 FR positive patients are expected to be enrolled in the study, with approximately 350 of those being FR(100%) patients.

- Unmet medical needs to be fulfilled.

PROC is an orphan condition with a high unmet medical need. Patients with PROC have very few therapeutic options. Importantly, the subset of women whose disease expresses the FR represents an epidemiologically small subset of PROC and an area of high unmet medical need, with an overall worse prognosis and no approved agents for selection or treatment.

- The benefits to public health of the immediate availability on the market of the medicinal product concerned outweigh the risk inherent in the fact that additional data are still required.

The available data from the phase 2 study indicate a positive risk-benefit balance for vintafolide for the proposed indication. Given the available results of the phase 2 study, the timelines of completion of the phase 3 study (EC-FV-06) and in view of the unmet medical need, the benefits to public health of the immediate availability on the market of the medicinal product concerned outweigh the risk inherent in the fact that additional data are still required.

New active Substance status

The applicant requested the active substance vintafolide contained in the above medicinal product to be considered as a new active substance in itself, as the applicant claims that it is not a constituent of a product previously authorised within the Union.

Scientific Advice

The applicant received Scientific Advice from the CHMP on 14 April 2011, 19 May 2011 and 22 September 2011. The Scientific Advice pertained to quality, non-clinical and clinical aspects of the dossier.

Licensing status

The product was not licensed in any country at the time of submission of the application.

1.2. Manufacturers

The manufacturing sites comply with the EU Good Manufacturing Practice requirements.

Manufacturer responsible for batch release

Schering-Plough (Brinny) Company
Innishannon
Co. Cork
Ireland

1.3. Steps taken for the assessment of the product

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

Rapporteur: Bengt Ljungberg Co-Rapporteur: Robert James Hemmings

- The application was received by the EMA on 26 October 2012.
- The procedure started on 21 November 2012.
- The CHMP adopted a report on similarity of Vynfinit with Yondelis (trabectedin) on date 17 January 2013
- The Rapporteur's first Assessment Report was circulated to all CHMP members on 11 February 2013. The Co-Rapporteur's first Assessment Report was circulated to all CHMP members on 8 February 2013.
- During the PRAC meeting on 7 March 2013, the PRAC adopted an RMP Advice and assessment overview.
- During the meeting on 21 March 2013, the CHMP agreed on the consolidated List of Questions to be sent to the applicant. The consolidated List of Questions was sent to the applicant on 22 March 2013.
- The applicant submitted the responses to the CHMP consolidated List of Questions on 13 September 2013.
- The summary report of the GCP inspection carried out between 22 April 2013 and 23 May 2013 at one site in Poland, one site in the United States and the sponsor site, was issued on 5 July 2013.
- The Rapporteurs circulated the Joint Assessment Report on the applicant's responses to the List of Questions to all CHMP members on 28 October 2013.
- During the PRAC meeting on 7 November 2013, the PRAC adopted an RMP Advice and assessment overview.
- During the CHMP meeting on 21 November 2013, the CHMP agreed on a list of outstanding

issues to be addressed in writing by the applicant.

- The applicant submitted the responses to the CHMP List of Outstanding Issues on 10 December 2013.
- The Rapporteurs circulated the Joint Assessment Report on the applicant's responses to the List of Questions to all CHMP members on 3 January 2014.
- During the PRAC meeting on 9 January 2014, the PRAC adopted an RMP Advice and assessment overview.
- During the CHMP meeting on 23 January 2014 the CHMP agreed on a second list of outstanding issues to be addressed in writing by the applicant.
- The applicant submitted the responses to the CHMP second List of Outstanding Issues on 29 January 2014.
- The Rapporteurs circulated the Joint Assessment Report on the responses provided by the applicant on 6 February 2014
- During the PRAC meeting on 6 February 2014, the PRAC adopted the PRAC Rapporteur's RMP Assessment Report.
- During the CHMP meeting on 18 February 2014, outstanding issues were addressed by the applicant during an oral explanation.
- During the meeting on 20 March 2014, 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 Vynfinit.

2. Scientific discussion

2.1. Introduction

Problem statement

An estimated 225,000 new cases of ovarian cancer were reported worldwide in 2008. In Europe, an estimated 65,538 new cases of ovarian cancer were reported in 2012 with 42,704 deaths (EUCAN Cancer factsheets: Ovary). Ovarian cancer is the fifth most common type of cancer in women and the fourth most common cause of cancer death in women. Epithelial ovarian carcinoma is the most common ovarian cancer accounting for approximately 90% of cases.

Several factors appear to affect the risk of developing ovarian cancer. 50% of cases occur in women older than 65 years. Approximately 5% to 10% of ovarian cancers are familial. The most important risk factor for ovarian cancer is a family history of a first-degree relative (e.g., mother, daughter, or sister) with the disease. Women who have had multiple pregnancies appear to have a lower risk than those with fewer pregnancies.

The most common symptoms of ovarian cancer arise from peritoneal spread and include abdominal pain, bloating, abdominal swelling (mainly due to ascites), nausea, anorexia and weight loss.

Prognosis factors include the histological grade and subtypes as well as the stage of the disease at diagnosis. The presence or absence of residual disease at the completion of the initial surgery, the patient's functional status and age, and the use or non-use of platin-based chemotherapy are also prognostic factors.

The FIGO (International Federation of Gynaecology and Obstetrics) staging system is used to classify the extent of disease and provide the basis for treatment considerations. According to the FIGO staging system, patients with newly diagnosed Stage I or II disease have limited ovarian carcinoma confined to the ovaries and pelvis; Patients diagnosed with Stage III or IV disease have advanced ovarian carcinoma that is intraperitoneal (IP) or involves distant metastases. Management of ovarian carcinoma depends on the extent of disease and prior therapy that the patient has received.

Advances in optimisation of cytoreductive surgery and platinum-based chemotherapy have resulted in a 5-year survival rate of approximately 45% (Bookman, 2005). Unfortunately, the majority of patients diagnosed with ovarian cancer will eventually develop disease that is resistant to platinum-based therapy. Women who initially respond to platinum-containing systemic therapy but progress after a treatment-free interval of less than 6 months are considered to have platinum-resistant ovarian cancer. Platinum-resistant ovarian cancer has a poor prognosis and patients have limited therapeutic options: topotecan, paclitaxel, pegylated liposomal doxorubicin (PLD). Other therapeutic options are urgently required to address the unmet medical need.

About the product

Folate (vitamin B9) is required by cells for normal metabolic activity as well as for DNA synthesis, and therefore essential for cell division. Folate is internalised by cells via two distinct mechanisms. The first is through the reduced folate carrier (RFC), a membrane transporter, present on almost all normal cells, that shuttles folate into the cell via a low affinity mechanism (Km~200 µM). The second mechanism involves the high affinity (Kd <1 nM) membrane folate receptor (FR) protein, which is expressed on many highly proliferative cancer cells. Following tight binding, internalisation, and a conformational change-induced intracellular release of folate, the receptor returns to the cell surface to resume its activity. The RFC is found in virtually all cells and constitutes the primary pathway responsible for uptake of physiological folates. The FR is found primarily on polarised epithelial cells and activated macrophages, and preferentially binds and internalises oxidised folates via receptor-mediated endocytosis. While low concentrations of the reduced folate carrier are probably sufficient to supply the folate requirements of most normal cells, the FR is frequently over-expressed on cancer cells, enabling the malignant cell to compete successfully for the vitamin when supplies are limited. At least three forms of the FR have been described (alpha, beta, gamma and truncated gamma).

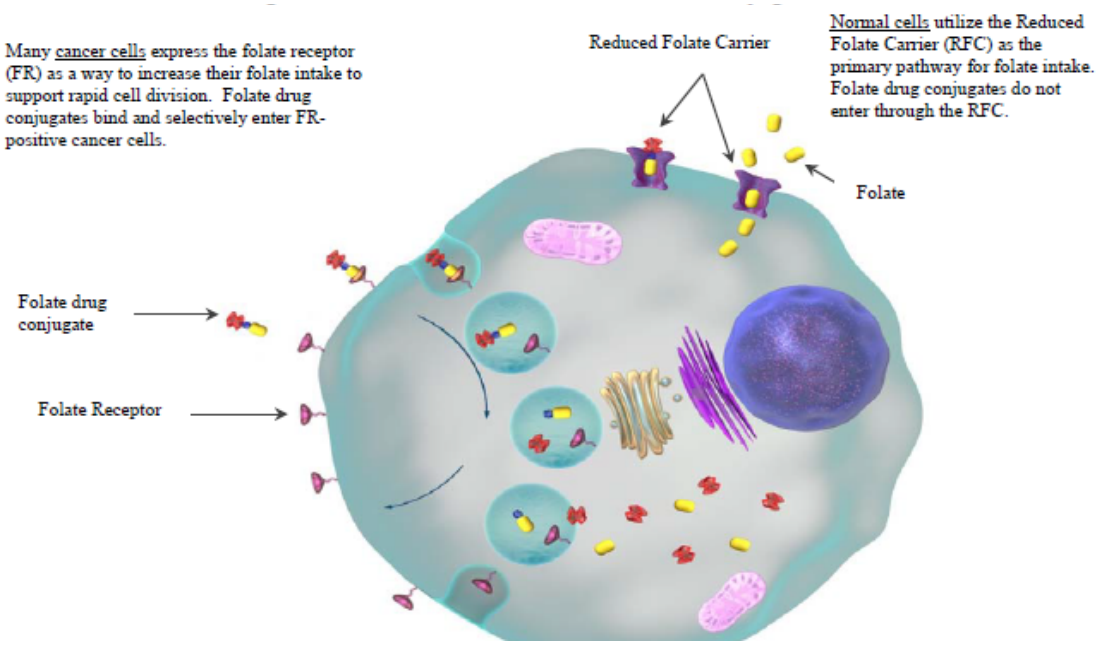

Figure 1: Mechanism of action of folate conjugates

A large number of cancers express high levels of the FR (Parker, 2005; Reddy, 2006; Vlahov, 2006; Leamon, 2007; Reddy, 2007) and FR expression is often associated with a worse overall prognosis. In ovarian cancer specifically, FR expression is known to increase with cancer stage, grade, and platinum resistant phenotype and be associated with a faster PFS and shorter OS (Toffoli, 1997; Toffoli, 1998; Chen, 2012).

Vynfinit (vintafolide, also referred as EC145) is a drug conjugate of folic acid chemically linked through a reducible bond to the vinca alkaloid desacetylvinblastine hydrazide (DAVLBH) to be used together with a companion diagnostic such as Folcepri, ^{99m}Tc etarfolatide (^{99m}Tc EC20) designed to detect tumour lesions which express active FRs.

Vintafolide was designed to specifically bind to the high affinity FR present on the surface of cancer cells and to release its active component, DAVLBH, once it enters the endosome of the target cell. DAVLBH, the drug payload, is a member of the vinca alkaloid class of antineoplastic agents (e.g. vinblastine, vincristine, vindesine) which act by inhibiting the polymerization of tubulin into microtubules, thus blocking spindle formation and arresting cells in metaphase of mitosis.

The applied indication was: Vynfinit in combination with pegylated liposomal doxorubicin (PLD) is indicated for the treatment of adult patients with platinum resistant ovarian cancer (PROC) who express the folate receptor (FR) on all target lesions as assessed by Folcepri.

Following review, the final indication for Vynfinit proposed was: Vynfinit in combination with pegylated liposomal doxorubicin (PLD) is indicated for the treatment of adult patients with platinum resistant ovarian cancer (PROC) who express the folate receptor (FR) on all target lesions. Folate receptor status should be assessed by a diagnostic medicinal product approved for the selection of adult patients for treatment with vintafolide, using single photon emission computed tomography

(SPECT) imaging, in combination with Computed Tomography (CT) or Magnetic Resonance Imaging (MRI).

2.2. Quality aspects

2.2.1. Introduction

The finished product is presented as a powder for solution for injection containing 2.5 mg of vintafolide as active substance.

Other ingredients are: Sodium citrate dihydrate, citric acid anhydrous and mannitol.

The product is available in Type I glass vial with a siliconised grey chlorobutyl stopper and an aluminium seal.

2.2.2. Active Substance

The active component vintafolide is composed of a vinblastine derivative tethered to a pentapeptide with a folic acid residue at its N-terminus. The folic acid residue acts as a targeting agent for tumour lines that over-express the folate receptor. The vinblastine derivative is the pharmacologically active species.

The chemical name of vintafolide is vincaleukoblastin-23-oic acid, O-4-deacetyl-2-[(2-mercaptoethoxy)carbonyl]hydrazide, disulfide with N-[4-[[(2-amino-3,4-dihydro-4-oxo-6-pteridinyl)methyl]amino]benzoyl]-L-γ-glutamyl-L-α-aspartyl-L-arginyl-L-α-aspartyl-L-α-aspartyl-L-cysteine and has the following structure:

Vintafolide is an amorphous yellow flocculent hygroscopic solid is presented as a solution.

Vintafolide exhibits stereoisomerism due to the presence of 15 chiral centres. Polymorphism has not been observed for vintafolide as it is a non-crystalline solid.

The characterisation of the active substance and its impurities are in accordance with the EU guideline on chemistry of new active substances. Potential and actual impurities were well discussed with regards to their origin and characterised.

Manufacture

Vintafolide is a semi-synthetic active substance produced in 6 main steps using well defined starting materials with acceptable specification. Subsequent purification by column chromatography and concentration as an aqueous solution provides commercial vintafolide active substance solution.

No stereocentres are modified in the process. The possibility of racemisation has been discussed and precautions have been taken to minimise this risk. All of the stereoisomers were found to be well controlled in the drug substance by control of the starting material/ intermediate.

The characterisation of the active substance and its impurities are in accordance with the EU guideline on chemistry of new active substances. Potential and actual impurities were well discussed with regards to their origin and characterised.

Adequate in-process controls are applied during the synthesis. The specifications and control methods for intermediate products, starting materials and reagents have been presented.

Specification

The active substance specification includes tests for appearance, identity (LC/ESI-MS), individual specified impurities (HPLC), individual unspecified impurities (HPLC), total impurities (HPLC), concentration (HPLC), residual solvents (GC-FID), and heavy metals (Ph Eur). Impurities present at higher than the qualification threshold according to ICH Q3A were qualified by toxicological and clinical studies and appropriate specifications have been set.

The analytical methods used have been adequately described and non-compendial methods appropriately validated in accordance with the ICH guidelines.

Batch analysis data for three primary stability batches and three validation batches of the active substance are provided. The results are within the specifications and consistent from batch to batch.

Stability

Stability data on 3 pilot scale batches of the active substance from the proposed manufacturer stored in the intended commercial package for 6 months at long term conditions -20 °C / ambient RH and for up 6 months under accelerate conditions -5 °C / ambient RH according to ICH guidelines were provided. The tests performed on stability are appearance, assay/concentration, individual specified and unspecified impurities. Microbiological and endotoxin tests were also carried out on an annual basis.

The results from stability studies conducted in accordance with ICH guidelines indicate that the active substance manufactured by the proposed supplier(s) is sufficiently stable. The stability results justify the proposed retest period in the proposed container.

2.2.3. Finished Medicinal Product

Pharmaceutical development

The proposed commercial finished product is a citrate buffered mannitol lyophilisate containing vintafolide reconstituted with sterile water for injection prior to administration.

All excipients are well known pharmaceutical ingredients and their quality is compliant with Ph. Eur standards. There are no novel excipients used in the finished product formulation. The list of excipients is included in section 6.1 of the SmPC.

The finished product is presented as a single use vial. Therefore, no antimicrobial preservatives are used in the formulation. The same container closure system intended for marketing was used throughout development and microbial contamination was not observed.

The primary container is a type I glass compliant with Ph Eur. An amber glass vial is used because the product is light sensitive. The stoppers are compliant with the chemical test requirements for Type I closures, as described in the Ph. Eur. and do not contain natural rubber.

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Adventitious agents

No excipients derived from animal or human origin have been used.

Manufacture of the product

The manufacturing process consists of mixing a solution of the active substance and excipients under nitrogen atmosphere, sterile filtration of the solution followed by aseptic filling operation and lyophilisation followed by stoppering and capping of the vials.

Major steps of the manufacturing process have been validated by a number of studies. Process validation data on three commercial scale batches and results from four media fills have been presented. It has been demonstrated that the manufacturing process is capable of producing the finished product of intended quality in a reproducible manner. The in-process controls are adequate for this type of manufacturing process

Product specification

The finished product release specifications include appropriate tests for this kind of dosage form: appearance, reconstitution time, identity (LC/ESI-MS), degradation products (RPLC), assay (RPLC), pH (Ph Eur), sterility (Ph Eur), endotoxin (Ph Eur), particulate matter (Ph Eur), uniformity of dosage .

Batch analysis results confirm the consistency of the manufacturing process and its ability to manufacture to the intended product specification.

Stability of the product

Stability data of 3 pilot batches of finished product stored under long term conditions for 18 months at 25 ºC / 60% RH and for up to 6 months under accelerated conditions at 40 ºC / 75% RH according to the ICH guidelines were provided. The batches are identical to those proposed for marketing and were packed in the primary packaging proposed for marketing. Additionally, a supportive stability batch manufactured with the intended market formulation and process and packaged in the proposed market container/closure systems was stored at 25°C / 60% RH for up to 24 months.

Samples were tested for solid appearance, reconstituted solution, appearance, reconstitution time, degradation products (RPLC), assay (RPLC pH (Ph Eur), sterility (Ph Eur), endotoxin (Ph Eur), particulate matter (Ph Eur). The analytical procedures used are stability indicating.

Two in-use stabilities studies were performed on the reconstituted vial and on a mini-bag. The reconstituted vial was stored at 25 ºC / 60% RH and 25 ºC for up 24 hours. The mini-bag was stored at room temperature (22 ± 3 ºC) and used within 24 hours of preparation.

One batch was subjected to photostability stress testing according to the conditions stated in ICH Q1B as foil wrapped (unexposed control samples) and non-foil wrapped (exposed samples) under illumination of a minimum 1.2 million lux hours cool white fluorescent light and 200 Watt hours/m2. The samples were tested for appearance and assay and degradation products. The results of the

photostability study showed no significant change and met acceptance criteria described in between the unexposed (control) and exposed samples when stored in the proposed amber vial/ultraviolet light at 5°C/ambient humidity.

Based on available stability data, the shelf-life and storage conditions as stated in the SmPC are acceptable.

2.2.4. Discussion on chemical, pharmaceutical and biological aspects

Information on development, manufacture and control of the active substance and finished product has been presented in a satisfactory manner. The results of tests carried out indicate 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 clinical use.

At the time of the CHMP opinion, there were a number of minor unresolved quality issues having no impact on the Benefit/Risk ratio of the product.

2.2.5. Conclusions on the chemical, pharmaceutical and biological aspects

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

2.3. Non-clinical aspects

2.3.1. Introduction

The non-clinical dossier consisted of primary pharmacodynamic, pharmacokinetics and toxicology studies conducted in mice, rats and dogs.

The pivotal repeat-dose toxicity studies, the genotoxicity studies and the phototoxicity study were conducted in compliance with Good Laboratory Practice (GLP). The toxicokinetics conducted as part of the rat repeated dose toxicity study were performed in compliance with GLP while the other pharmacokinetic studies were not.

Scientific advice was given by the CHMP on toxico-pharmacological aspects. The applicant was advised to discuss folate receptor mediated toxicity induced by vintafolide, and particularly justify the absence of renal toxicity despite high expression of folate receptors in the kidney of rats and humans, and explain why pre-clinical dose-limiting toxicities are not predictive of clinical toxicities (constipation, small bowel obstruction, peripheral neuropathy).

2.3.2. Pharmacology

Primary pharmacodynamic studies

In vitro pharmacodynamic studies

A summary of studies performed *in vitro* with vintafolide, including studies on combination with doxorubicin, is presented below.

Determination of vintafolide affinity to FR on KB cells (Study#0004-PR-0012)

Vintafolide was evaluated using an *in vitro* relative affinity assay that measures the ability of the folate-conjugate to directly compete with folic acid (FA) for binding to cell surface FRs at different serum concentrations. Nasopharyngeal carcinoma cell line (KB cells) were incubated in media plus 10% serum for 90 min at 37°C with 100 nM ^{3}H-folic acid in the presence and absence of increasing EC145. Serum did not seem to influence the ability of FA to bind to KB cells. EC145 was found to display its lowest competitive properties in the absence of serum (relative affinity ~0.2). EC145 was determined to have an affinity of ~0.5 relative to that of folic acid for binding to human FRs in the presence of serum (10-100%).

Determination of in vitro activity (IC_{50}) and FR specificity of EC145 on KB cells (Study#0004-PR-0013 & 0004-PR-0014)

The dose-response activity and specificity of vintafolide was evaluated in KB cells. KB cells were treated for 2 h (Panel A) with increasing concentrations of EC145 or with 100 nM EC145 for the indicated exposure times (Panel B) in the presence or absence of 0.1 mM folate competitor. Following either a 70- or 46-h chase period in fresh media (Panels A and B, respectively), cells were incubated with ^{3}H-thymidine for 2 h (Panel A) or 4 h (Panel B) and then counted for radiolabel incorporation into newly synthesized DNA.

The activity of vintafolide was found to be concentration dependent, with a IC_{50} of approximately 9 nM when cells were exposed for a 2 h period. The cytotoxic activity of EC145 was decreased in the presence of an excess folate indicating a FR specific activity of EC145. The toxicity of EC145 (100 nM) remained mediated via the FR during incubations up to 48 h.

The effect of vintafolide against FR-negative cells (Study#EC145-B-PR-0035)

FR-negative A549 cells were pulsed for 2 h with increasing concentrations of EC145 in the presence or absence of 0.1 mM folic acid (as a competitor). After a 68 h chase in fresh medium, cells were labelled with ^{3}H-thymidine for 2 h and then counted for radiolabel incorporation into newly synthesized DNA. The concentration of EC145 required for 50% inhibition of viability was greater than 100 nM in the FR negative cell lines used.

The effects of vintafolide on FR-positive KB cancer cells in combination with doxorubicin (Study#EC145-B-PR-0020)

Vintafolide was tested in FR-positive KB cancer cells in combination with doxorubicin. KB cells were treated with increasing concentrations of EC145 for 2 h in the absence or presence of doxorubicin at 12.5, 25, 50, 100 or 200 nM for 48 h. The effects on cell proliferation were then measured by analyzing ^{3}H-thymidine incorporation into newly synthesized DNA. The isobologram analysis of the IC_{80} values for the combination experiment was also performed.

EC145 and doxorubicin, when combined *in vitro*, displayed significant reduction in ^{3}H-thymidine incorporation compared with the individual drugs alone. Isobologram data showed synergy between EC145 and doxorubicin in KB cells.

The effects of DAVLBH in combination with doxorubicin in KB cells (Study#EC145-B-PR-0027 and EC145-B-PR-0028)

DAVLBH is a close structurally-related analogue of vindesine. Untargeted DAVLBH and vindesine were each tested in combination with doxorubicin against KB cells. Cells were exposed to doxorubicin, vindesine or DAVLBH for 72 h. The IC_{80} value (concentration of drug to inhibit cell growth by 80%) of each single agent was normalized to 1. Isobologram analysis of the IC_{80} values for the combination of untargeted DAVLBH + doxorubicin and for the combination of untargeted vindesine + doxorubicin were performed.

DAVLBH and doxorubicin as well as vindesine and doxorubicine, when combined *in vitro*, displayed significant reduction in ^{3}H-thymidine incorporation compared with the individual drugs alone. Isobologram data showed additive effects of both combinations in KB cells.

The effects of EC145 on cloned hERG potassium channels expressed in human embryonic kidney cells (study 120214FOH)

A hERG assay in a human embryonic kidney (HEK-293) cell-line was conducted under GLP conditions. Two concentrations of EC145 (9.7 and 253 µM) were tested at near physiological temperature. EC145 inhibited hERG current by (Mean ± SEM) 3.4 ± 0.4% at 9.7 µM (n = 4) and 10.4 ± 1.0% at 253 µM (n = 5) versus 0.9 ± 0.1% (n = 3) in control. The IC_{50} for the inhibitory effect of EC145 on hERG potassium current was not calculated but was estimated to be >253 µM which equated to ~8100-fold and ~3700-fold the clinical plasma C_{max} for unbound and total vintafolide, respectively.

Evaluate whether or not vintafolide is substrate for the reduced folate carrier (RFC) or proton-coupled folate transporter (PCFT) (s*tudy# EC145-B-PR-0038)*

Study# EC145-B-PR-0038 was designed to examine uptake of a radiolabeled form of vintafolide (^{3}H-vintafolide) in a set of isogenic Chinese hamster ovary cell lines which had been engineered from transporter-null R2 cells to express either the RFC, PCFT, or FR-alpha (FRα) (Deng, 2008; Deng, 2009; Wang, 2010; Wang, 2011). Uptake experiments for RFC or PCFT were carried out in PC43-10 (RFC+) or R2/PCFT4 (PCFT+) cells at a concentration of 0.5 µM ^{3}H-vintafolide for 5 min at 37°C at pH 7.2 (RFC), or pH 5.5 and 6.8 (PCFT). Transporter specificity was determined using the inhibitors PT523 for RFC, and AG94 for PCFT. To assess FR-mediated uptake, RT16 cells were incubated with 0.5 µM ^{3}H-vintafolide at pH 7.4 for 60 min at 37°C with or without 10 µM folic acid (FA) competitor. Cells were acid washed after the uptake period to remove any ^{3}H-vintafolide bound externally to the FR so that only internalised radioligand was evaluated. As a positive control for RFC and PCFT transport, ^{3}H-methotrexate (MTX) uptake was also determined under the same conditions.

^{3}H-MTX showed significant uptake by both the RFC and PCFT-expressing cells, and uptake was specifically inhibited by PT523 (for RFC) or AG94 (PCFT). ^{3}H-MTX, which is known to bind to the FR in the absence of higher affinity folates, also showed uptake in the FRα-expressing RT16 cells, and this FR-mediated uptake was mostly competable with excess FA. Conversely, ^{3}H-vintafolide exhibited no RFC- or PCFT-specific transport under the optimal conditions for transporter activity. As expected, very high uptake of ^{3}H-vintafolide was seen in the FRα-expressing RT16 cells, and these levels far exceeded those observed in the RFC- and PCFT-expressing cells. Moreover, FR-mediated uptake was predominantly competable (90% blocked) with excess FA. Though levels of ^{3}H-vintafolide taken up by the RFC-expressing cells were similar to that of ^{3}H-MTX, no inhibition

of ^{3}H-vintafolide uptake was seen with PT523, suggesting that this component is not mediated by RFC.

In vivo pharmacodynamic studies

Table 1: Summary of *in vivo* studies conducted with vintafolide

Type of Study, report	Test System	Test conditions	Results / Conclusion
Anti-tumour activity and FR-specificity of EC145 against human KB tumours in *nu/nu* mice. 0004-PR-0003 EC145-B-PR-0025 EC145-B-PR-0005	Balb/c derived *nu/nu* mice bearing FR-positive KB tumours 5 females/ group	Intravenous dosing 1, 2 or 5 µmol/kg (1.9, 3.8, 9.6 mg/kg) administered TIW (9, 6 and 5 doses respectively) with or without excess EC20 (folate analog) 1 µmol/kg, TIW, 6 doses ± EC20 in study EC145-B-PR-0025	1 and 2 µmol/kg EC145 has anti-KB tumour activity in *nu/nu* mice (with complete responses in some cases) without significant weight loss. The effect decreased in presence of an excess of folate analog (40 µmol/kg), but not in presence of folate (10µmol/kg). EC145 1 µmol/kg TIWx2 gave 5 of 5 complete responses and 3 of 5 cures in study EC145-B-PR-0025 and a decreased effect in presence of EC20 (100 µmol/kg) was seen.
Solid Human Ovarian Tumour Model EC145-B-PR-0034	*nu/nu* mice bearing OV90 tumours	Intravenous dosing 2 µmol/kg (3.8 mg/kg) TIW x 3 or qd5 x 3 ± DOXIL 4 mg/kg BIW x 3	Anti-tumour effect of EC145 was seen in all treated animals (PR, CR and/or cures reported for individual animals). Combined treatment with DOXIL seemed to potentiate the anti-tumour effect.
EC145 anti-tumour activity against syngenic M109 tumours in Balb/c mice. 0004-PR-0006	Balb/c mice bearing FR-positive M109 tumours 5 females/ group	Intravenous dosing 10 µmol/kg (19.2 mg/kg) BIW x 5 dose regimen	A BIW regimen (5 doses) was effective in this model with 4 of 5 CR's weight loss range was 8-14 %. Animals fully re-gained weight after dosing was ended. EC145 was effective in animals bearing FR-positive tumours.
Antitumour activity of EC145 against FR-negative tumour models SR # P-1219 & SR#P-1260	Balb/c mice bearing 4T1 tumours & Balb/c *derived* *nu/nu* mice bearing A549 tumours. 5 females/ group	Intravenous dosing 2 µmol/kg (3.8 mg/kg) EC145 TIW x 2 wk or 0.5, 1.0, 2.0 µmol/kg (0.38, 0.77, 1.54 mg/kg) DAVLBH TIW x 2 wk	4T1 tumours grew at the same rate in both the untreated and EC145-treated animals. In the A549 tumour model, little to no anti-tumour activity was observed. Weight loss in EC145 treated animals was 0-5.8%. FR-negative tumours are less responsive to EC145. DAVLBH showed little to no activity at the highest toxic dose (weight loss 8.2-13.2%)
Comparison of EC145 efficacy with that of unconjugated DAVLBH. 0004-PR-0003, EC145-B-PR-0017, EC145-B-PR-0004, EC145-B-PR-0005 & EC145-B-PR-0037	Balb/c derived *nu/nu* mice bearing KB tumours 5 females/ group	Intravenous dosing DAVLBH: 0.5, 0.75, 1, 2 µmol/kg (0.38, 0.58, 0.77, 1.54 mg/kg) TIW, 2 wk EC145: 0.5 (qdx5), 1.0 (TIW), 1.2 (qdx5), 2 (TIW), 4, 5 (TIW), 10 (BIW) µmol/kg (0.96, 1.9, 2.3, 3.8, 7.7, 9.6, 19 mg/kg) for 2 wk	DAVLBH (Vinblastine desacetylhydrazide) produced some anti-tumour activity at doses which were toxic to the animals. Unlike EC145, DAVLBH did not produce any Complete responses. EC145 had a higher anti-tumour activity and had less effect on weight loss as compared to DAVLBH.
Comparison of anti-tumour efficacy of EC145 and EC207 (non-FR binding despterin analog) EC145-B-PR-0004	Balb/c derived *nu/nu* mice bearing KB tumours 5 females/ group	Intravenous dosing (µg/kg) 5 µmol/kg, TIW	EC145 at 5 µmol/kg, TIW, 6 doses resulted in 5/5 CRs. The weight loss range observed in this group was between 1 and 11%. EC207, its non-binding counterpart, at 5 µmol/kg, TIW, 6 doses also gave 5/5 CRs with a weight loss range of 6 to 15%. No difference in anti-tumour efficacy was seen between EC145 and its non-FR binding counterpart at the dose and treatment schedule used.
In Vivo Evaluation of EC145/ DOXIL® Combination Therapy	Balb/c derived *nu/nu* mice bearing M109,	Intravenous dosing EC145: 1, 2 µmol/kg (1.9, 3.8 mg/kg) TIW x 2 weeks	Single agent vintafolide cured 3 of 5 mice without causing weight loss. Single agent PLD was found to yield 3 of 5 cures. Combination

EC145-B-PR-0019& EC145-B-PR-0030	KB or IGR-OV1 tumours 5 females/ group	DOXIL: 4 or 7 mg/kg biweekly x 2 weeks	of vintafolide + PLD was found to cure 100% (5 of 5) of treated animals. Furthermore, mice in this latter group only experienced a mild, transient weight loss of 0 to 5%. Combination of EC145 and Doxil displayed an increased anti-tumour effect.
In Vivo Evaluation of untargeted DAVLBH combination therapy EC145-B-PR-0031	*Nu/nu* mice bearing KB tumours. 4 animals/ cohort	Intravenous dosing DOXIL 4 mg/kg 2 times/week x 2 weeks DAVLBH 0.75 µmol/kg (1.44 mg/kg) three times/week x 2 weeks	DAVLBH alone did not result in anti-tumour activity when administered at a safe dose level (i.e. 75% of the MTD). In contrast, PLD resulted in 4/4 partial responses. The combination of DAVLBH + PLD was similar, or even less efficacious, than PLD alone.
Saturable tumour uptake of a folate conjugate *in vivo* Low072799	Balb/c mice bearing FR positive M109 or Line01 tumours 4 females/ group	Intravenous dosing ^{111}In-DTPA-folate M109; 54, 210, 455, 720, 1225, 1650, 2000 nmol/kg Line01; 100, 500, 1000, 1500, 2000 nmol/kg	Uptake of ^{111}In-DTPA-folate in both FR-positive tumour types, M109 and Line01 tumours, was saturated at 1500-1800 nmoles/kg (1287-1416 µg/kg) dose range.

Secondary pharmacodynamic studies

No secondary pharmacodynamic studies were submitted.

Safety pharmacology programme

Cardiovascular system

Male and female beagle dogs from the pivotal GLP 3-week toxicity study (study 0157-05269) were evaluated by ECG to assess the effects of vintafolide. This study was designed to characterise the potential toxicity of vintafolide when administered intravenously to Beagle dogs using 2 different dosing schemes: Groups 1 through 4 received vehicle or vintafolide intravenously every other day for 3 days/week every other week for 3 weeks at 0, 0.6, 0.12, and 0.24 mg/kg/injection, respectively; Groups 5 and 6 received vintafolide intravenously once a week for 3 weeks at 0.18 and 0.72 mg/kg/injection, respectively.

There were no toxicological ECG changes detected in dogs receiving up to 0.24 mg/kg/injection of vintafolide 3 days/week. On Study Day 19, the T wave was characterised by a large negative deflection in most dogs in Group 4 and 6. There were no toxicological ECG changes detected in dogs receiving 0.18 mg/kg/injection of vintafolide once per week for 3 weeks. Intravenous dosing with vintafolide at 0.72 mg/kg/injection once per week for 3 weeks led to a statistical increase in Heart Rate (HR) (and corresponding decrease in the RR interval and shortening of the QT interval) in male dogs and a statistical increase in HR in female dogs on Study Day 19.

The dose of 0.24 mg/kg represents a 26-fold increase relative to the human C_{max} at a dose of 2.5 mg. The bioanalytical methods differed between the 2 datasets, so the absolute C_{max} multiple was not conclusively defined.

Respiratory system

Clinical signs were collected in pivotal repeat-dose toxicity studies in rats and dogs and did not reveal any findings suggestive of adverse effects on the respiratory system.

Renal system

In all pivotal toxicology studies involving rats and dogs, renal tissue was evaluated for signs of adverse effects since this organ expresses the FR on the apical membrane of the proximal tubule. Vintafolide was concluded not to cause any kidney toxicity in preclinical toxicology studies.

Pharmacodynamic drug interactions

No pharmacodynamic drug interactions were submitted.

2.3.3. Pharmacokinetics

Non-clinical pharmacokinetic data were collected in mice (study 0004-PR-0017), rats (as part of study# 0157-11202), and dogs (as part of study# 0157-05269). The formulations of vintafolide used in these studies were nearly identical, as in most cases lyophilised vintafolide was solubilised in phosphate buffered saline, or water for injection prior to dosing.

Table 2: Comparative pharmacokinetic data and systemic exposure to vintafolide (EC145) after intravenous administration to Rats*, Dogs*, and Humans

Species (Formulation)	**Dose (mg/kg/day)**	**C_{max} (ng/mL)**	**AUC_{Last} (ng.h/mL)**
Rat (EC145 solution for injection)	0.32	4000	577
	0.64	5330	1060
	1.28	13100	2180
Dog (EC145 solution for injection)	0.06	866	1812.9
	0.12	1772	2468.1
	0.24	3366	4574
Human (EC145 solution for injection)	**(mg/day)**		
	1.2	61	28
	2.5	129	42
	4.0	179	80
	2.5 1 hour infusion	42	40
	3.0 1 hour infusion	54	50

*) PK parameters shown for females.
Note: Different bioanalytical methods were used, as improvements were made to the earlier methods in order to improve detection limits and establish the stability of vintafolide *ex vivo* in the biologic matrix.

Biodistribution in mice (Study 0004-PR-0017)

^{3}H-vintafolide predominantly accumulated within the FR-positive tumour and kidneys in tumour bearing Balb/c mice 4 hours after a single intravenous dose, and the extent of uptake in both tissues was ~ 3.8% of the injected dose (ID) per gram of wet weight mass (%ID/g). The tumour to blood ratio after 4 h was 38, a value consistent with previous reports of folate-conjugate uptake in this tumour model (Leamon et al., 2002). Uptake in most of the remaining normal tissues was typically >10-fold lower. However, uptake in liver (a non-FR expressing tissue) was also significant (2.4% ID/g).

Serum protein binding

Vintafolide was determined to be ~54% protein bound (i.e., 46% freely soluble) in solutions of human sera (analysed by ultrafiltration and subsequent HPLC-UV analysis). Protein binding values for the dog and monkey serum were comparable to human (61% and 62%, respectively). The Balb/c mouse sera demonstrated a slightly lower percent binding of vintafolide than the sera obtained from CD1/ICR mice (67% and 77%, respectively). Vintafolide was 74% bound to rat serum and 99% to rabbit sera.

In vitro determination of vintafolide stability in K3EDTA plasma from multiple species by LC-MS/MS detection (Non-GLP; Study EC145-B-PR-0032)

Appearance of DAVLBH, an indicator of vintafolide conjugate stability, in plasma was measured by LC-MS/MS to determine plasma stability of vintafolide. All plasma lots were prepared from fresh whole blood collected in K3EDTA tubes and then frozen at -20°C until use. Comparison of observed DAVLBH concentrations in plasma stability samples overtime to a tris(2-carboxyethyl)phosphine full release sample was used to determine vintafolide stability.

Table 3: Percent of DAVLBH release from vintafolide incubated in plasma from different species[1)]

Time (min.)	Human Plasma	Sprague-Dawley Rat Plasma	Balb/c Mouse Plasma	Beagle Dog Plasma	Phosphate Buffered Saline, pH 7.4
0	0.00	0.61	0.00	0.00	0.01
5	0.00	0.00	0.00	0.00	0.01
15	0.00	0.00	0.61	0.51	0.00
30	0.00	0.00	1.04	0.42	0.06
60	0.38	0.58	1.81	1.85	0.10
120	0.74	1.04	3.02	5.73	0.13
240	1.43	2.58	4.01	8.45	0.05
1440	0.02	3.67	4.58	9.42	0.10

[1)] Data generated by dividing the mean concentration of released DAVLBH from two stability samples spiked with vintafolide by the total DALVBH observed in vintafolide samples reduced with TCEP and multiplying by 100.

Urinary profiling of vintafolide in mice (study 0004-PR-0010)

Balb/c mice bearing established subcutaneous M109 tumours were injected intravenously with a therapeutic dose level (2 µmol/kg) of ^{3}H-vintafolide. Urine was collected from selected animals at 20 min and 60 min post injection, and the samples were analysed. At 20 min post injection, vintafolide was found intact at 52% in the urine, and it was accompanied by one major metabolite ("metabolite B") present at 46%. The metabolite was identified as EC119, the folate-peptide portion of vintafolide that is released following intramolecular disulfide reduction. At 60 min post injection, vintafolide was found intact at 38% and the EC119 metabolite at 60%. At both time points, ~ 2% of unidentified radiochemical material ("metabolite A") was detected.

Metabolism (report PK005)

In vivo metabolism of [^{3}H]-vintafolide was studied in bile duct-cannulated (BDC) Wistar rats following single 1 mg/kg IV dose. The average recoveries (as % of the radioactive dose, over a 0-72 hour collection period) in urine, bile, and faeces were 42%, 49%, and 3%, respectively. In bile, the radioactivity was predominantly composed of a methylated thiol (M3, formed via methylation following reduction of the disulfide bond) and oxidative metabolites of the methylated thiol (M4, M5, M6, and M7). The active drug (DAVLBH, M2), its hydrazone derivative (M2'), and oxidized DAVLBH (M8) were detected as relatively minor components in bile. In rat urine, unchanged vintafolide and its hydrolysis products (M12 and M13, formed via hydrolysis of the peptide spacer) were the predominant radioactive components. Although the active drug DAVLBH was not detected in urine, a product generated from DAVLBH conjugating with endogenous α-keto glutaric acid (M10) was identified. In rat plasma, vintafolide was the predominant circulating component.

Results from the evaluation of the metabolism of vintafolide in human and animal hepatocytes were also presented. The major route of metabolism of [^{3}H]-vintafolide in hepatocytes from rat, dog, and human was reduction of the disulfide bond to form the active drug DAVLBH (M2 and M2'). The oxidative derivative of DAVLBH (M8), the methylated thiol (M3) and its dehydrogenated product (M4) were also detected. A metabolite formed through hydrolysis of the peptide spacer (M12) was also present.

In vitro transporter studies (PK009)

The uptake of vintafolide in human MDCKII-OATP1B1 and 1B3 transfected cells was evaluated in study PK009. Uptake of [^{3}H]-vintafolide (0.5 µM) was not significantly different in MDCKII-OATP1B1 and 1B3 cells, compared to parental MDCKII cells. Under the conditions tested, vintafolide was not a substrate for OATP1B1 and OATP1B3.

In vivo tissue distribution study in rats (PK006)

This study used a radiolabeled vintafolide with ^{3}H-labeled at the active drug portion and with samples collected over 24-hour time period. The ability of ^{3}H-vintafolide to target tumours *in vivo* was assessed using a FR-positive M109 lung adenocarcinoma model. Balb/c mice bearing M109 tumours were injected intravenously with 0.2 mL (51.6 Ci/mL, 0.2 mM) of ^{3}H-vintafolide solution. Four hours after receiving a 2 µmol/kg i.v. dose, animals (3 per cohort) were euthanized, and approximately 100 mg of tissue samples (heart, lungs, liver, spleen, kidney, muscle, intestine, stomach, brain and tumour) or 100 µL of blood were removed and placed into pre-weighed vials.

The results showed that the biodistribution pattern of vintafolide was consistent with its targeting folate receptor expressed mainly in tissues outside the liver. At all time-points the radioactivity levels were highest in the kidney (specifically renal cortex), followed by the liver. Unlike in the kidney where the radioactivity levels declined very slowly with less than 2-fold change over 24 hour post intravenous dosing (which is consistent with the high expression of folate receptors in this tissue), the levels in the liver declined rapidly, approaching the limit of detection at 24-hr post dose. These findings were consistent with the fact that liver is a highly perfused organ which could contribute to the liver distribution (more obvious at an early time point) and clearance of vintafolide.

Uptake of vintafolide into Membrane Vesicles containing Human MDR1 Pgp and BCRP (PK008)

The uptake of 0.5µM vintafolide in human MDR1 Pgp and BCRP containing vesicles and control vesicles was evaluated. Uptake of [^{3}H]-vintafolide in MDR1 Pgp and BCRP containing vesicles was not ATP-dependent. Under the conditions tested, vintafolide was likely not a substrate for MDR1 Pgp and BCRP.

2.3.4. Toxicology

The toxicological profile of vintafolide was evaluated in single- and repeat-dose non-GLP studies, repeat-dose GLP pivotal toxicology studies in mice, rats, and dogs, and in genotoxicity, phototoxicity, and immunogenicity studies. The duration of the pivotal GLP repeat-dose toxicity studies was 3 weeks and 11 weeks in the rat, and 3 weeks in the dog. Recovery was assessed in all GLP repeat-dose toxicity studies.

Single dose toxicity

Table 4: Summary of single dose toxicity studies and major findings

Species / Sex / Number / Group Study ID	**Dose / Route**	**Approx. lethal dose / observed max non-lethal dose (mg/kg)**	**Major findings**
Mouse/Balb/C 2 females/group	19.2, 28.8, 38.3 mg/kg IV	>38.3/38.3	≥19.2 mg/kg: body wt ↓, pathology: heart (degeneration) 38.3 mg/kg: hunched posture, neutrophils ↑, monocytes ↑
Mouse/ICR N = 2/group (1 male/1 female) for 19.2, 28.8, 57.5, 76.7, 95.9 mg/kg; N = 4/group (2 males/2 females) for 38.3 mg/kg **0004-PR-0007**	19.2, 28.8, 38.3, 57.5, 76.7, 95.9 mg/kg IV	≥57.5/38.3	19.2 mg/kg: body wt ↓, pathology: heart (degeneration), brain (degeneration) 28.8 mg/kg: body wt ↓, hunched posture, straining to defecate, impaired mobility, neutrophils ↑, monocytes ↑, pathology: heart (degeneration), brain (degeneration), spleen (necrosis) 38.3 mg/kg: body wt ↓, hunched posture, impaired mobility, splayed hind limbs, AST ↑, ALT ↑ (males), BUN ↑, neutrophils ↑, monocytes ↑, eosinophils ↑ (males), basophils ↑, hematocrit ↓, pathology: heart (degeneration), liver (degeneration), bone marrow (necrosis) ≥ 57.5 mg/kg: hunched posture, severe hind-limb immobility, moribund by day 2-4 post dosing Based on weight loss (>20%) and impaired mobility, doses of ≥ 28.8 mg/kg were considered to exceed MTD.
Rat/Sprague Dawley N=2/group (1 males/1 female) **0004-PR-0007**	9.6, 14.4, 19.2 mg/kg IV	>19.2/19.2	9.6: body wt ↓, pathology: liver (inflammation), lung (inflammation), ALT ↑, WBC ↑, monocytes ↑, basophils ↑, lymphocytes ↑ 14.4: body wt ↓, hind limb paralysis, abscess in mouth (male), pathology: liver (inflammation), lung (inflammation), heart (degeneration), BUN ↑ (female), eosinophils ↑, basophils ↑, monocytes↑ 19.2: body wt ↓, hind limb paralysis, diarrhea, pathology: liver (inflammation), lung (inflammation), bone marrow (degeneration), heart (degeneration), ALT ↑, AST ↑ (male), BUN ↑ (male), basophils ↑, platelets ↓ Based on weight loss (>20%) and hind limb paralysis, doses of ≥ 14.4 mg/kg were considered to exceed MTD.

Repeat dose toxicity

Table 5: Summary of repeat-dose toxicity studies

Study type / GLP status / Study No	Study Title	Species / Sex / Number / Group	Dose / Route
Dose Escalation Study (range-finding) Non-GLP 0157-05110	An IV Dose Escalation Study of EC145 in Sprague Dawley Rats, a Non-GLP Study	Sprague Dawley rats	IV 2, 3, 5 mg/kg Dosing schedule: qdx5 or TIW
Dose Escalation Study (Range-finding) Non-GLP 0157-05111	An IV Dose Escalation Study of EC145 in Naive Beagle Dogs, a Non-GLP Study	Beagle dogs	IV 0.3, 0.6, 1.0, 1.4 mg/kg Dosing schedule: daily dosing, 3 times per week, or a once weekly dose
3 week Repeat-dose Toxicity GLP 0157-05300	A 3-Week GLP Toxicity Study of EC145 Given to Sprague Dawley Rats IV with a 2-Week Recovery Period	Sprague Dawley rats 10/sex/group (5/sex/group for recovery) TK animals: 6/sex/group (TK parameters not analysed)	IV Dosing schedule A: TIW 1 wk/rec 1 wk (6 doses) 0, 0.64, 1.28, 2.56 Dosing schedule B: q1wk 3 weeks (3 doses) 1.92, 7.68
Repeat-dose Toxicity (11-week) GLP 0157-11202	Multiple-Dose IV GLP Toxicity Study of EC145 in the Rat	Sprague Dawley rats 15/sex/group (5/sex/group for recovery) TK animals: 9/sex/group (6/sex/group in ctrl group)	IV TIW 1 wk/rec 1 wk (18 doses) 0, 0.32, 0.64, 1.28 mg/kg
3-week Repeat-dose Toxicity GLP 0157-05269	A 3-Week GLP Toxicity Study of EC145 IV with a 2-Week Recovery Period	Beagle dogs 3/sex/group (2/sex/group for recovery) 60 dogs in total TK performed on all groups in Dosing schedule A	IV Dosing schedule A: TIW 1 wk/rec 1 wk (6 doses) 0, 0.06, 0.12, 0.24 Dosing schedule B: q1wk 3 weeks (3 doses) 0.18, 0.72

In addition to the above studies, the applicant provided preliminary data from a 3-month repeat-dose toxicity study in dogs which was ongoing.

Results from non-GLP repeat dose toxicity studies

In the rat study (0157-05110), the daily, qdx5, doses were not well tolerated. In contrast, when 2 mg/kg was administered TIW for 1 week, all of the animals gained weight throughout the study. The reduction in exposure through the inclusion of treatment free days was critical to tolerability. The toxicity appeared likely related to lymphoid, hematopoietic, and gastrointestinal effects. Additionally, opportunistic infections were suspected as contributing to the muzzle edema, as well as a possible detriment to neuromotor function manifesting as recumbence and reduced activity. Cause of death was not determined in those animals found dead.

In the dog study (0157-05111), daily administration of vintafolide induced significant toxicity to the dogs at the dose levels tested. By changing the schedule to an every other day dose (TIW), 0.3 mg/kg vintafolide was tolerable when given for 1 week. The reduction in exposure through the inclusion of treatment free days was critical to tolerability. Observations (diarrhea, decreased activity, emesis) were consistent among the different dose levels/regimens when adverse findings were reported. The most frequent observation following gross pathology was intestinal hyperemia. Neutropenia was also noted in some dogs treated with vintafolide.

Results from GLP repeat dose toxicity studies

3-week study in rats (study 0157-05300)

The highest doses administered with either dosing schedule were associated with mortality and clearly exceeded the maximum tolerated dose (MTD). Vintafolide was better tolerated as a TIW dose rather than administered as a higher once per week dose. Vintafolide had effects on mortality, the hematopoietic system, the lymphoid system, the gastrointestinal tract, liver, and testes. There was no observed kidney toxicity at any dose or schedule. Except for slight reductions in testicular and epididymal weights (absolute and relative to body and brain weights) with corresponding degeneration/atrophy of the seminiferous tubules, there was no evidence of severe vintafolide-related toxicological alterations in any of the parameters examined in either sex when vintafolide was administered at dose levels of 0.64 and 1.28 mg/kg TIW every other week for 3 weeks. The toxicological effects (with the exception of testicular changes) were absent or reduced in severity following the recovery period. Due to the testicular changes, a NOAEL was not identified in this study.

11-week study in rats (study 0157-11202)

Vintafolide had effects on mortality, the hematopoietic system, the lymphoid system, and testes. The immunosuppressive effects of vintafolide were attributed as the likely cause of opportunistic infections resulting in mortality at 1.28 mg/kg. Decreased testes and epididymides size occurred in all males at all doses. The decreased testis and epididymides sizes correlated with testicular atrophy and epididymal hypospermia at histopathology. Decreased thymus weights were observed at 0.64 and 1.28 mg/kg and correlated with thymic lymphoid depletion at histopathology. Thymic lymphoid depletion was seen in some animals of both sexes at the low dose of 0.32 mg/kg. One high-dose female which was sacrificed as scheduled on day 77 was observed with a fibrosarcoma that was deemed incidental. Fibrosarcomas are rare but can be a spontaneous occurrence in Sprague Dawley rats (Prejean et al, 1973). Vinca alkaloids do not appear to be associated with fibrosarcomas, even in long term studies (vinblastine sulphate CARC review). However, because vintafolide was positive in the micronucleus assay and this single lesion occurred in a high dose group, a treatment relationship could not be excluded. Test article-related findings that persisted throughout the 1-month recovery period included testicular changes (decreased weight, atrophy, mineralization), epididymal hypospermia, and increased adipocytes in bone marrow.

3-week study in Beagle dogs (study 0157-05269)

Vintafolide was not well tolerated in the highest single weekly dose group (0.72 mg/kg) based on mortality. Clinical signs of toxicity noted at the highest dose of each dosing schedule included reduced activity, emesis, and mucoid diarrhea. Similar to rats, the TIW dosing schedule was better tolerated than the same total weekly dose given as a single administration (0.24 mg/kg/day vs. 0.72 mg/kg/day). Vintafolide-related effects were observed in the hematopoietic system, the gastrointestinal tract, liver, and testes. Except for slight reductions in splenic weight (males only), there was no evidence of vintafolide-related toxicological alterations in any of the parameters examined in either sex when vintafolide was administered at dose levels of 0.06 and 0.12 mg/kg TIW every other week for 3 weeks. The LOEL of vintafolide following at least 3 dosings/week given during Weeks 1 and 3 was 0.06 mg/kg and 0.18 mg/kg when dosed once weekly for 3 consecutive weeks.

The observed toxicological effects seen at the terminal sacrifice (with the exception of the changes in hematopoietic systems and testicular changes at 0.24 mg/kg/day and 0.72 mg/kg/day) were absent or reduced in severity following the recovery period.

3-month intravenous toxicity study in dogs (preliminary and non-audited post-mortem report TT# 13-1088)

In this study, vintafolide was dosed at 0.06, 0.12 and 0.18 mg/kg/dose with a dosing cycle identical to that used in the clinical study for ovarian cancer patients (three times per week every other week).

Histopathological changes in the optic nerve near the eye, within the optic tract between the optic chiasm and the midbrain area, and in the thymus were observed in dogs receiving 0.18 mg/kg/dose of vintafolide. In the optic nerve collected near the eye, there was very slight axonal degeneration in all 6 dogs at the high-dose (0.18 mg/kg). The axonal degeneration was distributed multifocally within the nerve and was characterised by rare swollen axons and occasional vacuolar spaces that contained individual necrotic cells, cell debris, or accumulations of eosinophilic hyalinized material. In the optic tract, there was similar very slight axonal degeneration in 3 of 3 males and 1 of 3 females. There were no histomorphologic changes in sections from the eye, other visual pathways of the brain (lateral geniculate, occipital cerebral cortex), occulomotor nerve (when present in brain sections), spinal cord, or peripheral nerves. These effects were observed at a dose level approximately 2.4 fold higher than the clinical dose based on body surface area (BSA) comparisons. No test article-related changes in the optic nerve or the optic tract were present at the mid-dose (0.12 mg/kg; 1.6 x the clinical dose based on BSA).

Genotoxicity

Table 6: Overview of genotoxicity studies performed with vintafolide

Type of test / study ID / GLP	**Test system**	**Concentrations / Concentration range / Metabolising system**	**Results**
Gene mutations in bacteria (in vitro) GLP Study AD36DS.503.BTL	Salmonella strains TA98, TA100, TA1535, TA1537 E. coli WP2uvrA	1.5–5000 µg +/- S9	Negative
Gene mutations in mammalian cells	Chinese hamster ovary cells	5–500 µg/ml	Negative

(in vitro) GLP Study AD36DS.331.BTL		+/- S9	
Chromosomal aberrations in vivo GLP Study AD36DS.123M.BTL	Mouse, micronuclei in bone marrow Mice/ ICR	6.25–25 mg/kg	Positive

Carcinogenicity

No studies assessing the carcinogenic potential of vintafolide were submitted.

Reproduction Toxicity

No reproductive and developmental toxicity studies were submitted.

Toxicokinetic data

Toxicokinetic data were collected in the 11-week GLP toxicity study in rats (study 0157-11202) and in the 3-week GLP study in Beagle dogs (study 0157-05269).

Table 7: Toxicokinetic parameters for vintafolide in beagle dogs following single intravenous bolus administration (Day 1) (study 0157-05269)

Parameter (units)	**Parameter value[1)]**					
Sex	**Males**			**Females**		
Dose (mg/kg)	0.06	0.12	0.24	0.06	0.12	0.24
C_{max}[2)](ng/mL)	946	2044	3430	866	1772	3366
$t_{1/2}$ (h)	3.21	1.79	2.08	3.60	2.28	1.88
AUC_{last} (ng*h/mL)	1627.1	2465.1	4765.8	1812.9	2468.1	4498.7
Vd (mL)	1429.6	1153.0	1256.1	1130.1	1035.9	1067.3
Cl (mL/h)	308.5	446.2	418.8	217.3	315.2	391.8
T_{last} (h)	4	4	4	4	4	4

1) 0.06 (n = 1M/1F); 0.12 (n = 1M/1F); 0.24 (n = 2M/2F; value represents average)
2) Based on samples taken at 2min after dosing

Free DAVLBH (a possible metabolite of vintafolide) were also analysed and was only detected in high-dose male dogs at 2 minutes after dosing. The concentrations of DAVLBH in the two samples were 76 and 84 ng/mL (~6% relative to vintafolide plasma concentration at this time point).

Table 8: Plasma vintafolide toxicokinetic parameters on Day 1 and Day 73 during Intravenous Administration to Male and Female Rats (11-week GLP study) (study 0157-11202)

Dose (mg/kg)a	Gender	Cmax (µg/mL)[1)]	tmax (min)	tlast (min)	AUClast (µg·min/mL)	AUC (µg·min/mL)	t1/2 (min)[2)]	Vz (mL/kg)	Cl (mL/min/kg)
Day 1									
0.32	M	2.70	0	240	44.4	44.6	35.2	364	7.17
	F	4.00	0	120	34.6	35.9	27.0	347	8.90
0.64	M	4.14	0	240	73.8	74.8	41.2	509	8.56
	F	5.33	0	240	63.8	64.1	34.0	489	9.98
1.28	M	15.1	0	240	152	154	45.7	547	8.31
	F	13.1	0	240	113	132	35.2	493	9.72
Day 73									

0.32	M	3.90	0	240	55.6	56.7	45.8	373	5.65
	F	1.82	3	240	42.0	42.5	41.4	450	7.54
0.64	M[3)]	4.95	0	240	97.8 (77.0)	99.9 (79.3)	47.2 (54.3)	436 (633)	6.41 (8.1)
	F	6.54	0	240	83.9	85.2	44.8	486	7.51
1.28	M[3)]	13.3	0	240	177 (153)	183 (159)	55.8	565 (648)	7.01 (8.1)
	F	9.99	0	240	158	161	45.0	516	7.95

1) The concentration at the end of dosing (time zero) was estimated by extrapolation of observed data.
2) Range of timepoints used to estimate half-life were 30-240 min except for LD F for which the range was 15-120 min.
3) Day 73 results were derived using mean concentrations that excluded data for one animal, which appeared to have been mis-dosed. Numbers in parentheses were derived using all concentration data.

In the 11 week rat study DAVLBH was also only detected in two samples (all other samples being below LOQ). The plasma concentration of DAVLBH was 9.80 and 4.29 ng/mL (as compared to 218 and 49.8 ng/mL vintafolide) in two rats from the high dose group 15 min. and 4 h after treatment at day 73, respectively.

Exposure margins for vintafolide in pivotal GLP repeated dose toxicity studies at the MTD as compared to the human dose were calculated using allometric scaling. The clinical dose of vintafolide is 2.5 mg, which equates to a 1.47 mg/m^2 dose in a patient with an average body surface area of 1.7 m^2. The calculated margins for a single cycle of exposure to vintafolide on a TIW, every other week schedule in non-clinical studies are presented below.

Table 9: Exposure margins for vintafolide in 3-week pivotal non-clinical toxicity studies at the MTD as compared to the clinical dose

	3-Week Rat (0157-05300)	**11-Week Rat (0157-11202)**	**3-Week Dog (0157-05269)**	**Human**
MTD	0.64 mg/kg	0.64 mg/kg	0.24 mg/kg	2.5 mg
Conversion to mg/m^2 *	3.84 mg/m^2	3.84 mg/m^2	4.8 mg/m^2	1.47 mg/m^2
Margin	2.61X	2.61X	3.27X	-

Note: The schedule of administration was TIW, every other week
* Allometric conversion to mg/m^2 using a factor of 6 (rat) and 20 (dog).

Local Tolerance

No local tolerance data were provided.

Other toxicity studies

Antigenicity

The potential of vintafolide to induce an immune response in Balb/c mice was evaluated in a non-GLP immunogenicity study (0004-PR-0008). The reason for the study being non-GLP was that the analytical methods for the enzyme-linked immunosorbent assay (ELISA) were not validated. The mice were administered vintafolide IV at 2 µmol/kg (3.8 mg/kg), three times per week, for two consecutive weeks. Serum samples were taken at D1, D14 and D28 post final injection with vintafolide, and analyzed with ELISA for the presence of antibodies against folic acid and the peptide

linker. The results were negative. There was no significant increase in antibody titer at any time-point post injection with vintafolide.

Phototoxicity

The phototoxic potential of vintafolide was measured by evaluating the relative reduction in viability of Balb/c 3T3 mouse fibroblasts exposed to vintafolide and ultraviolet radiation (+UVR), as compared with the viability of fibroblasts exposed to vintafolide in the absence of ultraviolet radiation (-UVR) (study 20019799). Chlorpromazine was used as a positive control.

Vintafolide at the highest achievable concentration of 1000 mg/L in Dulbecco's phosphate buffered saline (DPBS) demonstrated no cytotoxic response (absence of UVR exposure) or photocytotoxic effect (with UVR exposure) in this assay. All optical density, cell survival and Chlorpromazine results were within the OECD-432- required limits, demonstrating the validity of the assay conditions.

All optical density, cell survival and Chlorpromazine results were within the OECD-432-required limits, demonstrating the validity of the assay conditions. The results showed that vintafolide is not phototoxic at concentrations up to 1000 mg/L.

Studies on impurities

A qualifying 3-week toxicology study was conducted in rats to provide a 3.27 x level of qualification for the 12 substances (impurities at levels above the qualification threshold) based on a mg/m^2 basis compared to a human dose of 2.5 mg in a patient with an average body surface area of 1.7 m^2. The general pattern and severity of toxicity of vintafolide in the 3-week rat qualification study was considered to be similar to results at a comparable dose level (0.64 mg/kg) in the previous 3-week toxicity study in rats (0157-05300). There were no new effects that could be discernable from the toxicity caused by vintafolide.

Expression of the folate receptor (FR)

To justify the relevance of the species used for evaluation of possible folate receptor mediated toxicity induced by vintafolide, the applicant presented data on FR expression.

Using a radioligand binding assay, major organs other than the kidney and lung showed negligible or very low expression, specifically when considered within the context of known receptor levels in tumour tissue and with respect to the response to vintafolide therapy in controlled non-clinical studies (Parker, 2005).

Table 10: Average FR levels in some normal tissues using a radioligand binding assay

Tissue	**Rat *(Sprague Dawley)***		**Dog *(Beagle)***		**Human**	
	Avg. FR[a]	**Category[b]**	**Avg. FR**	**Category**	**Avg. FR**	**Category**
Heart	0.00 ± 0.00 (2)	negligible	0.18 ± 0.12 (3)	negligible	1.87 ± 1.05 (5)	negligible
Lung	0.00 ± 0.00 (2)	negligible	0.19 ± 0.17 (3)	negligible	7.79 ± 2.99 (12)	high[c]
Liver	0.02 ± 0.03 (2)	negligible	0.23 ± 0.18 (2)	negligible	1.23 ± 0.42 (4)	negligible
Intestine	0.60 ± 0.06 (2)	negligible	0.07 ± 0.09 (2)	negligible	2.74 ± 1.10 (3)	low
Kidney	6.00 ± 1.50 (3)	high[c]	1.25 ± 0.43 (3)	negligible	14.40 ± 6.70 (8)	high
Spleen	0.44 ± 0.12 (2)	negligible	0.52 ± 0.40 (2)	negligible	0.55 ± 0.43 (3)	negligible
Muscle	0.57 ± 0.57 (2)	negligible	0.00 ± 0.00 (2)	negligible	0.97 ± 0.41 (3)	negligible
Brain	0.20 ± 0.28 (2)	negligible	0.03 ± 0.04 (2)	negligible	0.32 ± 0.28 (3)	negligible

Tissue	Rat *(Sprague Dawley)*		Dog *(Beagle)*		Human	
	Avg. FR[a]	**Category[b]**	**Avg. FR**	**Category**	**Avg. FR**	**Category**
Ovarian Carcinoma (serous)	-	-	-	-	34.31 ± 22.87 (7)	high

a) Each value represents the average of separate determinations ± standard deviation, and are expressed in picomoles FR/milligram solubilized membrane protein.
b) Category of positivity reflects the level of positivity in relation to FR-positive and negative xenografts and the response to vintafolide therapy (> 6 pmol FR/mg protein = high; > 2.5 but < 6.0 pmol FR/mg protein = low; < 2.5 pmol FR/mg protein = negligible).
c) Receptor expression is limited to the apical, not basolateral membrane.

2.3.5. Ecotoxicity / environmental risk assessment

Phase 1: Estimation of Exposure

Screening for Persistence, Bioaccumulation and Toxicity (PBT)

The log octanol-water partition coefficients (log Kow) have been experimentally determined for vintafolide and for the active moiety DAVLBH. Their values were -0.61 and 0.15, respectively.

Calculation of the Predicted Environmental Concentration (PEC)

The proposed posology is 2.5 mg vintafolide administered IV three times weekly on weeks 1 and 3 every 28 days. The mean duration of cycles of treatment has been shown to be 4.8 months for folate-receptor positive ovarian cancer patients. However, some patients have been treated for greater than one year.

The number of treatment periods per year (n treatment) was calculated assuming the worst-case treatment scenario i.e. six individual doses in every 28 day cycle for a full year, equal to 78 individual doses of vintafolide 2.5 mg powder for solution for injection. The prevalence of ovarian cancer per 10,000 persons in the EU, Norway, Iceland and Liechtenstein has been calculated to be between 3.1-3.8. Considering expression of the folate receptor on ~90% of ovarian cancers, the prevalence of ovarian cancer expressing the functional folate receptor was calculated to be between 2.8-3.4 per 10,000 persons in the EU, Norway, Iceland and Liechtenstein.

Calculation of refined F_{pen}:

Refined $F_{pen} = P_{region} \times t_{treatment} \times n_{treatment} \div N_d$ (number of days per year)

Refined $F_{pen} = 0.00034 \times 1 \times 78 \div 365$

Refined $F_{pen} = 0.0000727$

Calculation of $PEC_{SURFACE\ WATER}$ for vintafolide:

$PEC_{SURFACE\ WATER} = (DOSE_{ai} \times \text{Refined } F_{pen}) / (WASTEW_{inhab} \times DILUTION)$

where, Maximum daily dose ($DOSE_{ai}$) = 2.5 mg; Refined $F_{pen} = 0.0000727$; $WASTEW_{inhab}$ = Amount of wastewater per inhabitant per day (= 200 L/inh/day); Dilution = dilution factor (= 10)

$PEC_{SURFACE\ WATER} = (2.5 \times 0.0000727) \div (200 \times 10)$ mg/L = 0.000091 µg/L.

Table 11: Summary of main study results

Substance (INN/Invented Name): vintafolide			
CAS-number (if available): n/a			
PBT screening		Result	**Conclusion**
Bioaccumulation potential- log K_{ow}	OECD107	K_{OW} = -0.61 and 0.15	Potential PBT (N)
Phase I			
Calculation	**Value**	**Unit**	**Conclusion**
$PEC_{surfacewater}$, default or refined (e.g. prevalence, literature)	0.000091	µg/L	> 0.01 threshold (N)
Other concerns (e.g. chemical class)			(N)

Vintafolide $PEC_{surfacewater}$ value is below the action limit of 0.01 µg/L and is not a PBT substance as log K_{ow} does not exceed 4.5.

2.3.6. Discussion on non-clinical aspects

A potent (IC_{50} 9 nM) and FR-dependent cytotoxic mechanism of vintafolide was supported by the results obtained *in vitro*. *In vivo* studies with established xenograft models showed a dose-dependent anti-tumour effect of vintafolide against FR-positive tumours, and also that FR-negative tumours did not respond. The presence of a folate analogue decreased the anti-tumour activity, supporting the conclusion of a FR-dependent mechanism. In addition, it was also observed that vintafolide had a greater anti-tumour effect and was better tolerated compared to treatment with vinblastine-desacetylhydrazide that only had an effect at high and toxic doses. A synergistic effect of vintafolide and pegylated liposomal doxorubicin was also indicated by the non-clinical pharmacological data presented.

Study EC145-B-PR-0038 showed with reasonable certainty that vintafolide is substrate for the folate receptor (FR) and not the reduced folate carrier (RFC) or the proton-coupled folate transporter (PCFT).

No secondary pharmacodynamics data were presented which was considered acceptable since the pharmacological effect has been shown to be mediated via the folate receptor and thus can be considered to be specific. No secondary pharmacological effects are expected.

The safety pharmacology data showed no signs of effects on the central nervous system or respiratory tract, while some cardiovascular effects were noted. However, the IC_{50} for the inhibitory effect of vintafolide on the hERG current was indicated to be >260 µM and the cardiovascular effects observed were not considered to be clinically relevant. Based on the available data, the CHMP concludes that there are no non-clinical cardiovascular safety signals.

Pharmacodynamic drug interaction studies were not submitted, however combination studies were conducted with doxorubicin and pegylated liposomal doxorubicin *in vitro* and *in vivo*, respectively, in order to evaluate the anti-tumour effect and tolerability of these combinations (see non-clinical primary pharmacodynamic studies), which was considered acceptable.

Results from plasma pharmacokinetic studies suggested that greater exposure to vintafolide was achieved in both rats and dogs after intravenous administration as compared to humans, reflected as increases in C_{max} and AUC_{last}, although no proper systemic exposure comparison could be made due to differences in sensitivity and accuracy in the different bioanalytical methods used. A single-dose GLP pharmacokinetic study in dogs using the current bioanalytical method is planned to be performed and will allow comparison of the exposures between humans and dogs. The interspecies comparison and the final report are expected to be available by June 2014 and March 2015 respectively. The CHMP recommends that the applicant provides these data as soon as available.

Pharmacokinetics data showed that vintafolide is rapidly cleared from the circulation and has a relative short half-life in both dog (2-3h) and rat (0.5-1 h), as well as human (0.5-1 h). A significant plasma stability of the disulfide bond was indicated *in vivo* since no or only low levels of free, unconjugated DAVLBH was detected in the toxicokinetic studies, which was also supported by *in vitro* analysis of plasma stability. A biodistribution study conducted with ^{3}H-vintafolide in Balb/C mice bearing FR-positive tumours showed the highest distribution to the tumour xenograft, kidneys and liver. An additional study in rat showed that the radioactivity levels declined very slowly in kidney, with less than 2-fold change over 24-hour post intravenous dosing, which was consistent with the high expression of folate receptors in this tissue, while the levels in the liver declined rapidly, approaching the limit of detection at 24-hour post dose. These findings were considered consistent with folate receptor distribution. Vintafolide was extensively metabolised in rats, with ~10% excreted as unchanged drug in excreta. Vintafolide was metabolised via multiple pathways including hydrolysis, reduction of the disulfide bond and oxidation. Formation of the active DAVLBH appeared to be a relatively minor pathway in rats. Metabolite profiles obtained *in vitro* in hepatocytes were qualitatively similar across species and indicated that there is no human specific metabolite.

The toxicity profile of vintafolide was evaluated for 11 weeks in rats, but only 3 weeks in dogs which is not in line with ICH S9 guidance that requires non-clinical studies of 3 months duration in both rodent and non-rodent species unless justified based on the relevance of the species and other scientific considerations. The applicant argued that, given similar toxicity profile in the rat study and the 3-week study in dogs, and considering that the expression pattern of folate receptors appears to be similar between the two species, the rodent was considered a relevant species to support the development program. Some effects upon the cardiovascular system were observed in dogs and were absent in rats. However, these effects were not observed when dogs were given vintafolide according to the clinical dosing schedule. The final report of the 3-month toxicity study in dogs is expected by 2Q2014 and the applicant is recommended to submit it soon as available.

The applicant was also requested to justify whether the species used are appropriate for evaluating possible folate receptor mediated toxicity induced by vintafolide. The applicant discussed possible species differences regarding the expression and affinity of the folate receptor, and metabolism of vintafolide. The provided data suggested a similar expression profile in rats, dogs and humans. A notable exception was the high expression of FR expression in human lung. Although there is a lack of knowledge about potential differences in affinity to the folate receptor between species, the overall toxicity profile of vintafolide in the repeat dose toxicity studies (see below) was consistent with that reported for non-targeted vinca alkaloids. From that perspective, the choice of rats and dogs as preclinical species was considered adequate.

The mechanism behind the lack of renal toxicity with vintafolide has been thoroughly discussed as recommended in the scientific advice. A key observation is the fact that expression of the proximal

tubule folate receptor is restricted to the apical membrane, thus being accessible only to vintafolide that has been cleared into the urine and not that which is in circulation (Birn et al 1997). Vinca alkaloids are not typically categorised as nephrotoxic, which further supports a lack of kidney toxicity related to vintafolide. In addition, in contrast to the situation in growing tumours, folate receptors in the kidney seem to be involved in folate transport and reuptake, returning excreted folates back into circulation (Sandoval et al 2004).

Regarding the potential lack of predictivity for clinical toxicities (such as constipation, small bowel obstruction, peripheral neuropathy) in the non-clinical models, the applicant suggested that the sensitivity of the non-clinical animal models to vinca-related proliferative toxicities may preclude the induction of neuropathic effects that could occur at higher doses, or following different treatment regimens. This explanation was considered plausible, although it was noted that it is not uncommon for some clinical effects not to be manifested in animals, and vice versa.

A comparison between animals and humans showed only 2-3 fold margins to maximum tolerated dose (MTD) in the non-clinical studies, and no margin to NOAEL in the 3-week dog study. In contrast, cross-species exposure comparison gave higher margins. However, the toxicokinetics values should be regarded with caution due to differences in analytical methods between non-clinical and human pharmacokinetic studies. Nevertheless, even by assuming considerable variability in the methods employed, plasma levels in terms of C_{max} and AUC were still considered to be higher in the non-clinical species.

The overall toxicological profile of vintafolide was similar to that shown for other vinca alkaloids and was largely ascribed to expected and exaggerated pharmacological effects. The primary pharmacologic mechanism of action as an inhibitor of microtubule formation is responsible for vinca alkaloid-related toxicity and vintafolide-related toxicity as well. The toxicity of vintafolide can be divided into primary and secondary effects, as follows: Primary effects: targeting of rapidly dividing cells in various tissues (bone marrow, lymphoid organs, intestine, testes); Secondary effects: increased occurrence of bacterial infections due to immunosuppression, alterations in bone marrow and peripheral blood reflecting bacterial infection, compensatory extramedullary haematopoiesis in the spleen and liver. Hypospermia in the epididymides as well as atrophy and/or decreased secretion of seminal vesicles and prostate gland were secondary effects due to testes toxicity.

In view of the pronounced testicular toxicity in rats, as well as the potential for delayed toxicity in the testis, the applicant is recommended to evaluate the testes in the 3-month dog study.

More unspecific effects such as hyperkeratosis of the squamous epithelium in the oesophagus and stomach, and various electrolyte alterations, may be due to reduction in food consumption and/or gastrointestinal disturbances.

In addition, some hemodynamic effects were observed in dogs treated at 0.72 mg/kg once weekly administration. It seems likely that these effects were not due to primary targeting of the heart but rather to functional cardiovascular mechanisms related to the vinca alkaloid component.

In general, vintafolide-related toxic effects were either fully reversible or showed decreased incidence and/or severity after the recovery periods. Haematological alterations tended to normalise during treatment-free weeks. Testes and epididymides effects in the rat studies were not reversible, probably because a longer recovery period than 4 weeks is needed to evaluate reversibility of testes toxicity.

Histopathological changes in the optic nerve near the eye, within the optic tract between the optic chiasm and the midbrain area, and in the thymus were seen in dogs receiving 0.18 mg/kg/dose of vintafolide. The risk for translation of the non-clinical finding of optic neuropathy in dogs to humans was assessed (see clinical safety section) and it appeared not to be translated into any clinical major safety concern. Optic nerve abnormalities are reflected in the Risk Management plan. In addition, it is recommended that all patients should have visual acuity and ophthalmological history documented prior to vintafolide administration and that ophthalmological evaluation should be considered if vision disorder develops or worsens in severity (see section 4.4 of SmPC, section Special warnings and precautions for use).

The genotoxic potential of vintafolide was studied with respect to gene mutations in bacteria, and chromosomal aberrations *in vitro* and *in vivo*. Vintafolide was found to be negative in the Ames test, negative in the *in vitro* chromosomal aberration test, and positive in the *in vivo* micronucleus test. The positive result of the *in vivo* micronucleus test was considered related to the mechanism of action of the drug, and was in line with other anti-mitotic therapies.

No studies assessing the carcinogenic potential of vintafolide have been performed which was acceptable and in line with the ICH S9 guidance. No studies evaluating the effects on fertility, reproductive and developmental toxicity were conducted since vintafolide was shown to target rapidly dividing cells in general toxicity studies, and the pharmaco-active moiety belongs to a class that is well characterised as causing reproductive and developmental toxicity. The genotoxic potential and anti-proliferative effects of vintafolide warrant caution with respect to pregnancy. Therefore, vintafolide is not recommended during pregnancy and in women of childbearing potential not using contraception. Women of childbearing potential who are receiving vintafolide should be advised to avoid becoming pregnant, and to inform the treating physician immediately should this occur. An effective method of contraception should be used during treatment and for at least 3 months after treatment.

Vintafolide was not phototoxic in the neutral red uptake assay in Balb/c 3T3 mouse fibroblasts. The antigenicity study showed no immune response to vintafolide treatment. No immunotoxicity study was submitted. However, since the bone marrow and lymphoid organs were found to be primary targets of vintafolide-related toxicity in the repeat-dose toxicity studies, vintafolide has clearly been shown to have immunotoxic potential.

Local tolerance studies were not submitted for IV administered vintafolide. However, the routine examination of injection sites during repeat dose studies in rats did not reveal any signs of local toxicity.

2.3.7. Conclusion on the non-clinical aspects

In vitro studies showed that vintafolide binds to folate receptor (FR) with high affinity and that the toxic effect of vintafolide had an IC_{50} of 9 nM on FR-positive KB cells. *In vivo* studies with established xenograft models (e.g., ovarian, cervical, breast) showed that vintafolide exerted dose-dependent anti-tumour effect against FR-positive tumours, and that FR-negative tumours did not respond to vintafolide. Results obtained in an FR-positive tumour model also showed that vintafolide had a greater anti-tumour effect and was better tolerated compared to treatment with untargeted vinblastine desacetylhydrazide (DAVLBH) which only had an effect at high and toxic doses.

The toxicological profile of Vynfinit was assessed in single- and repeat-dose toxicology studies, in which vintafolide was administered intravenously to mice, rats or dogs. Overall, the toxicological effects of vintafolide were similar to that observed for other vinca-alkaloids agents and included toxicity to rapidly dividing cells in various tissues (bone marrow, lymphoid organs, intestine, and testes). Consistently with other anti-mitotic therapies, genotoxicity assessment revealed positive result of the *in vivo* micronucleus test while results were negative in the Ames test and in the *in vitro* chromosomal aberration test.

The applicant was recommended to submit study reports from the single-dose GLP pharmacokinetic study and the 3-month GLP dog studies as soon as they are available.

2.3.8. Introduction

Clinical data were provided from one phase I study (EC-FV-01), two single-arm phase II studies (lung (EC-FV-03) and ovarian cancer (EC-FV-02)) and one pivotal randomised phase II study in patients with primary or secondary platinum resistant ovarian cancer (EC-FV-04).

Scientific advice was given by CHMP on clinical aspects in relation to the criteria for conditional marketing authorisation.

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

Study Identifier	Study Objective	Study Design and Type of Control	Dosage Regimen	Number of Subjects Entered / Completed	Healthy Subjects or Diagnosis of Patients	Treatment Duration
EC-FV-04	Compare PFS between participants who receive combination therapy with vintafolide and PLD versus PLD alone	Phase 2, open-label, randomized (2:1 ratio of vintafolide+PLD vs PLD alone), international, multicenter oncology study	20-25 mCi of ^{99m}Tc-etarfolatide, followed by treatment with either 1) 2.5 mg IV of vintafolide on Weeks 1 and 3 every 28 days + 50 mg/m^2 IV of PLD on Day 1 every 28 days (Arm A) or 2) 50 mg/m2 IV of PLD on Day 1 every 28 days (Arm B)	Arm A mITT: 100 / 77 Arm B mITT: 49 / 43	Patients with primary or secondary platinum resistant ovarian cancer	Arm A: Up to 20 cycles Arm B: Up to total allowable cumulative PLD dose
EC-FV-01	Determine MTD of a bolus dose and 1-hour IV infusion dose, characterize toxicity, characterize antitumor activity, and analyze archived tissue samples for FR expression	Phase 1, open-label, dose-escalation	Either an IV bolus dose or 1-hr IV infusion of vintafolide, administered M-W-F, wks 1 and 3 of a 4-wk cycle	32 / 24a	Patients with refractory or metastatic cancer (solid tumors)	Until PD or unacceptable toxicity

EC-FV-02	Collect data on clinical benefit, collect data on tumor response, collect data on PFS, response duration, and OS endpoints, and further assess the safety and tolerability	Phase 2, open-label, non-randomized, within-subject evaluation, single agent, multicenter oncology study	20-25 mCi of ^{99m}Tc-etarfolatide, followed at least 7 days later by vintafolide administered as a 1.0 mg IV bolus injection on Monday through Friday for 3 weeks of a 4-week cycle for 2 cycles (induction phase). For Cycles 3 and beyond (maintenance phase), vintafolide administered as a 2.5 mg IV bolus injection on Monday, Wednesday, and Friday, during Weeks 1 and 3 of a 4-week cycle. Following an interim analysis, the induction phase was removed.	49 / 49	Patients with 1) epithelial ovarian cancer (serous or endometrioid histology) or 2) ^{99m}Tc-etarfolatide positive ovarian cancer, primary peritoneal cancer or adenocarcinoma of the endometrium	Until PD or unacceptable toxicity
EC-FV-03	Collect data on clinical benefit, collect data on tumor response, collect data on PFS, response duration, and OS endpoints, and further assess the safety and tolerability	Phase 2, open-label, non-randomized, within-subject evaluation, single agent, multi-center oncology study	20-25 mCi of ^{99m}Tc-etarfolatide, followed at least 7 days later by vintafolide administered as a 1.0 mg IV bolus injection on Monday through Friday for 3 weeks out of a 4-week cycle for 2 cycles (induction phase). For Cycles 3 and beyond (maintenance phase), vintafolide is administered as a 2.5 mg IV bolus injection on Monday, Wednesday, and Friday, during Weeks 1 and 3 of a 4-week cycle.	43 / 43	Patients with adenocarcinoma of the lung who have previously received ≥2 cytotoxic-containing chemothera-peutic regimens	2 induction cycles and 6 maintenance cycles
EC-FV-06 (enrolling)	Primary analysis: Compare PFS (based on RECIST V 1.1 criteria) in participants with platinum-resistant ovarian cancer with 1) all target lesions etarfolatide positive [FR(100%)] and who receive combination therapy with vintafolide and PLD to subjects with platinum-resistant ovarian cancer who receive PLD and placebo	Phase 3, double-blinded, randomized (1:1 ratio of vintafolide+PLD vs PLD + placebo), international, multicenter oncology study	20-25 mCi of ^{99m}Tc-etarfolatide, followed by treatment with either 1) 2.5 mg IV of vintafolide on Weeks 1 and 3 every 28 days + 50 mg/m^2 IV of PLD on Day 1 every 28 days or 2) 50 mg/m^2 IV of PLD on Day 1 every 28 days + 2.5 mg IV of placebo on Weeks 1 and 3 every 28 days	Up to 600 patients with platinum resistant ovarian cancer to obtain 350 patients with FR (100%) target lesions (planned)	Patients with primary or secondary platinum resistant ovarian cancer	Participants will continue treatment until progressive disease (PD) or until unacceptable toxicity occurs.

2.3.9. Pharmacokinetics

Clinical pharmacokinetic data for vintafolide are available from one phase 1 dose escalating multiple dose study (EC-FV-01). *In vitro* data on CYP inhibition (GEN-B-PR-0001), plasma stability (EC-145-B-PR-0032) and plasma protein binding (0004-AR-0003) were also submitted.

Distribution

Study 0004-AR-0003 - Serum protein binding of EC145 (non-GLP study)

The objective of the study was to examine the *in vitro* binding of vintafolide (at 50 µM) to the protein fraction of serum from a variety of species (including man). Peak areas from the HPLC-UV analysis were used to determine % protein binding. Human sera demonstrated the lowest protein binding at 54%.

Volume of distribution

Based on data from the phase I study (EC-FV-01) the mean volume of distribution after an intravenous injection was 36 L, and population PK analysis of the same data with a two-compartment model suggested a central volume of 16.4 L and a peripheral volume of 9.7 L.

Elimination

In study EC-FV-01, vintafolide was rapidly cleared from the circulation, population estimate of terminal half-life was 26 minutes from the population PK modelling, and the corresponding value of clearance was 56 L/h.

In vitro stability in human plasma as well as phosphate buffer was determined in study EC145-B-PR-0032, where human plasma (2 samples/time-point) was spiked with 1 µM vintafolide and incubated at 37C in up to 24 hours. In all samples incubated 60 minutes or more, DALVBH was detected, in human plasma the highest amount detected was 1.4% and in PBS 0.1% of maximum theoretical amount (generated by adding tris(2-carboxyethyl)phosphine (TCEP) to reduce all vintafolide to DAVLBH).

Dose proportionality and time dependencies

Study EC-FV-01 was a dose-escalating phase 1 study of vintafolide given as intravenous (IV) injection or 1-h infusion on day 1, 3 and 5 on weeks 1 and 3 of a 4-weeks cycle to refractory solid tumor patients. The study was performed at two centres in the US and its primary objective was to establish the maximum tolerated dose of vintafolide as bolus IV injection or 1-h IV infusion. 32 patients were included, 16 were treated with bolus injection (1.2 mg n=3, 2.5 mg n=10, 4 mg n=3) and 16 with 1-h infusion (2.5 mg n=10, 3 mg n=6). Blood samples for PK analysis were collected on day 1 and 3 of the first cycle of therapy, 6 samples were collected on each day for each individual; up to 90 minutes after IV bolus dose and during infusion and for 60 minutes after the IV infusion. Vintafolide and DAVLBH were quantified using LC-MS/MS.

Concentration-time data from study EC-FV-01 were analysed by non-compartmental methods. Additionally, a population PK (PPK) analysis was conducted using nonlinear mixed effects modeling in NONMEM.

Data from the non-compartmental analysis are summarised in the tables below.

Table 12: Summary statistics of PK parameters following bolus injection

Dose (mg)	Day	Cmax (ng/ml)	AUC0-C (hr*ng/ml)	CL (L/hr)	t1/2 (min)	Vz (l)
1.2	1 (N = 2)	73.2 (53.39)[1]	NE[2]	NE	NE	NE
	3 (N = 2)	49.7 (29.49)	37.3	32.2	16.8	13.0
2.5	1 (N = 10)	134.3 (58.89)	50.1 (19.09)	46.9 (23.53)	19.9 (10.52)	21.9 (8.35)
	3 (N = 10)	123.2 (34.02)	49.9 (18.98)	56.8 (21.19)	21.5 (8.29)	26.6 (8.68)
4.0	1 (N = 3)	212.3 (237.22)	130.7 (106.49)	45.8 (37.34)	42.0 (1.20)	45.8 (36.49)
	3 (N = 2)	145.7 (89.52)	56.4 (29.27)	81.9 (42.50)	26.8 (9.40)	48.1 (8.91)

1 Values represent the mean (standard deviation).

2 NE = not evaluable; too few points exist to determine pharmacokinetic parameters.

Table 13: Summary statistics of PK parameters following 1-h intravenous infusion

Dose (mg)	Day	Tmax (min)	Cmax (ng/ml)	AUC0-C (hr*ng/ml)	CL (L/hr)	t1/2 (min)	Vz (l)
2.5	1 (N =10)	1.1 (0.13)	38.6 (24.97)	57.6 (41.17)	59.7 (32.60)	25.3 (7.77)	32.7 (13.52)
	3 (N =10)	1.1 (0.27)	44.8 (27.51)	70.9 (33.53)	41.6 (17.86	33.6 (19.90)	32.9 (18.66)
3.0	1 (N = 6)	1.1 (0.20)	47.4 (19.32)	61.7 (17.91)	52.2 (16.52)	27.1 (11.51)	34.6 (19.94)
	3 (N = 6)	0.9 (0.32)	59.8 (27.80)	80.3 (24.34)	39.7 (10.39)	26.1 (7.75)	24.0 (7.07)

1 Values represent the mean (standard deviation).

Special populations

No studies in special populations were submitted.

Pharmacokinetic interaction studies

Following IV injection of 2.5 mg vintafolide in the phase 1 study, mean C_{max} was 128 ng/mL = 68 nM. With 54% protein binding, unbound C_{max}, was around 30 nM.

One human *in vitro* CYP inhibition study (GEN-B-PR-001) was performed using pooled liver microsomes with conventional substrate for CYP1A2, 2C9, 2C19, 2D6 and 3A4 (and positive controls. In addition to vintafolide (EC145), five metabolites were also tested (EC0489, EC0225, DAVLBH, EC119, EC0746) in concentrations up to 100 µM.

No CYP inhibition was detected. IC_{50} was estimated to be >100 µM in all cases, except for the metabolite EC0225 which showed some inhibition of CYP1A2 and CYP2C19. EC145 and DAVLBH were shown to be stable under assay conditions, whereas there were problems in the assay of the folate linker EC119.

2.3.10. Pharmacodynamics

No pharmacodynamics studies were submitted.

2.3.11. Discussion on clinical pharmacology

Clinical pharmacokinetic data are available only from one study, a phase 1 dose escalating multiple-dose study (EC-FV-01, n=32). In this phase 1 study, bioanalysis of vintafolide and DAVLBH was performed with a LC-MS/MS method. The data suggested reasonable performance of the method used. The applicant clarified that an improved bioanalysis method with lower LLOQ for DAVLBH will be used in future PK assessment.

Vintafolide showed a relatively limited overall distribution (mean V 36 L). *In vitro* data showed a low plasma protein binding (54%) at high plasma concentrations. Protein binding data at clinically relevant concentrations using ultrafiltration without a density gradient showed similar results (57% bound).

Vintafolide is designed to target FR-expressing tumours. No data were provided to evaluate to what extent the drug distributes to the tumour cells in the patient and it is not known whether the extent of distribution into tumour cells has an influence on systemic volume or clearance. The applicant referred to data from mice xenografted with FR-expressing tumours showing that only a minor fraction of the drug dose was distributed to the tumour (<1%) suggesting that FR-positive tumour load is unlikely to have clinically meaningful impact on systemic clearance and distribution of vintafolide.

In the non-clinical studies, accumulation in liver was observed in mice. *In vitro* studies were performed to investigate whether vintafolide is a substrate of hepatic transporters. Data from MDCK cell lines suggested that vintafolide is unlikely to be a substrate for OATP1B1 and OATP1B3, but the efflux transporters Pgp and BCRP could not be studied in these cells due to low permeability of the substance. Experiments in membrane vesicles suggested that vintafolide is not a substrate for Pgp and BCRP. No transporter data are available for DAVLBH.

The terminal plasma half-life of vintafolide was short (around 30 minutes) and the plasma levels of DAVLBH seemed to be low after vintafolide administration (not quantifiable in most subjects in the phase 1 study).

The routes of metabolism and excretion of vintafolide and its active metabolite DAVLBH have not been adequately characterised. There is no information (pharmacological activity or pharmacokinetics) on other human metabolites than DALVBH and it is unclear which entities (parent compound and/or vinca-containing metabolites) contributed to the systemic toxicity of vintafolide. Data on metabolites in plasma and urine is also lacking. The applicant suggested that DAVLBH is released from EC145 conjugate within the acidic milieu of the endosome once the folic acid-drug conjugate binds to the FR and endocytosis occurs. The low levels of DAVLBH in the systemic circulation compared to tolerable levels of similar vinca alkaloids suggested that systemic DAVLBH may not be responsible for the systemic toxicity observed after vintafolide administration. DAVLBH was the main metabolite observed in human hepatocyte incubations, but the low plasma levels of this metabolite suggested a lower formation *in vivo* and/or fast elimination of this metabolite. In rat, a variety of other metabolites were found, many of them retaining the vinca

alkaloid and parts of the peptide component. Depending on distribution, some of these could contribute to efficacy or toxicity.

Pharmacokinetics data from rat showed multiple elimination pathways in this species, with mostly non-CYP metabolism and drug related material found both in bile and urine. This suggested a low interaction risk, but the relevance for human is unclear. Considering the short exposure after each dose, the drug administration in cycles and the possibility to dose-adjust based on toxicity, routine risk minimisation activities were considered acceptable to handle the risk of increased exposure until further information is available through the additional pharmacovigilance activities. The major elimination pathways and main metabolites of vintafolide and DAVLBH will be clarified including the identification of the main metabolising enzymes and transporters through additional pharmacovigilance activities as reflected in the risk management plan. Results are expected by Q1 2015. The CHMP recommended that the applicant performs a mass balance study to collect these data if feasible. If the results of the mass-balance study indicate a role of biliary excretion or if mass balance data is lacking, the applicant will study biliary transport further *in vitro*, and clarify if vintafolide and its metabolites are substrates for hepatic uptake and efflux transporters.

No studies were performed in special populations, e.g. in patients with renal or hepatic impairment. The proposed posology of Vynfinit includes the same starting dose for all (2.5 mg three times weekly) and a possibility for dose reduction based on adverse reactions. Therefore, special attention should be paid to signs of vintafolide-related toxicity in these patients (such as haematological toxicity during first cycle and cumulative neurotoxicity). The impact of renal and hepatic functions on vintafolide pharmacokinetics will be explored using population PK analysis in study EC-FV-06 as reflected in the risk management plan.

There is a lack of data on the influence of race and age, which is considered acceptable at this stage. Based on the performed population PK analysis using the phase 1 data, a strong influence of BSA on clearance was identified. However, insufficient information was provided to allow a thorough assessment of the model and the conclusions. Further pharmacokinetics information will be available from the phase 3 study (EC-FV-06) in which PK in special populations (e.g. renal impairment, sex, age, weight/BSA and race) will be evaluated. This is adequately addressed in the risk management plan.

No *in vivo* interaction data are available. *In vitro* data indicate no CYP inhibitory activity of vintafolide or DAVLBH on CYP1A2, 2C9, 2C19, 2D6 or 3A4. There are also experimental data for the folate linker (EC119), but the validity of these data is unclear due to questionable stability. *In vitro*, vintafolide is neither an inhibitor of Pgp or BCRP nor an inducer of CYP3A4, 2B6 or 1A2. Vintafolide will be used in combination with liposomal doxorubicin, but no PK data of the compounds used together are available so far. The ongoing phase 3 study (EC-FV-06) includes sampling of vintafolide in the presence of liposomal doxorubicin in a subset of the patients and the effects of doxorubicin on vintafolide elimination will be evaluated with a population PK approach. Pharmacokinetics results from study EC-FV-06 are expected by Q4 2015.

Overall, there is limited knowledge on how vintafolide or the active metabolite is eliminated, and the role of metabolising enzymes and transporters is not known. Therefore, when co-treatment with a drug known to be an inhibitor of enzymes or transporters is initiated, special attention should be paid to a potential increase in vintafolide-related side effects (such as haematological toxicity or neurotoxicity). Also medicinal products inducing metabolising enzymes could theoretically influence

plasma levels of vintafolide or active metabolite, possibly resulting in decreased drug efficacy, but a risk of increased toxicity cannot be excluded.

In addition, concurrent administration of vintafolide with other medicinal products that may bind to the folate receptor or alter the folate pathway (e.g., folic acid supplements, vitamins enriched in folic acid, or anti-folate therapy, e.g., methotrexate) may decrease efficacy and thus should be avoided (see section 4.5 of the SmPC, Interaction with other medicinal products and other forms of interaction).

2.3.12. Conclusions on clinical pharmacology

Due to the lack of knowledge about the elimination routes of vintafolide and DAVLBH, the potential effects of impaired organ function on vintafolide and DAVLBH pharmacokinetics cannot be predicted.

Considering the short exposure after each dose and drug administration in cycles and the possibility to dose-adjust based on toxicity, the risks are considered manageable and are also adequately addressed in the risk management plan (see additional pharmacovigilance activities).

2.4. Clinical efficacy

2.4.1. Dose response study

Dose finding study EC-FV-01

Methods

EC-FV-01 was a two-centre, open-label, dose-escalating study in patients who had refractory or metastatic cancer (solid tumours) for which no effective standard therapy existed. The study comprised a dose escalation phase to determine the dose-limiting toxicity (DLT) and maximum tolerated dose (MTD), and an extension phase, dosing until disease progression or unacceptable toxicity.

The first patient was enrolled on 13 March 2006. The primary objective of the study was to determine the MTD of vintafolide when administered as a bolus intravenous (IV) dose and when administered as a 1-hour IV infusion. The secondary objectives were:

- Develop a PK/PD model to aid in the determination of the phase 2 dose
- Characterise the toxicity profile of vintafolide
- Characterise the anti-tumour activity of vintafolide (assessed according to RECIST)
- Investigate archived, paraffin-embedded tissue samples for the level of FR (exploratory)

The MTD was defined as the dose at which no more than 1 of 6 patients had DLT. DLT was predefined as any of the following drug-related toxicities during the first cycle of therapy (graded according to NCI CTCAE, version 3.0):

- Grade 2 non-haematologic toxicity (except alopecia) that failed to recover to grade 1 by the time the second cycle of therapy was due to be administered,
- Grade 3 non-haematologic toxicity (except for nausea/vomiting without maximal symptomatic/prophylactic treatment),
- Grade 4 haematological toxicity,
- Any other toxicity that, in the investigator's judgment, would prevent use of the drug dose or regimen by the general oncology community.

Vintafolide was administered on days 1, 3, and 5 (of week 1) and on days 15, 17, and 19 (of week 3) of a 4-week cycle. The planned doses were 1.2, 2.5, 4.0, 6.0, 8.5, 11 and 14.5 mg for the bolus IV injection route of administration (1.2 mg represented one-sixth of the human equivalent dose in the dog, the most sensitive species tested non-clinically) and 2.5, 3.0, 4.0, 5.0 and 6.0 mg for the 1-hour IV infusion route of administration. During the dose escalation phase, a minimum of 3 patients were treated at each dose level, and all of the patients in a cohort completed the first cycle of therapy before patients were enrolled at the next higher dose level. If DLT was observed in 1 of the first 3 patients who were treated at a given dose, an additional 3 patients were treated at that dose level. If DLT was observed in 0 or 1 of the 6 patients who were treated at the dose level, enrolment proceeded at the next higher dose level. If DLT was observed in 2 or more of the 6 patients who were treated at a dose level, the MTD was considered to have been exceeded, and further escalation of the vintafolide dose ceased. Additional patients were then entered at the next lower dose level to further characterise the toxicity at that dose level.

Results

34 patients were screened and 32 patients were enrolled (18 men and 14 women, all patients had metastatic disease). The distribution of patients among the dose cohorts was as follows:

- Bolus IV injection (16 patients): 1.2 mg, 3 patients; 2.5 mg, 10 patients; 4.0 mg, 3 patients
- 1-Hour IV infusion: (16 patients): 2.5 mg, 10 patients; 3.0 mg, 6 patients

The primary reason for discontinuation from the study was disease progression.

Bolus IV injection

The 1.2 mg and 2.5 mg doses were generally well tolerated during the first cycle of therapy, with no DLTs observed. Administration of 4.0 mg was associated with the development of grade 2 constipation after 1 week of therapy (3 doses of vintafolide) in 1 patient and with reversible grade 2 small intestinal obstruction after 1 dose in 1 patient, both considered to be related to vintafolide. Further dose escalation was not undertaken because of the emergence of these toxicities. Subsequently, an additional 7 patients were enrolled at the 2.5 mg dose level (total of 10 patients). No first cycle DLT was observed in any of the 10 patients. The MTD of vintafolide when administered as a bolus IV injection on days 1, 3, and 5 (week 1) and days 15, 17, and 19 (week 3) of a 4 week cycle was considered to be 2.5 mg.

1-Hour IV Infusion

The 2.5 mg dose was generally well tolerated. Administration of 3.0 mg was associated with the development of grade 3 constipation in 2 patients (with 1 of these also having grade 3 abdominal

pain) out of a total of 6 treated. No further dose escalation was undertaken because of the emergence of these toxicities. An additional 7 patients were treated with 2.5 mg (total of 10 patients). A first cycle DLT was observed in 1 out 10 patients (grade 3 ileus). The MTD of vintafolide, when administered as a 1-hour IV infusion on days 1, 3, and 5 (week 1) and days 15, 17, and 19 (week 3) of a 4 week cycle was determined to be 2.5 mg.

No clinically important differences in the incidence of drug-related adverse events were observed between the bolus injection and 1-hour infusion routes of administration. The bolus administration of 2.5 mg resulted in a 3-fold higher C_{max} (mean, 129 vs. 42 ng/mL) but equivalent AUC (42 vs. 40 h*ng/mL) compared to the 1-hour infusion of the same 2.5 mg dose. However, the bolus administration, with its significantly higher C_{max}, was not associated with a greater incidence of constipation. The dose schedule of 2.5 mg of vintafolide as a bolus IV injection on days 1, 3, and 5 (week 1) and days 15, 17, and 19 (week 3) of a 4 week cycle was selected for the phase 2 study (EC-FV-04).

2.4.2. Main studies

The efficacy of vintafolide was evaluated in three phase 2 studies: One pivotal randomised phase 2 study was performed in patients with platinum resistant ovarian cancer, EC-FV-04 (n=162); Supporting studies included two phase II studies of single-agent vintafolide, one in advanced platinum resistant or refractory ovarian cancer, EC-FV-02 (n=49) and one in advanced recurrent NSCLC, EC-FV-03 (n=43).

Study EC-FV-04 (PRECEDENT)

Study EC-FV-04 was a randomised phase 2 trial comparing EC145 and pegylated liposomal doxorubicin (PLD/Doxil/Caelyx) in combination, versus PLD alone, in patients with platinum-resistant ovarian cancer.

This study was a multicentre study conducted at sites in the United States, Canada, and Poland.

Methods

Study Participants

Main inclusion criteria

- Platinum-resistant ovarian cancer, where platinum-resistant was defined as disease that responded to primary (first line) platinum therapy and then progressed within 6 months or disease that progressed during or within 6 months of completing secondary (second line) platinum therapy
- Measurable disease: at least a single (RECIST-defined) measurable lesion on a radiological evaluation that was conducted no more than 4 weeks prior to beginning study therapy (EC145 and/or PLD). Measurable lesions were defined as those that could be accurately measured in at least one dimension with the longest diameter ≥ 20 mm when measured using conventional techniques or ≥ 10 mm when measured with spiral CT scan.
- Prior debulking surgery
- Not received more than 2 prior systemic cytotoxic regimens
- Eastern Cooperative Oncology Group (ECOG) performance status of 0 to 2
- Adequate organ function

Main exclusion criteria

- Tumour of low malignancy potential
- Prior exposure to anthracycline therapy to FR-targeted therapy (EC145, EC0225, farletuzumab, etc.) or vinca-containing compounds
- Prior abdominal or pelvic radiation therapy, to >10% of the bone marrow, or within the past 3 years to the breast/sternum, head, or neck.
- Serious co-morbidities (as determined by the investigator)
- Antifolate therapy
- Symptomatic central nervous system metastases

^{99m}Tc-EC20 (^{99m}Tc-etarfolatide) scan was not required for trial eligibility. At clinical centres that lacked ^{99m}Tc-EC20 nuclear imaging capabilities, patients were enrolled for treatment without undergoing scanning with ^{99m}Tc-EC20. All clinical centres that had ^{99m}Tc-EC20 nuclear imaging capabilities were required to scan patients prior to enrolment.

The nuclear medicine radiologists at these sites were required to complete the qualification training prior to reading images. Before starting study treatment, target lesions were selected by the site radiologists according to RECIST 1.0 criteria. This allowed the site nuclear medicine radiologists to determine the appropriate anatomical regions for the SPECT scan. Patients then underwent ^{99m}Tc-EC20 imaging and the nuclear medicine radiologists reviewed the CT and SPECT scans to evaluate the ^{99m}Tc-EC20 uptake. Each patient score was then calculated by the study statistician. Patient level FR status was determined using the number of FR-positive target lesions divided by total number of target lesions.

Prior to the ^{99m}Tc-EC20 imaging procedure, subjects received one intravenous injection of 0.5 mg of folic acid to reduce background and improve image quality, followed within 1-3 minutes by a 1-2 mL injection of 0.1 mg of EC20 labelled with 20-25 mCi of technetium-99m. Folic acid was administered as a slow IV push followed by 5-10 mL of normal saline. ^{99m}Tc-EC20 was administered over a period of approximately 30 seconds followed by 5-10 mL of normal saline.

Treatments

The doses of the study drugs were adjusted according to the guidelines for haematologic toxicities (absolute neutrophil count and platelets) and for other toxicities (CTCAE grading). In addition, the dose of PLD was adjusted according to the guidelines for the occurrence of palmar plantar erythrodysesthesia (PPE)/hand-foot syndrome (HFS), for the occurrence of stomatitis and for hepatic insufficiency. Patients were to be discontinued from study treatment for any of the following reasons: progressive disease (PD), unacceptable toxicity, patient non-compliance or voluntary withdrawal and pregnancy or breastfeeding. Study-related drugs were administered only under the direction of the investigator. No cross-over was allowed.

Control arm: PLD IV injection of 50 mg/m^2 once every 28 days (for a recommended minimum of 4 courses) until the maximum allowable cumulative dose of 550 mg/m^2 (as long as the patient did not exhibit disease progression, did not show evidence of cardiotoxicity, and continued to tolerate treatment PD).

Experimental arm: Bolus IV injection of 2.5 mg of EC145 on Monday, Wednesday, and Friday of Weeks 1 and 3 of a 4-week cycle. PLD was administered as in the control arm. On the days when patients receive EC145 and PLD, EC145 was to be administered at least 45 minutes prior to administration of PLD.

Patients who received the maximum allowable cumulative dose of 550 mg/m^2 PLD as well as those who discontinued treatment with PLD (after >2 cycles) because of unacceptable toxicity were allowed to continue therapy with EC145 as a single agent for the remainder of the cycles.

Eligible patients received treatment for a minimum of 6 weeks (i.e. through the time of the second CT scan).

Objectives

The primary objective of the study was to compare progression-free survival (PFS), based upon investigator assessment using Response Evaluation Criteria in Solid Tumors (RECIST) Version 1.0 and pre-specified clinical findings, in patients with platinum-resistant ovarian cancer who received combination therapy with EC145 and pegylated liposomal doxorubicin (PLD) (EC145+PLD) compared to patients who received PLD alone.

A secondary objective of the study was to evaluate the correlation between therapeutic response (e.g. PFS, radiologic response, etc) and ^{99m}Tc-EC20 levels, i.e. FR Positivity. Other secondary objectives of the study were to compare overall survival (OS) of patients between the 2 treatment arms; to evaluate the safety and tolerability of EC145 in combination with PLD; to compare the objective response rate (ORR) and disease control rate (DCR) based on investigator assessment when analyzed using RECIST; to compare the duration of response and duration of disease control of EC145 in combination with PLD, versus PLD alone.

The exploratory objectives of the study were to analyse treatment effect by evaluating tumour size as a continuous variable at computed tomography (CT) scan intervals and to explore the impact of certain prognostic factors (e.g., age, number of prior platinum/taxane containing regimens, baseline cancer antigen 125 [CA-125], baseline performance status) on PFS.

Outcomes/endpoints

Primary endpoint: Progression Free Survival (PFS)

PFS was defined as the number of weeks from randomisation to the date the patient experienced an event of radiographically or clinically defined disease progression as assessed by the investigator or to the date of death, whichever occurred first.

Progressive disease was defined on the basis of RECIST criteria or pre-specified clinical events only: Escalating pain not referable to another cause; Increased ascites; Protracted nausea/vomiting despite treatment; Declining performance status; Examination findings consistent with disease progression. If any of these events occurred and was interpreted by the treating physician as indicating disease progression, then an objective imaging assessment (either scheduled or unscheduled) was conducted, whenever medically feasible, to evaluate disease progression by RECIST criteria.

Tumour size was measured by radiographic assessment at baseline, every 6 weeks for 24 weeks (weeks 6, 12, 18, and 24), and every 8 weeks thereafter (weeks 32, 40, etc).

Secondary endpoints:

Therapeutic response	Correlation between therapeutic response and FR status
Overall Survival (OS)	OS defined as the number of weeks from the date of randomisation to the date of death from any cause
Objective response rate (ORR)	ORR defined as the percent of patients who achieve PR or CR
Overall disease control rate (DCR)	DCR defined best overall response of either CR, PR or SD
Duration of response	Duration of response (measured from the first day of a tumour response until the day on which PD or death occurred), based on investigator assessment analysed using RECIST criteria
Duration of DCR	Duration of DCR (measured from the first day of a randomisation until the day on which PD or death occurred), based on investigator assessment analysed using RECIST criteria

Sample size

Study EC-FV-04 was originally designed with a primary analysis based on the intent-to-treat (ITT) population. Ninety-five events (PD or deaths) in this population were expected to provide approximately 70% power to detect a significant difference between the two treatment arms. This calculation was made based on a generalisation of the Freedman (1982) formula in order to account for the 2:1 randomisation; sample size calculations for the number of subjects was based on the method of Lachin and Foulkes (1986). Based on a one-sided alpha = 0.10 significance level, 95 events provided 70% power to detect a PFS hazard ratio of approximately 0.68. Assuming an exponential distribution, this hazard ratio is associated with an improvement in median PFS from 13 weeks in the PLD alone arm to 19 weeks in the vintafolide+PLD arm.

However, the final statistical analysis plan (SAP) specified that the ITT population of patients with measurable disease (mITT) would be used for the primary efficacy analyses, so that 95 events were needed among this subset of study patients. Enrolment of approximately 119 patients in the mITT population was expected to result in a 20% censoring rate for the primary analysis. To also accommodate a 10% early dropout/withdrawal rate, a total of approximately 131 patients with measurable disease were planned for enrolment. Including the 13 patients with non-measurable disease who were randomised before the study design was amended; the final overall study enrolment targeted approximately 143 patients.

Randomisation

Each patient was centrally randomised in a 2:1 sequential manner by stratum according to the randomisation schedule provided by the study statistician. Patients were stratified by:

1. Primary versus secondary platinum failure

2. Geographic treatment region (North America vs. other)

3. Baseline CA-125 (< 200 U/ml vs ≥ 200 U/ml)

Blinding (masking)

This was an open label study.

Statistical methods

The statistical methods presented in the protocol were amended in the statistical analysis plan (SAP) three times prior to data lock including: a change in the definition of events to be included in the efficacy analysis; a change to the primary analysis population (see above); the addition of further analyses.

The following populations were defined for the efficacy analyses:

• Intent-to-treat (ITT): all randomised patients regardless of whether they had received their randomised treatment;

• ITT of all measureable patients (mITT): all patients in the ITT population with measurable disease regardless of EC20 scan status, used for the primary analysis.

The mITT population was divided into three subgroups depending on the degree of FR positivity as follows:

- FR(+): patients with at least one FR positive tumour (also referred to as FR(10-100%));
- FR(++): patients with a percentage of FR positive tumours greater than or equal to the upper threshold of FR positivity (also referred to as FR(100%));
- FR(-): patients with no FR positive lesions (also referred to as FR(0%)).

The primary analysis of PFS was conducted on the mITT population. The PFS curve was estimated for each treatment arm using the Kaplan-Meier method with the primary analysis comparing the two treatment arms using a one-sided log-rank test at the 0.10 level of significance. Cox proportional-hazard model was used to estimate the hazard ratio in terms of the magnitude of treatment effect and the 95% confidence interval (CI).

For patients who did not experience disease progression or death, the data were censored at the time of the last objective (radiographic) tumour assessment (or, if no tumour assessment was performed after the baseline visit, at the time of randomisation plus one day). Data from patients who were lost to follow-up were included in the analysis as censored observations on the last date that the patient was known to be progression-free (defined as the date of the last objective tumour assessment). Patients who missed one or more assessments and who showed disease progression at the assessment that immediately followed the missed assessment were considered to have progressed at the date of the first missed assessment. The data for patients who discontinued treatment without showing disease progression and who received subsequent anticancer therapy were censored at the date of the last objective progression-free assessment prior to start of the anticancer therapy.

Pre-specified sensitivity analyses were conducted as follows: Stratified analysis based on strata formed by CA-125 (<200 U/mL vs ≥ 200 U/mL) and prior platinum failure (primary vs secondary); Adjusted analysis using a Cox proportional hazards model including age, platinum failure, CA-125 level, region, tumour size, months since last platinum treatment and ECOG as baseline factors; Analysis with clinical progression censored at the date of last radiological assessment.

Post-hoc sensitivity analyses were conducted as follows: Analysis with clinical progression censored at the date of clinical progression; Analysis with all PFS events considered regardless of violations, discontinuation of study drug or change of therapy; Analyses excluding all non-eligible patients, non-waivers and waivers; Sensitivity analysis for unscheduled assessments; Sensitivity analysis including patients with non-measureable disease.

P-values for tests of secondary endpoints, exploratory analyses, and sensitivity analyses were not adjusted for multiplicity.

The interim monitoring plan for the study included a single pre-specified interim analysis of PFS for futility only. The interim analysis was conducted under the auspices of an external and independent Data Safety Monitoring Board (DSMB). Interim safety analyses were also conducted by the DSMB. The trial was to remain open for survival follow-up until the overall survival censoring rate reached approximately 20%.

Results

Participant flow

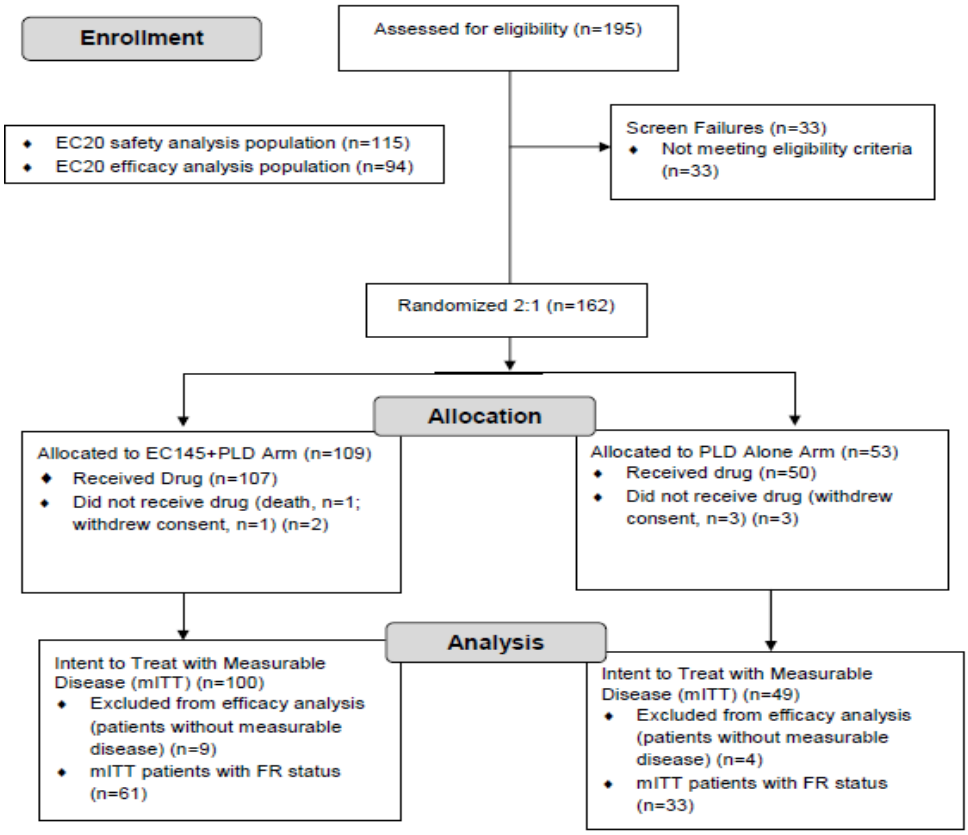


Table 14: Reason for withdrawal for patients without a PFS event and considered no longer at risk for a PFS event, by treatment group

	Vintafolide + PLD N=109	PLD N=53	Combined N=162

Non-compliant	1	0	1
Adverse events	5	2	7
Physician decision	3	2	5
Withdraw consent	5	5	10

Recruitment

The study was conducted at 50 sites in the United States, 6 sites in Canada, and 5 sites in Poland. 28 patients in total were included in the analysis from the EU (Poland). The date of the first patient enrolled was 18 September 2008 and the date of the last patient completed (for data cut-off) was 13 September 2010.

Conduct of the study

Protocol amendments (summary of main changes):

The original protocol (Version 1.0, dated 9 July 2008) was amended four times:

- No 1. (implemented before any patients were enrolled, dated 22 August 2008): addition of information regarding toxicity and monitoring, addition of interim analysis (futility), updated primary efficacy analysis, secondary analysis and sample size.
- No 2. (after 7 patients had been enrolled, dated 27 January 2009): ^{99m}Tc mandatory only at sites with SPECT facility.
- No 3. (after 67 patients had been enrolled, dated 3 August 2009): Data external to the study prompted a change in inclusion criteria from measurable and evaluable to measurable disease. Progressive Disease based on RECIST and not RECIST or Gynaecologic Cancer Intergroup (GCIG) as GCIG pertained to the use of CA-125 as an indicator of progression. Change of stratification variables from measurable versus evaluable to CA-125 ≥ 200 U/mL versus 200 U/mL <CA-125.
- No 4. (after data base lock, dated 30 September 2011): modification to follow patients for death until the overall survival censoring rate reaches 20%.

Protocol Deviations

Fourteen of the 162 randomised patients were granted waivers from study entry eligibility criteria by the medical monitor (e.g. laboratory values slightly above the normal ranges).

The following protocol deviations were to be identified through a review of source data, a review of the clinical database, and medical monitoring: Overdose; clinically significant deviations in study drug administration /dosing; errors in dosing that resulted in doses of study-related drug (vintafolide or PLD) administered at > 10% below the level mandated by the study and without a prior history of toxicity or safety concern; errors in mode of administration (e.g. IM instead of IV; bolus administration vs infusion, etc.); errors in schedule that resulted in greater exposure or more frequent exposure than directed by the protocol (e.g., PLD administered every 21 days, not every 28 days, etc.); dose was not dose adjusted for patient when it should have been, patients who should have been withdrawn, but were not; patients enrolled in violation of eligibility criteria; patients who received exclusionary concomitant medications; Failure to obtain proper informed consent; Significant investigator non-compliance with protocol or scientific misconduct; Laboratory

assessments for study drug dosing not obtained and/or reviewed prior to dose administration; Failure to report serious adverse event in specified time frame.

GCP inspection

A GCP inspection was carried out at the sponsor site and two investigator sites: one in Poland and one in the USA. Overall, there were no areas for concern identified at the Polish investigator site and at the sponsor site. The US investigator site showed poor compliance with the protocol and lack of correct identification and documentation of the protocol deviations which resulted in sub-standard data being generated that could not always be verified.

The observed protocol deviations were further evaluated and a number of sensitivity analyses were carried out to take account of observed deviations. Overall, the quality assurance system (monitoring and auditing) and actions undertaken by the applicant supported reliability of the data.

Baseline data

Table 15: Demographic and Baseline Characteristics (mITT Population)

Variable	EC145+PLD Arm (N=100)	PLD Alone Arm (N=49)
Race n (%)		
White	95 (95.0%)	47 (95.9%)
Asian	0 (0.0%)	1 (2.0%)
Black or African American	3 (3.0%)	1 (2.0%)
Other	2 (2.0%)	0 (0.0%)
Age – years		
Mean	60	61.2
Median	60	62
ECOG Performance Status, n (%)		
0	68 (68.0%)	26 (53.1%)
1	28 (28.0%)	22 (44.9%)
2	4 (4.0%)	1 (2.0%)
Disease Characteristic		
Sum of LD (mm)		
Mean	120.4	74.1
Median	92.5	56
Min - Max	15 - 487	12 - 394
Bulky disease single lesion>5cm	30 (30%)	4 (8.2%)
CA-125, n (%)		
<200 U/mL	58 (59.2%)	31 (64.6%)
>= 200 U/mL	40 (40.8%)	17 (35.4%)
Missing	2	1
CA-125 Level		
Mean	408.87	1111.83
Min - Max	2.0 - 4411.0	6.0 - 19310
Prior Therapy		
Number of Prior Regimens		
1	60 (60.0%)	27 (55.1%)
2	36 (36.0%)	18 (36.7%)
3	4 (4.0%)	4 (8.2%)
Number of Prior Platinum-Containing Regimens		
1	65 (65.0%)	30 (61.2%)
2	34 (34.0%)	18 (36.7%)

3	1 (1.0%)	1 (2.0%)
Primary/Secondary Platinum Failure		
Primary	65 (65.0%)	30 (61.2%)
Secondary	35 (35.0%)	19 (38.8%)
Treatment-Free Interval from Last Platinum Dose to Randomisation, months		
Mean	5.32	5.29
Median	4.70	5.19
Min - Max	0.5 - 34.1	0.9 - 13.0
Type of Cancer, n (%)		
Ovarian	90 (90.0%)	46 (93.9%)
Primary Peritoneal	8 (8.0%)	3 (6.1%)
Fallopian Tube	2 (2.0%)	0 (0.0%)
Months Since Diagnosis		
Mean	19.6	18.9
Median	12.7	12.7
Stage of Cancer at diagnosis, n (%)		
Stage IIIC	67 (67.0%)	30 (61.2%)
Stage IV	12 (12.0%)	8 (16.3%)

The main reason for ending last platinum regimen was completed regimen (not PD or intolerability), about 75% in both study arms.

Baseline data in relation to Folate Receptor expression

Table 16: Disease Characteristics at Screening (FR(++) Population)

	FR(++) Population	
Disease Characteristic	**EC145+PLD Arm (N=23)**	**PLD Alone Arm (N=15)**
Sum of LD (mm)		
N	23	15
Mean	89.7	48.7
STD	59.06	21.23
Median	77.0	45.0
Min - Max	21 – 223	17 – 85
Participants with Measurable Disease, n (%)	23 (100%)	15 (100%)
CA-125, n (%)		
<200 U/mL	11 (47.8%)	7 (50.0%)
>= 200 U/mL	12 (52.2%)	7 (50.0%)
Missing	0	1
CA-125 Level		
N	23	14
Mean	672.13	1841.67
STD	1099.254	5064.885
Median	222.50	203.40
Min - Max	9.0 – 4411.0	11.0 – 19310
Receipt of Neoadjuvant Therapy, n (%)		
Yes	2 (8.7%)	1 (6.7%)
No	21 (91.3%)	14 (93.3%)

Primary / Secondary Platinum Failure, n (%)		
Primary	16 (69.6%)	9 (60.0%)
Secondary	7 (30.4%)	6 (40.0%)
Best Response to Last Platinum Therapy, n (%)		
CR	9 (39.1%)	8 (53.3%)
PR	5 (21.7%)	5 (33.3%)
SD	9 (39.1%)	0 (0.0%)
PD	0 (0.0%)	2 (13.3%)
Time from Last Platinum Dose to Progression, mo.		
N	23	15
Mean	3.61	3.68
STD	2.591	1.516
Median	3.68	3.42
Min - Max	0.4 – 11.5	0.6 – 5.8
Treatment-Free Interval from Last Platinum Dose to Randomization, mo.		
N	23	15
Mean	4.66	5.74
STD	2.511	2.675
Median	4.73	5.91
Min - Max	1.1 – 12.0	0.9 – 13.0
Duration of Exposure to Last Platinum-Containing Regimen, mo.		
N	23	15
Mean	3.94	3.92
STD	1.611	1.845
Median	3.48	3.94
Min - Max	0.0 – 7.8	0.7 – 8.0
Reason Last Platinum Therapy Ended, n (%)		
PD	4 (17.4%)	2 (13.3%)
Toxicity	3 (13.0%)	1 (6.7%)
Completed Regimen	16 (69.6%)	11 (73.3%)
Other	0 (0.0%)	1 (6.7%)

Abbreviations: Sum of LD = Sum of the longest diameters of all target lesions using RECIST criteria; STD = standard deviatio
Notes: Percentages are based on the number of participants with nonmissing data in each treatment arm.
Duration of Exposure to Last Platinum-Containing Regimen = (therapy stop date) - (therapy start date) + 1.

Table 17: Initial Cancer Diagnosis and Tumour Staging (FR(++) Population)

Initial Cancer Diagnosis	EC145+PLD Arm (N=23)	PLD Alone Arm (N=15)
Type of Cancer, n (%)		
Ovarian	19 (82.6%)	14 (93.3%)
Primary Peritoneal	3 (13.0%)	1 (6.7%)
Fallopian Tube	1 (4.3%)	0 (0.0%)
Histopathologic Classification, n (%)		
Serous	9 (39.1%)	6 (40.0%)
Clear Cell	1 (4.3%)	1 (6.7%)
Papillary Serous	9 (39.1%)	6 (40.0%)
Mixed	3 (13.0%)	0 (0.0%)
Other	1 (4.3%)	2 (13.3%)
Histopathologic Grade, n (%)		
G1	2 (8.7%)	0 (0.0%)
G2	3 (13.0%)	1 (6.7%)
G3	14 (60.9%)	8 (53.3%)
G3-4	2 (8.7%)	1 (6.7%)
Unknown	2 (8.7%)	5 (33.3%)
Months Since Diagnosis[1]		
N	23	15
Mean	15.5	20.0
STD	9.86	15.11
Median	11.3	12.1
Min-Max	4.9-44.0	9.0-56.8
Stage of Cancer at diagnosis, n (%)		
Stage II	0 (0.0%)	1 (6.7%)
Stage IIA	1 (4.3%)	0 (0.0%)
Stage III	1 (4.3%)	1 (6.7%)
Stage IIIA	0 (0.0%)	1 (6.7%)
Stage IIIB	1 (4.3%)	1 (6.7%)
Stage IIIC	16 (69.6%)	9 (60.0%)
Stage IV	4 (17.4%)	2 (13.3%)
Residual Tumor Size After Primary Debulking (cm)		
N	21	14
Mean	0.99	1.30
SD	1.326	1.664
Median	0.40	0.50
Min-Max	0.0 – 5.0	0.0 – 5.0

Numbers analysed

A total of 162 were randomised, 109 to vintafolide+PLD and 53 to PLD. Of these randomised patients, 100 vintafolide+PLD treated patients and 49 PLD treated patients were included in the analysis. Patients were excluded from the analysis because they did not have measurable disease. This dataset is referred to as modified intention to treat (mITT) and all patients in this population had measurable disease.

Table 18: Number of patients included in each analysis set and FR subgroup

Analysis Set	Analysis Population	EC145+PLD Arm Number of Patients	PLD Arm Number of Patients	Combined Treatment Arms Number of Patients
Intent-to-treat Population with measurable disease[1] (mITT)	Primary efficacy population	100	49	149
EC20 efficacy analysis population[2]	mITT population with FR status	61	33	94
EC145/PLD FR(++) Population[3]	FR(++) subgroup of mITT Population	23	15	38
EC145/PLD FR(+) Population[4]	FR(+) subgroup of mITT Population	48	26	74
EC145/PLD FR(-) Population[5]	FR(-) subgroup of mITT Population	13	7	20

[1] The intent-to-treat population of all randomized patients with measurable disease (mITT), regardless of whether they received their randomized treatment.
[2] mITT population with FR status
[3] Patients who have all (100%) FR positive lesions [FR(++)]
[4] Patients who have at least 1 FR positive lesion [FR(+)]
[5] Patients who have 0% FR positive lesions [FR(-)]

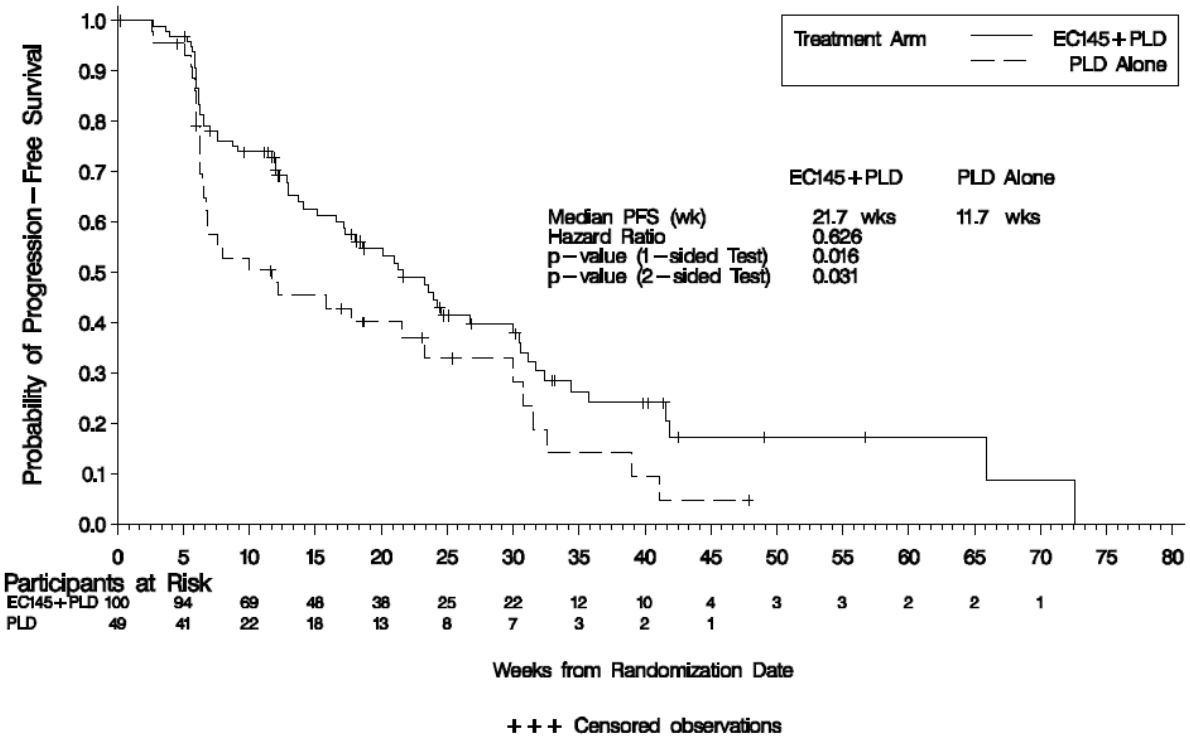
Outcomes and estimation

Primary endpoint: Progression free survival

Table 19: Progression Free Survival by Treatment Arm (mITT Population, investigator assessment)

Statistic	**EC145+PLD Arm (N=100)**	**PLD Alone Arm (N=49)**
Assessed	100	49
Patients with Disease Progression	60	30
Deaths	2	3
Censored	38	16
PFS[1] (weeks)		
25th Percentile (95% CI)	8.7 (6.3, 13.7)	6.3 (6.0, 6.9)
Median (95% CI)	21.7 (16.6, 30.0)	11.7 (6.7, 23.3)
75th Percentile (95% CI)	35.7 (30.4, 65.9)	30.7 (21.6, 39.0)
PFS Rate at 12 Weeks (95% CI)	0.704 (0.601, 0.786)	0.479 (0.322, 0.619)
PFS Rate at 18 Weeks (95% CI)	0.561 (0.449, 0.659)	0.401 (0.253, 0.546)
PFS Rate at 24 Weeks (95% CI)	0.445 (0.333, 0.551)	0.329 (0.184, 0.482)
Hazard Ratio (95% CI)	0.626 (0.409, 0.959)	
Log-Rank p-value (One-Sided Test)	0.016	
Log-Rank p-value (Two-Sided Test)	0.031	

[1] Progression-Free Survival is the number of weeks from the randomization date to the date the patients experienced an event of radiographically or clinically defined disease progression or death, or to the date of the last RECIST evaluation for censored observations.
NOTE:
Progression-Free Survival and PFS Rate based on Kaplan-Meier estimates.



Source: Figure 14.12.1

Figure 2: Kaplan-Meier Curve of PFS by treatment arm EV-FV-04 (mITT Population)

Table 20: Robustness analyses of PFS comparing the EC145+PLD and PLD alone arms (mITT Population [n=149])

Analysis	HR (95% CI)	P-value
Unadjusted	0.626 (0.409, 0.959)	0.031 [1]
Stratified [2]	0.605 (0.383, 0.942)	0.026 [3]
Adjusted [4]	0.597 (0.371, 0.961)	0.034 [5]
Clinical Progression Censored at time of progression	0.597 (0.374, 0.954)	0.030 [1]
Clinical Progression Censored at time of last radiological assessment	0.601 (0.382, 0.943)	0.026 [1]
EMA defined PFS [6]	0.610 (0.403, 0.921)	0.018 [1]
Excluding all non-eligible pts [7]	0.565 (0.358, 0.890)	0.013 [1]
Excluding non-waivers[8]	0.578 (0.374, 0.892)	0.013 [1]
Excluding waivers[9]	0.616 (0.394, 0.963)	0.033 [1]
Sensitivity analysis for unscheduled assessments	0.629 (0.411, 0.964)	0.033 [1]
Sensitivity analysis including pts with non-measurable disease	0.743 (0.492, 1.121)	0.161 [1]

[1] P-value based on the log-rank test.
[2] Analysis stratified on platinum failure and CA-125 level.
[3] P-value based on stratified logrank test.
[4] Results from Cox proportional hazards model with age, platinum failure, CA-125 level, geography, tumour size, months since last platinum treatment, and ECOG as baseline factors included in the model.

[5] P-value based on the Wald test.
[6] All PFS events considered regardless of violations, discontinuation of study drug or change of therapy, as per EMA Guideline, Annex 1: Methodological Considerations for using PFS as a Primary Endpoint in Confirmatory Trials for Registration.
[7] Excluded from analysis 20 non-eligible patients.
[8] Excluded from analysis 6 non-eligible patients who did not receive eligibility waivers.
[9] Excluded from analysis 14 non-eligible patients who received eligibility waivers.

As PFS is a composite endpoint, data were also reported in relation to type of event.

Table 21: Summary of Progression-Free Survival components (mITT Population)

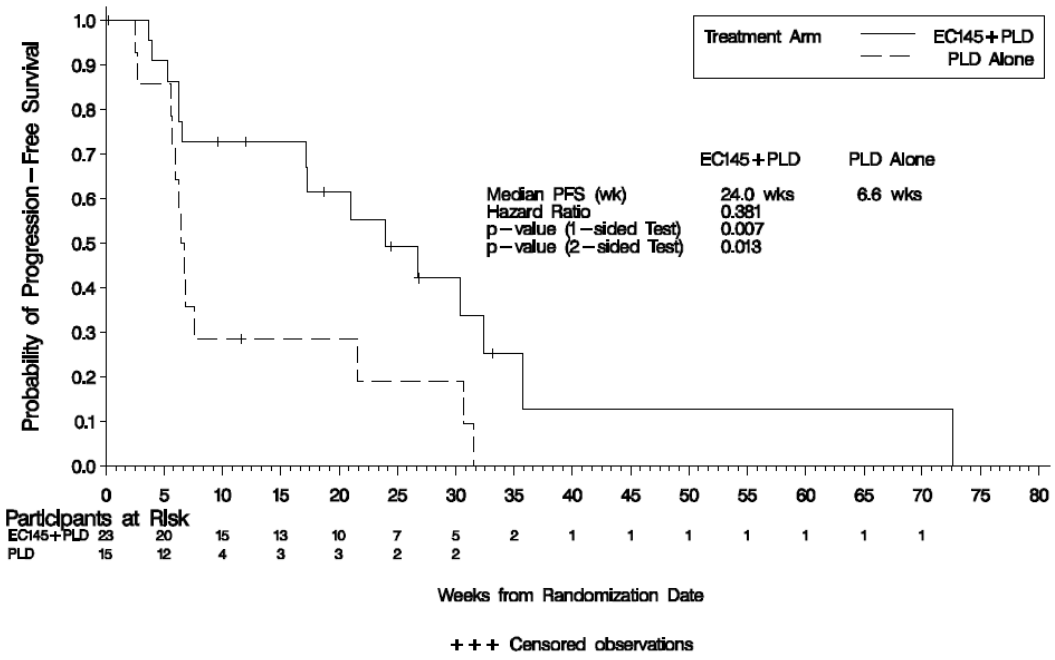
Components	Vintafolide+PLD Arm N	(%)	PLD Alone Arm N	(%)
Number Assessed	100		49	
Number of Deaths	2	(2.0)	3	(6.1)
Number Censored	38	(38.0)	16	(32.7)
Number with Disease Progression	60	(60.0)	30	(61.2)
Only New Lesions	14	(14.0)	10	(20.4)
Progression of Lesions and New Lesions	8	(8.0)	7	(14.3)
Progression of Lesions and No New Lesions	26	(26.0)	8	(16.3)
Clinical Progression	12	(12.0)	5	(10.2)

PFS results in FR(++) population

Table 22: Progression Free Survival by Treatment Arm (FR(++) Population)

	EC145+PLD Arm (N=23)	**PLD Alone Arm (N=15)**
Assessed	23	15
Patients with Disease Progression	15	12
Deaths	0	1
Censored	8	2
PFS[1] (weeks)		
25th Percentile (95% CI)	6.6 (5.3, 24.0)	5.7 (2.7, 6.7)
Median (95% CI)	24.0 (17.1, 32.4)	6.6 (5.7, 21.6)
75th Percentile (95% CI)	35.7 (26.7, 72.6)	21.6 (6.4, 31.6)
PFS Rate at 12 Weeks (95% CI)	0.727 (0.491, 0.867)	0.286 (0.088, 0.524)
PFS Rate at 18 Weeks (95% CI)	0.615 (0.372, 0.788)	0.286 (0.088, 0.524)
PFS Rate at 24 Weeks (95% CI)	0.492 (0.256, 0.692)	0.190 (0.036, 0.437)
Hazard Ratio (95% CI)	0.381 (0.172, 0.845)	
Log-Rank p-value (One-Sided Test)	0.007	
Log-Rank p-value (Two-Sided Test)	0.013	

PFS = progression free survival
NOTES:
[1]Progression free survival is the number of weeks from the randomization date to the date the patient experienced an event of radiographically or clinically defined disease progression or death, or to the date of the last RECIST evaluation for censored observations.
Progression-Free Survival and PFS Rate based on Kaplan-Meier estimates.



Source: Figure 14.16.1

Figure 3: Kaplan-Meier curve of PFS by treatment arm (FR (++) Population)

Table 23: Robustness Analyses of PFS Comparing the EC145+PLD and PLD Alone Arms, (FR(++) Population [N=38])

Analysis	HR (95% CI)	P-value
Unadjusted	0.381 (0.172, 0.845)	0.013 [1]
Stratified[2]	0.366 (0.153, 0.880)	0.020 [3]
Adjusted[4]	0.302 (0.113, 0.804)	0.017 [5]
Clinical Progression Censored at time of progression	0.284 (0.115, 0.702)	0.004 [1]

[1] P-value based on the logrank test.
[2] Analysis stratified on platinum failure and CA-125 level.
[3] P-value based on stratified logrank test.
[4] Results from Cox proportional hazards model with age, platinum failure, CA-125 level, geography, tumor size, months since last platinum treatment, and ECOG as baseline factors included in the model.
[5] P-value based on the Wald test.

A comparison of PFS across folate receptor groups within the PLD alone arm showed that PFS was shorter for FR (100%) patients compared to FR(0%) patients (HR: 3.49; 95% CI: 0.77, 15.86).

Secondary endpoints

Table 24: Overall Response Rate and Disease Control Rate by treatment arm (mITT Population)

	EC145+PLD Arm (N=100)		PLD Alone Arm (N=49)	
	Confirmed	Unconfirmed	Confirmed	Unconfirmed
Best Response	n (%)	n (%)	n (%)	n (%)
Complete Response (CR)	1 (1.0%)	1 (1.0%)	1 (2.0%)	1 (2.0%)
Partial Response (PR)	17 (17.0%)	27 (27.0%)	5 (10.2%)	7 (14.3%)
Stable Disease (SD)	55 (55.0%)	45 (45.0%)	20 (40.8%)	18 (36.7%)
Progressive Disease (PD)	23 (23.0%)	23 (23.0%)	15 (30.6%)	15 (30.6%)
Insufficient Evaluation (IE) / No Assessment[1]	4 (4.0%)	4 (4.0%)	8 (16.3%)	8 (16.3%)
Overall Response Rate[2] (ORR)	18 (18.0%)	28 (28.0%)	6 (12.2%)	8 (16.3%)
95% Confidence Interval[3]	(11.0%, 27.0%)	(19.5%, 37.9%)	(4.6%, 24.8%)	(7.3%, 29.7%)
p-value[4]			0.479	0.154
Disease Control Rate[5] (DCR)		73 (73.0%)		26 (53.1%)
95% Confidence Interval[3]		(63.2%, 81.4%)		(38.3%, 67.5%)
p-value[4]				0.018

[2] A patient with a best response of complete response (CR) or partial response (PR) was considered as having an overall response.

[3] The confidence interval for the percent of patients with an overall response or disease control was based on the exact binomial distribution (the Clopper-Pearson method).

[4] The comparison between treatment arms was based on Fisher's Exact test.

[5] A patient with a best response at or beyond the initial scheduled follow-up scan (ie, 6 week scan within a minus six day tolerance) of complete response (CR), partial response (PR), or stable disease (SD) was considered as having disease control. One exception occurred as follows: Patient 001-201 had SD less than 6 weeks (was 31 days) that was included with a best response of SD instead of it being called an insufficient evaluation (IE).

Table 25: Overall Response Rate and Disease Control Rate by treatment arm (FR(++) Population)

	EC145+PLD Arm (N=23)		PLD Alone Arm (N=15)	
	Confirmed	Unconfirmed	Confirmed	Unconfirmed
Best Response	**n (%)**	**n (%)**	**n (%)**	**n (%)**
Complete Response (CR)	0 (0.0%)	0 (0.0%)	1 (6.7%)	1 (6.7%)
Partial Response (PR)	4 (17.4%)	4 (17.4%)	0 (0.0%)	0 (0.0%)
Stable Disease (SD)	13 (56.5%)	13 (56.5%)	3 (20.0%)	3 (20.0%)
Progressive Disease (PD)	5 (21.7%)	5 (21.7%)	9 (60.0%)	9 (60.0%)
Insufficient Evaluation (IE) / No Assessment[1]	1 (4.3%)	1 (4.3%)	2 (13.3%)	2 (13.3%)
Overall Response Rate[2] (ORR)	4 (17.4%)	4 (17.4%)	1 (6.7%)	1 (6.7%)
95% Confidence Interval[3]	(5.0%, 38.8%)	(5.0%, 38.8%)	(0.2%, 32.0%)	(0.2%, 32.0%)
p-value[4]			0.630	0.630
Disease Control Rate[5] (DCR)		17 (73.9%)		4 (26.7%)
95% Confidence Interval[3]		(51.6%, 89.8%)		(7.8%, 55.1%)
p-value[4]				0.007

[1] Confirmed response is not applicable if best response was stable disease, progressive disease, or insufficient evaluation / no assessment.
[2] A patient with a best response of complete response (CR) or partial response (PR) was considered as having an overall response.
[3] The confidence interval for the percent of patients with an overall response or disease control was based on the exact binomial distribution (the Clopper-Pearson method).
[4] The comparison between treatment arms was based on Fisher's Exact test.
[5] A patient with a best response at or beyond the initial scheduled follow-up scan (ie, 6 week scan within a minus six day tolerance) of complete response (CR), partial response (PR), or stable disease (SD) was considered as having disease control.

Overall survival (cut-off: 22 February 2012)

In this analysis there were altogether 39 censored observations, 10 patients had withdrawn consent or were lost to follow up leaving 29 patients (19.5% censored) at risk for a death event in this survival update.

Table 26: Summary of patients remaining at risk for death as of 22 February 2012 (Survival Update EC-FV-04)

	N (% censored)		Median (months)	
Population	**EC154+PLD**	**PLD**	**EC145+PLD**	**PLD**
mITT	21 (21.0%)	8 (16.3%)	23.9	21.4

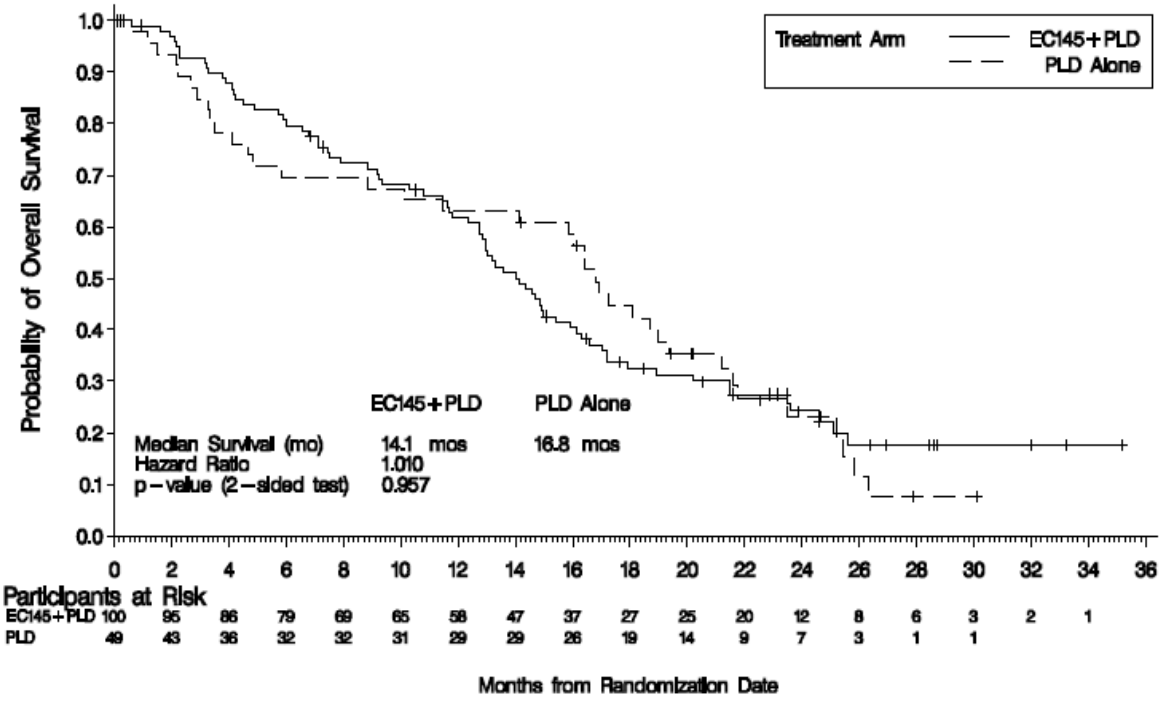


Figure 4: Kaplan-Meier curve of Overall Survival (mITT) EC-FV-04 (Data cut-off date: February 22, 2012)

Table 27: Updated Overall Survival (mITT) EC-FV-04 (Data cut-off date: February 22, 2012)

	Unadjusted Analyses		Adjusted Analyses[1]		Stratified Analyses[2]	
	HR (95% CI)	P-value[3]	HR (95% CI)	P-value[4]	HR (95% CI)	P-value[5]
mITT, n=149 (Events: 73, 37)	**1.010** (0.679, 1.503)	0.957	**0.864** (0.564, 1.324)	0.503	**0.936** (0.623, 1.406)	0.756

[1] Results from Cox proportional hazards model with age, platinum failure, CA-125 level, geography, tumor size, months since last platinum treatment, and ECOG as baseline factors included in the model
[2] Analysis stratified on platinum failure and CA-125 level.
[3] P-value based on the logrank test.
[4] P-value based on the Wald test.
[5] P-value based on stratified logrank test.

Table 28: Unadjusted, Adjusted, and Stratified Analyses of updated OS by FR status (EC-FV-04)

	Unadjusted Analyses		Adjusted Analyses[1]		Stratified Analyses[2]	
	HR (95% CI)	P-value[3]	HR (95% CI)	P-value[4]	HR (95% CI)	P-value[5]
mITT, n=149 (Events: 73, 37)	**1.010** (0.679, 1.503)	0.957	**0.864** (0.564, 1.324)	0.503	**0.936** (0.623, 1.406)	0.756
FR(100%), n=38 (Events: 18, 12)	**1.097** (0.525, 2.296)	0.805	**0.481** (0.169, 1.370)	0.171	**0.945** (0.434, 2.058)	0.887
FR(10-100%), n=74 (Events: 38, 20)	**1.094** (0.634, 1.887)	0.750	**0.884** (0.478, 1.635)	0.695	**0.990** (0.561, 1.748)	0.973
FR(0%), n=20 (Events: 10, 4)	**1.529** (0.468, 4.998)	0.479	**1.698** (0.293, 9.847)	0.555	**1.092** (0.310, 3.847)	0.892

[1] Results from Cox proportional hazards model with age, platinum failure, CA-125 level, geography, tumor size, months since last platinum treatment, and ECOG as baseline factors included in the model
[2] Analysis stratified on platinum failure and CA-125 level.
[3] P-value based on the logrank test.
[4] P-value based on the Wald test.
[5] P-value based on stratified logrank test.

Ancillary analyses

Independent review analysis

An independent review of imaging (IRC) was retrospectively undertaken. Results are reported by FR status (FR(++) all lesions FR positive, FR(+) at least one lesion positive, FR(-) all lesions FR negative, by default liver lesions were set to be FR positive).

Table 29: Analysis of PFS based on IRC assessment by FR status

Population	Vintafolide+PLD		PLD		HR (95% CI)	Log Rank P-value
	N (events)	Median (weeks)	N (events)	Median (weeks)		
mITT	100 (65)	18.1	49 (31)	8.6	0.768 (0.499, 1.182)	0.223
FR (100%)	23 (16)	17.3	15 (12)	6.6	0.465 (0.209, 1.034)	0.050
FR (10-100%)	48 (33)	17.3	26 (18)	6.7	0.652 (0.364, 1.168)	0.145
FR (0%)	13 (8)	18.1	7 (2)	NA	2.146 (0.441, 10.432)	0.333

Table 30: Analysis of PFS based on site assessment by FR status

Population	EC145+PLD		PLD		HR (95% CI)	Log Rank P-value
	N (events)	Median (weeks)	N (events)	Median (weeks)		
mITT	100 (62)	21.7	49 (33)	11.7	0.626 (0.409, 0.959)	0.031
FR(++)	23 (15)	24.0	15 (13)	6.6	0.381 (0.172, 0.845)	0.013
FR(+)	48 (30)	24.6	26 (19)	7.6	0.547 (0.304, 0.983)	0.041
FR(-)	13 (8)	16.6	7 (2)	23.3	1.806 (0.369, 8.833)	0.468

PFS events and time-points determined by site investigators and the IRC were analysed together to calculate early discrepancy rates (EDR = rate that investigators assessed as PFS events, but not confirmed by the IRC) and late discrepancy rates (LDR = rate that IRC had earlier assessed as PFS events as a proportion of total number of discrepancies).

Table 31: Early and Late Discrepancy Rates by Arm, mITT (n=149) EC-FV-04

	EC145+PLD (n=100)	PLD Alone (n=49)	Differential Discordance	Fisher's Exact Test P-Value
Early Discrepancy Rate	17.7%	15.2%	2.5%	>0.999
Late Discrepancy Rate	74.4%	68.8%	5.6%	0.746

Table 32: Agreement rates for PFS times by arm, mITT (n=149)

Site Results	EC145+PLD (n=100)	PLD Alone (n=49)
PFS Times Agree	72 (72.0%)	38 (77.6%)
IRC Time Shorter	28 (28.0%)	11 (22.4%)
Fisher's Exact Test P-value	0.554	
Median Difference	-7.9 weeks	-5.9 weeks

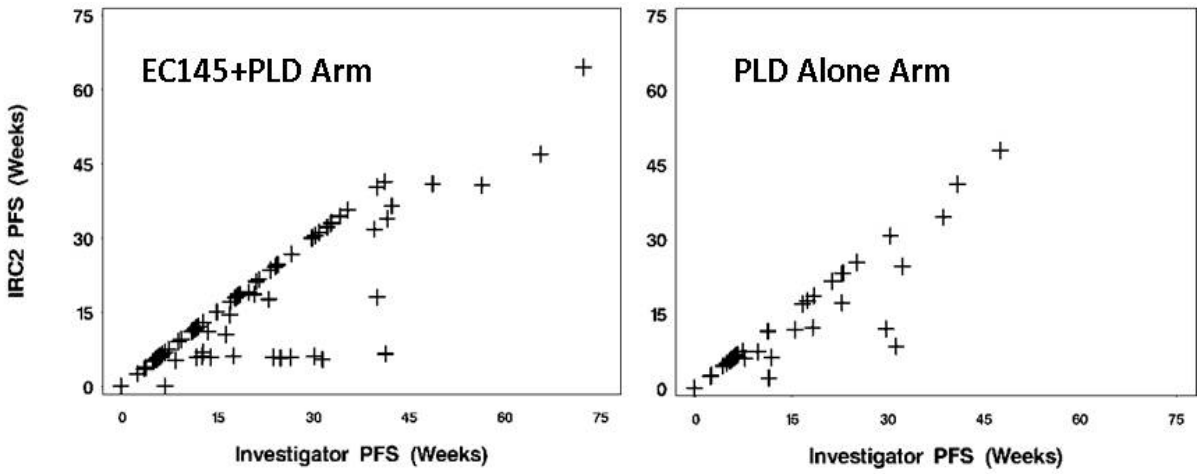


Figure 5: IRC versus site agreement by arm

PFS2 analysis

For the purposes of the PFS2 analysis, the date of event was defined as the following, whichever occurred first:

- Date the patient experienced an event of radiological or clinical disease progression reported on the long-term subject follow-up form
- Date of death
- Date of end or discontinuation of next-line treatment

For next-line treatment, any therapy other than radiotherapy was considered. Any event of disease progression reported during the long-term follow-up evaluation was considered, including disease progression in patients who did not receive subsequent next-line treatment.

Patients who did not experience disease progression during the study period but who had disease progression documented on the follow-up form were also considered as having an event of PFS2.

For patients who did not receive subsequent therapy, nor experience disease progression or death during long-term follow-up, the data were censored at the time of the last follow-up contact.

Data from patients lost to follow-up after at least one follow-up assessment were included in the analysis as censored observations on the date the patient was last known to be alive. Patients who did not have follow-up contacts were censored on the date of study discontinuation.

From a total of 140 PFS2 events, the median PFS2 was 35.6 weeks for the vintafolide+PLD arm and 20.7 weeks for the PLD alone arm in the mITT population. The hazard ratio for time to PFS2 was 0.715 (95% CI: 0.501, 1.021; log-rank 2 sided p=0.066).

In the target population for the application (FR(100%) patients), there were 23 patients in the vintafolide+PLD arm, and 15 patients in the PLD arm. Twelve patients underwent subsequent therapy in the vintafolide+PLD arm versus 8 patients in the PLD alone arm. The median PFS2 was 39.4 weeks for the vintafolide+PLD arm, compared to 17.9 weeks for the PLD alone arm, with a HR of 0.484 (95% CI 0.244, 0.961, p=0.036).

Subgroup analyses of investigator assessed PFS

Table 33: Subgroup analysis of PFS by baseline patient and disease characteristics, (mITT Population [n=149])

Factor	EC145+PLD N=100 n (events)	PLD Alone N=49 n (events)	HR (95% CI)	Interaction P-value[1]
Age (years)				
≥ 65	32 (21)	20 (13)	0.713 (0.347, 1.462)	--
< 65	68 (41)	29 (20)	0.578 (0.338, 0.987)	0.644
Country				
USA/Canada	80 (53)	41 (30)	0.597 (0.379, 0.939)	--
Poland	20 (9)	8 (3)	0.794 (0.203, 3.105)	0.619
ECOG (PS)				
0	68 (44)	26 (17)	0.594 (0.337, 1.048)	--
1 or 2	32 (18)	23 (16)	0.678 (0.341, 1.345)	0.694
Platinum failure				
Primary	65 (40)	30 (20)	0.664 (0.387, 1.139)	--
Secondary	35 (22)	19 (13)	0.598 (0.297, 1.205)	0.713
Months since treatment2				
≥ 4.90 (median)	47 (28)	28 (15)	0.761 (0.402, 1.441)	--
< 4.90 (median)	53 (34)	21 (18)	0.526 (0.295, 0.937)	0.439
PFI3 (months)				
>3 to 6	56 (32)	37 (24)	0.618 (0.362, 1.055)	--
0 to 3	44 (30)	12 (9)	0.621 (0.292, 1.320)	0.988
Number of Prior cytotoxic containing therapies				
> 1	40 (25)	22 (15)	0.524 (0.272, 1.010)	--
= 1	60 (37)	27 (18)	0.748 (0.425, 1.315)	0.361
CA-125 (U/mL)				
≥ 200	40 (30)	17 (9)	0.757 (0.349, 1.643)	--
< 200	58 (31)	31 (23)	0.543 (0.315, 0.936)	0.573
Sum of LD (mm)				
≥ 50	77 (49)	30 (20)	0.687 (0.406, 1.161)	--
< 50	23 (13)	19 (13)	0.594 (0.272, 1.296)	0.652
Number of Lesions				
> 3	52 (33)	14 (7)	0.678 (0.293, 1.568)	--
≤ 3	48 (29)	35 (26)	0.628 (0.368, 1.072)	0.861
Lung/Liver lesions				

Yes	38 (26)	11 (7)	0.624 (0.269, 1.449)	--
No	62 (36)	38 (26)	0.630 (0.377, 1.052)	0.986
[1]Wald-based p-value for treatment by baseline factor interaction. [2]Number of months from date of last platinum containing dose until randomization [3]PFI (Platinum Free Interval): Time from last platinum dose to PD prior to study entry				

Summary of main study

The following tables summarise 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 34: Summary of Efficacy for trial EC-FV-04

Title: A randomised phase II trial comparing EC145 and pegylated liposomal doxorubicin (PLD/Doxil/Caelyx) in combination, versus PLD alone, in patients with platinum-resistant ovarian cancer.			
Study identifier	EC-FV-04 (PRECEDENT)		
Design	International, multicentre, open-label, randomised phase II		
Hypothesis	Superiority		
Treatments groups	Experimental		PLD + vintafolide, mITT: 100
	Control		PLD, mITT:49
Endpoints and definitions	PFS	investigator	
	PFS FR (100%)	investigator	
Database lock	13 September 2010 (95 PFS events)		
Progression-free survival, mITT			
n	100	49	
PD	60	30	
Deaths	2	3	
Censored	38	16	
Median (95% CI)	22 weeks (17; 30)	12 weeks (7; 23)	
Hazard ratio (95% CI),	0.63 (0.41; 0.96), p-value 0.03		
Progression-free survival, FR(100%)			
n	23	15	
PD	15	12	
Deaths	0	1	
Censored	8	2	
Median (95% CI)	24 weeks	7 weeks	
Hazard ratio (95% CI)	0.38 (0.172; 0845), p-value 0.01		
Overall survival, mITT, updated analyses			
n	100	49	
Deaths	73	37	
HR (stratified analyses) (95% CI),	0.94 (0.62; 1.41), p-value 0.76		

Overall survival, FR(100%), updated analyses		
n	23	15
Deaths	18	12
HR (stratified analyses) (95% CI),	HR 0.95 (0.43; 2.06), p-value 0.89	

Supportive studies

Study EC-FV-02

EC-FV-02 was a phase 2, multicentre, open-label, non-randomised study of the companion imaging diagnostic agent EC20 and the therapeutic agent vintafolide in adult patients with advanced epithelial ovarian, primary peritoneal, fallopian tube, or endometrial cancer.

The study was carried out between the 28 August 2007 and the 27 April 2009. The data lock was 17 July 2009.

The study was conducted in 2 parts. In Part A of the study (patients enrolled before March 2008), patients with EC20-positive tumours and patients with EC20-negative tumours were enrolled. There was no limit on the maximum number of prior therapies. There was an induction phase and a maintenance phase. In Part B of the study, the protocol was amended to include only patients with EC20-positive tumours and prior therapies ≤4. The change resulted from an interim review of data for the first 44 treated patients (better activity observed in patients who received ≤3 prior therapies and had EC20-positive tumours). The induction phase was removed due to patient inconvenience.

Objectives

• Primary objective of the study was to collect data on the clinical benefit, defined as the ability of a patient to receive 6 or more cycles of therapy and to identify a target population

• Secondary objectives were to collect data on PFS, tumour responses and duration of response, DCR and OS; to further assess the safety and tolerability of therapy; and the exploratory objective of analysing archived, paraffin-embedded tissue samples for levels of FR expression and correlate with response.

A total of 80 patients underwent preliminary screening for study eligibility and 16 patients were identified as screen failures (e.g. no measurable disease by RECIST, consent withdrawn).

A total of 64 heavily pre-treated patients received a pre-injection of 0.5 mg of folic acid, followed by 0.1 mg of ^{99m}Tc-etarfolatide and underwent planar and SPECT imaging approximately 1 to 2 hours post injection.

Of these 64, 49 patients were determined to be eligible to be dosed with vintafolide (vintafolide analysis set).

Of the 49 EC145-treated patients, 43 patients met the pre-specified criteria for inclusion in the EC145 mITT analysis set. Six patients were excluded from the efficacy analyses because they failed to complete 1 cycle of therapy (3 patients), had baseline computed tomography (CT) scans >28 days before the start of EC145 therapy (2 patients), or did not have platinum resistant/refractory disease (1 patient). Among the 43 patients who were included in the EC145 mITT analysis set, 15 patients had received ≤ 3 prior therapies and were included in an EC145 mITT≤ 3 analysis set.

Table 35: Efficacy Results in EC145 mITT Analysis Set by FR Status and Overall

Parameter	FR (100%) (N=14)		FR (10-90%) (N=22)		FR (0%) (N=3)		All Patients[1] (N=43)	
	n	%	n	%	N	%	n	%
Clinical Benefit (≥6 cycles of EC145)	2	14.3	1	4.5	0	0.0	3	7.0
Complete Response (CR)	0	0.0	0	0.0	0	0.0	0	0.0
Partial Response (PR)	1	7.1	1	4.5	0	0.0	2	4.7
Stable Disease (SD)	7	50.0	7	31.8	1	33.3	16	37.2
Progressive Disease (PD)	6	42.9	14	63.6	2	66.7	25	58.1
Overall Response Rate (CR + PR)	1	7.1	1	4.5	0	0.0	2	4.7
Disease Control Rate (CR + PR + SD)	8	57.1	8	36.4	1	33.3	18	41.9
Median Progression Free Survival (weeks)	15.2		7.4		NA[2]		7.4	
HR, logrank p-value	0.797, (0.362, 1.756) p=0.302							
Median Overall Survival (weeks)	63.6		41.7		12.9		50.6	
HR, logrank p-value	0.574 (0.213, 1.542), p=0.135							

[1]Includes 4 patients with unknown FR status; [2]NA: Not available due to only 3 patients with 1 event

Study EC-FV-03

EC-FV-03 was a phase 2, multicentre, open-label, non-randomised study of vintafolide in adult patients with histologically confirmed adenocarcinoma of the lung that had previously been treated with ≥2 cytotoxic containing chemotherapeutic regimens. Patients were required to have radiographic evidence of measurable disease.

The study was carried out between 7 September 2007 and 10 November 2009. The database lock was 12 February 2010.

The primary objective of the study was to collect data on the clinical benefit, defined as the ability of a patient to receive 4 or more cycles of vintafolide therapy. The secondary objectives of the study were to collect data on tumour responses, DCR, PFS, response duration and OS, and to assess safety and tolerability. An exploratory objective of the study was to evaluate response to vintafolide therapy and uptake of the companion imaging diagnostic agent, ^{99m}Tc-EC20. Entry requirements included radiographic evidence of measurable disease and at least one EC20-positive tumour.

A total of 60 patients with NSCLC received a pre-injection of 0.5 mg of folic acid, followed by 0.1 mg of ^{99m} Tc-etarfolatide and underwent planar and SPECT imaging approximately 1 to 2 hours post injection.

Of these 60, 43 patients were determined to be eligible to be dosed with vintafolide (vintafolide analysis set).

Of the 43 patients who received treatment with vintafolide, 29 patients met the pre-specified criteria for the mITT analysis set and were included in the primary efficacy analyses.

Table 36: Study EC-FV-03: Efficacy Results in EC145 modified Intent-to-Treat (mITT) analysis set by ^{99m}Tc etarfolatide Status and Overall

Parameter	FR (100%) (N=14)		FR (10-90%) (N=14)		All Patients (N=29)[1]	
	n	%	N	%	n	%
Clinical Benefit (≥ 4 cycles of EC145)	7	50.0	2	14.3	9	31.0
Complete Response (CR)	0	0.0	0	0.0	0	0.0
Partial Response (PR)	1	7.1	0	0.0	1	3.4
Stable Disease (SD)	7	50.0	2	14.3	9	31.0
Progressive Disease (PD)	6	42.9	12	85.7	19	65.5
Overall Response Rate (CR + PR)	1	7.1	0	0.0	1	3.4
Disease Control Rate (CR +PR + SD)	8	57.1	2	14.3	10	34.5
Median Progression Free Survival (weeks)	31.1		7.3		7.4	
HR, logrank p-value	0.326, p=0.014					
Median Overall Survival (weeks)	47.2		14.9		32.1	
HR, logrank p-value	0.539, p= 0.104					

[1] One patient was included in the FR(0%) group; this patient had progressive disease.

2.4.3. Discussion on clinical efficacy

Design and conduct of clinical studies

The pivotal study (EC-FV-04) for this application was an add-on study to PLD with 2:1 randomisation and with investigator assessed PFS as primary endpoint.

In order to identify patients for treatment a companion diagnostic ^{99m}Tc-etarfolatide (^{99m}Tc-EC20) was co-developed. It is accepted based on the exploratory studies conducted prior to the pivotal study that the likelihood of tumour response to vintafolide (EC145) in FR negative tumours is too low to be of clinical relevance, a notion corroborated by the findings in the pivotal study for this application.

Apart from the FR detection objectives, study EC-FV-04 was a conventionally designed, randomised phase 2 study. PLD is a reasonable background regimen in patients with platinum resistant ovarian cancer. The main inclusion and exclusion criteria were also reasonable. Based on the findings from study EC-FV-01 the recommended phase 2 dose for vintafolide was determined to be 2.5 mg which is considered acceptable. The dose of PLD was standard and due to mainly non-overlapping toxicity the dose of vintafolide was not reduced from the RPIID in the experimental arm.

A GCP inspection was conducted in three sites in relation to study EC-FV-04 and revealed poor compliance with the principles of GCP and with the protocol at the investigator site in the US inspected site which enrolled a total of 9 patients. None of the subjects recruited to this site were included in the FR(100%) analysis. The applicant undertook analyses in relation to secondary endpoints, including adjusted analyses, all showing consistent and favourable results when this US site was excluded. In addition, the applicant audited sites that randomised 112 of 162 (69%) patients and 25 of 38 (66%) FR(100%) patients. Overall, the CHMP concluded that the quality assurance system (monitoring and auditing) and actions undertaken should produce reliable data. In addition, a number of sensitivity analyses (including censoring of "clinical progression") and subgroup analyses were compatible with robustness and internal consistency.

Efficacy data and additional analyses

The folate receptor (FR), the target for vintafolide, is (over)expressed in many tumours, among them ovarian cancer, and is prognostic for poor outcome. As platinum resistant tumours per se has a poor prognosis it was a reasonable first step to focus the development of vintafolide on FR positive, platinum resistant, ovarian cancer. Based on this and considering the pharmacology of the product it is considered justified to focus on FR expressing tumours. Nevertheless, the results in the full study population are also discussed, mainly as some of the relevant subgroups become too small and as ^{99m}Tc-etarfolatide scanning was not undertaken in all patients. Furthermore, this is a conservative estimate as FR(0%) tumours are included in the mITT population.

Efficacy data in the mITT population

Based on investigators' assessment of PFS, the results were statistically significant in the mITT and the FR(100%) population and mITT results were robust in a wide variety of sensitivity analyses (HR 0.57-0.63, p-value 0.01-0.03).

The sensitivity analyses undertaken all showed consistent results, except for the analysis where patients without measurable disease were included (HR: 0.74) (enrolment stopped by amendment 3). There were no signs of bias based on differences in scheduled versus non-scheduled tumour assessments. Similarly, censoring of patients at time of clinical progression indicated no bias (HR: 0.60) and there were no signs of bias with respect to imaging sessions.

In line with Appendix 1 of the anti-cancer guideline, an independent review of imaging (IRC) was retrospectively undertaken to support the investigator analysis of PFS, normally being the preferred analysis. In all analyses conducted, the HR was more favourable according to the investigator analyses. The IRC assessment was borderline significant positive only in the FR(100%) subgroup.

This might at least partly be explained by the discrepancy observed at the week 6 analysis since the large proportion of disagreements related to the first scheduled assessment at week 6 and in the experimental arm. In quite a few patients in both study arms, the difference between IRC progression and investigator progression was large, 3 to 5 months, which is not compatible with reasonable tumour progression rates. A total of 8 patients (7 vintafolide, 1 placebo) had a delay of 18 weeks or more from IRC PD to investigator PD. If these patients were censored in the IRC analysis at time of site progression or IRC progression the HRs became 0.66 and 0.70, respectively to be compared with 0.77. Similar results were obtained if 12 weeks or more was used as cut-off.

In addition and due to rapid progression in the control arm, more patients underwent more than one post baseline assessment in the experimental arm (69% versus 43% in the control arm). As the IRC

can only shorten time to progression, but not prior to the first scheduled assessment, more patients in the experimental arm were at risk of shortened time to progression in the IRC analysis of PFS.

Based on conventional analyses such as early and late discrepancy rates and agreement rates for PFS by study arm, there were no obvious signs of investigator bias. These analyses supported the credibility of investigator reported PFS.

Therefore, altogether there were no good reasons to assume that investigator reported PFS results were biased to a relevant extent and, importantly, IRC analyses replicated the relationship between FR positivity and outcome.

There were, however, a non-trivial number of patients withdrawn from the study (n=14+9) prior to an event of PFS. This was compensated for in a PFS2 analysis conducted post hoc, but in principle in accordance with the anti-cancer notes for guidance, where almost complete data show an HR in the mITT group of 0.72 and in the FR(100%) of 0.48.

Regarding the summary of PFS component in the mITT population, there were too few events to support any conclusions.

With respect to potential differences in types of progressive lesions, FR+ or FR- in the study arms, data were much too limited to support any notions.

In relation to subgroup analysis of investigator assessed PFS, all differences in point estimate were considered minor and there was no apparent pattern in relation to likely prognostic factors.

Survival point estimates did not replicate findings in the PFS analyses. The absence of favourable trend in OS in study EC-FV-04 was considered of concern and rather extensive analyses were undertaken with the aim to try to identify whether causes of death might be attributed to study therapies. No such relationship was identified, but it was fully acknowledged that whether causes of death should be attributed to the underlying disease, co-morbidities, study therapies or interactions between therapy and underlying conditions is frequently not possible to ascertain.

Median time from progression to death was about 12 months versus about 5.5 months on therapy and a total of 8 deaths occurred on experimental therapy (+30 days) whilst there were altogether 74 deaths reported in the survival analysis. It was also not possible to identify any mechanistic grounds for a vinca-alkaloid to give rise to late toxic events leading to death >30 days after end of therapy.

In the mITT analyses (investigator) the PFS HR was 0.63 (95% CI 0.41; 0.96) and the OS HR 1.01 (95% CI 0.68; 1.50). In the retrospectively conducted covariate adjusted analyses the corresponding data were PFS HR 0.60 (0.37; 0.96) and OS HR 0.86 (0.56; 1.32), meaning that the covariate imbalance mainly was of importance for time from progression to death.

It was noted that outcomes with respect to OS and PFS were particularly poor in the FR 0% stratum (PFS HR 1.8 and OS HR 1.5). However, when OS was analysed by baseline stratification factors, the HR moved towards 1 (HR 1.1). This is compatible with imbalances in baseline stratification factors of importance not least as a negative anti-tumour effect (PFS) of Vynfinit as add-on to PLD is non-plausible from a mechanistic perspective.

Updated survival data per April 2013 were also submitted. The analysis provides 4 additional deaths. However, this latest updated OS analysis reflected all additional survival data that was

provided by sites after the 22 February 2012 data cut-off, but did not represent a comprehensive "sweep" of the sites, which is the standard process for survival updates. As with the updated analysis from February 2012, this update provided a slightly lower HR for the mITT (HR=0.987, 95% CI: 0.667, 1.461).

Efficacy data in the FR(100%) population

Regarding baseline data in the FR(100%) population, the tumour burden at baseline, measured by the summary of tumour diameters (STD), appeared larger in the experimental arm as in the full study population. Performance status, however, appeared favourable in the experimental group. Similar proportions of patients were first-line platinum resistant. Response to last-line therapy was hard to draw any conclusions from due to the very small sample size, but it was noticed that best response was PD in two patients in the control group versus none in the experimental arm.

In the FR(100%) population an early progression rate in the control group was observed where about 70% progressed at the first scheduled assessment at 6 weeks. Altogether 8 out of 23 patients were censored in the PFS analysis in the experimental arm. Due to the small sample size, this is not optimal, but PFS2 data (HR of 0.484) are considered supportive (see above).

Data from studies EC-FV-02 and EC-FV-03 provide some support for increased activity in case of FR positive ovarian tumours.

Additional efficacy data needed in the context of a conditional MA

Platinum resistant ovarian cancer is a serious orphan condition and FR expression is a recognised prognostic factor for poor outcome, a notion confirmed in the pivotal trial EC-FV-04 where PFS and OS were distinctly poorer for the control group in the FR(100%) group compared with the complementary set of patients whilst this was not observed in the experimental group.

Due to the poor prognosis in general for platinum resistant ovarian cancer, there is an unmet medical need in this patient population that could be fulfilled with the proposed medicinal product. Patients with platinum resistant ovarian cancer have currently limited therapeutic options: topotecan, paclitaxel and pegylated liposomal doxorubicin (PLD). FR(100%) patients represent a small subpopulation of this orphan condition that have a poorer overall prognosis and there are currently no means for patient selection and treatment.

Efficacy data are currently available mainly from one phase 2 study in 38 patients enrolled in the target population and 149 in the mITT population. Therefore, additional efficacy data is needed in the context of a conditional MA in order to confirm the benefit of vintafolide in combination with PLD in the intended indication.

Additional comprehensive clinical data can be provided from study EC-FV-06, a randomised double-blind phase 3 trial comparing vintafolide and PLD in combination versus PLD in patients with PROC. As of the end of October 2013, Study EC-FV-06 had a total of 250 participants randomised, regardless of FR status. Approximately 350 FR(100%) patients will be enrolled in the study. Assuming maximum impact of marketing authorisation on enrolment, it is still estimated that full enrolment of the requisite 350 FR(100%) patients will occur by May 2015 and comprehensive data on efficacy in terms of PFS and OS are likely to be available after conditional approval. The final analysis of the primary endpoint of PFS in FR (100%) patients (245 PFS events) and interim OS analysis is expected to be submitted in December 2015 while the final OS analysis is expected to be

available in March 2017 as reflected in the RMP. This study should be conducted by the applicant as a specific obligation for approval.

2.4.4. Conclusions on the clinical efficacy

Clinically meaningful efficacy results in terms of PFS benefit have been demonstrated in patients with platinum resistant ovarian cancer expressing the folate receptor in all tumour lesions as assessed by ^{99m}Tc-etarfolatide.

In the FR(100%) population, the PFS HR was about 0.4 (p=0.01) and the observed median difference was about 4 months based on site assessment. According to IRC assessment, the PFS HR in the same population was about 0.5 (p=0.05) and the observed median difference about 2.5 months. Irrespective of analyses, this is regarded as meaningful results in this target population.

Overall Survival point estimates did not replicate findings in the PFS analyses. However, comprehensive reasons for death analyses indicated that there was no excess of treatment related deaths in the experimental arm. Altogether the diluting effect of long post-progression survival, about 1 year, in combination with baseline imbalances, and wide confidence intervals are considered to be the most likely explanations to the absence of favourable trends in terms of survival.

Overall, the CHMP concludes that clinically meaningful efficacy results in terms of PFS benefit have been demonstrated in patients with platinum resistant ovarian cancer expressing the folate receptor in all tumour lesions as assessed by ^{99m}Tc-etarfolatide. The CHMP considers the following measures necessary to address the missing efficacy data in the context of a conditional MA:

- To submit clinical efficacy results from study EC-FV-06, a randomised double-blind phase 3 trial comparing vintafolide in combination with PLD versus PLD + placebo in patients with platinum-resistant ovarian cancer who express the folate receptor on all target lesions as assessed by the ^{99m}Tc-etarfolatide imaging procedure
    - Final clinical study report: March 2017

The benefit to public health of the immediate availability on the market of vintafolide outweighs the risk in the fact that additional data are still required.

2.5. Clinical safety

Patient exposure

Safety data derives from three completed studies: EC-FV-04 (pivotal), EC-FV-02 and EC-FV-03 (both supportive), and a summary of safety data from one phase 1 dose escalation study (EC-FV-01).

Table 37: Tabulation of patients contributing to the safety analysis

Study Number	EC145+PLD (Vintafolide+PLD)	EC145 (Vintafolide)	PLD	Total
EC-FV-04 (Ovarian Cancer)	107	NA	50	157
EC-FV-02 (Ovarian	NA	49	NA	49

Cancer)				
EC-FV-03 (NSCLC)	NA	43	NA	43
EC-FV-01 (Solid tumours)	NA	32	NA	32
TOTAL	**107**	**124**	**50**	**281**

Table 38: Patient exposure (Data cut-off: 13 September 2010)

	Patients enrolled[1]	**Patients exposed[2]**	**Patients exposed to the proposed dose range[3]**	**Patients with long term* safety data**
Placebo-controlled	0	0	0	0
Active –controlled[4]	195	107	107	40
Open studies[5]	178	124	**53**	16
Post marketing	0	0	0	0
Compassionate use	0	0	0	0

1 Number of patients that signed informed consent
2 Number of patients that received at least one dose of vintafolide
3 Number of patients that received at least one dose of 2.5 mg vintafolide days 1, 3, 5, 15, 17, 19 of a 28 day cycle
4 EC-FV-04 study: vintafolide+PLD vs PLD alone
5 Vintafolide single-agent studies (EC-FV-01, EC-FV-02, EC-FV-03)
* In general this refers to 6 months and 12 months (9 Active Controlled patients and 4 open study patients) continuous exposure data, or intermittent exposure.

In EC-FV-04, the total mean cumulative actual dose per patient was 60.30 mg of vintafolide and 201.43 mg/m2 of PLD for the vintafolide+PLD arm. The total mean cumulative actual dose of PLD was 191.88 mg/m2 in the PLD arm. The mean number of treatment cycles in the vintafolide+PLD arm was 4.9 cycles with a median of 4.0 cycles. In the PLD arm, the mean number of treatment cycles was 4.0, with a median of 2.0 cycles. The mean total treatment duration was slightly longer in the combination arm: vintafolide+PLD arm was 18.6 ± 14.7 weeks and the PLD arm was 15.0 ± 12.2 weeks.

Table 39: EC-FV-04 Patient exposure in relation to FR status

	Vintafolide+PLD arm				**PLD Alone arm**	
	vintafolide mg		PLD mg/ m^2		PLD mg/ m^2	
	FR (100 %)	FR (0 %)	FR (100 %)	FR (0 %)	FR (100 %)	FR (0 %)
Total Mean cumulative actual dose per patient	68.45	66.73	218.52	215.26	133.33	250.0
Total median cumulative dose per patient	62.5	47.50	212.5	200.0	100.0	275.0
Mean value for Dose intensity per participant	88.1 -97.2	75.4-97.4	86.5-105.9	76.4-98.8	75-98.3	100.0-105.0

Range (%)						
Mean Overall dose intensity for PLD			94.4	90.0	95.0	103.3
Mean Overall dose intensity for vintafolide	85.2	85.5				
	Vintafolide+PLD arm				**PLD Alone arm**	
	FR (100 %)	FR (0 %)			FR (100 %)	FR (0 %)
Mean No of cycles	5.2	5.2			2.9	4.3
Median No of cycles	5.0	4.0			2.0	5.0
No of weeks on treatment (range)	0-72	5-43			2-30	0-24
Mean total treatment duration (weeks)	19.9±16.1	20.1±12.5			9.9±9.2	16.2±9.6

Adverse events

Adverse events from study EC-FV-04

Table 40: Overall Summary of adverse events by treatment arm (EC145/PLD Safety Population)

Number (%) of Patients With:	EC145+PLD Arm (N=107) n (%)	PLD Alone Arm (N=50) n (%)	P-Value (Fisher's Exact)
At Least 1 TEAE[1]	106 (99.1%)	49 (98.0%)	0.537
At least 1 TEAE of Grade 3 or 4	81 (75.7%)	27 (54.0%)	0.009
At Least 1 Serious TEAE	51 (47.7%)	17 (34.0%)	0.122
At Least 1 Drug-Related[2] TEAE	101 (94.4%)	43 (86.0%)	0.116
At Least 1 Drug-Related TEAE Resulting In Withdrawal Of PLD	13 (12.1%)	2 (4.0%)	0.147
At Least 1 Drug-Related TEAE Resulting In Withdrawal Of EC145	6 (5.6%)	0 (0.0%)	0.178
At Least 1 Drug-Related Serious TEAE	22 (20.6%)	6 (12.0%)	0.264
At Least 1 Drug-Related Serious TEAE Resulting In Withdrawal Of PLD	0 (0.0%)	2 (4.0%)	
At Least 1 Drug-Related Serious TEAE Resulting In Withdrawal Of EC145	1 (0.9%)	0 (0.0%)	
At Least 1 Drug-Related TEAE of Grade 3 or 4	56 (52.3%)	18 (36.0%)	0.061
At Least 1 Drug-Related Serious TEAE of Grade 3 or 4	19 (17.8%)	5 (10.0%)	0.242
Hospitalized	47 (43.9%)	15 (30.0%)	0.116
Death (Grade 5)[3]	3 (2.8%)	2 (4.0%)	0.654
Death (Grade 5) within 30 days Post-EC145/PLD	3 (2.8%)	2 (4.0%) [4]	0.654

[1] Treatment-Emergent Adverse Events are adverse events starting after administration of EC145+PLD or PLD and within 30 days of the last dose of EC145+PLD or PLD, unless otherwise indicated.

[2] Drug-related adverse events include those with a definite, probable, or possible drug-relationship.

[3] All deaths after first dose of EC145+PLD or PLD, including those occurring > 30 days after the last dose.

[4] Patient 400-214 (PLD Alone Arm) died 47 days after the last dose of PLD; since the AE started within 30 days post-PLD it is included as a serious non-drug related AE (malignant pericardial effusion) resulting in Death (Grade 5) within 30 days post-PLD but is not included as a death that occurred within 30 days post-PLD.

NOTES:
Adverse events were coded in accordance with Medical Dictionary for Regulatory Activities Version 11.1.
Grades are based on Common Terminology Criteria for Adverse Events (CTCAE) V3.0, with the exception of hand-foot syndrome, stomatitis and hematologic toxicity. Grades for these AEs are defined in the protocol.
Source: Tables 14.6.4, 14.6.5.9, 14.11

Table 41: Overall summary of AEs by cycle by treatment arm EC-FV-04 (EC145/PLD Safety Population)

Number (%)[1] of Cycles With Adverse Events:	EC145+PLD Arm (N=518 cycles) n (%)	PLD Alone Arm (N=202 cycles) n (%)
At Least 1 TEAE[2]	445 (85.9)	162 (80.2)
At Least 1 Serious TEAE	63 (12.2)	23 (11.4)
At Least 1 Grade 3 or Grade 4 TEAE	152 (29.3)	37 (18.3)
Hospitalized	56 (10.8)	18 (8.9)

[1] N is the total number of patient cycles with study treatment; n is the number of these cycles in which an adverse experience was noted and % is the percentage for n/N.

[2] Treatment-Emergent Adverse Events are adverse events starting after administration of EC145+PLD or PLD and within 30 days of the last dose of EC145+PLD or PLD, unless otherwise indicated.

Treatment-Emergent Adverse Events (TEAEs) regardless of causality

The most common TEAEs (occurring in ≥20% of patients) in the vintafolide+PLD Arm were fatigue (56.1%), anemia (45.8%), stomatitis (45.8%), nausea (44.9%), neutropenia (43.9%), Palmar-plantar erythrodysesthesia (PPE) syndrome (43.0%), constipation (41.1%), abdominal pain (35.5%), vomiting (34.6%), rash (33.6%), peripheral sensory neuropathy (29.0%), diarrhea (27.1%), anorexia (25.2%), and leucopenia (23.4%). The most common TEAEs in the PLD Alone arm were nausea (58.0%), Fatigue (44.0%), Stomatitis and palmar-plantar erythrodysesthesia syndrome (42.0% each), Constipation (38.0%), Anaemia (34.0%), Vomiting (28.0%), Neutropenia (24.0%), Diarrhoea (20.0%).

Treatment-emergent adverse events by cycle

The 157 subjects in the safety population received a total of 720 cycles of treatment (518 in vintafolide+PLD arm vs. 202 in PLD Alone arm). When the longer duration of therapy was taken into account and TEAEs were evaluated by cycle, a decrease in the difference as regards Grade 3 or 4 TEAEs, serious TEAEs and hospitalizations were observed. Subjects in the vintafolide+PLD arm experienced TEAEs most commonly within the SOC of blood and lymphatic system disorders (primarily anemia and neutropenia) compared to the PLD Alone arm. Overall, anemia, neutropenia and thrombocytopenia AEs, as reported by the investigator, were observed in 16.6% (vs. 10.4% administered PLD alone), 19.1% (vs.10.4%), and 2.7% (vs. 3.0%) of all cycles, respectively. Stomatitis and PPE occurred in 16.6% (vs. 22.8%) and 19.1% (vs. 15.8%) of cycles, respectively. Peripheral sensory, motor, sensorimotor or polyneuropathy occurred in 10.4% (vs. 2.5%) of cycles, and constipation and small intestinal obstruction/ileus were observed in 12.7% (vs. 10.4%) and 2.9% (vs. 4.0%) of cycles, respectively.

After accounting for the number of cycles of treatment, anaemia, neutropenia and neuropathy were numerically greater (>5% more) in patients administered vintafolide+PLD. Thrombocytopenia, constipation and small intestinal obstruction/ileus, fatigue and PPE were similar (within 5%) between treatment arms. Stomatitis was numerically greater (>5% more) in patients administered PLD alone. All AEs were non-cumulative except for PPE, which increased in frequency with subsequent cycles.

Adverse reactions

An overview of all treatment-emergent adverse events considered related to the treatments (adverse reactions) reported in study EC-FV-04 in the vintafolide+PLD combination arm is presented in the table below.

Table 42: Adverse reactions reported in patients in study EC-FV-04

System organ class	Frequency	Adverse reaction
Infections and infestations	Common	fungal infection
		candida infection
		oral candidiasis
		vulvovaginal mycotic infection
	Uncommon	fungal skin infection
		oral herpes
Blood and lymphatic system disorders	Very common	neutropenia*
		thrombocytopenia*
		anaemia*
		leukopenia*
		lymphopenia*
	Uncommon	febrile neutropenia
Immune system disorders	Uncommon	hypersensitivity
Metabolism and nutrition disorders	Very common	anorexia
	Common	dehydration
		hypoalbuminaemia*
		decreased appetite
	Uncommon	malnutrition
Psychiatric disorders	Common	depression
		insomnia
	Uncommon	anxiety
Nervous system disorders	Very common	peripheral sensory neuropathy
	Common	extrapyramidal disorder
		polyneuropathy
		peripheral sensorimotor neuropathy
		neuropathy peripheral
		dizziness
		paraesthesia
		dysgeusia
	Uncommon	ataxia
		balance disorder
		syncope
		memory impairment
		peripheral motor neuropathy
		neuralgia
		dysaesthesia
		hypoaesthesia
		parosmia
		restless leg syndrome
		vocal cord paralysis
Eye disorders	Common	vision blurred
	Uncommon	visual impairment
		eye irritation
Vascular disorders	Uncommon	hypertension
		periphlebitis
		flushing
		hot flush
Respiratory, thoracic and mediastinal disorders	Common	dyspnoea
		dyspnoea exertional
		epistaxis
		dysphonia
Gastrointestinal disorders	Very common	stomatitis
		vomiting
		diarrhoea
		constipation
		nausea
	Common	small intestinal obstruction
		abdominal pain
		abdominal pain upper
		abdominal discomfort
		abdominal distension
		dysphagia
		oral pain
		gastrooesophageal reflux disease
		sensitivity of teeth
		paraesthesia oral
		hypoaesthesia oral
		oral pruritus
		dry mouth

		flatulence
	Uncommon	rectal haemorrhage
		abdominal pain lower
		retching
		eructation
		gingival pain
Hepatobiliary disorders	Common	hyperbilirubinaemia*
Skin and subcutaneous disorders	Very common	palmar-plantar erythrodysaesthesia syndrome
		rash
	Common	rash papular
		erythema
		pruritus
		skin hyperpigmentation
		skin discolouration
		alopecia
	Uncommon	skin exfoliation
		dermatitis exfoliative
		urticaria
		petechiae
		rash generalised
		rash maculo-papular
		rash erythematous
		skin ulcer
		nail disorder
		nail discolouration
		nail pigmentation
Musculoskeletal and connective tissue disorders	Common	muscular weakness
		pain in extremity
		back pain
		myalgia
		arthralgia
		muscle spasms
Renal and urinary disorders	Common	urinary incontinence
	Uncommon	dysuria
Reproductive system and breast disorders	Common	pelvic pain
General disorders and administrative site conditions	Very common	fatigue
		asthenia
	Common	chest discomfort
		pyrexia
		pain
		malaise
	Uncommon	gait disturbance
		infusion related reaction
		oedema peripheral
		oedema
		infusion site extravasation
		chills
		early satiety
Investigations	Very common	gamma-glutamyltransferase increased*
		aspartate aminotransferase (AST) increased*
		blood alkaline phosphatase increased*
	Common	weight decreased
Injury, poisoning and procedural complications	Uncommon	fall
		procedural site reaction
*Derived from available laboratory data. For the chemistry data, N=104 and for the hematology data, N=103 in the Vynfinit + PLD treatment arm.		

The table below presents the adverse reactions reported in ≥ 5% of patients with PROC randomised to receive Vynfinit in combination with PLD versus PLD alone, and who received at least one dose of Vynfinit and/or PLD. The frequency and severity of the adverse reactions reported are based on the treatment emergent adverse events (regardless of causality).

Table 43: Adverse reactions reported in ≥ 5% of patients in study EC-FV-04 by treatment arm

System organ class	Frequency	Adverse reaction*	Vynfinit + PLD n= 107		PLD n= 50	
			All Grades n (%)	Grade 3-4 n (%)	All Grades n (%)	Grade 3-4 n (%)
Infections and infestations	Very common	urinary tract infection	16 (15)	0	6 (12)	0
Blood and lymphatic system disorders	Very common	anaemia†	86 (83)	6 (6)	36 (78)	2 (4)
		leukopenia†	85 (83)	16 (16)	33 (72)	2 (4)
		lymphopenia†	72 (70)	18 (17)	29 (63)	9 (20)
		neutropenia†	47 (46)	13 (13)	15 (33)	2 (4)
		thrombocytopenia†	14 (14)	2 (2)	9 (20)	1 (2)
Metabolism and nutrition disorders	Very common	anorexia	27 (25)	2 (2)	6 (12)	1 (2)
		hypokalemia†	13 (13)	4 (4)	8 (17)	0
		hypoalbuminaemia†	10 (10)	0	3 (7)	0
		dehydration	16 (15)	2 (2)	7 (14)	2 (4)
Nervous system disorders	Very common	peripheral sensory neuropathy	31 (29)	4 (4)	6 (12)	0
		dizziness	15 (14)	1 (1)	4 (8)	0
Vascular disorders	Common	hypotension	3 (3)	0	3 (6)	0
		flushing	1 (1)	0	4 (8)	0
Gastrointestinal disorders	Very common	stomatitis	49 (46)	9 (8)	21 (42)	2 (4)
		nausea	48 (45)	1 (1)	29 (58)	4 (8)
		constipation	44 (41)	2 (2)	19 (38)	0
		vomiting	37 (35)	1 (1)	14 (28)	1 (2)
		diarrhoea	29 (27)	2 (2)	10 (20)	0
		abdominal pain	38 (36)	8 (7)	9 (18)	1 (2)
	Common	dysphagia	9 (8)	0	1 (2)	0
		oral pain	6 (6)	0	0	0
Skin and subcutaneous disorders	Very common	palmar-plantar erythrodysaesthesia syndrome	46 (43)	12 (11)	21 (42)	1 (2)
		rash	36 (34)	2 (2)	9 (18)	0
		dry skin	13 (12)	0	4 (8)	0
	Common	alopecia	9 (8)	0	2 (4)	0
		erythema	10 (9)	0	3 (6)	0
Musculoskeletal and connective tissue disorders	Very common	myalgia	12 (11)	0	2 (4)	0
		pain in extremity	11 (10)	0	1 (2)	0
	Common	muscular weakness	10 (9)	2 (2)	3 (6)	0
General disorders and administrative site conditions	Very common	fatigue	60 (56)	10 (9)	22 (44)	3 (6)
		asthenia	18 (17)	0	3 (6)	0
		pyrexia	21 (20)	0	7 (14)	0
	Common	infusion related reaction	2 (2)	0	3 (6)	2 (4)
Investigations	Very common	gamma-glutamyltransferase increased†	52 (50)	7 (7)	21 (46)	2 (4)
		aspartate aminotransferase (AST) increased†	22 (21)	1 (1)	4 (9)	0
		blood alkaline phosphatase increased†	22 (21)	1 (1)	16 (35)	0

*Adverse reaction terms reported in ≥ 5% of patients in PRECEDENT
†Derived from available laboratory data. For the chemistry data, N=104 and N=46 in the Vynfinit + PLD and PLD treatment arms, respectively. For the hematology data, N=103 and N=46 in the Vynfinit + PLD and PLD treatment arms, respectively.
Note: The frequency and severity of the adverse reactions reported are based on the treatment emergent adverse events (regardless of causality)

Adverse events by FR-status

Table 44: Summary of Adverse Events by FR Status (0 % vs. 100 %) Compared to the Overall Safety Population - EC-FV-04

	Overall Safety Population		FR(100%)Safety Population		FR(0%)Safety Population	
Number of Patients with:	EC145+PLD Arm (N=107) n (%)	PLD Alone Arm (N=50) n (%)	EC145+PLD Arm (N=22) n (%)	PLD Alone Arm (N=15) n (%)	EC145+PLD Arm (N=13) n (%)	PLD Alone Arm (N=6) n (%)
At Least 1 TEAE[1]	106 (99.1)	49 (98.0)	22 (100)	15 (100)	13 (100)	6 (100)
At Least 1 Drug-Related[2] TEAE	101 (94.4)	43 (86.0)	22 (100)	11 (73.3)	12 (92.3)	6 (100)
At Least 1 Drug-Related TEAE Resulting in Withdrawal of PLD	13 (12.1)	2 (4.0)	1 (4.5)	0 (0.0)	3 (23.1)	0 (0.0)
At Least 1 Drug-Related TEAE Resulting in Withdrawal of EC145	6 (5.6)	0 (0.0)	0 (0.0)	0 (0.0)	1 (7.7)	0 (0.0)
At Least 1 Serious TEAE	51 (47.7)	17 (34.0)	9 (40.9)	6 (40.0)	7 (53.8)	1 (16.7)
At Least 1 Drug-Related Serious TEAE	22 (20.6)	6 (12.0)	1 (4.5)	0 (0.0)	1 (7.7)	0 (0.0)
At Least 1 Drug-Related Serious TEAE Resulting in Withdrawal of PLD	0 (0.0)	2 (4.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
At Least 1 Drug-Related Serious TEAE Resulting in Withdrawal of EC145	1 (0.9)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
At Least 1 Drug-Related TEAE of Grade 3 or 4	56 (52.3)	18 (36.0)	8 (36.4)	4 (26.7)	6 (46.2)	1 (16.7)
At Least 1Drug-Related Serious TEAE of Grade 3 or 4	19 (17.8)	5 (10.0)	1 (4.5)	0 (0.0)	1 (7.7)	0 (0.0)
Deaths[3] (Grade 5)	3 (2.8)	2 (4.0)	0 (0.0)	2 (13.3)	0 (0.0)	0 (0.0)
Deaths (Grade 5) within 30 days post-EC145/PLD	3 (2.8)	2 (4.0)[4]	0 (0.0)	2 (13.3)[4]	0 (0.0)	0 (0.0)

[1] Treatment-Emergent Adverse Events are adverse events starting after administration of EC145+PLD or PLD and within 30 days of the last dose of EC145+PLD or PLD, unless otherwise indicated.
[2] Drug-related adverse events include those with a definite, probable, or possible drug-relationship.
[3] All deaths after first dose of EC145+PLD or PLD, including those occurring > 30 days after the last dose.
[4] Patient 400-214 (PLD Alone Arm) died 47 days after the last dose of PLD; since the AE started within 30 days post-PLD it is included as a serious non-drug related AE (malignant pericardial effusion) resulting in Death (Grade 5) within 30 days post-PLD but is not included as a death that occurred within 30 days post-PLD.
Source: EC-FV-04 CSR Table 14.6.4; Table 14.14.1; Table 14.15.1

The safety profile of the FR(100%) population treated with Vynfinit + PLD was similar to that of the primary safety population. The only differences were that myalgia, muscular weakness, and dry skin were reported as very common treatment emergent adverse reactions (13.6% each) in the FR(100%) population while they were reported as common (8%, 7%, and 7%, respectively) in the primary safety patient population.

Cardiac adverse events - Left Ventricular Ejection Fraction (LVEF)

The protocol specified that LVEF assessments were to be performed in accordance with the PLD package insert, i.e. all patients were to receive a baseline LVEF assessment along with an assessment following 550 mg/m^2 cumulative PLD doses. As a further safety measure, patients in the vintafolide+PLD treatment arm were to receive an additional LVEF assessment at half the maximum permitted cumulative PLD dose (275 mg/m^2). If a patient had progressive disease and discontinued from the study prior to a cumulative PLD dose of 550 mg/m^2, a post-baseline LVEF assessment was not likely to be performed. Only a limited number of subjects reached the level of the first post-baseline LVEF assessment.

A total of 25 subjects out of the 26 subjects that reached a cumulative PLD dose of $\geq$ 275 mg/m^2 had a LVEF assessment. Only one subjects reached the cumulative PLD dose of $\geq$ 550 mg/m^2.

Table 45: Left Ventricular Ejection Fraction (EC145/PLD Safety Population)

Time Point	Statistics	EC145+PLD Arm (N=107)		PLD Alone Arm (N=50)	
		Observed Values	Change From Baseline	Observed Values	Change From Baseline
Baseline[1]	**N**	**105**		**48**	
	Mean	63.27		63.76	
	STD	7.284		7.65	
	Median	61.90		60.00	
	Min-Max	49.0 - 81.3		50.0 - 82.5	
During Treatment[2]	**N**	**34**	34	**7**	7
	Mean	63.18	-0.18	64.86	-0.14
	STD	7.245	5.414	9.94	5.146
	Median	63.10	-0.50	64.00	0.00
	Min-Max	53.3 - 84.0	-11.0 – 15.0	55.0 - 86.0	-5.0 – 8.0
End of Treatment[3]	**N**	**10**	10	**6**	6
	Mean	59.09	-9.09	64.17	-0.83
	STD	14.980	18.984	9.326	8.329
	Median	60	-8	63	0.5
	Min-Max	25.0 - 79.0	-55.0 – 12.0	50.0 - 75.0	-15.0 – 10.0

[1] Baseline = Screening. [2] Includes patients with an assessment at baseline and the last value available during treatment. [3] Includes patients with an assessment at baseline and the last value available after treatment.

Table 46: Left Ventricular Ejection Fraction, EC-FV-04 (Vintafolide/PLD Safety Population)

	Vintafolide+PLD Arm (N=107)	PLD Alone Arm (N=50)
Number of patients with Left Ventricular Ejection Fraction baseline measurement	105/107 (98.1%)	48/50 (96.0)
Number of patients who received a cumulative PLD dose of ≥ 275 mg/m^2	**26**	15
Number of patients who received a cumulative PLD dose of ≥ 275 mg/m^2 and had a follow-up Left Ventricular Ejection Fraction measurement	**25/26** (96.2%)	N/A*
Number of patients who received a cumulative PLD dose of ≥ 550 mg/m^2	**1**	2
Number of patients who received a cumulative PLD dose of ≥ 550 mg/m^2 and had a Left Ventricular Ejection Fraction baseline measurement and 2 follow-up measurements	**0/1** (0.0%)	0/2 (0.0%)

N = Number of patients.

*Follow-up LVEF not mandated per protocol. Although not mandated, 8/15 received a follow-up LVEF assessment

Eye Disorder adverse events

Further to optic nerve toxicity reported in dogs, an analysis of all treatment-emergent eye disorder adverse events across the vintafolide program was performed.

The incidence of visual acuity AEs of special interest was 1.15% (all causality) and 0.45% (drug-related) per month of vintafolide exposure. A greater incidence of visual acuity AEs was not seen with cumulative vintafolide exposures (i.e. few events after >3 months of chronic vintafolide exposure). Furthermore, all visual acuity AEs were low grade 1 or 2, did not result in dose modification or study drug discontinuation, and either resolved or did not progress.

Among the 451 patients assumed to have been exposed to vintafolide 2.5 mg three times a week every other week (all of the patients of study EC-FV-06 were included and therefore assumed to be exposed to vintafolide), there were 42 patients who experienced an AE in the Eye Disorder SOC as of 10 December 2013. Most (33/42, 78.6%) of these patients experienced the AE within 3 months from the start of treatment with vintafolide, with few patients experiencing an eye disorder AE after 6 months of vintafolide exposure (total of 5 patients).

Table 47: Number of patients with an AE in the Eye Disorder SOC (Regardless of Causality) by Preferred Term Studies EC-FV-01§, EC-FV-02, EC-FV-03, EC-FV-04, EC-FV-06*

	Any Time (N=451) n (%)		AE onset date within: [0-3 months] (N=451) n (%)		[3-6 months] (N=189) n (%)		[6-9 months] (N=89) n (%)		[9-12 months] (N=40) n (%)		>12 months (N=14) n (%)	
Subjects with one or more AEs	**42**	**(9.3)**	**33**	**(7.3)**	**7**	**(3.7)**	**1**	**(1.1)**	**3**	**(7.5)**	**1**	**(7.1)**
Amaurosis fugax	1	(0.2)	1	(0.2)	0	(0.0)	0	(0.0)	0	(0.0)	0	(0.0)
Blepharitis	1	(0.2)	0	(0.0)	1	(0.5)	0	(0.0)	0	(0.0)	0	(0.0)
Cataract	1	(0.2)	0	(0.0)	0	(0.0)	0	(0.0)	1	(2.5)	0	(0.0)
Conjunctival haemorrhage	1	(0.2)	1	(0.2)	0	(0.0)	0	(0.0)	0	(0.0)	0	(0.0)
Conjunctivitis	5	(1.1)	5	(1.1)	0	(0.0)	0	(0.0)	0	(0.0)	0	(0.0)
Corneal deposits	1	(0.2)	0	(0.0)	1	(0.5)	0	(0.0)	0	(0.0)	0	(0.0)
Dry eye	5	(1.1)	4	(0.9)	1	(0.5)	0	(0.0)	0	(0.0)	0	(0.0)
Eye irritation	1	(0.2)	1	(0.2)	0	(0.0)	0	(0.0)	0	(0.0)	0	(0.0)
Eye pain	1	(0.2)	1	(0.2)	0	(0.0)	0	(0.0)	0	(0.0)	0	(0.0)
Eye pruritus	7	(1.6)	7	(1.6)	1	(0.5)	0	(0.0)	0	(0.0)	0	(0.0)
Eye swelling	1	(0.2)	0	(0.0)	0	(0.0)	0	(0.0)	1	(2.5)	0	(0.0)
Eyelid oedema	2	(0.4)	1	(0.2)	1	(0.5)	0	(0.0)	0	(0.0)	0	(0.0)
Lacrimation increased	10	(2.2)	8	(1.8)	3	(1.6)	0	(0.0)	0	(0.0)	0	(0.0)
Ocular hyperaemia	2	(0.4)	1	(0.2)	1	(0.5)	0	(0.0)	0	(0.0)	0	(0.0)
Photopsia	1	(0.2)	1	(0.2)	0	(0.0)	0	(0.0)	0	(0.0)	0	(0.0)
Retinal haemorrhage	1	(0.2)	1	(0.2)	0	(0.0)	0	(0.0)	0	(0.0)	0	(0.0)
Vision blurred	10	(2.2)	7	(1.6)	1	(0.5)	1	(1.1)	1	(2.5)	0	(0.0)
Visual acuity reduced	2	(0.4)	1	(0.2)	0	(0.0)	0	(0.0)	0	(0.0)	1	(7.1)
Visual disturbance	1	(0.2)	1	(0.2)	0	(0.0)	0	(0.0)	0	(0.0)	0	(0.0)
Visual impairment	2	(0.4)	2	(0.4)	0	(0.0)	0	(0.0)	0	(0.0)	0	(0.0)

§ Only subjects who received vintafolide 2.5mg bolus
* EC-FV-06: all of the subjects are assumed to be exposed to vintafolide and all eye disorder AEs are considered.
N= Number of subjects exposed to vintafolide at beginning of interval
n= Number of subjects with AE onset in interval

In EC-FV-04 study, there were no cases of optic nerve disorder or blindness reported. For eye disorder AEs regardless of causality, out of a total of 11 patients with any eye disorder AEs, there

were 7 AEs of blurred vision, reduced visual acuity, and vision impairment (7/107, 6.5%), all Grade 1 or 2. Of these AEs, only 3 were thought to be related to drug (3/107, 2.8%).

Pooled Data from studies EC-FV-02 and EC-FV-03

Drug-related TEAEs (as judged by the investigator) were reported for 80 patients (87.0%). The most common (≥1/10) being constipation (39.1%), fatigue (37.0%), nausea (21.7%), anorexia (17.4%), neuropathy (12.0%), vomiting (12.0%) and abdominal pain (10.9%). There was limited bone marrow suppression activity, with 5.4% anemia (2.2% Grade 3 and no Grade 4) and neutropenia in < 5% of patients. Most of drug-related TEAEs were Grade 1 or 2, with few Grade 3 events reported. No Grade 4 or Grade 5 drug-related TEAEs were reported.

Adverse Events of special interest (EC-FV-04, EC-FV-02, and EC-FV-03)

These adverse events were selected because they are common or serious adverse events reported for other vinca-alkaloids.

Anaemia

Anaemia was the second most frequently reported adverse events in subjects receiving vintafolide+PLD, exceeded only by fatigue. The difference in incidence between the vintafolide+PLD treatment group and the PLD-alone treatment group was 11.8%. Anaemia was reported more frequently among subjects receiving combination therapy (vintafolide+PLD) than in patients receiving vintafolide monotherapy in protocols EC-FV-02 and EC-FV-03 which is expected due to the combination therapy. Among the 49 adverse events reported in the vintafolide+PLD treatment group, most were Grade 1 (9.3%) or Grade 2 (27.1%). Severe anaemia was less common, with 8.4% of vintafolide+PLD-treated patients reporting Grade 3 anaemia, and a single (0.9%) vintafolide+PLD-treated subject reporting Grade 4 anaemia.

Decreased White Blood Cells

Almost 50 % of the subjects in the combination arm experienced at least one TEAE whereof 25 % were of Grade 3 or 4 as compared to PLD alone (28.0% and 10 % respectively). Likewise, the incidence of neutropenia among the subjects showed similar proportions. Most events were considered to be drug related in both treatment groups. A low number of patients subject to febrile neutropenia were observed (one patient in each treatment arm).

Decreased Gastrointestinal Motility

Constipation was the most common adverse event of decreased gastrointestinal motility and was generally responsible for most of the overall incidence rates across all 3 Phase II studies and in both treatment arms of EC-FV-04. There were few Grade 3 events of constipation and no grade 4 events among patients receiving either vintafolide+PLD or vintafolide monotherapy. There were no Grade 3 or 4 events of constipation in the PLD Alone Arm of EC-FV- 04.

In study EC-FV-04, in patients being treated with Vynfinit + PLD compared to PLD alone, 32.7% versus 14.0% had constipation and 1.9% versus 2.0% had small intestinal obstruction.

There were four (3.7%) events of ileus reported in the vintafolide+PLD Arm and one (2.0%) event reported with vintafolide monotherapy in the EC-FV-02 study. There were no reports of ileus in neither the PLD Alone arm of EC-FV-04 or in the EC-FV-03 study.

Peripheral Neuropathy

Peripheral sensory neuropathy is a class effect of vinca alkaloids. Grades 3 and 4 peripheral sensory neuropathy were experienced by 3.7% patients treated with Vynfinit + PLD compared to 0% treated with PLD alone.

Most events were considered to be drug-related and persistent. There was one serious event each in EC-FV-04 and EC-FV-03 but no fatal cases were reported in any of the trials. The most common adverse event of neuropathy in EC-FV-04 was peripheral sensory neuropathy though the majority of events were grade 1 and grade 2. All events in the PLD group were Grade 1 or 2. In EC-FV-02, neuropathy was most common while neuropathy peripheral was more common in EC-FV-03. Few events occurred across studies in peripheral motor neuropathy, peripheral sensorimotor neuropathy, or polyneuropathy.

EC-FV-04 Serious adverse event / deaths / other significant events

Serious Adverse Events

More patients reported at least one serious TEAE regardless of causality (occurring in ≥2%) in vintafolide+PLD Arm (47.7%) compared with the PLD Alone Arm (34.0%). However, when taking into account the number of cycles, the percent of cycles with at least one serious TEAE was similar between the treatment arms (12.2% in the vintafolide+PLD arm vs. 11.4% in the PLD Alone arm). The incidence of these most common serious TEAEs was similar between the treatment arms.

In addition, more patients in the vintafolide+PLD Arm experienced drug-related serious TEAEs (20.6% vs. 12.0% in PLD Alone arm). The serious drug-related TEAEs occurring in more than one patient after administration of combination treatment were neutropenia (reported in four patients); anaemia, leukopenia, and abdominal pain (reported in three patients each); nausea, small intestinal obstruction, stomatitis, and PPE syndrome (reported in two patients each). Only one serious drug-related TEAE occurred in more than one patient after administration of PLD alone: infusion related reaction (reported in two patients).

Table 48: Drug-Related Serious TEAEs by Treatment Arm EC-FV-04 (EC145/PLD Safety Population)

System Organ Class Preferred Term	EC145+PLD Arm (N=107) n (%)	PLD Alone Arm (N=50) n (%)	P-Value (Fisher's Exact)[1]
Number of Patients Reporting at Least 1 Drug-Related Serious TEAE	22 (20.6)	6 (12.0)	0.264
Blood and lymphatic system disorders	7 (6.5)	1 (2.0)	0.437
Anaemia	3 (2.8)	0 (0.0)	
Febrile neutropenia	1 (0.9)	1 (2.0)	
Leukopenia	3 (2.8)	0 (0.0)	
Neutropenia	4 (3.7)	1 (2.0)	1.000
Gastrointestinal disorders	11 (10.3)	1 (2.0)	0.105
Abdominal pain	3 (2.8)	0 (0.0)	
Abdominal pain lower	1 (0.9)	0 (0.0)	
Aphthous stomatitis	1 (0.9)	0 (0.0)	
Constipation	1 (0.9)	0 (0.0)	
Diarrhoea	1 (0.9)	0 (0.0)	
Nausea	2 (1.9)	0 (0.0)	
Small intestinal obstruction	2 (1.9)	1 (2.0)	
Stomatitis	2 (1.9)	0 (0.0)	
Vomiting	1 (0.9)	0 (0.0)	
General disorders and administration site conditions	2 (1.9)	2 (4.0)	
Fatigue	1 (0.9)	0 (0.0)	
Infusion related reaction	0 (0.0)	2 (4.0)	
Infusion site extravasation	1 (0.9)	0 (0.0)	
Infections and infestations	0 (0.0)	1 (2.0)	
Gastroenteritis	0 (0.0)	1 (2.0)	
Metabolism and nutrition disorders	1 (0.9)	0 (0.0)	
Anorexia	1 (0.9)	0 (0.0)	
Musculoskeletal and connective tissue disorders	1 (0.9)	0 (0.0)	
Back pain	1 (0.9)	0 (0.0)	
Nervous system disorders	1 (0.9)	0 (0.0)	
Peripheral sensory neuropathy	1 (0.9)	0 (0.0)	
Renal and urinary disorders	1 (0.9)	0 (0.0)	
Haematuria	1 (0.9)	0 (0.0)	
Respiratory, thoracic and mediastinal disorders	0 (0.0)	1 (2.0)	
Pulmonary embolism	0 (0.0)	1 (2.0)	
Skin and subcutaneous tissue disorders	2 (1.9)	0 (0.0)	
Palmar-plantar erythrodysaesthesia syndrome	2 (1.9)	0 (0.0)	

[1] *P*-value presented if at least 5 events occurred.

NOTES:

Treatment-Emergent Adverse Events are adverse events starting after administration of EC145 or PLD and within 30 days of the last dose of EC145 or PLD, unless otherwise indicated.

Drug-related adverse events include those with a definite, probable, or possible drug-relationship.

Patients are counted once for each system organ class and for each preferred term.

Adverse events were coded in accordance with Medical Dictionary for Regulatory Activities V11.1.

Source: EC-FV-04 CSR Table 14.6.5.4

Deaths

Deaths were defined as those occurring within 30 days after the last dose or before subsequent therapy, whichever was earlier.

Table 49: Deaths during the Study (EC145/PLD Safety Population)

	EC145+PLD (n=107) n (%)	PLD Alone (n=50) n (%)
Total Number of Patients Who Died During Treatment	8 (7.5%)	2 (4.0%)
Progressive Disease	5 (4.7%)	1 (2.0%)
Adverse Events	3 (2.8%)	0 (0.0%)
Drug-Related	0 (0.0%)	0 (0.0%)
Not Drug-Related	3 (2.8%)	0 (0.0%)
Other	0 (0.0%)	0 (0.0%)
Unknown [1]	0 (0.0%)	1 (2.0%)

[1] Patient 500-405 experienced an AE of sudden death (not drug-related); however, no autopsy or further information was available to determine the primary reason of death so the reason was listed as "unknown."

NOTES: Percentages are based on the number of patients in the treatment groups. Death during treatment, within 30 days after last dose or before subsequent therapy whichever is earlier. Drug-related adverse events include those with a definite, probable, or possible drug-relationship.

Table 50: Listing of Deaths during the Study (Within 30 Days of Last Dose)

Patient	Cause of Death	AE Relationship to Study Drug
EC145+PLD Arm		
002-128	AEs: rhabdomyolysis; hyperkalaemia	Definitely not related; definitely not
002-245	Disease progression	Not applicable
017-222	AE: pulmonary embolism	Probably not related
160-619	Disease progression	Not applicable
161-112	AE: respiratory failure	Definitely not related
300-130	Disease progression	Not applicable
400-235	Disease progression	Not applicable
505-406	Disease progression	Not applicable
PLD Alone Arm		
180-111	Disease progression	Not applicable
500-405	Unknown	Not applicable

Source: EC-FV-04 CSR Listing 16.2.6.2

Laboratory findings

Hematologic assessments

The majority of the hematologic toxicities were Grade 1 and 2 in both treatment arms. An increase in toxicity including Grade 3/4 with respect to white blood cells were observed in the combination arm while an increase in toxicity regarding platelets (though few Grade 3 or 4) were observed in the PLD arm. Haemoglobin toxicities in the vintafolide+PLD treatment arm as compared with the PLD

alone arm were 83.5% versus 78.3% with similar rates of grade 3/4. The incidence of absolute neutrophil count toxicities overall and grade 3/4 was higher in the vintafolide+PLD Arm.

Clinical chemistry assessments

Table 51: Results from the clinical chemistry analysis (for all grade events)

Laboratory Test Name	EC145 + PLD Arm (N=104*) All Grades n (%)	PLD Alone (N=46*) All Grades n (%)
Albumin (low)	10 (9.6)	3 (6.5)
Alkaline phosphatase	22 (21.2)	16 (34.8)
ALT	17 (16.3)	5 (10.9)
AST	22 (21.2)	4 (8.7)
Bicarbonate	26 (25.0)	9 (19.6)
Bilirubin, total	2 (1.9)	0 (0.0)
Calcium	29 (27.9)	8 (17.4)
Creatinine	22 (21.2)	16 (34.8)
GGTP	52 (50.0)	21 (45.7)
Glucose	67 (64.4)	30 (65.2)
Potassium	20 (19.2)	11 (23.9)
Sodium	16 (15.4)	10 (21.7)
NOTES: Lab values starting after administration of EC145 or PLD and within 30 days of the last dose of EC145 or PLD. Central laboratory data only for Albumin, Alkaline Phosphatase, ALT, AST, Bicarbonate, Total Bilirubin, Calcium, Creatinine, GGTP, Glucose. * 4 patients did not have post baseline labs		

Safety in special populations

Age

The age group of < 65 years comprised 72 subjects while the age group of ≥ 65 years consisted of 35 subjects.

Table 52: Overall summary of TEAEs by age category, Vintafolide/PLD Safety Population; Vintafolide+PLD arm

	Age <65 yrs (N=72)	65-74 yrs (N=24)	75-84 yrs (N=10)	85 + (N=1)
Total (at least one AE)	71 (98.6)	24 (100.0)	10 (100.0)	1 (100.0)
Fatal	3 (4.2)	0 (0.0)	0 (0.0)	0 (0.0)
Serious	31 (43.1)	11 (45.8)	8 (80.0)	1 (100.0)
Withdrawal of vintafolide	4 (5.6)	2 (8.3)	2 (20.0)	0 (0.0)

Withdrawal of PLD	8 (11.1)	4 (16.7)	3 (30.0)	0 (0.0)
CNS (confusion/extrapyramidal)	2 (2.8)	2 (8.3)	2 (20.0)	1 (100.0)
AE related to falling	3 (4.2)	0 (0.0)	2 (20.0)	1 (100.0)
CV events	8 (11.1)	2 (8.3)	1 (10.0)	1 (100.0)
Cerebrovascular events	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Infections	35 (48.6)	13 (54.2)	5 (50.0)	1 (100.0)

The number of patients reporting at least one TEAE in each treatment arm was similar for patients ≥65 years of age compared with patients <65 years of age. However, in the PLD Alone Arm, patients ≥65 years of age experienced a slightly higher incidence of TEAEs (90.5%) compared with patients <65 years of age (82.8%), with anaemia, neutropenia and peripheral sensory neuropathy as the most common events. For patients <65 years of age the incidences of drug-related neutropenia and drug-related peripheral sensory neuropathy were statistically significantly higher in the vintafolide+PLD Arm vs. the PLD alone arm.

For patients <65 years of age, the incidence of drug-related Grade 3 or 4 TEAEs was 50.0% in the vintafolide+PLD Arm vs. 31.0% in the PLD Alone Arm and for patients ≥65 years of age, the corresponding incidence was 57.1% in the vintafolide+PLD Arm and 42.9% in the PLD Alone Arm.

Overdose

Single intravenous doses of up to 2.5 mg have been tolerated. Doses of 3 mg (1 hour infusion) and 4 mg (bolus) resulted in severe constipation and ileus occurring within 12 hours of dose administration. The gastrointestinal toxicity was spontaneously reversible. Higher dose have not been tested.

Discontinuation due to adverse events

Patients who experienced TEAEs that resulted in withdrawal of vintafolide or PLD (i.e. PLD in the comparator arm) were discontinued from the study while patients in the vintafolide + PLD arm who experienced AEs resulting in withdrawal of PLD only, were allowed to continue in the study.

Overall, in the vintafolide+PLD arm, at least one TEAE resulting in withdrawal of PLD was reported in 15 (14.0%) subjects, with PPE syndrome and skin exfoliation (6.5%) as the most common events. At least one TEAE resulting in withdrawal of vintafolide was reported in eight (7.5%) subjects, with peripheral sensory and sensorimotor neuropathy (2.8%) and stomatitis (1.9%) as the most common events. In the PLD Alone Arm, the only TEAE resulting in withdrawal of PLD was infusion related reaction reported in 2 patients (4%) and were considered drug-related.

As regards drug related TEAEs resulting in withdrawal of PLD, thirteen subjects (12.1%) in the vintafolide+PLD Arm reported one or more of the following: PPE syndrome, neutropenia, stomatitis, peripheral sensory neuropathy, anaemia, fatigue, peripheral sensorimotor neuropathy and skin exfoliation. Six subjects (5.6%) in the vintafolide+PLD Arm reported one or more of the following drug-related TEAEs resulting in withdrawal of vintafolide: stomatitis, peripheral sensory neuropathy, anaemia, neutropenia, fatigue, peripheral sensorimotor neuropathy and oropharyngeal pain.

Dose delays

More patients reported at least one drug-related TEAE resulting in delay of vintafolide (54.2%) compared with the percentages of subjects reporting a drug-related TEAE resulting in PLD delay in

either treatment arm (39.3% vintafolide+PLD, 32.0% PLD Alone arm). The most common drug-related TEAE resulting in delay of vintafolide or PLD (occurring in ≥ 20% of patients) was neutropenia (33.6% vintafolide delay, 20.6% PLD delay in vintafolide+PLD arm, and 18.0% PLD delay in PLD Alone arm).

Dose reductions

The incidence of drug-related TEAEs resulting in reduction of vintafolide or PLD was similar to that observed regardless of causality thus indicating that most of these TEAEs were considered drug-related. A reduction of vintafolide was reported in only 2 subjects (1.9%). As expected, the two most frequent AEs resulting in reduction of PLD in either treatment arms were skin and subcutaneous disorders, and stomatitis.

2.5.1. Discussion on clinical safety

The toxicity profile of vinca-alkaloids encompasses primarily myelosuppression (with neutropenia as the principal dose-limiting toxicity), peripheral neurotoxicity (mainly characterized by a peripheral, symmetric mixed sensory-motor, and autonomic polyneuropathy) and gastrointestinal autonomic dysfunction (as manifested by bloating, constipation, ileus, and abdominal pain). Mucositis occurs frequently while nausea, vomiting, and diarrhoea occur to a lesser extent. For PLD it is mainly palmar-plantar erythrodysesthesia (PPE), stomatitis/mucositis, nausea and myelosuppression though sepsis related to neutropenia is rare.

The core safety data comprised 281 subjects derived from three completed studies: EC-FV-04 (pivotal), EC-FV-02 and EC-FV-03 (both supportive) and a summary of safety data from one Phase 1 dose escalation study. Of the safety population, only about 40 % had received vintafolide and PLD which is the proposed treatment combination in this application (107 patients in the pivotal study). Moreover, the proposed indication is vintafolide in combination with PLD for the treatment of patients with platinum resistant ovarian cancer who express the folate receptor (FR) on all target lesions i.e. 100 %. This subset consisted of only 22 subjects in the pivotal study hence the exposure provides only modest experience for safety assessment of a new chemical entity in combination with a known cytotoxic agent.

Comparisons between extent of exposure in relation to FR status with respect to the PLD Alone arm indicates that the FR (0 %) subjects were able to receive more treatment as compared to the FR (100 %) subpopulation. This may reflect the more aggressive course of disease in FR (100 %) positive tumours. Overall dose intensity was highest for PLD compared with vintafolide.

TEAEs were frequently reported and with a similarity between the two treatment arms although when duration of treatment was taken into account (518 cycles in vintafolide+PLD arm vs. 202 in the PLD arm), the incidence of TEAEs was lower.

The frequency of drug related TEAE reports were high in both treatment arms. As expected, more drug-related TEAEs occurred in general in the combination arm with an emphasis on haematological, neurological and gastrointestinal toxicity.

Furthermore, more patients reported serious TEAEs and were hospitalized during treatment in vintafolide+PLD arm than in the PLD alone arm. These differences may, however, reflect the longer

duration of treatment with the combination therapy as the differences diminished when the number of cycles was taken into account.

The incidence of TEAEs and drug-related adverse events of PPE and stomatitis which are associated with PLD treatment were reported in similar frequency between treatment arms though the incidence of drug-related Grade 3 or 4 was numerically higher in the vintafolide+PLD arm.

In the PLD alone arm hospitalisation, serious TEAEs and Grade 3 or 4 TEAEs by cycle occurred mainly during the first courses. It is recognised that a higher incidence of adverse reactions during the initial courses of a chemotherapy regimen is what is usually observed in clinical practice. For completeness the Applicant provided an account of the TEAES reported during the first cycles of treatment (cycle 1 through cycle 3) which did not reveal anything unexpected and were mainly consistent with the overall safety findings (data not shown).

The adverse events most frequently occurring in the two supportive studies were mainly in line with the safety profile in the pivotal study.

A total of ten deaths occurred within 30 days of last dose (8 (7.5%) in the vintafolide+PLD arm vs. 2 (4.0%) in the PLD Alone arm) mainly due to disease progression. None were considered drug-related. Two deaths in the combination arm were considered as caused by recurrence and progression of deep vein thrombosis which both had in their medical history prior to study enrolment. The increased risk of thrombotic events in relation to cancer as well as in conjunction with chemotherapy treatment is well known. However, due to the limited number of events it is not possible to draw any conclusions as to the implications on a potential thrombogenicity by vintafolide compared to vinca-alkaloids conventionally used. The Incidences of adverse reactions pertaining to thrombo-embolic events were found to be roughly equivalent between the two arms (data not shown). Given that the subjects in the vintafolide/PLD arm received more treatment cycles compared to the control arm and the similarity in events between the two arms as regards vascular disorders, it is concluded that the risk of thrombotic events may not be increased by the combination of vintafolide and PLD.

With regards to laboratory findings, ALT and AST all grade events were higher in the combination arm. The majority of AST/ALT laboratory findings, however, were considered to be clinically insignificant, with a relatively short duration. No AST/ALT changes led to study discontinuation or dose modification. Moreover, preclinical investigations did not demonstrate evidence of hepatotoxicity in animal model species.

All grade events were similar for potassium abnormalities in both arms (19.2% vs. 23.9%). However, in the PLD arm, these events were all grade 1 but an increased incidence of Grade 3/4 potassium abnormalities were seen in the vintafolide+PLD combination arm. The majority of the abnormalities, however, were not considered clinically significant and there were no treatment emergent adverse events that led to study drug discontinuation or dose modification.

In terms of discontinuation due to adverse events, the rate may imply that the combination of Vintafolide+PLD is moderately tolerable. The observed difference in dose delays may be interpreted in the context that vintafolide was administered six times per cycle as compared to PLD being administered only once per cycle.

Dose adjustments should be considered for haematologic toxicities, palmar-plantar erythrodysaesthesia (PPE)/hand-foot syndrome (HFS), stomatitis, hyperbilirubinaemia, and other toxicities (see section 4.2 of the SmPC, Posology and method of administration).

In terms of cardiac function, all patients were expected to have assessment of LVEF at baseline. At the end of treatment, observed values were based on just ten subjects in the combination arm and six subjects in the PLD arm. Overall, none of the LVEF assessments performed in the pivotal study is suggestive of a detrimental effect by vintafolide. The cardiac AEs reported in the pivotal study were of low grade and did not lead to dose modification or study drug discontinuation.

Although ECG monitoring was not required in the protocol, ECG information is currently being collected in the ongoing Phase 3 study (EC-FV-06). No QTc study has been carried out but a formal QTc assessment is being performed in the Phase I study PN001. In addition, potential risk of QTc prolongation is included in the RMP. To date, ECGs have been collected from six subjects in study PN001 without indications of any evidence of QTc prolongation. No other evidence of cardiac toxicity has been detected thus far. From a non-clinical perspective, vintafolide administered in clinically applicable doses did not inhibit hERG conductivity and vintafolide did not prolong the QT interval at any dose or schedule in dogs. Overall, the lack of a signal for cardiotoxicity in clinical studies is supported by the targeted nature of vintafolide and that normal heart tissue is considered to be a folate receptor negative organ.

As adverse events of special interest, anaemia, decrease in white blood cells, decrease in gastrointestinal motility and peripheral neuropathy were selected due to their association with the toxicity profile of vinca-alkaloids.

As expected, the incidence of drug related TEAEs of anaemia and neutropenia were higher in the vintafolide+PLD arm as compared with PLD Alone. Grade 3 or 4 neutropenia was reported in 23.4 % in the vintafolide+PLD arm versus 10.0% in the PLD Alone arm. Febrile neutropenia, however, were few (<1 %). The incidence of thrombocytopenia was similar between the treatment groups. The results are clearly suggestive of the potentiating effect on bone marrow suppression by the combination of vintafolide and PLD and beyond what is associated with PLD or vintafolide alone (since there were limited events of decreased white blood cells reported among patients receiving monotherapy vintafolide in ECFV- 02 (4.1%) and none in EC-FV-03).

The incidence and severity of TEAEs related to a decrease in GI motility were in general similar across the treatment arms. An increase in drug-related constipation was reported and furthermore, ileus regardless of causality were reported in about 4 % of the subjects in the vintafolide+PLD arm while none occurred in the PLD alone arm. To help decrease the occurrence of constipation, patients should start a bowel regimen that consists of increased fluid intake, and the addition of a fibre supplement prior to administration. Other measures should be instituted as necessary.

The incidence of drug related peripheral neuropathy (including Grade 3 or 4) was substantially higher in the vintafolide+PLD arm as compared to the PLD Alone arm (no Grade 3 or 4 event was reported in the PLD arm). Events of neuropathy are commonly associated with vinca alkaloids due to their mechanism of action. Thus, it is not unexpected that neuropathy adverse events were primarily seen among patients in the vintafolide+PLD and vintafolide monotherapy treatment groups. Patients should be followed for signs of neuropathy and treatment with Vynfinit should be discontinued in case of clinically relevant neuropathy.

During the evaluation, non-clinical findings pertaining to the optic nerve damage in dogs were reported (see non-clinical section) and the applicant was requested to assess whether this finding translates into any clinical safety concern and provided a review of the available clinical safety data. The applicant concluded that most of the AEs occurred within three months from treatment initiation which may be the case and even probable although difficult to ascertain at this point since there were few patients remaining on treatment at later time-points.

The findings as regards specific AEs within the eye disorder SOC and their incidences in the EC-FV-06 study were consistent with the overall findings from all studies mainly conjunctivitis, lacrimation increased, vision blurred, dry eye, eye pruritus. No events of particular concern were identified in the full study population of the study.

The incidence of visual acuity AEs of special interest was low and no greater incidence of visual acuity AEs was observed with cumulative vintafolide exposures although the number of subjects still on treatment beyond 3 months is deemed limited. It was recognised that visual acuity AEs were low grade 1 or 2 and did not result in dose modification or study drug discontinuation.

As regards the likelihood and biological rationale for off target effects in the vital systems of man, the applicant provided a discussion on some of the safety issues surrounding the use of vintafolide. It is agreed that the targeted nature of vintafolide allows for uptake of the drug into FR expressing tissues, but does not eliminate the potential for off-target effects related to the cytotoxic moiety. It is possible for non-specific exposure to occur via free DAVLBH, or through other non-specific means of interaction of vintafolide with normal cells. Adverse events occurring in tissues that are considered negative for FR expression such as peripheral neuropathy, haematologic toxicities or constipation, are the result of either passive diffusion, or uptake through an alternate mechanism of transport. Thus, the view of the Applicant is that the observed axonal degeneration in the optic nerve is likely to be the result of a non-specific mechanism.

Overall, the CHMP considered that the non-clinical concern with respect to optic nerve toxicity as observed in the dog study appears not to be translated into any clinical major safety concern. Optic nerve abnormalities are addressed in the RMP. In addition, it is recommended that all patients should have visual acuity and ophthalmological history documented prior to vintafolide administration and ophthalmological evaluation should be considered if vision disorder develops or worsens in severity.

As regards a potential impact in relation to tumour expression of FR or lack thereof, the safety profile for the FR (100%) and FR (0%) subpopulations was in general similar to that of the overall safety population. However, it appears that the FR (0 %) population experienced more drug-related TEAE resulting in treatment withdrawal and serious TEAE compared to both mITT- and FR (100 %) populations. In addition, more drug-related TEAE of Grade 3 or 4 and drug-related serious TEAE of Grade 3 or 4 were reported compared to the FR (100 %) population. Though recognising the very limited numbers of subjects in each of the FR status subpopulations, this may be indicative of a higher drug exposure leading to an increased risk of toxicity in subjects with FR- negative tumours. However, treatment with vintafolide is relevant to FR (100%) patients only as per the proposed indication. Assessment of folate receptor (FR) status using diagnostic medicinal products approved for the selection of adult patients for treatment with vintafolide, such as ^{99m}Tc-etarfolatide and folic acid, must be performed within 28 days prior to Vynfinit + PLD therapy. In addition, vintafolide is

contraindicated in patients assessed as folate receptor negative (FR[0%]) by the imaging procedure.

For patients ≥ 65 years of age compared to patients <65 years of age, the percentage of patients reporting at least one drug-related TEAE was similar in the vintafolide+PLD arm. In the PLD arm, patients ≥ 65 years of age experienced a slightly higher incidence of TEAEs compared with patients <65 years of age, with anaemia, neutropenia and peripheral sensory neuropathy as the most common events. However, since the elderly age group only consisted of 35 subjects, it is difficult to draw any firm conclusions as to the toxicity profile.

There are no data with the use of vintafolide in pregnant women. Any formal reproductive toxicity studies have not been conducted with vintafolide in animals. However, multiple-dose toxicology studies with vintafolide in animals have shown male reproductive toxicity. Vintafolide is not recommended during pregnancy and in women of childbearing potential not using contraception .

It is not known whether vintafolide is excreted in human milk. Therefore a risk to the newborns/infants cannot be excluded. It is recommended that breast-feeding should be discontinued during treatment with vintafolide. A decision must be made whether to discontinue breast-feeding or to discontinue/abstain from vintafolide therapy taking into account the benefit of breast-feeding for the child and the benefit of therapy for the woman.

There are limited data on the effects of vintafolide overdose. In the event of an overdose patients should be closely monitored and symptomatic supportive care measures instituted as required.

Vintafolide is for intravenous use only and should not be given by intrathecal, intramuscular or subcutaneous injection. The risk of medication error is addressed in the RMP.

As vintafolide is a member of the vinca alkaloid class, it may cause a severe local reaction in case of extravasation. If leakage into the surrounding tissue occurs, the injection should be discontinued immediately and any remaining portion of the dose should be introduced into another vein. For other vinca alkaloids, local injection of hyaluronidase with the application of heat has been used to disperse the medicinal product to minimise the discomfort and the possibility of tissue damage. However, this approach has not been studied for vintafolide.

From the safety database all the adverse reactions reported in clinical trials have been included in the Summary of Product Characteristics.

Additional safety data needed in the context of a conditional MA

Considering the size safety database is modest, the safety profile of vintafolide need to be further characterised. It is expected that the phase III study EC-FV-06 to be completed as a specific obligation to better characterise the efficacy will also provide further information on the safety of vintafolide in combination with PLD.

2.5.2. Conclusions on the clinical safety

Overall, as expected more TEAEs were reported in the vintafolide+PLD arm compared to PLD alone. The treatment combination presents a safety profile that mainly reflects the known toxicities associated with PLD and the vinca alkaloid class of agents with a higher degree of suppression of the bone marrow, constipation, neurotoxicity and PPE. The lack of a signal for cardiotoxicity in clinical

studies is supported by the targeted nature of vintafolide and that normal heart tissue is considered to be a folate receptor negative organ.

As the drug-related discontinuation rate was low and no drug-related deaths were reported, it is considered from a safety perspective, that vintafolide in combination with PLD is moderately tolerable and that the adverse reactions are manageable.

The CHMP considers the following measures necessary to address the missing safety data in the context of a conditional MA:

- Submit clinical safety results from study EC-FV-06, a randomised double-blind phase 3 trial comparing vintafolide in combination with PLD versus PLD + placebo in patients with platinum-resistant ovarian cancer who express the folate receptor on all target lesions as assessed by the ^{99m}Tc-etarfolatide imaging procedure
    - Final clinical study report: March 2017

The benefit to public health of the immediate availability on the market of vintafolide outweighs the risk implicit in the fact that additional data are still required.

2.6. Pharmacovigilance

Detailed description of the pharmacovigilance system

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

2.7. Risk Management Plan

The CHMP received the following PRAC Advice on the submitted Risk Management Plan:

PRAC advice

Based on the PRAC review of the Risk Management Plan version 2.2, the PRAC considers by consensus that the risk management system for vintafolide (Vynfinit) in the following indication, *"Vynfinit in combination with pegylated liposomal doxorubicin (PLD) is indicated for the treatment of adult patients with platinum resistant ovarian cancer (PROC) who express the folate receptor (FR) on all target lesions. Folate receptor status should be assessed by a diagnostic medicinal product approved for the selection of adult patients for treatment with vintafolide, using single photon emission computed tomography (SPECT) imaging, in combination with Computed Tomography (CT) or Magnetic Resonance Imaging (MRI)."* is acceptable.

The CHMP endorsed this advice with slight changes to add further details on the agreed RMP measures.

The MAH implemented the changes requested in the RMP by CHMP. The CHMP endorsed the changes to the Risk Management Plan (version 2.4) with the following content.

- **Safety concerns**

Table 53: Summary of the Safety Concerns

Important identified risks	Anaemia White blood cells decreased Peripheral neuropathy
Important potential risks	Drug interaction(s) with antifolate therapies Drug interaction(s) with folic acid supplements Medication error: Intrathecal administration Testicular toxicity* Optic nerve abnormalities
Missing information	Use in pediatric patients Use in pregnant and lactating women Use in patients with renal impairment Use in patients with hepatic impairment Use in patients with cardiac impairment QTc prolongation and tachyarrhythmias Use in the elderly (≥ 65 years of age) Pharmacokinetic missing information to include: - Main elimination pathways of vintafolide and its main metabolites including the active metabolite DAVLBH (including identification of main metabolizing enzymes and drug transporters) - Influence of doxorubicin on the PK of vintafolide - The potential risk for drug-drug interactions with enzyme or transporter inhibitors

*Testicular toxicity is not applicable for the proposed indication, as the target population is women with ovarian cancer. However, testicular toxicity is a well-known class effect of vinca alkaloid agents and has an antimitotic mechanism of action in human and preclinical species.

- **Pharmacovigilance plans**

Table 54: Ongoing and planned studies in the Post-authorisation PhV development plan

Study/activity Type, title and category (1-3)	Objectives	Safety concerns addressed	Status (planned, started)	Date for submission of interim or final reports (planned or actual)
Category 3: Protocol Number EC-FV06 Protocol Title: A Randomized Double-Blind Phase 3 Trial Comparing Vintafolide (vintafolide) and Pegylated Liposomal Doxorubicin	Primary Objective: Compare progression-free survival (PFS), based upon investigator assessment using RECIST v 1.1 in the FR (100%) patient population who receive combination therapy with vintafolide and pegylated liposomal doxorubicin (PLD) (i.e., vintafolide + PLD) with that of participants with	Potential for optic nerve abnormalities Potential for vintafolide use in patients with renal impairment Potential for vintafolide use in patients with	Study is ongoing	PFS Study Report: 4Q2015 Final Study Report (including OS data): 1Q2017

(PLD/Doxil/Caelyx) in Combination Versus PLD in Participants With Platinum-Resistant Ovarian Cancer Activities: The following additional actions will be undertaken in EC-FV06 to address specific safety concerns as indicated below: 1. Report the results of the planned population PK analysis of Phase III data, in particular, the effect of mild renal impairment on vintafolide PK, and the impact of body weight on vintafolide PK; 2. Report the results of planned interaction study with doxorubicin based on PK data from the Phase III study.	platinum resistant ovarian cancer who receive PLD and placebo. Secondary Objectives: 1. Compare overall survival (OS), the single secondary endpoint of the phase 3 study, between treatment arms in the FR (100%) population. 2. Compare PFS and OS between treatment arms for other populations based on percentage of target lesions that are etarfolatide (FR) positive. A hierarchical stepdown analysis will be conducted in a nested fashion to determine if there is a lower FR threshold that maintains statistical significance. Additionally, analyses of individual and mutually exclusive subgroups defined by FR levels will be conducted. Safety Objective: Evaluate the safety and tolerability of vintafolide in combination with PLD.	hepatic impairment Missing information: Pharmacokinetic information		
Category 3: Protocol Number PN001 Protocol Title: A Phase I Dose Escalation Study Evaluating Vintafolide (MK-8109) Chemotherapy Alone or in Combination in Adult Subjects with Advanced Cancers The following additional actions will be undertaken in PN001 to address specific safety	Primary Objectives: 1. Determine the safety and tolerability of vintafolide when coadministered with additional chemotherapies in subjects with advanced cancers; 2. Establish a maximum tolerated dose (MTD) of vintafolide when coadministered with additional chemotherapies; 3. Establish a maximum single tolerated dose of vintafolide when	Missing information: QTc prolongation and tachyarrhythmias Missing information: Pharmacokinetic information	Study is ongoing	QTc Report: 4Q2015 Final Study Report: 3Q2016

concerns as indicated below: 1. Evaluation of the effect of a single intravenous dose of vintafolide on the QTc interval will be performed; 2. Metabolite profiling in plasma and excreta (if possible both urine and faeces), as well as quantification of vintafolide and DAVLBH in plasma and urine, will be performed; 3. Based on the results from PN001, as appropriate, the following will be done: a. Identification of relevant metabolizing enzymes b. Identification of drug transporters. Provide data on whether DAVLBH is a substrate for Pgp. Clarification on the role of folate receptor in drug uptake into the liver.	administered as monotherapy; 4. Evaluate the effect of a maximum single tolerated dose of vintafolide on the QTc interval; 5. Determine the safety and tolerability of vintafolide administered on a weekly schedule in subjects with advanced cancers; and 6. Establish a maximum tolerated dose (MTD) of vintafolide when administered on a weekly schedule. Secondary Objectives 1. Provide a preliminary evaluation of response to vintafolide incombination with other chemotherapy regimens or vintafolide, alone, using RECIST 1.1 criteria. Analyses will be performed on a by-subject and by-target-lesion basis using both categorical and continuous measures of response; 2. Assess the pharmacokinetic profile of vintafolide in combination with chemotherapy regimens in subjects with solid tumors; 3. Assess the pharmacokinetic profile of the vinca alkaloid desacetylvinblastine hydrazide (DAVLBH) after treatment with vintafolide in combination with chemotherapy regimens in subjects with solid tumors; 4. Assess the pharmacokinetic profile and urinary excretion of maximum single tolerated dose of vintafolide following IV infusion in subjects with solid tumors;			

	5. Assess the pharmacokinetic profile and urinary excretion of the DAVLBH moiety of vintafolide after single dose vintafolide in subjects with solid tumors; 6. Identify metabolites of vintafolide in plasma and urine; and 7. Determine duration of response in subjects with advanced cancers treated with weekly vintafolide.			
In Vitro Metabolism Studies Relevant metabolizing enzymes and drug transporters will be identified. Based on these results, the need for additional PK and/or DDI studies will be discussed. Data on whether DAVLBH is a substrate for Pgp, and clarification on the role of folate receptor in drug uptake into the liver, will also be provided.	Identification of relevant metabolizing enzymes and drug transporters	Missing information: Pharmacokinetic information	Ongoing	1Q2015

- **Risk minimisation measures**

Table 55: Summary Table of Safety Concerns and Risk Minimisation Measures

Safety Concern	Routine Risk Minimization Measures	Additional Risk Minimization Measures
Important Identified Risks		
Anaemia	SmPC: Section 4.2 Posology and method of administration Section 4.8. Undesirable effects; Section 5.3. Preclinical safety data Package leaflet: Section 4, Possible side effects	None
White blood cells decreased	SmPC:	None

Safety Concern	Routine Risk Minimization Measures	Additional Risk Minimization Measures
	Section 4.2 Posology and method of administration; Section 4.8. Undesirable effects; Section 5.3. Preclinical safety data Package leaflet: Section 4, Possible side effects	
Peripheral neuropathy	SmPC: Section 4.8. Undesirable effects Package leaflet: Section 4, Possible side effects	None
Important Potential Risks		
Drug interaction(s) with antifolate therapies	SmPC: Section 4.2. Posology and method of administration; Section 4.5 Interaction with other medicinal products and other forms of interaction Package leaflet: Section 2, What you need to know before you are given Vynfinit	None
Drug interaction(s) with folic acid supplements	SmPC: Section 4.2 Posology and method of administration; Section 4.5 Interaction with other medicinal products and other forms of interaction Package leaflet: Section 2, What you need to know before you are given Vynfinit	None
Medication error: Intrathecal administration	SmPC: Section 4.2 Posology and method of administration; Section 4.4 Special warnings and precautions for use Package leaflet: Section 2, What you need to know before you are given Vynfinit Text on the outer packaging: Section 5. Method and route of administration Text on small immediate packaging units: Section 2. Method of administration	None

Safety Concern	Routine Risk Minimization Measures	Additional Risk Minimization Measures
Testicular toxicity	SmPC: Section 4.6 Fertility, pregnancy and lactation; Section 5.3 Preclinical safety data	None
Optic nerve abnormalities	SmPC: Section 4.4 Special Warning and precautions Section 4.8 Undesirable Effects; Section 5.3. Preclinical safety data Package leaflet: Section 2, What you need to know before you are given Vynfinit Section 4. Possible side effects	None
Missing Information		
Use in paediatric patients	SmPC: Section 4.2 Posology and method of administration Section 5.1 Package leaflet: Section 2 What you need to know before you are given Vynfinit	None
Use in pregnant and lactating women	SmPC: Section 4.6 Fertility, pregnancy and lactation Package leaflet: Section 2 What you need to know before you are given Vynfinit	None
Use in patients with renal impairment	SmPC: Section 4.2 Posology and method of administration	None
Use in patients with hepatic impairment	SmPC: Section 4.2 Posology and method of administration	None
Use in patients with cardiac impairment	Package leaflet: Section 4. Reporting of side effects	None
QTc prolongation and tachyarrhythmias	Package leaflet: Section 4. Reporting of side effects	None
Use in the elderly (≥ 65 years of age)	SmPC: Section 4.2 Posology and method of administration; Section 5.2 Pharmacokinetic properties	None
Missing Pharmacokinetic	SmPC:	None

Safety Concern	Routine Risk Minimization Measures	Additional Risk Minimization Measures
information including • Main elimination pathways of vintafolide and its main metabolites including the active metabolite DAVLBH (including identification of main metabolizing enzymes and drug transporters) • Influence of doxorubicin on the PK of vintafolide • The potential risk for drug-drug interactions with enzyme or transporter inhibitors	Section 4.5 Interaction with other medicinal products and other forms of interaction	

The PRAC considered that the proposed risk minimisation measures are sufficient to minimise the risks of the product in the proposed indication.

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

The folate receptor (FR), the target for vintafolide, is known to be over-expressed in many tumours, among them platinum resistant ovarian cancer, and FR expression is considered to be prognostic for poor outcome. In order to identify patients for treatment, a companion diagnostic, ^{99m}Tc-etarfolatide was co-developed. It is accepted based on the exploratory studies conducted prior to the pivotal study that the likelihood of tumour response to vintafolide in tumours not expressing the FR is too low to be of clinical relevance, a notion corroborated by the findings in the pivotal study for this application.

The pivotal study for this application, study EC-FV-04, was an open-label phase 2 add-on study to PLD with 2:1 randomisation (mITT, n= 100 + 49) in patients with PROC. In this study, each patient was assigned an FR score ranging from 0% to 100% based on the percentage of target lesions that were FR-positive. Since the mITT population also included patients with all tumour lesions being FR-negative, the efficacy results in the mITT population are thus highly likely to be underestimated.

Based on investigators' assessment, the PFS HR was 0.626 (95%CI: 0.409-0.959) in the mITT population and 0.381 (95%CI: 0.172, 0.845) in the FR(100%) population where FR(100%) denotes

that all tumour lesions are FR positive. These results were statistically significant in both the mITT population (p=0.031) and the FR(100%) (p=0.01) populations. mITT results were robust in a wide variety of sensitivity analyses (HR 0.57-0.63, p-value 0.01-0.03) and there were no signs of bias, e.g. based on differences in scheduled versus non-scheduled tumour assessments.

The median PFS benefit in the mITT population was about 2½ months, whilst in the target population for this application, FR(100%), the median benefit was about 4 months.

A retrospective IRC assessment was undertaken and was statistically significant (borderline, p=0.05) only in the FR(100%) subgroup. Based on conventional analyses such as early and late discrepancy rates and agreement rates for PFS by study arm, there were no signs of investigator bias. Plots showing IRC versus site agreement indicated that there were a number of early disagreements (week 6, first RECIST assessment) where time from IRC defined disease progression to investigator defined disease progression was too long to be plausible. In addition and due to rapid progression in the control arm, more patients underwent more than one scan in the experimental arm increasing the risk for downgrading time to progression in the IRC analysis of PFS.

A benefit in terms of OS was not demonstrated. A post-hoc covariate adjusted analysis was compatible with an OS HR of 0.85 compared with 1.0 in the non-adjusted analysis. Of interest, the adjusted HR was the same as the HR derived from simply propagating the PFS benefit forward.

A PFS2 analysis in line with the European guideline was also undertaken. Based on very complete data (about 5% censored in the experimental arm) the HR for PFS2 was found to be 0.72 in the mITT population and 0.48 in the target population FR(100%).

Platinum-resistant ovarian carcinoma has a poor prognosis and the expected overall survival is about 1 year. The most persuasive measure of patient benefit in this situation would be improved survival, but a relevant increase in PFS with reasonably good tolerability can be considered as a clinical benefit.

Uncertainty in the knowledge about the beneficial effects

The application is supported by non-comprehensive data from a single pivotal phase 2 trial. Thus, the magnitude of the PFS benefit is less well defined due to the small sample size. However it cannot be questioned that a PFS benefit has been shown in the FR (100%) population. Additional efficacy data will be provided in the context of a conditional marketing authorisation, to define more precisely the magnitude of the effect.

Risks

Unfavourable effects

The safety database consisted of subjects enrolled in four clinical studies. The total number of patients exposed to the combination therapy was 107 patients in the pivotal phase 2 study.

Almost all subjects reported a TEAE in the pivotal study with a similarity between the two arms. There were more reports of TEAEs of Grade 3 or 4, serious TEAEs and TEAEs leading to withdrawal of study medication in the vintafolide+ PLD arm compared to PLD alone arm. However, when duration of therapy was taken into account and adverse events were evaluated by cycle, the differences diminished since subjects in the combination arm did receive more cycles and

subsequently had a longer duration on treatment. This is presumed due to a benefit from the combination treatment compared to PLD alone.

The most common TEAEs in the vintafolide+PLD Arm were fatigue, anaemia, stomatitis, nausea, neutropenia, PPE syndrome, constipation, abdominal pain, vomiting, rash, peripheral sensory neuropathy, diarrhea, anorexia, and leucopenia while in the PLD alone arm they were nausea, fatigue, stomatitis, PPE syndrome, constipation, anaemia, vomiting, neutropenia and diarrhoea. The incidences of both PPE and stomatitis were similar between treatment arms, and were in the range of what has been previously reported with PLD.

The findings as regards specific AEs within the eye disorder SOC and their incidences in the EC-FV-06 study were consistent between studies with the most common preferred terms being conjunctivitis, lacrimation increased, vision blurred, dry eye, eye pruritus. The incidence of visual acuity AEs of special interest was low. Moreover, the AEs were of grade 1 or 2 and did not result in dose modification or study drug discontinuation.

While recognising the low rate of deaths in the pivotal study, two of the subjects died due to recurrent and progressive deep vein thrombosis events. Given, however, that the subjects in the vintafolide/PLD arm received more treatment cycles as compared to the control arm and the similarity in events between the two arms as regards vascular disorders, it is concluded that the risk of thrombotic events is not increased by vintafolide.

None of the deaths occurring on study was considered drug-related to vintafolide+PLD or PLD alone and no post study deaths have been attributed to adverse events from treatment with vintafolide. Deaths were primarily due to progressive disease in both arms.

Uncertainty in the knowledge about the unfavourable effects

Only about 40 % of the safety population did receive the combination of vintafolide and PLD thus limiting a comprehensive safety assessment of the treatment relevant to the proposed indication. The total number of patients exposed to the combination therapy was 107 patients, which provides only modest experience for a thorough safety assessment of a new chemical entity in combination with a known cytotoxic agent. The clinical safety profile of vintafolide is expected to be further characterised in the confirmatory phase 3 trial, EC-FV-06.

The age group ≥ 65 years consisted of a limited number of subjects (n=35) which hampers a safety assessment in this age group. It is therefore included as missing information in the risk management plan.

In terms of cardiac function, the amount of missing data mid-treatment and end of treatment makes the interpretation of the effect of vintafolide in combination with PLD on LVEF difficult. Based on the available information, there is no observation suggestive of a detrimental effect by vintafolide on LVEF. ECG monitoring was not required in the protocol of study EC-FV-04 but will be collected in the ongoing Phase 3 study (EC-FV-06) as outlined in the risk management plan. No QTc study has been carried out. However, a formal QTc assessment is being performed in the Phase 1 study PN001 which is part of the pharmacovigilance activities. The use of vintafolide in patients with cardiac impairment is included in the RMP as missing information.

Furthermore, the non-clinical finding of optic nerve toxicity reported in dogs appears not to be translated into any clinical major safety concern and is adequately addressed in the risk management plan.

At present, there are uncertainties regarding the routes of metabolism and excretion of vintafolide or the vinca-alkaloid DAVLBH. Thus it is not possible to identify situations (impaired organ function, drug-drug interactions) with risk for increased exposure to vintafolide or DAVLBH. This is addressed in the risk minimisation through several pharmacovigilance activities.

Benefit-risk balance

Importance of favourable and unfavourable effects

Due to the small sample size there were uncertainties in relation to the magnitude of the PFS benefit in patients with FR(100%) tumour lesions, i.e. the targeted indication. However, a median PFS benefit of about 4 months (investigator assessment) and 2½ months (IRC assessment) have been shown. In the targeted population of poor prognosis patients, this is considered to be clinically relevant per se.

Adverse events were common and as expected an increased frequency was seen in the combination arm. The adverse events observed in the safety population were consistent with the known toxicity profiles of vinca alkaloids and PLD. There were no strong data (pre-clinical or clinical) suggestive of an increased risk of cardiotoxicity by vintafolide. Overall, the lack of a signal for cardiotoxicity in clinical studies is supported by the targeted nature of vintafolide and that normal heart tissue is considered to be a folate receptor negative organ.

Benefit-risk balance

The demonstrated benefit in terms of PFS is considered to outweigh the risk associated with treatment with vintafolide in combination with PLD based on available data from the clinical safety database.

Discussion on the benefit-risk balance

Clinically meaningful efficacy results in terms of PFS benefit have been shown in patients with platinum resistant ovarian cancer expressing the folate receptor in all tumour lesions as assessed by ^{99m}Tc-etarfolatide.

From a safety perspective, vintafolide in combination with PLD appears tolerable with manageable adverse reactions. Considering the size of the safety database, additional safety data from the ongoing phase 3 study are required to further characterise the safety profile of vintafolide.

Considering the clinical efficacy data are currently available mainly from one phase 2 study in 38 patients enrolled in the target population and 149 in the mITT population, additional efficacy data is needed in the context of a conditional MA.

The CHMP considered that vintafolide falls under the scope of Article 2 of Commission Regulation (EC) No. 507/2006 as eligible for a Conditional Marketing Authorisation as it belongs to:

a) Medicinal products which aim at the treatment, the prevention or the medical diagnosis of seriously debilitating diseases or life-threatening diseases;

b) Medicinal products designated as orphan medicinal products in accordance with Article 3 of Regulation (EC) No 141/2000.

Furthermore, the requirements listed in Article 4 of the Regulation apply to vintafolide on the basis of the following reasons:

a) The risk-benefit balance of the product is positive:

The demonstrated benefit in terms of PFS is considered to outweigh the risk associated with treatment with vintafolide in combination with PLD based on available data from the clinical safety database. The effect on PFS was supported by a convincing pharmacological rationale and a number of supportive sensitivity analyses and is not affected by subsequent therapies. A post-hoc covariate adjusted analysis was compatible with an OS HR of 0.85, which was similar to the effect observed on PFS.

b) It is likely that the applicant will be able to provide comprehensive clinical data:

Additional comprehensive clinical efficacy and safety data will be available from study EC-FV-06, a randomised double-blind phase 3 trial comparing vintafolide in combination with PLD versus PLD alone in patients with platinum-resistant ovarian cancer who express the folate receptor on all target lesions as assessed by the ^{99m}Tc-etarfolatide imaging procedure.

c) Fulfilment of unmet medical need in the proposed indications:

Due to the poor prognosis in general for platinum resistant ovarian cancer, there is an unmet medical need in this patient population that could be fulfilled with the proposed medicinal product. Importantly, the subpopulation of women whose disease expresses the FR represents an epidemiologically small subset of PROC with an overall worse prognosis and no approved agents for selection or treatment.

d) The benefits to patients of the immediate availability outweigh the risks inherent in the fact that additional data are still required:

The available data from the phase 2 study indicate a positive risk-benefit balance for vintafolide for the proposed indication. Given the positive benefit-risk balance and in view of the unmet medical need, the benefits to public health of the immediate availability outweigh the risks inherent in the fact that additional data are still required.

4. Recommendations

Similarity with authorised orphan medicinal products

The CHMP by consensus is of the opinion that Vynfinit (vintafolide) is not similar to Yondelis (trabectedin) within the meaning of Article 3 of Commission Regulation (EC) No. 847/200. See appendix 1.

Outcome

Based on the CHMP review of data on quality, safety and efficacy, the CHMP considers by consensus that the risk-benefit balance of Vynfinit in combination with pegylated liposomal doxorubicin (PLD) for the treatment of adult patients with platinum resistant ovarian cancer (PROC) who express the

folate receptor (FR) on all target lesions, is favourable and therefore recommends the granting of the conditional 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).

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 8 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 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.

If the dates for submission of a PSUR and the update of a RMP coincide, they can be submitted at the same time.

Specific Obligation to complete post-authorisation measures for the conditional marketing authorisation

This being a conditional marketing authorisation and pursuant to Article 14(7) of Regulation (EC) No 726/2004, the MAH shall complete, within the stated timeframe, the following measures:

Description	Due date
Submit clinical efficacy and safety results from study EC-FV-06, a randomised double-blind phase 3 trial comparing vintafolide in combination with PLD versus PLD + placebo in patients with platinum-resistant ovarian cancer who express the folate receptor on all target lesions as assessed by the ^{99m}Tc-etarfolatide imaging procedure Final clinical study report	March 2017

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

Not applicable.

New Active Substance Status

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