20 March 2014
EMA/CHMP/219148/2014
Committee for Medicinal Products for Human Use (CHMP)

Assessment report

Folcepri

International non-proprietary name: etarfolatide

Procedure No.: EMEA/H/C/002570/0000

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.

30 Churchill Place ● Canary Wharf ● London E14 5EU ● United Kingdom
Telephone +44 (0)20 3660 6000 **Facsimile** +44 (0)20 3660 5505
Send a question via our website www.ema.europa.eu/contact

An agency of the European Union

© European Medicines Agency, 2014. Reproduction is authorised provided the source is acknowledged.

Table of contents

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

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 Folcepri, 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 18 October 2012.

Folcepri was designated as an orphan medicinal product EU/3/12/1043 on 10 September 2012. Folcepri was designated as an orphan medicinal product in the following indication: Diagnosis of positive folate receptor status in ovarian cancer.

The applicant applied for the following indication: This medical product is for diagnostic use only. After radiolabelling with sodium pertechnetate (^{99m}Tc) solution, Folcepri is indicated for single photon emission computed tomography (SPECT) imaging, in combination with CT or MRI, for the selection of adult patients for whom treatment with Vynfinit, a folate receptor (FR) targeted therapeutic, is being considered.

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 etarfolatide 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 test(s) or studies.

Information on Paediatric requirements

Pursuant to Article 7 of Regulation (EC) No 1901/2006, the application included an EMA Decision P/0249/2012 on the granting of a product-specific waiver.

Information relating to orphan market exclusivity

Similarity

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

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 claim(s):

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

Study EC-FV-04 was a randomised, multicentre, open-label phase 2 study, in which patients with platinum resistant ovarian cancer (PROC) received treatment with vintafolide in combination with pegylated liposomal doxorubicin (PLD) versus PLD alone. In this study, patients underwent ^{99m}Tc-etarfolatide imaging and the ^{99m}Tc- etarfolatide uptake was evaluated for each patient before starting study treatment. Results of study EC-FV-04 showed 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 etarfolatide. Conversely, no benefit was observed in patients who had 0% FR positive lesions [FR(0%)]. ^{99m}Tc-etarfolatide has therefore demonstrated the ability to effectively select patients with the worse prognosis (FR(100%) population), thereby personalising vintafolide treatment for those patients most likely to benefit.

Balanced against the outlined benefit, the safety profile of etarfolatide, which was evaluated in more than 550 cancer patients, was benign with most adverse events transient and of mild intensity.

- 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. Additional data from this study are expected to further define the clinical utility of ^{99m}Tc-etarfolatide scan for selection of patients for treatment with the vintafolide in combination with PLD in a larger subset of 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 etarfolatide 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 etarfolatide 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

Almac Pharma Services
Seagoe Industrial Estate, Craigavon, BT63 5UA
United Kingdom

1.3. Steps taken for the assessment of the product

- The application was received by the EMA on 26 October 2012.
- The procedure started on 21 November 2012.
- 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 summary report of the GMP inspection carried out at one manufacturing site, between 22 and 26 July 2013 was issued on 21 august 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 Rapporteur's Risk Management Plan Assessment Report
- 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 Outstanding Issues to all CHMP members on 7 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 second CHMP List of Outstanding Issues on 29 January 2014.
- The Rapporteurs circulated the Joint Assessment Report on the applicant's responses to the second List of Outstanding Issues to all CHMP members on 6 February 2014
- During the CHMP meeting on 18 February 2014, outstanding issues were addressed by the applicant during an oral explanation.
- The PRAC adopted an updated Rapporteur's Risk Management Plan Assessment Report on 12 March 2014
- 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 Folcepri.

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).

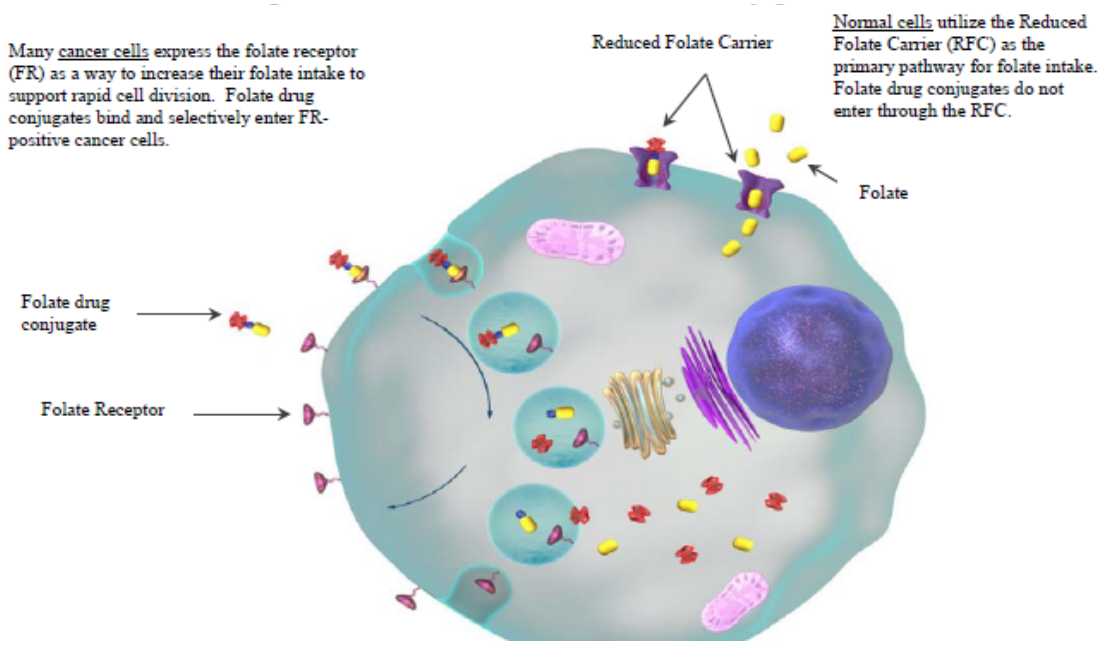


Figure 1: Mechanism of action of folate conjugates

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).

There is currently no radiopharmaceutical imaging agent approved in the EU that utilises the FR as a pharmacological target for diagnostic purposes.

Etarfolatide (also referred as EC20) is composed of a ^{99m}technetium chelating peptide covalently bonded to folic acid. The folic acid moiety is proposed to function as a targeting ligand that binds to folate receptors that may be present on the surface of many cancer cells. The peptide moiety of etarfolatide functions as a chelator of certain transition metals including ^{99m}technetium (^{99m}Tc). When formulated, ^{99m}Tc-etarfolatide is administered intravenously for the purpose of anatomically identifying malignant lesions that express functional folate receptors (FR). Positive lesion uptake of ^{99m}Tc-etarfolatide is a prerequisite for the administration of companion folate-targeted therapeutics, such as vintafolide (EC145, a folate-vinca alkaloid conjugate).

The applied indication was: This medical product is for diagnostic use only. After radiolabelling with sodium pertechnetate (^{99m}Tc) solution, Folcepri is indicated for single photon emission computed tomography (SPECT) imaging, in combination with CT or MRI, for the selection of adult patients for whom treatment with Vynfinit, a folate receptor (FR) targeted therapeutic.

Following review, the final indication for Folcepri proposed was:

This medicinal product is for diagnostic use only. After radiolabelling with sodium pertechnetate (^{99m}Tc) solution, Folcepri is indicated, after intravenously administered folic acid, for single photon

emission computed tomography (SPECT) imaging, in combination with Computed Tomography (CT) or Magnetic Resonance Imaging (MRI), for the selection of adult patients for treatment with vintafolide, a folate receptor (FR) targeted therapeutic for use in ovarian cancer.

2.2. Quality aspects

2.2.1. Introduction

Folcepri is a kit for radiopharmaceutical preparation containing 0.10 mg of lyophilisate etarfolatide as an active substance.

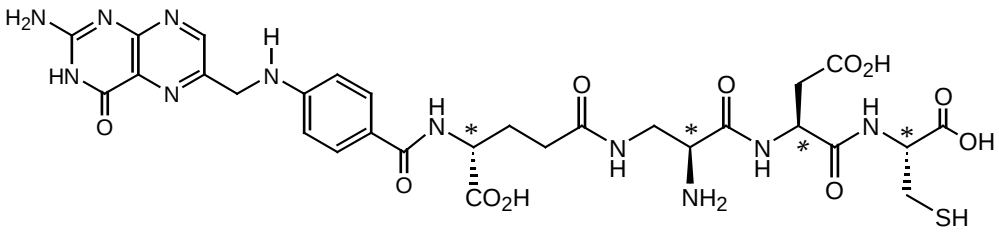
Other ingredients are: Sodium α-D-glucoheptonate dihydrate, stannous chloride (E512), hydrochloric acid (E507) (for pH adjustment) and/or sodium hydroxide (E524) (for pH adjustment), and water for Injections (E2).

Folcepri should be labelled with sodium pertechnetate ^{99m}Tc solution from an approved 99Mo/^{99m}Tc generator finished product. The sodium pertechnetate ^{99m}Tc solution for injection is not part of the kit.

The finished product is packed in glass vial with chlorobutyl stopper and an aluminium seal.

2.2.2. Active Substance

The chemical name of etarfolatide is N-[4-[[(2-amino-3,4-dihydro-4-oxo-6-pteridinyl) methyl]amino]benzoyl]-D-γ-glutamyl-(2S)-2-amino-β-alanyl-L-α-aspartyl-L-cysteine Pte-γ-D-Glu-β-Dap-Asp-Cys and has the following structure:



The active substance, etarfolatide is an amorphous, yellow, flocculent, hygroscopic solid. As a non-crystalline solid, no polymorphic screening of the active substance was conducted. No

Etarfolatide contains four stereocentres, marked in asterisks in the structure above. Three of the amino acid stereocentres possess configuration in the natural state (L-configuration).

Manufacture

Etarfolatide is prepared using standard fluorenylmethoxycarbonyl (Fmoc) solid phase synthetic methods with diisopropylcarbodiimide (DIC) and hydroxybenzotriazole (HOBt) as coupling (or 'activation') agents. Two mole equivalents of coupling agents and Fmoc protected amino acid residues are used relative to the Cys amino acid residue (active site) loading on the resin. Once synthesised and cleaved from the resin, the peptide is purified by HPLC and freeze-dried to produce the final solid drug substance. The manufacturing process consists of 6 steps.

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, impurities, active content, residual solvents, and heavy metals. Impurities present are in compliance with ICH Q3A.

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

The active substance specification includes tests for appearance, identity (LC/ESI-MS), individual specified impurities (RP-LC), individual unspecified impurities (RP-LC), total impurities (RP-LC) assay (RP-LC), moisture content (KF), and residual solvents (GC).

Impurities present are in compliance with ICH Q3A

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

Batch analysis data of three production scale batches of the active substance are provided. The results are within the specifications and consistent from batch to batch.

Stability

Stability data on three primary stability batches and one supporting batch from the proposed manufacturers stored in the intended commercial package for 24 months under long term conditions at - 20±5 ºC /Ambient RH and for up 6 months under accelerate conditions at 5±3 ºC /Ambient RH according to the ICH guidelines were provided. Eighteen months' data for a further supportive drug substance stability batches under long term and accelerated conditions have also been provided.

Photostability testing following the ICH guideline Q1B was performed on four batches. Results on stress conditions describe the stress conditions were also provide on four batches.

The following parameters were tested: appearance, assay and related substances by UPLC. Microbial enumeration and endotoxin are tested annually to demonstrate the microbiological profile does not change during the proposed retest period. The analytical methods used were the same as for release and were stability indicating.

The stability results conducted in accordance with ICH guidelines indicate that the drug substance manufactured by the proposed suppliers is sufficiently stable. The stability results justify the proposed retest period in the proposed container.

2.2.3. Finished Medicinal Product

Pharmaceutical development

The finished product is a lyophilisate containing sodium α-D-glucoheptonate, Tin(II) chloride and etarfolatide at a concentration of 0.10 mg/vial upon reconstitution with a ^{99m}Tc normal saline solution. The formulation was modelled after similar approved Technetium imaging agents. Additional optimization surrounding stoichiometry related to glucoheptonate and stannous chloride was also conducted during development.

Etarfolatide is an amorphous hygroscopic solid and that no physicochemical characteristics was identified that would influence the quality of the product. All excipients are well known pharmaceutical ingredients and their quality is compliant with Ph Eur standards except sodium α-D-glucoheptonate. There are no novel excipients used in the finished product formulation. The list of excipients is included in section 6.1 of the SmPC.

Relevant details with respect to the optimisation of each step of the manufacturing process were provided. The formulation used during clinical studies is the same that is used for marketing.

The primary packaging is a glass vial with chlorobutyl stopper and an aluminium seal The Type I glass is compliant with Ph Eur Chapter 3.2.1. The stoppers are compliant with the chemical test requirements for Type I closures, as described in the Ph Eur, Chapter 3.2.9 and do not contain natural rubber.

Adventitious agents

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

Manufacture of the product

A summary, a detailed description and a flow diagram of the manufacturing process were provided. Major steps of the manufacturing process have been validated by a number of studies on three commercial scale batches, with data available for review at the site, upon request. In addition batch data were presented for validation batches and the development and optimisation of the process is described in detail. 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 pharmaceutical form.

Product specification

The finished product release specifications include appropriate tests for this kind of dosage form: appearance, reconstituted solution appearance, reconstitution time, identity (LC/ESI-MS), individual specified degradation products (UPLC), individual unspecified degradation products (UPLC), total degradation products (UPLC), assay (UPLC), radiochemical purity (Radio HPLC), moisture content (thermogravimetric analysis), glucoheptonate (HPLC), Tin (II) content (ICP-MS), Total Tin (ICP-MS), pH (potenciometric), sterility (Ph Eur), endotoxin (Ph Eur), particulate matter (Ph Eur), uniformity of dosage units (Ph Eur).

Batch analysis results are provided for 3 registration batches confirm the consistency of the manufacturing process and its ability to manufacture to the intended product specification.

Stability of the product

Three commercial scale batches of the finished product manufactured with the intended formulation and process at the proposed site were stored under long term conditions for 12 months at 5 °C/Ambient RH, and for 6 months under accelerate conditions at 25 ± 2 °C / 60% ± 5% RH according to the ICH guidelines were provided. Stability data was also provided for three months at 40 ± 2 °C / 75% ±5% RH. The batches of medicinal product are identical to those proposed for marketing and were packed in the primary packaging proposed for marketing.

Samples were tested for appearance, purity, pH, sterility, bacterial endotoxins, particulate content, individual unspecified impurity, total degradation products, assay, moisture and reconstitution time. The analytical procedures used are stability indicating.

In-use stability of the ^{99m}Tc-etarfolatide solution was assessed by measuring radiochemical purity at room temperature over time.

In addition, two batches were exposed to light as defined in the ICH Guideline on Photostability Testing of New Drug Substances and Products.

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.

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.

2.2.6. Recommendation(s) for future quality development

Not applicable.

2.3. Non-clinical aspects

2.3.1. Introduction

The non-clinical dossier consisted of primary pharmacology studies, single- and repeat-dose toxicology studies in mice, rats and rabbits and assessments of potential genotoxicity, local irritation, and immunogenicity. A cardiovascular safety pharmacology study was also conducted in dogs. For all *in vivo* studies, except local tolerance, intravenous dosing was chosen as the route of

administration. In some cases, the test article was prepared by compounding the drug product with decayed ^{99m}Tc prior to use, in other studies non-chelated etarfolatide was used.

The single dose toxicity, the repeated dose toxicity, the genotoxicity, the local tolerance and the cardiovascular safety pharmacology study were conducted in compliance with Good Laboratory Practice (GLP). The antigenicity study was not conducted in compliance with GLP.

2.3.2. Pharmacology

Primary pharmacodynamic studies

In vitro pharmacodynamic studies

Table 1: Summary of primary pharmacodynamic studies performed *in vitro* with etarfolatide (EC20)

Type of Study, report	**Test System**	**Results / Conclusion**
Folate receptor affinity of etarfolatide SR#P-1013	KB cells incubated with ^{3}H-folic acid (100 nM) in the presence and absence of increasing doses of unlabeled competitor ligands.	Etarfolatide was determined to have an affinity of 0.92 relative to that of folic acid (FA) for FR. Both isomers of Rhenium-etarfolatide displayed relative affinity values that also were similar to that of folic acid.
Folate receptor specificity EC20-B-PR-0023 & EC20-B-PR-0026	FR-α expressing KB cella's, FR-β expressing CHO-β cells, IGROV, 4T1, A549 cells	Cells were incubated with 100 nM ^{99m}Tc-EC20 ± 10 µM Folic Acid. No specific uptake was observed in FR-negative cell lines e.g. the A549 and 4T1 cells lines. FA decreased the binding of EC20. Data suggest that KB, KB-DR300, N/A-R-Cl2, N/A-R, IGROV & MDAMB-231 all overexpress folate receptors at various levels *in vitro*. ^{99m}Tc-etarfolatide displayed dose-dependent, saturable uptake in FR-α positive KB cells and FR-α positive CHO-β cells. ^{99m}Tc-EC20 displayed high affinity (1-3.2 nM) to both FR isoforms. ^{99m}Tc-etarfolatid was shown to bind with equal affinity to the α- and β-folate receptors. No uptake was seen in FR-negative cell lines and decreased significantly in presence of folic acid.

An additional study was conducted with etarfolatide to determine whether or not it is a substrate for the reduced folate carrier (RFC) or proton-coupled folate transporter (PCFT). Uptake of a radiolabeled etarfolatide:rhenium chelate (^{3}H-EC20:Re) was examined 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) (Study# EC20-B-PR-0034).

^{3}H-EC20:Re uptake in the FRα-expressing RT16 cells was relatively specific for FRα and was mostly blocked by excess FA, although there was some non-competable cellular uptake (~38% non-specific uptake). The non-specific uptake was likely due the high concentration of ^{3}H-EC20:Re used in the study (0.5 µM) as well as a relatively low level of competitor (20-fold excess) since a subsequent study using a lower, yet FR-saturable concentration of 0.1 µM ^{3}H-EC20:Re, as well as

increased competitor (100 and 1000-fold molar excess) showed considerably lower non-specific uptake of ^{3}H-EC20:Re in the RT16 cells (~16% at 10 µM excess FA and ~5% at 100 µM excess FA).

Cellular uptake of ^{3}H-EC20:Re by RT16 cells far exceeded the uptake in the other Chinese hamster ovary cell lines that did not express FRα. In the RFC+, PCFT+ and transporter-null cells, there was some non-mediated cellular uptake of ^{3}H-EC20:Re. However, the amount was modest compared to the levels in the RT16 cells, and it was incompletely blocked by specific inhibitors for the individual transporters. This suggested that the observed cell-associated ^{3}H-EC20:Re was non-specific and not mediated via uptake of the RFC or PCFT.

In vivo pharmacodynamic studies

Table 2: Summary of primary pharmacodynamic studies performed *in vivo* with etarfolatide (EC20)

Type of Study, report	**Test System**	**Test conditions**	**Findings**
Time course of ^{99m}Tc-etarfolatide distribution among major organs and tissues in mice SR#P-1010	Balb/c mice 3 Female/ group	^{99m}Tc-etarfolatide 50 µg/kg Intravenous Biodistribution evaluated at 0.5, 1, 2, 4, 8 and 24 hours	Blood clearance of ^{99m}Tc-etarfolatide was rapid. Only 1.7% of the injected dose remained in circulation after 30 minutes. ^{99m}Tc-etarfolatide concentrated in the FR-positive kidneys with a peak at 1 hour post injection and then a slow decline over time. Kidney accumulated the highest concentration of ^{99m}Tc following intravenous administration of ^{99m}Tc-etarfolatide and the tissue with the next highest accumulation was the liver.
^{99m}Tc-etarfolatide specifically accumulates in folate receptor positive tumours EC20-B-PR-0025, -0029, -0001 (Parker, et al., 2005; Reddy et al., 2004)	*nu/nu* mice bearing A549, OV90 or KB tumours and Balb/c mice bearing M109 or 4T1 tumours 3 Female/ group	50 µg/kg ^{99m}Tc-etarfolatide ± 100-fold excess FA Intravenous	Uptake of ^{99m}Tc-EC20 was found to be restricted to FR-positive M109 tumour and FR-positive normal kidney tissue. A 100-fold excess of co-injected folic acid blocked ≥96% of ^{99m}Tc-EC20 uptake in tumour and kidney tissue and accumulation of ^{99m}Tc-EC28 (the nonbinding despteroyl analog of EC20) into these same tissues was negligible. Binding of EC20 with or without co-treatment with FA to tumours from clones with different FR levels supported that binding is FR-mediated. ^{99m}Tc-etarfolatide uptake was found to be proportional to the level of FR expression with FR-positive M109 tumours accumulating the greatest amount and A549 tumours (which express negligible levels of FR) accumulating the lowest amount of radiolabel.
Tumour uptake of	Balb/c mice bearing	75, 224, 746, 2237,	^{99m}Tc-etarfolatide uptake in the tumour

^{99m}Tc-etarfolatide is saturable EC20-B-PR-0030	M109 tumours 3 Female/ group	7457 µg/kg Intravenous	rapidly increased at lower doses but eventually plateaued at doses ≥3,000 nmol/kg. Mice pre-injected with 2 µmol/kg folate conjugates displayed reduction in the tumour uptake of ^{99m}Tc-EC20.
^{99m}Tc-etarfolatide uptake is directly proportional to lesion size Reddy et al., 2004, J Nucl Med 45, 857-66.	Balb/c mice bearing M109 tumours 3 Female/ group	50 µg/kg Intravenous	The % injected dose of ^{99m}Tc-etarfolatide in the tumours correlated with respect to the size of the tumour.
Tissue biodistribution of ^{99m}Tc etarfolatide in mice with KB tumours co-dosed with varying amounts of folic acid. SR#P-1004	*nu/nu* mice bearing KB tumours 3 Female/ group	20 µg/kg Intravenous + escalated doses of co-administered unlabeled folic acid (0, 10, 40, 80, 180, and 900 µg/kg, respectively)	^{99m}Tc-etarfolatide uptake was highest in FR-positive tumours and kidneys. Radiolabel uptake decreased in both of these tissues as the dose of co-administered non-radioactive folic acid was increased. Radiotracer uptake in most non-target tissues did not vary in groups administered 40 - 900 µg/kg of FA, while the tumour and kidney levels of tracer declined over these groups as the total dose of non-radioactive folic acid was escalated.
^{99m}Tc-etarfolatide and vintafolide (folate-vinca alkaloid conjugate) distribute equally EC145-B-PR-0033	*nu/nu* mice bearing KB tumours 3 Female/ group	0.5 µmol/kg ^{99m}Tc-EC20 ± 50 µmol/kg vintafolide (EC145) 373 µg/kg Intravenous	SPECT/CT scan showed that FR-positive KB tumour and kidneys showed strong positive uptake of ^{99m}Tc-etarfolatide. In contrast, little to no radiotracer uptake was observed in animals that received co-injected vintafolide (used as competitor).
Tumour to non-tumour ratios improve with co- or pre-injected low dose folic acid EC20-B-PR-0024	Balb/c mice bearing M109 tumours 3 Female/ group	Intravenous 50 µg/kg ^{99m}Tc-EC20 with or without co- or 5 minute pre-injection of non-radioactive folic acid (100, 10 or 1 equivalents of the EC20 dose).	A 5 min pre-injection or a co-injection of non-radioactive folic acid blocked tumour and kidney-associated ^{99m}Tc-EC20 in a dose-dependent fashion. Tumour to non-tumour (T:NT) uptake ratios of ^{99m}Tc-etarfolatide were found to improve if a small amount of folic acid was co- or pre-injected into the mice.

Secondary pharmacodynamic studies

No secondary pharmacodynamic studies were submitted.

Safety pharmacology programme

Cardiovascular system (CVS)

A GLP study was conducted in groups of 5 conscious telemetered male Beagle dogs to determine the acute effects of intravenously administered ^{99}Tc-etarfolatide on the CVS. Each dog was treated intravenously with the control (0.9%) saline, sodium alpha-D-glucoheptonate and tin (II) chloride vehicle and with 3 doses of ^{99}Tc-etarfolatide (0.0264, 0.0792, and 0.264 mg/kg) which represented doses that are 10, 30, and 100 times the human clinical dose. There was one treatment group and a washout period of at least 7 days was used between doses. One-minute means of hemodynamic parameters and ECG parameters were measured for a period of 24 hours following each dose.

There were no treatment related effects on heart rate or arterial pressure. An overall increase in pressure was observed for the mid-dose group (0.0792 mg/kg) throughout the 24 hour data collection period. There was no dose-response effect, and the values were still within normal physiological variation. There was no electrocardiographic evidence of test article action in this study. It was concluded that intravenous administration of ^{99}Tc-etarfolatide to dogs at doses of up to 0.264 mg/kg (approximately 90X the dose in humans based on body surface area) showed no effects on cardiac (ECG) or circulatory function (heart rate and diastolic, systolic, and mean arterial pressures).

Central nervous system (CNS)

Clinical signs collected in pivotal single and repeat-dose toxicity studies in mice, rats, and rabbits did not reveal any findings suggestive of adverse CNS effects.

Respiratory system

Clinical signs collected in pivotal single and repeat-dose toxicity studies in mice, rats, and rabbits did not reveal any findings suggestive of adverse effects on respiratory system.

Pharmacodynamic drug interactions

No pharmacodynamic drug interactions studies were submitted.

2.3.3. Pharmacokinetics

Non-clinical pharmacokinetic studies have been conducted with etarfolatide in mice, while serum protein binding was analysed in rat, mouse, dog and human plasma. The formulations of etarfolatide used in the *in vivo* studies were in most cases lyophilized etarfolatide solubilized in decayed sodium pertechnetate diluent, or in diluent alone.

Since ^{99m}Tc-etarfolatide is a radionuclide, the analytical procedure used for biodistribution, excretion studies and determination of serum protein binding involved the use of a gamma counter programmed to account for the decay of ^{99m}Tc sodium pertechnetate ($NaTcO_4$).

Radiochemical profiling of ^{99m}Tc-etarfolatide in urine was assessed using radiochemical High-performance liquid chromatography (HPLC) methods and a gamma radio-detector.

Table 3: Overview of pharmacokinetic studies

Type of Study	Test System	Method of Admin	Study No.
Distribution			
Biodistribution	Balb/c mice	IV	SR#P-1010
Biodistribution	Balb/c mice with KB xenografts	IV	SR#P-1004
Serum protein binding *in vitro*	Human and rat	Not applicable	SR#P-1002
Serum protein binding *in vitro*	Human, rat, dog, mouse	Not applicable	EC20-B-PR-0033
Metabolism			
Urinary profiling	Balb/c mice	IV	SR#P-1011
Enzyme interaction (*in vitro*)	Purified dihydrofolatereductase (DHFR)	Not applicable	EC20-B-PR-0021
Excretion			
Urinary excretion	Balb/c mice	IV	SR#P-1011

Results of the biodistribution studies (Study SR#P-1010 and Study SR#P-1004) are presented in the pharmacology section 2.3.2.

Serum protein binding (SR#P-1002)

^{99m}Tc-etarfolatide (0.1 mg/mL) was mixed with fresh rat serum, and commercial human serum from male type AB donors and the *in vitro* binding of ^{99m}Tc-etarfolatide to serum proteins was analysed by ultrafiltration.

Table 4: *In vitro* Serum binding of ^{99m}Tc-etarfolatide

Matrix	% free	corrected % free
Saline control	95.0 ± 2	100
Rat serum	31.2 ± 0.4	32.8 ± 0.4
Human serum	27.0 ± 1	28.0 ± 1.0

Note: The "corrected % free" is the normalized value correcting for any radiotracer that is non-specifically bound to the ultrafiltration membrane (using the data from a filtered control solution of ^{99m}Tc-etarfolatide).

Serum protein binding (EC20-B-PR-0033)

An additional non-GLP study evaluating *in vitro* serum binding was conducted with ^{99m}Tc-etarfolatide in human, dog, rat, and mouse serum. ^{99m}Tc-etarfolatide was spiked at a concentration of 50, 100, or 1000 nM into each type of sera equilibrated to 37^{O}C, incubated for 15 minutes at 37^{O}C and protein binding analysed by centrifugation using a 30k NMWL centrifugal filter to separate unbound analyte from analyte bound to serum proteins. Nonspecific binding was found to be negligible.

Results from the serum binding analysis showed that ^{99m}Tc-etarfolatide was 66, 68, and 69% bound to human serum at 1000, 100, and 50 nM spike concentrations, respectively, indicating that the serum binding was independent of ^{99m}Tc-etarfolatide concentration. This was also indicated in all other species examined. Using the same range of concentrations, rat serum bound ^{99m}Tc-etarfolatide with an average of 74%, while mouse showed an average of 43%, and dog an average of 32% serum protein binding.

Urinary metabolic profile (SR#P-1011)

Female mice (n = 2) were injected intravenously with 1 mCi (6.7 nmol) of ^{99m}Tc-etarfolatide, euthanized at 1, 4, and 6 hours post injection and then urine samples were collected from the bladder. The radiochemical purity of the ^{99m}Tc-etarfolatide control sample remained constant at ~93% over the 6 hour duration of the experiment, which was used as a control for determining *in vivo*-dependent changes, versus those which may occur over time.

There were four ^{99m}Tc-containing peaks detected in mouse urine following ^{99m}Tc-etarfolatide administration. These peaks were identified as free ^{99m}Tc, ^{99m}Tc chelated to etarfolatide at an undefined position, and the two ^{99m}Tc-etarfolatide isomers A and B. These same peaks were identified in the ^{99m}Tc-etarfolatide standard where they remained at similar levels through the course of 6 hours. An examination of these peaks in the recovered urine showed that ^{99m}Tc-etarfolatide does not appear to undergo significant metabolism. After one and four hours post injection into Balb/C mice, the radiochemical speciation profile of ^{99m}Tc-etarfolatide in the mouse urine did not change. However, the area percentage of peak 2 had decreased while the area percentage of peak 1 (free ^{99m}Tc) had actually increased with respect to time. Notably, the radioactivity present in the urine at 6 hours post injection was very low, thus accurate HPLC analysis and radiochemical speciation was not possible. The proportion of the two ^{99m}Tc-etarfolatide isomers (peaks 3 and 4) compared to the total recovered ^{99m}Tc-containing excretion products remained relatively constant at approximately 93% throughout the 4 hours during which it could be quantified.

Urinary excretion (SR#P-1011)

Approximately 30% of the injected dose (based on counts per minutes (CPMs)) was recovered in the urine after 1 hour, while only ~1% of the injected dose was recovered at 6 hour.

Enzyme interaction (in vitro) (EC20-B-PR-0021)

A range of doses of etarfolatide (0.1 to 100 µM), Rhenium-etarfolatide (0.1 to 100 µM), or methotrexate (positive control; 0.1, 1 µM) were incubated with purified DHFR enzyme to determine their inhibition potential of Dihydrofolate reductase (DHFR) activity.

Etarfolatide did not inhibit DHFR activity at the concentrations tested (up to 100 µM), while Re-etarfolatide caused less than a 50% decrease in DHFR activity at 100 µM but no inhibition at lower concentrations.

2.3.4. Toxicology

The toxicological profile of etarfolatide was evaluated in two single-dose toxicity studies in rats and rabbits, two repeated dose toxicity studies in mice and rabbits, and in genotoxicity, local tolerance and antigenicity studies.

Table 5: Summary of single dose toxicity studies

Study ID	**Species/ Sex/Nmbr/ Group**	**Dose/Route**	**Major findings**
An expanded Acute Intravenous Toxicity Study of EC20 in Albino Rats GLP WIL-291003	Rat 10/sex/group (3 and 14 day observation periods n=5)	Intravenous 0(saline), 0(vehicle) 0.057, 0.57 mg/kg*) ^{99m}Tc etarfolatide, prepared with decayed technetium-99m (in 80 mg/ml sodium alpha-D-glucoheptonate; 0.08 mg/ml Tin (II) chloride dihydrate)	Mortalities: none Clinical findings: none BW or BW gain: no effects Food cons: no effects Haematology: not affected Clin-Chem: no changes Macroscopic: no findings Organ w: non-significant Histopath: no sign changes **NOEL 0.57 mg/kg**
An Expanded Acute Intravenous Toxicity Study of EC20 in Albino Rabbits GLP WIL-291004	NZW Rabbit 8/sex/group (3 and 14 day observation periods n=4)	Intravenous 0(saline), 0(vehicle) 0.0358, 0.358 mg/kg*) ^{99m}Tc etarfolatide, prepared with decayed technetium-99m (in 80 mg/ml sodium alpha-D-glucoheptonate; 0.08 mg/ml Tin (II) chloride dihydrate)	Mortalities: none Clinical findings: none BW or BW gain: no effects Food cons: no sign effects Haematology: no sign diff ClinChem: no sign diff Macroscopic: no findings Organ w: non-significant Histopath: no sign. changes (pulmonary congestion with concurrent haemorrhage and/or edema seen in several animals - not considered test article-related) **NOEL 0.358 mg/kg**

*) dose levels chosen to represent 10 and 100 times the clinical dose (based on body surface area)

Table 6: Summary of repeat dose toxicity studies

Study ID / Duration	Species / Sex / Number / Group	Dose / Route	Major findings
A 2-Week Intravenous Toxicity Study of EC20 in Mice GLP 0437ME18.002	Mouse 10/sex/group (saline, HD-vehicle, HD n=20)	Intravenous once daily 0(saline), 0(vehicle HD), 0(vehicle MD) 0.2, 0.6, 2.0 mg/kg*) ^{99m}Tc etarfolatide (radiochemical purity 87.1% and purity 91.1%) in 160mg/ml sodium alpha-D-glucohepto-nate; 0.16 mg/ml Tin (II) chloride dihydrate	Mortalities: D1: 4M+2F in HD-vehicle and 2M+2F in HD; D2-7 11M+7F in HD vehicle and 6M+6F in HD (these groups were terminated on day 8) Clinical findings: none BW or BWgain: no effects Food cons: no sign effects Hematology: no sign diff (or within historical control). Polychromasia was noted for most of the animals in HD-vehicle & HD ClinChem: no sign diff (or within historical control) Macroscopic: no findings (pale livers and gallbladders, discoloration at inj. site in HD-vehicle and HD) Organ w: non-significant Histopath: test formulation related lesions not evident **NOAEL 0.6 mg/kg** (based on vehicle dependent toxicity induced at the high dose)
A 2-week Intravenous Toxicity Study of EC20 in Rabbits GLP 0437LE18.001	NZW Rabbit 9/sex/group	0(saline), 0(vehicle HD), 0.044, 0.132, 0.441 mg/kg*) ^{99m}Tc etarfolatide (radiochemical purity 87.1% and purity 91.1%) in 160mg/ml sodium alpha-D-glucohepto-nate; 0.16 mg/ml Tin (II) chloride dihydrate	Mortalities: none Clinical findings: no test-article related findings BW or BWgain: no effects Food cons: no sign effects Hematology: no sign diff ClinChem: no sign diff Macroscopic: no test-article related findings Organ w: non-significant Histopath: no test-article related findings **NOEL 0.441 mg/kg**

*) dose levels chosen to represent 10, 30 and 100 times the clinical dose (based on body surface area)

Genotoxicity

Table 7: Summary of genotoxicity results

Type of test / study ID / GLP	Test System	Concentrations / Metabolising system	Results Positive / negative / equivocal
Bacterial Reverse Mutation Assay GLP AA51EK.502.BTL	*Salmonella typhimurium,* TA98, TA100, TA1535, TA1537 *Escherichia coli* WP2*uvr*A	^{99}Tc-etarfolatide 100 µg ± S9 Purity 94.2% Radiochem pur. 92.9%	No toxicity and no precipitate seen. No increase in revertants per plate seen in any of strains used. **Negative**
In vitro Mammalian Chromosome Aberration Test GLP AB08FU.331001.BTL	CHO cells	etarfolatide 500 µg/ml 1700 µg/ml 5000 µg/ml ± S9 Purity 95.1% Analysis of test solution: conc. 114% and purity 90.2%	No increase in structural or numerical aberrations seen. Significant effects seen in positive controls. **Negative**

Type of test / study ID / GLP	Test System	Concentrations / Metabolising system	Results Positive / negative / equivocal
Mammalian Erythrocyte Micronucleus Test GLP AB08FU.123M.BTL	Male IRC mice –erythrocytes 5 male/group	etarfolatide 2, 20, 333 mg/kg Purity 95.1%	No toxicity seen at 333 mg/kg in pilot toxicity study. **Negative**

Carcinogenicity

No studies assessing the carcinogenic potential of etarfolatide were submitted.

Reproduction Toxicity

No reproductive and developmental toxicity studies were submitted.

Toxicokinetic data

The clinical dose of etarfolatide is 0.1 mg, which equates to a 0.059 mg/m^2 dose in a patient with an average body surface area (BSA) of 1.7 m^2. The calculated margins for a single dose of exposure to etarfolatide in non-clinical studies using modern body surface area conversion factors are presented below.

Table 8: Exposure margins for etarfolatide in the non-clinical toxicity studies as compared to the clinical dose

Species (Type of study)	No observed effect level (NOEL)	NOEL in mg/m^2 [a]	Exposure margin
Rat (single dose)	0.57 mg/kg	3.4	57X
Rabbit (single dose)	0.358 mg/kg	4.3	73X
Mouse (repeat dose) [b]	0.6 mg/kg	1.8	31X
Rabbit (repeat dose)	0.441 mg/kg	5.3	90X
Human (clinical dose)	0.1 mg	0.059[c]	-

[a] conversion to mg/m^2 using a factor of 6 (rat), 12 (rabbit) and 3 (mouse).
[b] NOAEL for repeat dose study in mice.
[c] Human conversion of mg/m^2 is based on average body surface area of 1.7 m^2.

Local Tolerance

Since intravenous dosing is the intended route of administration in patients, a GLP study was conducted to assess the acute irritation potential of ^{99}Tc-etarfolatide when injected intramuscularly and perivenously into rats (Study 0452RE18.001). Twelve rats were administered ^{99}Tc-etarfolatide as a single, intramuscular injection at dose levels of 0, 0.0882, and 0.2645 mg/kg, and an additional group of 12 rats were injected with the vehicle at a dose level of 30 times the human dose equivalent. The rats that were injected intramuscularly were sacrificed at 72 hours post-dose whilst those injected perivenously were sacrificed at 1, 24, or 72 hours post-dose.

A single perivenous or intramuscular injection of decayed ^{99}Tc-etarfolatide or its vehicle was not associated with any specific gross or microscopic lesions in rats at doses up to 2.645 mg/kg (260 times the human dose based on body surface area). The only treatment-related effect was a

slightly greater observation of moderate to severe erythema or very slight to slight oedema following a single perivenous injection, which was no longer evident by 72 hours post-dose.

Other toxicity studies

Antigenicity

The potential for etarfolatide to generate an immune response was studied in Balb/C mice (EC20-B-PR-0022). Balb/C mice were dosed intravenously with 1.49 mg/kg etarfolatide, three times per week for 2 consecutive weeks, and sera from were analysed for antibody titres using an ELISA assay. No antibody titer developed, as compared to untreated mice.

2.3.5. Ecotoxicity / environmental risk assessment

Table 9: Summary of main study results

Substance (INN / Invented Name): etarfolatide (Folcepri)			
CAS-number (if available): n/a			
PBT screening		Result	Conclusion
Bioaccumulation potential- log K_{ow}	OECD107	high solubility and peptide molecular structure	Potential PBT (N)
Phase I			
Calculation	Value	Unit	Conclusion
$PEC_{surfacewater}$, default or refined (e.g. prevalence, literature)	0.00000005	µg/L	> 0.01 threshold (N)
Other concerns (e.g. chemical class)	Environmental release of radioactivity and technetium.		(N)
$PEC_{surfacewater}$ of radioactivity	0.0000125	µCi/L	(N)
$PEC_{surfacewater}$ of ^{99}Tc	0.000000065	ng/L	(N)

Etarfolatide $PEC_{surfacewater}$ value is below the action limit of 0.01 µg/L.

LogK_{ow} for etarfolatide has not been established but is expected to be below 4.5 based on the high solubility in water at pH 7 and peptide molecular structure. Etarfolatide is thus not considered to be a PBT substance.

Etarfolatide is to be radiolabelled with sodium pertechnetate (^{99m}Tc) solution before administration. ^{99m}Tc disintegrates with the emission of gamma radiation and a half- life of 6 hours to ^{99}Tc which can be regarded as quasi stable. The release of radioactivity and technetium is neither expected to pose a risk to the environment. ^{99m}Tc-etarfolatide should be used according to the special precautions for disposal and other handling stated in the SmPC.

2.3.6. Discussion on non-clinical aspects

^{99m}Tc-etarfolatide was found to bind equally well to cells that express FRα or FRβ and etarfolatide was shown to bind with high and similar affinity as Folic Acid (FA). There was no uptake of ^{99m}Tc-etarfolatide in FR negative cell lines, and in FR positive cells the uptake was shown to be decreased in presence of FA, suggesting specificity for the FR present on cell membranes.

Similar to all normal cells in the body, the FR-negative cell lines A549 and 4T1 used expressed the reduced folate carrier (RFC), which is a ubiquitously expressed anion transporter responsible for the uptake of unconjugated folates in all tissues (Matherly et al., 2007). Since no uptake of ^{99m}Tc-etarfolatide was seen in these cell lines, it was considered that Tc-etarfolatide was not a substrate for the RFC and that there may be a specificity of Tc-etarfolatide for FR-expressing tissues. The additional study conducted (Study# EC20-B-PR-0034) showed with reasonable certainty that etarfolatide is substrate for the folate receptor (FR) and not the reduced folate carrier (RFC) or the proton-coupled folate transporter (PCFT). There was also some non-specific uptake of etarfolatide that could not be completely blocked by excess of folic acid.

In vivo studies showed that blood clearance of ^{99m}Tc-etarfolatide was rapid and that etarfolatide predominantly binds to kidney and FR-expressing tumour tissue. Overall, the uptake was found to be dependent on the level of FR expression, and the uptake of ^{99m}Tc-etarfolatide was reduced to low levels (< 1%) when an excess of unmodified folic acid had been co-injected along with the test article. Uptake in tumours rapidly increased with increasing dose at lower doses but eventually plateaued at doses ≥3,000 nmol/kg. Therefore, *in vivo* data also supported a FR-dependent mechanism for the uptake of etarfolatide in tissues. The percentage of injected dose of ^{99m}Tc-etarfolatide in tumours correlated with respect to the size of the tumour indicating that tumour-associated FRs remains accessible to blood-borne etarfolatide even when tumour size increases. Tumour to non-tumour uptake ratios of ^{99m}Tc-etarfolatide were found to improve in mice if a small amount of folic acid was co- or pre-injected.

No safety pharmacology studies were performed with etarfolatide except for an intravenous cardiovascular study in dogs where ^{99}Tc-etarfolatide was administrated at doses of up to 0.264 mg/kg. This was considered acceptable. Etarfolatide had no effects on cardiac (ECG) or circulatory function (heart rate and diastolic, systolic, and mean arterial pressures). Furthermore, clinical signs collected in pivotal single and repeat-dose toxicity studies in mice, rats, and rabbits did not reveal any findings suggestive of adverse effects on the CNS or respiratory system. It should also be noted that etarfolatide is intended to be administered as a single dose of 0.1 mg.

Limited pharmacokinetic evaluations were conducted in mice, in which distribution, excretion and urinary metabolic profile were analysed after administration of ^{99m}Tc-etarfolatide. ^{99m}Tc-etarfolatide is an intravenously administered radio-diagnostic and therefore no absorption or bioavailability studies were conducted. In mice it was shown that ^{99m}Tc-etarfolatide is rapidly cleared from circulation and that ~30% of the injected dose was found in the urine within the first hour post treatment while only ~1% of the dose was recovered after 6 hours.

The primary organ of uptake in mice was the kidney, which is to be expected as ^{99m}Tc-etarfolatide is small and water soluble, and the kidney expresses the folate receptor on the apical membrane of the proximal tubule. Biodistribution study in tumour bearing mice showed that ^{99m}Tc-etarfolatide predominantly concentrated in FR-positive tumours and kidneys and that the uptake decreased in both of these tissues when folic acid was co-administered. Radiotracer uptake in most non-target tissues did not vary with respect to the added folic acid.

Serum protein binding was determined *in vitro* in rat, mice, dog and human serum and ^{99m}Tc-etarfolatide was found to be 74%, 43%, 32% and 68% bound to serum proteins,

respectively. Results indicated that the serum binding was independent of ^{99m}Tc-etarfolatide concentration.

Preliminary urinary metabolite profile was evaluated in mice and two principle ^{99m}Tc-labeled compounds detected in urine following treatment were identified as the two ^{99m}Tc-etarfolatide isomers. These accounted for more than 90% of the labelled material excreted (free ^{99m}Tc and ^{99m}Tc chelated to etarfolatide at an undefined position, were also identified in smaller amounts). A similar pattern was seen in an un-metabolised standard, indicating that ^{99m}Tc-etarfolatide does not undergo significant metabolism in mice prior to urinary excretion. However, no excretion studies were performed and the fraction excreted via the urine has thus not been established.

The use of Re-etarfolatide instead of Tc-etarfolatide and analysis of its inhibitory potential in study SR-PR-0021 was not clear. However, no DHFR-related toxicity such as inhibitory effects on proliferative tissues has been evident in any of the preclinical toxicological studies even at doses more than 100x the clinical dose. The results obtained paired with the low clinical dose of etarfolatide used suggested that an inhibition of DHFR activity is not likely in patients.

Although the pharmacokinetic evaluations performed with ^{99m}Tc-etarfolatide are limited, this is considered to be sufficient taking into account the peptide structure and the low single dose used (0.1 mg).

The non-clinical toxicology program included single- and repeat-dose toxicology studies in mice, rats and rabbits with doses up to 100 times the clinical dose based on body surface area. No significant toxicological findings were observed in single dose toxicological studies performed in rats and rabbits or repeat dose toxicological studies performed in mice and rabbits; except for vehicle dependent toxicity observed at the highest dose used in the repeat dose study in mice 2.0 mg/kg (100 times the clinical dose based on body surface area). Studies of two week duration were performed instead of the recommended 1 month. Considering the low dose of ^{99m}Tc-etarfolatide used and the intended use is for the selection of adult patients for whom treatment with vintafolide is being considered and thus that ^{99m}Tc-etarfolatide will be administered at only one occasion, this is considered acceptable.

Carcinogenicity studies were not submitted as etarfolatide is intended for single use in the clinic and it is not mutagenic or clastogenic which is considered acceptable. Reproductive and developmental toxicity studies have not been submitted. The use of ^{99m}Tc-etarfolatide is contraindicated in pregnant women. The SmPC also reflects that there are no animal data on potential effects on fertility.

No severe effects were observed after single intramuscular or perivenous administration of ^{99}Tc-etarfolatide to rats at a dose up to 30 times the clinical dose (based on body surface area).

Antigenicity results obtained indicated that etarfolatide is not immunogenic in mice when dosed three times per week for 2 consecutive weeks.

2.3.7. Conclusion on the non-clinical aspects

In vitro and *in vivo* studies have shown that ^{99m}Tc-etarfolatide binds with high affinity to FR expressed on cancer cells while no such uptake was evident on cells that did not express FR. In direct-binding experiments with human KB cells, ^{99m}Tc-etarfolatide was shown to bind to FR with a

dissociation constant of 3 nM, a value consistent with previously published Kd values for high affinity folate ligands.

The toxicological profile of Folcepri was assessed in single- and repeat-dose toxicology studies, in which etarfolatide, decayed ^{99m}Tc-etarfolatide, or ^{99}Tc-etarfolatide were administered intravenously to mice, rats or rabbits. No evidence of local or systemic toxicity, which could be associated with any of these three entities, was observed at any dose level tested. Etarfolatide was negative in the battery of genotoxicity assays and negative for antigenicity. It elicited a transient local intolerance response following perivenous administration. There are no non-clinical data available on potential effects of ^{99m}Tc-etarfolatide on fertility and the use of ^{99m}Tc-etarfolatide is contraindicated in pregnant women.

The ERA indicates that the intended use of the product is not expected to pose a risk to the environment. ^{99m}Tc-etarfolatide, like other radiopharmaceuticals, should be received, used and administered only by authorised persons in designated clinical settings. Their receipt, storage, use, transfer and disposal are subject to the regulations and/or appropriate licenses of the competent official organisation.

Overall, non-clinical data revealed no special hazard for humans based on conventional studies of safety pharmacology, repeated dose toxicity and genotoxicity.

2.4. Clinical aspects

Clinical data were provided from two phase 1 studies (EC20.1, EC20.2), one pivotal randomised phase 2 study in patients with platinum resistant ovarian cancer (EC-FV-04) and two supportive single-arm phase 2 studies (lung (EC-FV-03) and ovarian cancer (EC-FV-02)). Additional pharmacokinetic data were also provided from one phase 1 study (EC20.11) in healthy volunteers.

2.4.1. Introduction

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.

Table 10: Tabular overview of clinical studies supporting the use of ^{99m}Tc-etafolatide in selecting patients for treatment with the therapeutic agent vintafolide

Study Identifier	Study Objective	Study Design and Type of Control	Dosage Regimen	Number of Subjects [1]	Healthy Subjects or Diagnosis of Patients	Treatment Duration [2]
EC20.1 (etarfolatide only)	Safety, biodistribution, metabolism, protein binding, excretion, and dosimetry	Phase 1, open-label, non randomized, within subject evaluation, single dose	Single intravenous injection of 0.1 mg etarfolatide labeled with 15-20 mCi of ^{99m}Tc. Planar image acquisition	8 (4 normal volunteer; 4 ovarian cancer)	Normal female volunteers and females with known or suspected ovarian cancer age ≥ 18 years	24 hours

			at 5 min and 1, 4, 6-8, and 18-24 Hours post-injection. Two (2) of 4 normal volunteers and 2 of 4 ovarian cancer patients received 0.5-2.0 mg folic acid 2-3 min. prior to injection of ^{99m}Tc-etarfolatide.			
EC20.11 (etarfolatide only)	Safety, biodistribution, excretion, dosimetry	A Phase 1, open-label, Clinical Study to Evaluate the Biodistribution and Safety of ^{99m}Tc-etarfolatide (EC20) in Normal Volunteers	0.5 mg or 1.0 mg IV injection of Folic Acid IV injection of 0.1 mg of etarfolatide labeled with 20 to 25 mCi of ^{99m}Tc-Etarfolatide	20	Healthy volunteers, age ≥ 18 years of age	20-24 hours
EC20.2 (etarfolatide only)	Safety, determine optimal imaging time and acquisition technique, efficacy data, and assay masses for presence of folate receptor	Phase 1, open-label, non-randomized, within subject evaluation, single dose	Intravenous injection of 1.0 mg folic acid followed 1-3 minutes later by a single dose of 0.1 mg etarfolatide labeled with 15-25 mCi of ^{99m}Tc. Planar image acquisition at 1 hour and 2-4 hours post-injection	12	Females age ≥ 18 years of age with suspected ovarian cancer, metastatic or recurrent ovarian cancer, or endometrial cancer	12-72 hours
EC-FV-04	Compare PFS between participants who receive combination therapy with vintafolide and PLD versus PLD alone Evaluate the correlation between therapeutic response and ^{99m}Tc-etarfolatide FR status; OS, ORR, DCR, duration of response, and duration of disease control	Phase 2, open-label, randomized (2:1 ratio of vintafolide+PLD vs PLD alone), international, multicenter oncology study	Intravenous injection of 0.5 mg folic acid followed 1-3 minutes later by a single dose of 0.1 mg etarfolatide labeled with 20-25 mCi of ^{99m}Tc. Planar image acquisition at 1-2 hours post-injection followed immediately by SPECT image acquisition. 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/m^2 IV	115 safety / 94 efficacy	Patients with primary or secondary platinum resistant ovarian cancer	Minimum 7 days

			of PLD on Day 1 every 28 days (Arm B)			
EC-FV-02	Collect data on clinical benefit, collect data on tumour 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	Intravenous injection of 0.5 mg folic acid followed 1-3 minutes later by a single dose of 0.1 mg etarfolatide labeled with 20-25 mCi of ^{99m}Tc. Planar image acquisition at 1-2 hours post-injection followed immediately by SPECT image acquisition. 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.	64 safety / 43 efficacy	Female patients > 18 years of age with 1) epithelial ovarian cancer (serous or endometrioid histology) or 2) ^{99m}Tcetarfolatide positive ovarian cancer, primary peritoneal cancer or adenocarcinoma of the endometrium	Minimum 7 days
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	Intravenous injection of 0.5 mg folic acid followed 1-3 minutes later by a single dose of 0.1 mg etarfolatide labeled with 20-25 mCi of ^{99m}Tc. Planar image acquisition at 1-2 hours post-injection	60 safety / 29 efficacy	Adult patients age ≥ 18 years with histologically confirmed adenocarcinoma of the lung who have previously received ≥ 2 cytotoxic containing chemotherapeutic regimens with 1) radiographic evidence of measurable disease, and	Minimum 7 days

			followed immediately by SPECT image acquisition. 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.		2) "^{99m}Tc etarfolatide positive" tumours.	

[1] Number of participants who received at least one dose of ^{99m}Tc-etarfolatide.
[2] Duration of ^{99m}Tc-etarfolatide safety monitoring after administration of ^{99m}Tc-etarfolatide screening dose.

Five ^{99m}Tc-etarfolatide exploratory phase II studies EC20.3, EC20.4, EC20.7, EC20.8 and EC20.9 were conducted using ^{99m}Tc-etarfolatide to image a variety of solid tumours. These studies did not include treatment with vintafolide. Besides the collection of additional safety data, the primary objective of these studies was to obtain data on the percentage of patients with different cancer types who showed uptake of ^{99m}Tc-etarfolatide in tumours. These studies were included in the safety analysis only (see section 2.6).

2.4.2. Pharmacokinetics

Pharmacokinetics data were collected in two phase I trials (EC20.1, EC20.11). In addition, *ex vivo* studies on plasma protein binding were submitted.

Clinical study EC20.1

The primary objectives of this study were:

1) to determine the biodistribution and excretion of ^{99m}Tc-etarfolatide and estimate the radiation absorbed dose;
2) to evaluate the metabolism and protein binding of ^{99m}Tc-etarfolatide; and
3) to monitor safety parameters following administration of ^{99m}Tc-etarfolatide.

Participants (N=8) received a single injection of etarfolatide labelled with 15 to 20 mCi of ^{99m}Tc. Additionally, 2 of the normal volunteers and 2 of the participants with known or suspected ovarian cancer received an injection of 0.5 to 2.0 mg of folic acid 1 to 2 minutes before the injection of ^{99m}Tc-etarfolatide.

The conducted study used an IV formulation which was prepared as a 0.1 mg etarfolatide labelled with 15 to 20 mCi of ^{99m}Tc injected solution, the same concentration in the proposed final formulation.

Blood samples were drawn 5 minutes before injection and at 5, 10, 20, and 30 minutes and 1, 2, 4, 6 to 8, and 18 to 24 hours post-injection. A 12-hour sample was also drawn from participants who agreed. Urine was collected for approximately 24 hours post-injection. Blood and urine were counted in a gamma counter using standard methods to determine and calculate blood clearance half-lives and cumulative urinary excretion. Blood clearance, urinary excretion, and biodistribution data were evaluated to determine human radiation dose estimates in accordance with the medical internal radiation dose (MIRD) schema.

Serial whole body images were acquired during the 24 hour post dose period for dosimetry evaluations, to estimate the biodistribution of the radiopharmaceutical in different tissues/ regions of interest (ROI).

Plasma protein binding was measured in the participants' blood samples collected at 5 min, 30 minutes, 1 hour and 2 hours after injection of ^{99m}Tc-etarfolatide.

Distribution

A two-compartment model was fitted to the plasma data from each individual. The estimated mean Vss for ^{99m}Tc-etarfolatide was 2.8 L/kg (SD 1.3).

Table 11: Summary statistics for pharmacokinetic parameters estimates for decay-corrected radioactivity following injection of ^{99m}Tc-etarfolatide

Parameter	N	MIN.	MAX.	MED.	MEAN	STD. DEV.
V_{ss}(liter/kg)	8	0.8	4.5	3.20	2.87	1.29
CL(mL/min/kg)	8	0.2	1.4	1.15	1.00	0.43

Source: EC20.1 CSR Table 11.4.3

Biodistribution data for the ^{99m}Tc-etarfolatide within the whole body and within specific organs were obtained from whole-body conjugate anterior and posterior planar scintigrams.

Table 12: Percentage of injected dose (%ID) in whole body and selected ROIs in 24 hour period following injection of ^{99m}Tc-EC20

ROI and Imaging Session	Median (%)	Range (%)
Whole Body		
5 min	98.69	75.6 – 116.6
1 hour	92.97	77.9 – 99.0
4 hour	74.84	65.3 – 94.4
6 – 8 hour	82.99	60.9 – 94.0
18 – 24 hour	62.88	39.5 – 72.7
Liver, including gall bladder		
5 min	22.91	12.8 – 42.6
1 hour	18.79	8.7 – 39.8
4 hour	13.17	8.6 – 31.8
6 – 8 hour	12.90	7.1 – 29.3
18 – 24 hour	8.76	4.4 – 19.5
Left Kidney		
5 min	6.06	4.6 – 7.5
1 hour	5.32	3.9 – 6.8
4 hour	3.97	3.1 – 6.1
6 – 8 hour	4.33	2.7 – 6.6
18 – 24 hour	3.83	2.4 – 5.8
Intestines, excluding liver and kidneys, N=7 at all times		
5 min	6.53	0 – 29.8
1 hour	5.11	0 – 34.8
4 hour	8.70	2.8 – 30.8
6 – 8 hour	8.59	2.5 – 36.0
18 – 24 hour	4.18	1.6 – 24.4
From Section 14.4, Table S104 [1] Unless noted otherwise		

Decay-corrected radioactivity disappeared from the whole-body region of interest (ROI) gradually throughout the 24-hour observation interval. At approximately 24 hours after injection, about one-third of the radioactive injected dose had been eliminated from the body when averaged over all healthy volunteers and patients examined.

The liver was the organ that contained the greatest amount of radioactivity among the ROIs presented. Decay-corrected radioactivity in the liver appeared to decline more quickly than radioactivity in whole-body ROI, so that by the end of the first day after injection, about two-thirds had disappeared from this organ, compared to one-third in the whole body. There was no obvious influence of disease on the time course of radioactivity in the liver, but there appeared to be an influence of folic acid.

The kidney was the organ with the next-highest amount of radioactivity. The time course of decay-corrected radioactivity in the left kidney was similar to the whole body, i.e., about one third of the % ID seen at 5 minutes post-injection disappeared over the 24-hour observation interval. Disease appeared to have slowed egress of radioactivity from the left kidney. Folic acid pre-treatment did not appear to exert a profound influence on the time course in this organ.

The intestinal tract and surrounding abdominal area excluding the kidneys and liver (and gall bladder) was the last anatomic region that consistently contained identifiable radioactivity accumulation. Unlike the ROIs described above, decay-corrected radioactivity in the intestinal region fluctuated over the observation interval. Confirmed presence of ovarian cancer appeared to

have dramatically increased the radioactivity in intestinal ROI. Folic acid pre-treatment did not exert any obvious influence on the time course in this ROI.

Elimination

Plasma radioactivity-time curves with a faster radioactivity decay in for the first hour followed by a more shallow phase were observed after a single dose of ^{99m}Tc-etarfolatide. The harmonic mean of the two half-lives were 25 minutes (90% CI 15-68 min) and 29 hours (20-58 h), respectively, and mean Cl 1 ml/min/kg (SD 0.43).

Over the 24 hour collection interval, 42.1 ± 11.8 % of the injected radioactivity was recovered in urine.

Urine samples from four of the patients were separated using HPLC before radiometric detection. In the 1h sample most radioactivity represented unchanged ^{99m}Tc-etarfolatide, but in the 4h sample there was a substantial amount of metabolite (34-81% unchanged). The most abundant metabolite (1.45-45%) had the same retention time as free ^{99m} Tc. In plasma, only the 30 minutes sample could be analysed, and at this time point the percentage of intact ^{99m}Tc-etarfolatide was generally more than 85%.

Plasma protein binding

Plasma protein binding was studied in samples by precipitating the plasma proteins with acetone and measuring radioactivity in supernatant and precipitate. The percentage of plasma protein binding varied between subjects between 11% and 63%, average 37%. The highest protein binding was found in samples from patients that had received folic acid pre-treatment.

Table 13: Plasma protein binding of ^{99m}Tc-etarfolatide

Patient #	Folic Acid (mg)	Protein Binding (%)				
		5 min	30 min	1 hr	2 hr	Average
1	0	15.38	14.62	7.67	13.34	12.8
2	0	12.00	9.65	11.89	9.45	10.8
3	0	29.86	35.09	34.63	20.90	30.1
4	0.5	36.41	42.67	46.65	52.08	44.5
5	2.0	41.80	57.17	69.40	64.38	58.2
8	1.0	59.33	73.21	62.82	56.78	63.0

Clinical study EC20.11

Study EC20.11 was a phase 1 clinical study to evaluate the biodistribution and safety of ^{99m}Tc-etarfolatide in healthy volunteers (N=20).

Approximately half of the participants entered into the study received 0.5 mg of folic acid. The remaining participants received 1.0 mg of folic acid (approximately split evenly between male and female within each dose group). The first imaging time point was done without folic acid. The second imaging time point was done with folic acid 1 to 3 minutes prior to ^{99m}Tc-etarfolatide injection, within 1 week but no sooner than 4 days after the first imaging time point.

Distribution

The organs/tissues receiving the largest dose equivalent were the urinary bladder wall followed by the kidneys and liver. The mean effective dose was 0.028 rem/mCi (0.0076 mSv/MBq), and there was no difference between the 2 groups of 0.5 mg and 1.0 mg folic acid pre-injection.

Dosimetry estimates for ^{99m}Tc-etarfolatide based on data from participants who received 0.5 mg folic acid pre-injection are presented under section 2.6, Clinical Safety.

Elimination

The overall α- and β-clearance half-lives were 26.9 minutes and 50.95 hours, respectively, and the overall steady state volume of distribution was 2.24 L/kg with a clearance of 1.38 ml/(min kg). Kidneys were the major route of elimination, 41% of the injected radioactivity was recovered in the urine within the first 24 hours of administration. Folic acid pre-injection at either 0.5 mg or 1.0 mg decreased radiation exposure in most organs.

The urinary excretion of radioactivity at 8-24 hours post injection of ^{99m}Tc-etarfolatide in all participants (N=20) was 40.9 ± 10.0 % injected dose, and there was no major difference between those who received 0.5 mg folic acid from those who received 1.0 mg folic acid pre-injection.

Special populations

No studies in special populations were submitted.

Pharmacokinetic interaction studies

In study EC20.1, four patients did not receive folic acid pre-treatment, two received 0.5 mg, one 1 mg and one 2 mg. ^{99m}Tc-etarfolatide Vss decreased with increasing folic acid pre-treatment. Participants with the lowest clearance were found in the group without folic acid pre-treatment.

2.4.3. Pharmacodynamics

Mechanism of action

^{99m}Tc-etarfolatide is a companion imaging agent to vintafolide designed to identify FR-positive lesions. This is accomplished by chemical linkage of the ^{99m}Tc chelating agent to folate, i.e. the same targeting moiety as in vintafolide. The FR is expressed on some, mainly low differentiated and highly proliferative tumours. ^{99m}Tc-etarfolatide is uptaken by the FR and ^{99m}Tc is internalised thereby providing the basis for SPECT imaging detection of FR positive tumours.

Primary and Secondary pharmacology

Only *in vitro* and *in vivo* pharmacology data in animals were submitted (see section 2.3.2).

2.4.4. Discussion on clinical pharmacology

The substance applied for is the chelating agent etarfolatide, but all clinical pharmacology studies have been performed on the complex ^{99m}Tc-etarfolatide, which is considered to be the actual drug substance, and has been measured with radioactivity detection. No analytical method for unlabelled etarfolatide was developed and the fate of unbound excess etarfolatide has not been addressed, which is acceptable considering the low toxicity in both preclinical and clinical studies.

Pharmacokinetic data derived from the two phase 1 studies, EC20.1 and EC20.11.

In study EC20.1, drug related components appeared to distribute extensively in the body and a considerable proportion of the dose (63%) remained in the body at 24 hour, the time of the last scan. The claim that pre-treatment with folic acid accelerated loss of whole body radioactivity was not adequately supported by clinical data. The data provided suggested an effect of folic acid on the Vss, but not on weight-normalised clearance.

In the whole body scintigraphy, the highest levels of radioactivity were found in the liver, but declined fast. The kidney also showed a substantial drug uptake, and the gastrointestinal tract, especially in cancer patients. The applicant suggested that the relatively high hepatic distribution observed was attributable to the highly perfused nature of the organ together with the possible presence of folate receptors on activated Kupffer cells in the liver. As the compound is to be administered as a low single dose agent and the drug does not appear to persist in the liver it is considered that interactions in the liver are unlikely.

^{99m}Tc-etarfolatide was only modestly bound to plasma proteins based on the precipitation method used. However, the methodology used to assess protein binding in the patient samples involved the addition of folic acid to the samples and acetone to denature the samples which is not considered to be an accepted methodology to measure free drug in plasma. Therefore, conclusions to be drawn from this data are limited. *In vitro* data showed that ^{99m}Tc-etarfolatide is 74% and 68% bound to serum proteins in rat and humans (at 50 – 1000 nM), respectively.

Elimination of radioactivity after administration of ^{99m}Tc-etarfolatide was initially fast, followed by a slower phase with a half-life of 29 hours. The longer half-life might reflect slow redistribution from peripheral compartment binding sites to the central compartment from which relatively rapid urinary excretion takes place. It should however be noted that 63% of the dose remained in the body at 24 hours and the clearance value of 1.0 ml/min/kg was low.

EC20.1 study included 24 hour urine collection, which should account for more than half of the excreted drug considering the terminal half-life of around 29 hours. Data suggested that most of the ^{99m}Tc-etarfolatide is excreted in the urine, mainly as unchanged drug but also as metabolites.

Based on pharmacokinetics data from study EC20.11, the distribution and elimination half-lives of ^{99m}Tc-etarfolatide were estimated to be 27 minutes and 51 hours, respectively. The radioactive half-life of ^{99m}Tc is 6 hours. Results from study EC20.11 showed that elimination occurred primarily by clearance through the kidney and liver, with excretion into the bladder and intestines. About 41% of the decay-corrected radioactive dose was recovered in urine within the first 24 hours after injection. Kidneys were the major route of elimination of radioactivity from the body.

There is no data available in patients with impaired organ function. Due to the single dose administration and thus short exposure to ^{99m}Tc-etarfolatide, and considering the benign safety profile observed in both non-clinical and clinical studies, the lack of pharmacokinetic data in special populations can be accepted. However, due to the high renal excretion of ^{99m}Tc-etarfolatide, patients with renal impairment are likely to have an increased drug/radioactivity exposure. Therefore, care must be exercised in patients with impaired renal function (see SmPC section 4.4 Special warnings and precautions for use).

No *in vitro* drug-drug interaction studies were submitted. Since etarfolatide is given as a single dose, and no pharmacological effect has been observed, it is considered that the risk of

pharmacokinetic interactions is low, and no data are required. Based on data from 8 patients in the EC20.1 study, pre-treatment with folic acid may decrease the distribution volume of ^{99m}Tc-etarfolatide, which may be due to blocking of peripheral FR binding sites. Therefore, co-administration of folate supplement or anti-folate therapy must be avoided for 24 hours prior to receiving Folcepri due to the risk of competitive affinity to the folate receptor which may compromise the imaging quality (see SmPC section 4.4 Special warnings and precautions for use).

Etarfolatide is a diagnostic agent given at a low dose (0.1 mg) and is not expected to have a pharmacodynamic effect. No clinical studies have been submitted to contribute to the knowledge of etarfolatide's pharmacodynamics. From the pre-clinical pharmacology data, it appears that the mechanism of action for Folcepri is based on its binding capacity to folate receptors (see section 2.3.6 on non-clinical aspects).

2.4.5. Conclusions on clinical pharmacology

Pharmacokinetic data showed that radiolabelled Folcepri is distributed throughout the body within several minutes of injection. Normal organs that show significant uptake of radiolabelled Folcepri are the kidneys, spleen, liver and urinary bladder contents. Only minor accumulation and retention were visible in other organ systems. Also, there appears to be greater diffused abdominopelvic uptake in women with ovarian cancer versus that seen in normal volunteers. This observation may reflect either an increase in tumour-specific FR binding sites (i.e. diffuse FR-positive peritoneal tumour deposits) or an increased leakage of Folcepri into the peritoneal space due to neoplasia-related inflammation of the peritoneal membrane and the presence of ascites.

There is limited knowledge on the elimination of etarfolatide. However, since the drug will be given as a single administration only, the dose is low (0.1 mg) and the compound has shown a benign safety profile both non-clinically and clinically, additional studies are not required.

2.5. Clinical efficacy

The clinical efficacy dossier consisted of six clinical studies which can be grouped according to their objectives:

1. Use of folic acid pre-injection: Studies EC20.1 and EC20.11
2. Optimal Imaging time point: Study EC20.2
3. Studies supporting the clinical utility of ^{99m}Tc-etarfolatide:
    - Study EC-FV-02 (single-agent vintafolide for advanced ovarian cancer),
    - Study EC-FV-03 (single-agent vintafolide for advanced NSCLC)
    - Study EC-FV-04 (randomised trial of vintafolide+PLD versus PLD alone for treatment of platinum-resistant ovarian cancer)

The clinical evidence provided to support the clinical utility of ^{99m}Tc-etarfolatide to select patients for treatment with vintafolide was based on clinical studies evaluating the clinical efficacy of vintafolide in treating patients who are SPECT/planar image positive following injection of ^{99m}Tc-etarfolatide.

2.5.1. Dose response study

No study evaluating the relationship between plasma concentration and effect was submitted. The dose was selected based on a formulation study looking at radiochemical purity.

A single dose level of 0.1 mg has been used in clinical studies. The activity dose of the 740-925 MBq (20-25 mCi) was chosen to maximize image quality, and to keep radiation exposure within acceptable limits.

Since only a small fraction of etarfolatide chelates the technetium-99m, the objective in selecting an etarfolatide dose was to identify the lowest dose of etarfolatide that meets specification of radiochemical purity or percentage of technetium-99m chelated to etarfolatide. Higher amounts of etarfolatide did not significantly improve radiochemical purity. According to the formulation study, 0.1 mg was the minimum amount of etarfolatide that provided the proper ratio for efficient radiolabeling with technetium-99m.

Table 14: Etarfolatide Radiochemical Purity by Dose

etarfolatide (mg)	Radiochemical Purity
0.01	85.6
0.05	91.7
0.10	93.7
0.20	94.5
0.50	95.0
1.00	94.2
2.00	95.4

2.5.2. Exploratory studies

The use of folic acid pre-injection (studies EC20.1 and EC20.11)

Study EC20.1

In non-clinical studies, pre-injection of a small amount of folic acid improved tumour-to-background ratios. As part of study EC20.1, a visual assessment by readers of ^{99m}Tc-etarfolatide nuclear scan images with different doses of folic acid was explored.

Two healthy volunteers and 2 ovarian cancer patients received injections of 0.5, 1.0, or 2.0 mg IV folic acid 1–2 minutes prior to the injection of ^{99m}Tc-etarfolatide (1 healthy volunteer each received 0.5 mg and 2.0 mg, 1 ovarian cancer patient each received 0.5 mg and 1.0 mg folic acid).

The planar images of a healthy volunteer and a patient without pre-injection of folic acid compared to a healthy volunteer and a patient with a 0.5 mg folic acid pre-injection are presented below. Single Photon Emission Computed Tomography (SPECT) scans were obtained 1-4 hours post ^{99m}Tc-etarfolatide administration.

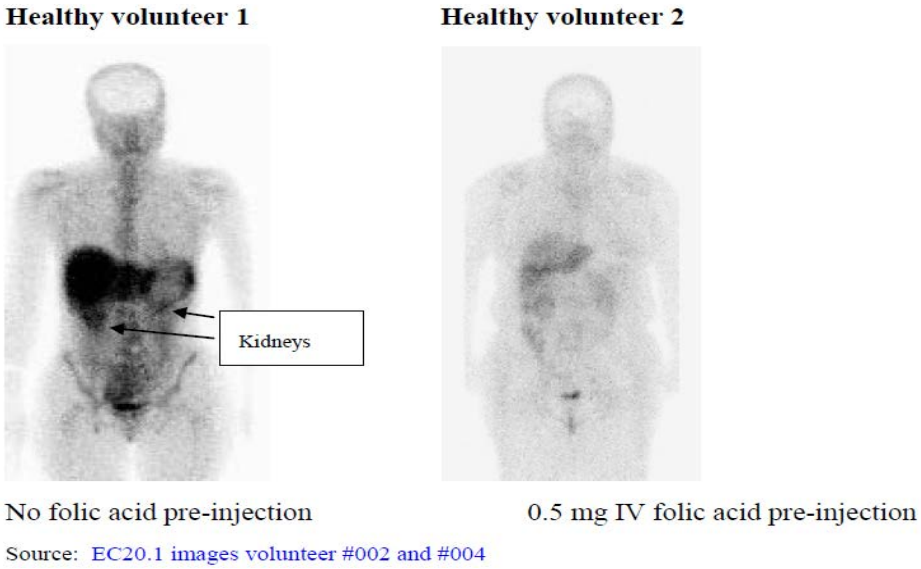


Figure 2: Planar Images Without and With Folic Acid Pre-injection in healthy volunteers

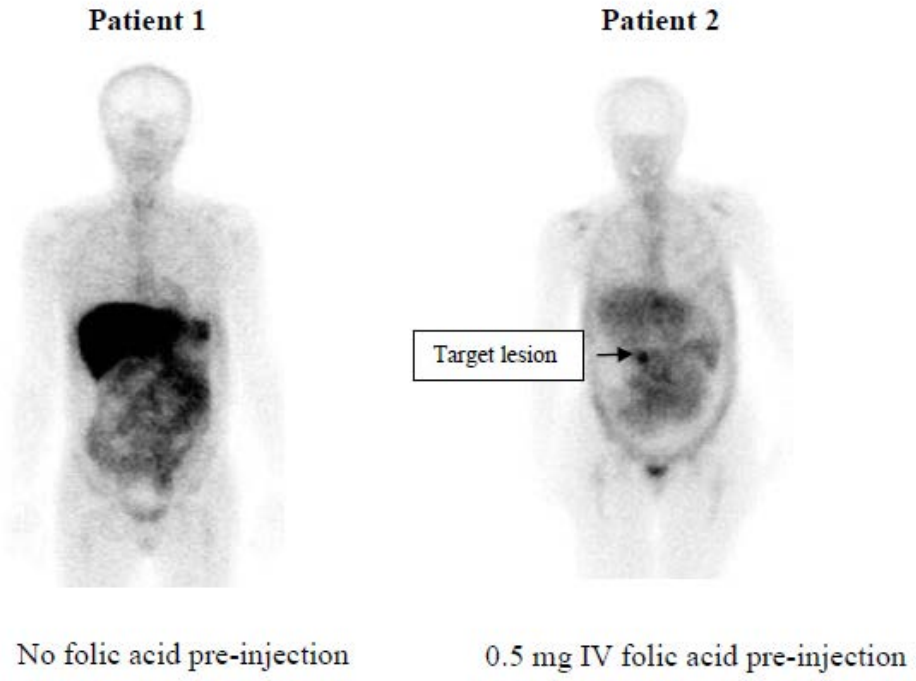


Figure 3: Planar images with and without Folic Acid Pre-injection in Ovarian Cancer Patients

Study EC20.11

In this study, all participants (N=20) first received ^{99m}Tc-etarfolatide SPECT and planar imaging without folic acid. Participants then underwent a second ^{99m}Tc-etarfolatide imaging procedure after being allocated to receive either a 0.5 (N=11) or 1.0 mg (N=9) folic acid 1 to 3 minutes pre-injection. The protocol stipulated that the second scan must occur within 4 to 7 days of the first scan in order for an acceptable wash-out period of any residual ^{99m}Tc-etarfolatide.

For the Qualitative Image Analysis, two sets of SPECT images for each participant were evaluated by an independent nuclear medicine radiologist blinded to folic acid pre-injection. The blinded nuclear medicine radiologist evaluated each set of images and determined a global assessment of activity/distribution by choosing the image showing the least amount of ^{99m}Tc-etarfolatide background activity/distribution or selecting the option in which both images are equivocal. In 11 out of 11 and 9 out of 9 cases from the 0.5 and 1.0 mg folic acid cohorts, respectively, the blinded independent nuclear medicine radiologist selected the SPECT image associated with folic acid pre-injection as showing lower ^{99m}Tc-etarfolatide activity.

The quantitative assessment results of the study showed that there was a decreased %ID in the abdominal normal organs/tissue (intestines, left kidney, liver, spleen) following pre-injection of folic acid, compared to scans without any pre-injection of folic acid.

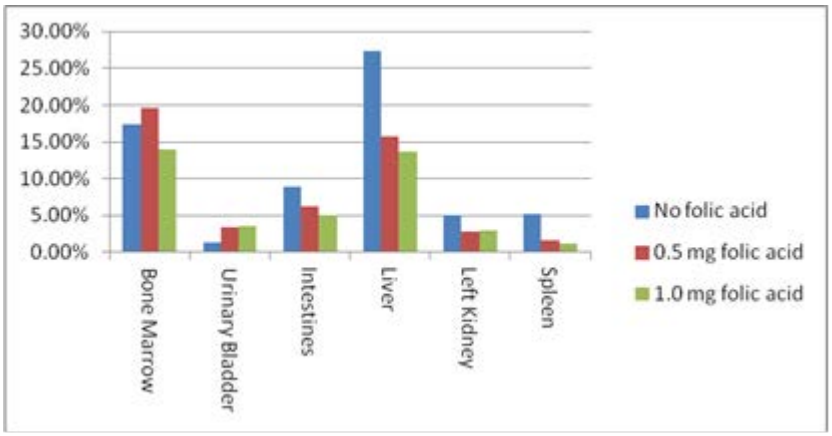


Figure 4: Change in mean ^{99m}Tc-etarfolatide activity for selected organs based on pre-injected folic acid dose level

Optimal Imaging time point (Study EC20.2)

The EC20.2 study was a multi-centre study to evaluate the safety and efficacy of ^{99m}Tc-etarfolatide in women with suspected ovarian or endometrial cancer.

The objectives of this study were to

- Expand the safety database,
- Optimise the timing and techniques for image acquisition,
- Gather preliminary efficacy data for the radioactive drug product (^{99m}Tc-etarfolatide), and
- Assay masses for the presence of folate receptor.

The study population included female patients 18 years and older who had suspected ovarian cancer or metastatic or recurrent ovarian or endometrial cancer with a known pelvic mass diagnosed by ultrasound, magnetic resonance imagining (MRI), or computed tomography (CT); who either (1) had a fixed paraffin-embedded tissue sample available for immunohistochemical (IHC) staining from a previous pathological evaluation or (2) were scheduled for a procedure to obtain tissue for pathological evaluation that could be fixed and paraffin embedded for IHC staining; and who had adequate kidney function (creatinine value of <2.0 mg/dL within the previous 30 days) were eligible for the study.

At the time of surgery, representative fresh tissue samples (e.g., uterus, ovary, omentum, peritoneal surfaces, endometrium) from each specimen were collected by the gynaecologist/oncologist or pathologist. An IHC stain was used to test each tissue sample for folate receptor. The results of the folate receptor assays were compared with the blinded-to-outcome reads.

At approximately 1 hour post-injection and at 2 to 4 hours post-injection, mid-thigh to head, anterior and posterior planar scintigrams were to be acquired for each patient. Single-photon emission computed tomography (SPECT) images of the pelvis and lower abdomen were obtained at the last imaging time point (i.e., 2 to 4 hours post-injection) immediately before the final mid-thigh to head planar images was acquired.

Off-site image evaluations were to be performed at a centralised location that was not involved in the study and were to be done by readers who had not had contact with the patients, investigators, or other individuals involved in the study; the off-site image evaluations were to be used to demonstrate efficacy. On-site image evaluations were to be performed to support the off-site image evaluations. Sequential unblinding was performed: a fully blinded image evaluation and a blinded-to-outcome image evaluation. The results of the 2 blinded evaluations were compared with the results of the evaluation of the truth standard (pathological diagnosis [benign versus malignant] at the time of surgery) to determine the diagnostic performance of ^{99m}Tc-etarfolatide.

Efficacy was to be evaluated using Bayesian 2-by-2 analyses to compare the results of ^{99m}Tc-etarfolatide imaging (both fully blinded and outcome blinded reads) to histopathology in terms of sensitivity, specificity, accuracy, positive predictive value (PPV), and negative predictive value (NPV).

The study was initiated on 06 August 2002 and the last patient visit occurred on 23 May 2003.

Twelve (12) patients received a bolus IV injection of folic acid, followed 1 to 3 minutes later by a bolus intravenous injection of etarfolatide labelled with 15-25 mCi of ^{99m}Tc-etarfolatide.

The on-site nuclear medicine physician visually evaluated the images from the 1 and 2 to 4 hour time points and determined that the 1 hour time point yielded optimal image quality in the majority of patients (8/12).

Table 15: Optimal Planar Imaging Time Point

Time Point	Fully Blinded Evaluation		Blinded to Outcome Evaluation	
	N=12		N=12	
	n	%	n	%
1 Hour	8	66.7	8	66.7
2 to 4 Hour	4	33.3	4	33.3

Overall, based on blinded-to-outcome evaluation of the ^{99m}Tc-etarfolatide images, the investigator's evaluation of the ^{99m}Tc-etarfolatide images agreed with the IHC assay results for 58.3% (7/12) of the patients. The Kappa coefficient was 0.063, indicating poor agreement between the two methods.

Table 16: Distribution of Principal Investigator's ^{99m}Tc-etarfolatide Blinded-to-Outcome Evaluations and Immunohistochemical Results

	IHC Positive	IHC Negative	Total
FolateScan FR+	6	4	10
FolateScan FR-	1	1	2
Type of Agreement			
Overall Percent Agreement of FolateScan With IHC			58.3% (7/12)
Agreement of FolateScan Positive With IHC Positive			85.7% (6/7)
Agreement of FolateScan Negative With IHC Negative			20.0% (1/5)
Kappa Coefficient			0.063

FR, folate receptor; IHC, immunohistochemical

2.5.3. Main studies

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.

The 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/hand-foot syndrome, 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 20 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,

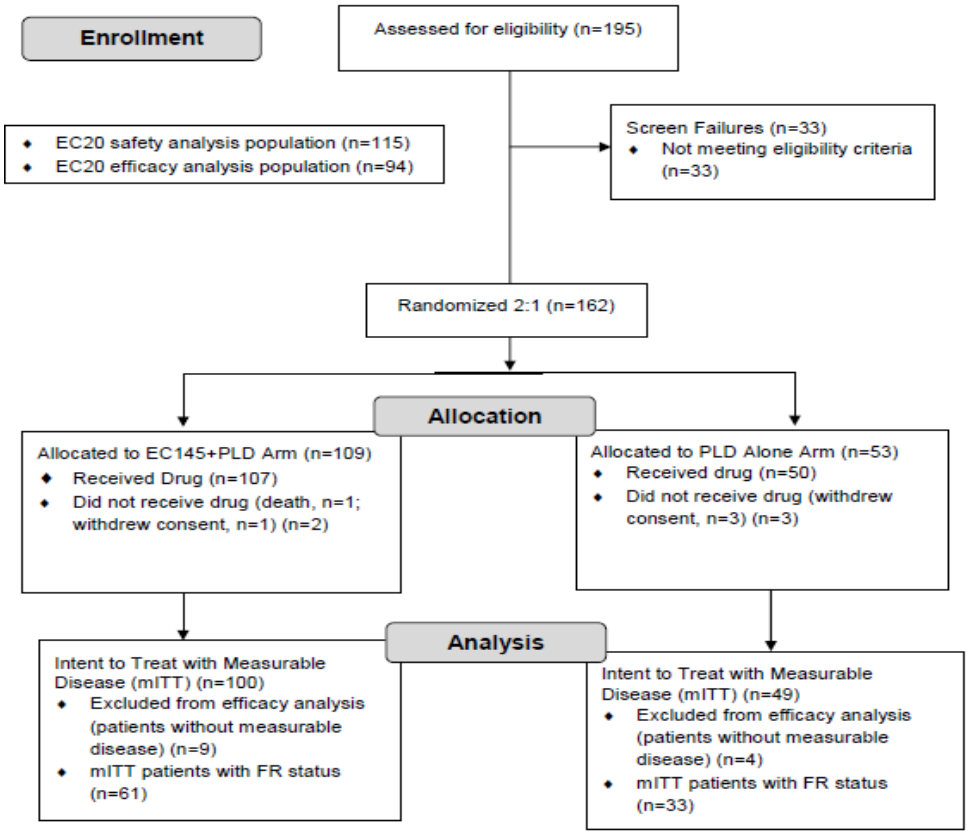
non-waivers and waivers; Sensitivity analysis for unscheduled assessments; Sensitivity analysis including patients with non-measureable disease.

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 17: 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 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 18: 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 19: Disease Characteristics at Screening (FR(++) Population)

Disease Characteristic	FR(++) Population	
	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 20: 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 21: 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

Table 22: Summary of efficacy results of study EC-FV-04 (mITT, FR(100%), FR(0%))

FR status	mITT N=149		FR(100%) N=38		FR(0%) N=20	
	EC145+ PLD N=100	PLD Alone N=49	EC145+ PLD N=23	PLD Alone N=15	EC145+ PLD N=13	PLD Alone N=7
ORR (%)	28.0	16.3	17.4	6.7	30.8	0.0
DCR (%)	73.0	53.1	73.9	26.7	84.6	71.4
Median PFS (wks)	21.7	11.7	24.0	6.6	16.6	23.3
PFS HR (95% CI) log rank p-value	0.626 (0.409, 0.959) p=0.031		0.381 (0.172, 0.845) p=0.013		1.806 (0.369, 8.833) p=0.468	
Median OS (mos)	14.1	16.8	14.0	16.0	15.9	21.2
OS HR (95% CI) log rank p-value	1.010 (0.679, 1.503) p=0.957		1.097 (0.525, 2.296) p=0.805		1.529 (0.468, 4.998) p=0.479	

ORR=Overall Response Rate; DCR=CR+PR+SD

Primary endpoint: PFS

Two sensitivity analyses were undertaken, the first evaluating the impact of considering lesions in the liver as being non-evaluable instead of FR positive, the second of using a maximum of 5 target lesions (RECIST 1.1) instead of 10 (RECIST 1.1). For those patients with more than 5 lesions, the five largest lesions were used in the sensitivity analyses.

Sensitivity Analysis	PFS Hazard Ratio (p-value) [95% Confidence Interval]	
	FR(100%)	FR(10-100%)
Base Case -Target lesion located in liver are considered FR-positive -Up to 10 target lesions selected for ^{99m}Tc-etarfolatide assessment.	(N=38) 0.381 (0.013) [0.172,0.845]	(N=74) 0.547 (0.041) [0.304,0.983]
Sensitivity 1: Target lesions located in liver are considered non-evaluable	(N=30) 0.360 (0.021) [0.145,0.893]	(N=63) 0.665 (0.193) [0.358,1.236]
Sensitivity 2: 5 target lesions (RECIST 1.1)	(N=39) 0.381 (0.013) [0.172,0.845]	(N=74) 0.547 (0.041) [0.304,0.983]

PFS effect size as a function of etarfolatide scan positivity (mITT)

Analyses of PFS incorporating FR status were conducted with the aim to evaluate the relationship between the vintafolide/PLD PFS effect size and the level of FR/scan positivity. The 94 mITT patients with ^{99m}Tc-EC20 scan results were included in these correlative analyses.

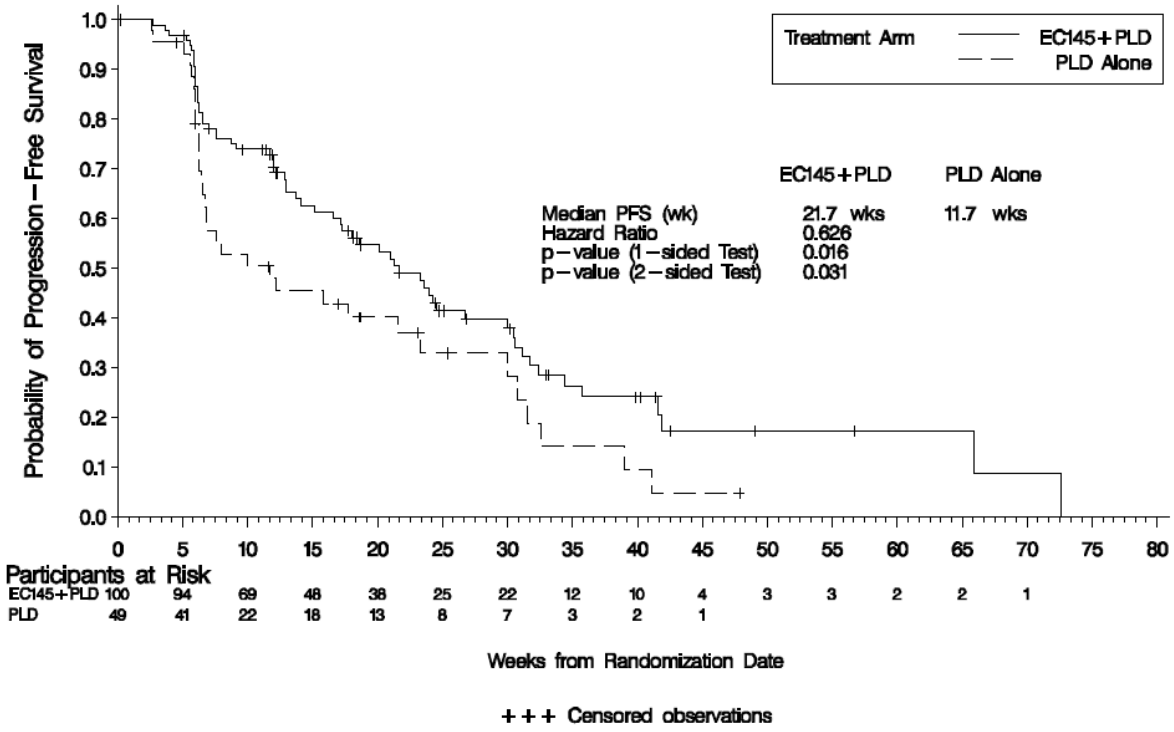
The distribution of the number of patients and PFS events by level of FR positivity is shown in the table below. The two largest subgroups of patients and events occurred for the FR(100%) and FR(0%) status levels. The range of positivity exclusive of these two subgroups (i.e., >0% and <100%) was supported by a total of 36 patients and 21 PFS events for both arms combined.

Table 23: Distribution of Patients and PFS Events by FR Status (N=94) – EC-FV-04

	Percent of Positive Lesions						
	0	> 0 – 20	> 20 – 40	> 40 - 60	> 60 – 80	> 80 – 99	100
N	20	2	11	13	10	0	38
PFS Events	10	1	9	6	5	0	28

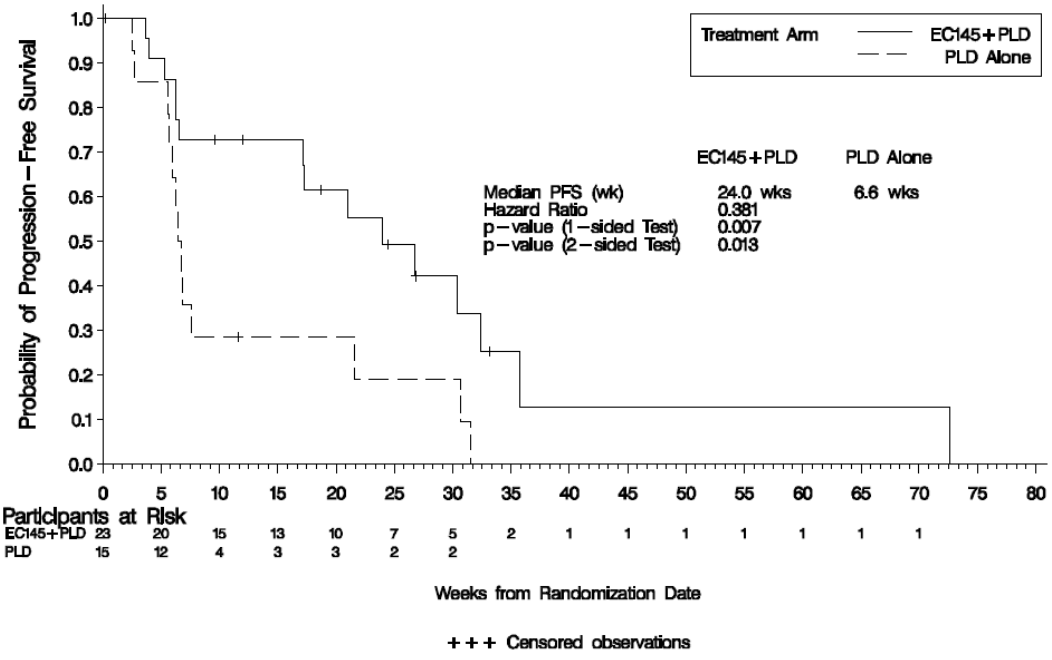
Table 24: Threshold analysis of PFS based on FR Status/ EC20 scan (N=94) - EC-FV-04

	EC145+PLD		PLD		
	N (events)	Median (Wks)	N (events)	Median (Wks)	HR (95% CI)
mITT	100 (62)	21.7	49 (33)	11.7	0.626 (0.409, 0.959)
FR(100%)	23 (15)	24.0	15 (13)	6.6	0.381 (0.172, 0.845)
FR(10-100%)	48 (30)	24.6	26 (19)	7.6	0.547 (0.304, 0.983)
FR(10-90%)	25 (15)	24.6	11 (6)	30.0	0.873 (0.334, 2.277)
FR(0%)	13 (8)	16.6	7 (2)	23.3	1.806 (0.369, 8.833)



Source: Figure 14.12.1

Figure 5: Kaplan-Meier Curve of PFS by treatment arm EV-FV-04 (mITT Population)



Source: Figure 14.16.1

Figure 6: Kaplan-Meier curve of PFS by treatment arm (FR (100%) Population)

Table 25: 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.

Table 26: 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.

Secondary endpoints:

Table 27: 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 28: Overall Response Rate and Disease Control Rate by treatment arm (FR(100%) 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.

Inter-reader and intra-reader variability study (Study 203119)

Study 203119 was a sub-study of the pivotal study EC-FV-04 assessing inter- and intra-reader variability based on ^{99m}Tc-etarfolatide SPECT images obtained.

Inter-reader agreement

Three nuclear medicine physicians were selected by the independent core imaging lab to provide a blinded independent assessment of the ^{99m}Tc-etarfolatide (EC20) scan images. The readers were trained by the core imaging lab using a training program which utilised a representative subset of images from study EC-FV-04. These training images were not included in the variability study.

All readers were blinded to study arm (vintafolide/PLD or PLD only), outcome (response to therapy) and patient clinical information such as height, weight, ascites, CA-125, etc.).

Prior to images being presented to the Nuclear Medicine physicians for review, the images underwent a thorough quality control process. CT images along with corresponding ^{99m}Tc-etarfolatide SPECT images were then randomly presented to the blinded independent readers for review. There was no communication between the readers during the review process or between the SPECT readers and the CT reader.

For all participants who received the ^{99m}Tc-etarfolatide scan, a blinded independent radiologist used RECIST v1.1 to select up to 10 measurable target lesions, with up to 5 designated as primary target lesions by the radiologist. The 5 primary target lesions were used for the 5 lesion agreement assessment. The target lesions identified on CT scans were provided to the two blinded independent

readers, and adjudicator as necessary; thus, all readers used the same set of target lesions for each patient.

The readers assessed preselected target lesions on SPECT scans for ^{99m}Tc-etarfolatide uptake. Target lesions not evaluable for ^{99m}Tc-etarfolatide uptake were identified as follows:

- Target lesions < 15 mm in size not classified as definitely positive
- Target lesions where no SPECT data were available
- Missing anatomy
- Poor image quality

Lesions in or in close proximity to areas of high background such as the kidney, bladder, or spleen may have been considered non-evaluable by the reader. Lesions in close proximity to the liver may also have been considered non-evaluable.

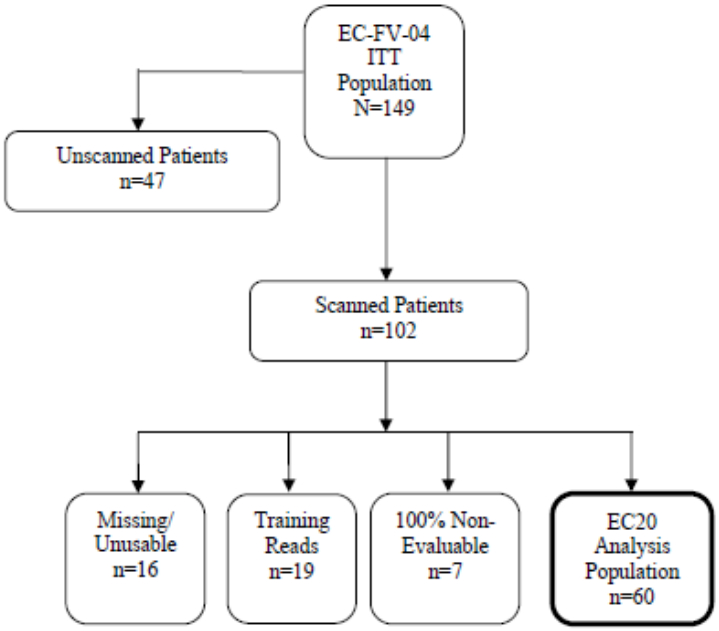
All lesions in the liver were considered FR-positive. This was based on lesion response data from the vintafolide single-agent study for patients with advanced ovarian cancer (EC-FV-02) which showed that liver lesions had a response (16.1%) to EC145 similar to FR-positive lesions (9.4%) as opposed to FR-negative lesions (0.0%). It was considered that although liver lesions cannot be evaluated for FR expression because of high background, these lesions can be considered FR-positive based on response to vintafolide.

If the two independent readers disagreed on the number of positive lesions, adjudication was conducted by a third qualified reader. The adjudicator was required to agree with one of the two independent readers. For purposes of calculating the agreement, the agreement rate was based on thresholds of positivity. The independent blinded assessment and adjudication was conducted under an independent review charter.

In this study the following threshold definitions were used:

- FR(++) threshold means 100% target lesions ^{99m}Tc-etarfolatide positive vs. less than 100% positive.
- FR(50) threshold means at least 50% target lesions ^{99m}Tc-etarfolatide positive vs. less than 50% of the target lesions ^{99m}Tc-etarfolatide positive.
- FR(+) threshold means at least one target lesion ^{99m}Tc-etarfolatide positive versus all target lesions ^{99m}Tc-etarfolatide negative

Patient Disposition



The EC20/^{99m}Tc-etarfolatide analysis population consisted of all Study EC-FV-04 participants where both readers agreed the patient had an evaluable ^{99m}Tc-etarfolatide scan.

Table 29: Patient Agreement Summary and PABAK Results for FR-positive thresholds

FR-Positive Threshold	Agreement (95% CI)	Kappa (95% CI)	PABAK (95% CI)
FR(+) threshold	87% (75.4%, 94.1%)	0.4783 (0.17, 0.79)	0.73 (0.51, 0.88)
FR(50) threshold	68% (55.0%, 79.7%)	0.3736 (0.16, 0.59)	0.37 (0.10, 0.59)
FR(++) threshold	85% (73.4%, 92.9%)	0.6098 (0.38, 0.84)	0.70 (0.47, 0.86)

Table 30: Agreement Summary on a Maximum of 5 lesions

	Agreement (95% CI)	Kappa (95% CI)
Patient Agreement		
FR(+) vs. FR(-) (10 lesions)	87% (75.4%, 94.1%)	0.4783 (0.17, 0.79)
FR(+) vs. FR(-) (5 lesions)	85% (73.4%, 92.9%)	0.4398 (0.14, 0.74)
FR(++) vs. FR(<++) (10 lesions)	85% (73.4%, 92.9%)	0.6098 (0.38, 0.84)
FR(++) vs. FR(<++) (5 lesions)	82% (69.6%, 90.5%)	0.5576 (0.33, 0.79)
Lesion Agreement		
Evaluable vs. Non-Evaluable (10)	89% (85.5%, 92.7%)	0.6872 (0.59, 0.79)
Evaluable vs. Non-Evaluable (5)	90% (85.5%, 93.9%)	0.6379 (0.50, 0.78)
Positive vs. Negative (10)	78% (71.5%, 82.9%)	0.5136 (0.40, 0.62)
Positive vs. Negative (5)	78% (70.5%, 83.6%)	0.4770 (0.34, 0.61)

The consequences of a misread in relation to treatment decision was evaluated based on the misread type. As part of the two-reader inter-reader study, adjudication was performed when readers disagreed in order to determine the correct or "truth read". The read not accepted by the adjudicator was considered a "misread". There were 60 cases in the study and 9 "misreads". Misreads were divided into three categories as shown in the table below. The data indicated the most likely misread is an FR(100%) patient being assessed as FR(<100%), thus resulting in not being eligible for vintafolide treatment. This occurred 12% of the time. 3% of the time was an FR(0%) patient misread as FR(100%), thus resulting in a patient potentially considered eligible but who won't benefit from being treated.

Table 31: EC-FV-04: Clinical Implication for Potential Misread and Observed Rates

Misreads	**% (n)**	**Clinical Implication**
FR(0%) misread as FR(100%)	3% (2/60)	Patient who won't benefit from treatment, FR(0%), is misread as FR(100%) and treated with vintafolide+PLD
FR(10-90%) misread as FR(100%)	0% (0/60)	Patient who may benefit from treatment is misread as FR(100%) and treated with vintafolide+PLD
FR(100%) misread as FR(0-90%)	12% (7/60)	Patient who will benefit, FR(100%) and not treated with vintafolide+PLD.
Total Misreads	15% (9/60)	

Intra-reader study

The objective of the intra-reader study was to determine the reproducibility of the ^{99m}Tc-etarfolatide reads as assessed by the same reader after a period of time. The pre-specified threshold for positivity was 1 or more positive lesions out of a maximum of 10 evaluated lesions.

The primary objective of the intra-reader study was to validate the reproducibility of ^{99m}Tc-etarfolatide to select FR(+) patients. The study assessed the within-reader agreement rate for determinations of FR(+) versus FR(-) based on images from the EC-FV-04 study in platinum resistant ovarian cancer.

Secondary objectives were to

1. Assess intra-reader agreement on determination of FR status using kappa statistics.

2. Measure intra-reader agreement on a lesion basis.

3. Measure intra-reader agreement for other FR thresholds such as at least half (1/2) lesions positive [FR(50)] and all lesions positive [FR(++)].

4. Assess the agreement when only a maximum of 5 lesions are evaluated.

The secondary analysis based on a maximum of 5 lesions was added to determine if the agreement rates at the various thresholds were at least as high as the agreement rates based on a maximum of 10 lesions.

Following the completion of the inter-reader study, the intra-reader component of the study was performed using two readers from the inter-reader study. There was a 28-day minimum "washout" period before each reader performed the second reads. Each reader re-read the same 20 randomly selected cases, but each ^{99m}Tc-etarfolatide image was presented to each reader in a random order. The readers did not reference any read results from the prior study. The readers did not discuss any of these cases at any time during the read process. Each reader used the same method to read the randomly presented cases as they had utilised in the previous read process: readers identified ^{99m}Tc-etarfolatide-evaluable target lesions for selection of ^{99m}Tc-etarfolatide scores. Evaluable lesions were identified as either positive or negative.

Table 32: Patient Agreement Summary and PABAK results for FR-positive thresholds

	FR-Positive Threshold	Agreement (95% CI)	Kappa (95% CI)	PABAK (95% CI)
Reader 1	FR(+) threshold	90% (68.3%, 98.8%)	0.4444 (-0.20, 1.00)	0.80 (0.34, 0.98)
	FR(50) threshold	85% (62.1%, 96.8%)	0.6591 (0.31, 1.00)	0.70 (0.24, 0.94)
	FR(++) threshold	100% (83.2%, 100%)	1.0000 (1.00, 1.00)	1.00 (0.66, 1.00)
Reader 2	FR(+) threshold	95% (75.1%, 99.9%)	0.6429 (0.01, 1.00)	0.90 (0.50, 1.00)
	FR(50) threshold	95% (75.1%, 99.9%)	0.6429 (0.01, 1.00)	0.90 (0.50, 1.00)
	FR(++) threshold	95% (75.1%, 99.9%)	0.9000 (0.71, 1.00)	0.90 (0.50, 1.00)

Table 33: Agreement Summary of a Maximum of 5 lesions

		Agreement (95% CI)	Kappa (95% CI)
Reader 1	**Patient Agreement**		
	FR(+) vs. FR(-) (10 lesions)	90% (68.3%, 98.8%)	0.4444 (-0.20, 1.00)
	FR(+) vs. FR(-) (5 lesions)	90% (68.3%, 98.8%)	0.4444 (-0.20, 1.00)
	FR(++) vs. FR(<++) (10 lesions)	100% (83.2%, 100%)	1.0000 (1.00, 1.00)
	FR(++) vs. FR(<++) (5 lesions)	90% (68.3%, 98.8%)	0.7980 (0.53, 1.00)
	Lesion Agreement		
	Evaluable vs. Non-Evaluable (10)	89% (80.5%, 94.5%)	0.70 (0.53, 0.87)
	Evaluable vs. Non-Evaluable (5)	92% (82.7%, 97.4%)	0.69 (0.44, 0.94)
	Positive vs. Negative (10)	90% (80.4%, 96.4%)	0.76 (0.58, 0.94)
	Positive vs. Negative (5)	90% (79.0%, 96.8%)	0.68 (0.42, 0.94)
Reader 2	**Patient Agreement**		
	FR(+) vs. FR(-) (10 lesions)	95% (75.1%, 99.9%)	0.6429 (0.01, 1.00)
	FR(+) vs. FR(-) (5 lesions)	95% (75.1%, 99.9%)	0.6429 (0.01, 1.00)
	FR(++) vs. FR(<++) (10 lesions)	95% (75.1%, 99.9%)	0.9000 (0.71, 1.00)
	FR(++) vs. FR(<++) (5 lesions)	95% (75.1%, 99.9%)	0.8936 (0.69, 1.00)
	Lesion Agreement		
	Evaluable vs. Non-Evaluable (10)	97% (90.1%, 99.3%)	0.87 (0.72, 1.00)
	Evaluable vs. Non-Evaluable (5)	97% (89.2%, 99.6%)	0.65 (0.20, 1.00)
	Positive vs. Negative (10)	99% (92.8%, 100.0%)	0.93 (0.78, 1.00)
	Positive vs. Negative (5)	98% (91.1%, 100.0%)	0.92 (0.78, 1.00)

Ancillary analyses

Not applicable.

Supportive studies

EC-FV-02

EC-FV-02 was a Phase 2, multicentre, open-label, non-randomised study of the companion imaging diagnostic agent ^{99m}Tc-etarfolatide (EC20) and the therapeutic agent vintafolide (EC145), administered as a single agent, in a broad spectrum of adult patients with advanced epithelial ovarian, primary peritoneal, fallopian tube, or endometrial cancer.

The patients were heavily pre-treated (1-14 prior therapies) and had a large tumour burden (mean sum of longest diameters of target lesions was 13.9 cm). Patients were imaged with ^{99m}Tc-etarfolatide prior to treatment with single-agent vintafolide.

The study was conducted in 2 parts (Part A and Part B). In Part A of the study, both patients with EC20-positive tumours and patients with EC20-negative tumours were enrolled, and no limit was placed on the maximum number of prior therapies. After an interim review of data for the first 44 EC145-treated patients showed better activity for EC145 in patients with EC20-positive tumours

who had received ≤3 prior therapies, the protocol was amended to include only patients with EC20-positive tumours and to limit the number of prior therapies to ≤4 (Part B). An additional 5 patients were treated with EC145 under Part B of the study. The final analysis of the study was conducted on combined data from Part A and Part B of the study.

Following the completion of all screening procedures and confirmation of eligibility, all patients received a single intravenous (IV) injection of 0.5 mg of folic acid, followed within 1 to 3 minutes by a 1- to 2-ml injection of 0.1 mg of EC20 labelled with 20 to 25 mCi of technetium-99m. Patients then underwent planar imaging (mid-thigh to head, posterior and anterior images) 1 to 2 hours after injection of ^{99m}Tc-EC20. Single photon emission computed tomography (SPECT) images of the region(s) known to contain the target lesion(s) were obtained immediately following the acquisition of the planar images.

Target lesions were selected by the radiologist according to Response Evaluation Criteria in Solid Tumours (RECIST), Version 1.0 (Therasse 2000). Nuclear medicine physicians then visually assessed ^{99m}Tc-EC20 uptake for each target lesion and classified the uptake as positive (marked or mild uptake) or negative (no uptake). Lesions <1.5 cm in size were considered non-evaluable unless the reader marked them as having ^{99m}Tc-EC20 uptake. A lesion was considered non-evaluable if it was not imaged, negative and <15 mm in diameter, or located in organs in which high background compromised evaluation (e.g., kidney, liver, spleen or bladder).

Based on ^{99m}Tc-EC20 scan results, patients were placed into 1 of 4 groups:

Group	Patient Score	Description
$EC20^{++}$	100%	All target lesions EC20 positive
$EC20^{+}$	1%-99%	At least one EC20-positive target lesion but not all target lesions EC20 positive
$EC20^{-}$	0%	No EC20-positive target lesions with at least one evaluable target lesion
$EC20^{?}$	Nonevaluable	No evaluable target lesions

The ^{99m}Tc-etarfolatide status was calculated based on percentage of ^{99m}Tc-etarfolatide positive target lesions. Thirty-nine (39) patients in the modified intent-to-treat population (N=43) had an evaluable ^{99m}Tc-etarfolatide/EC20 status: 14 (32.6%) patients were classified as EC20++ (FR100%), 22 patients (51.2%) as EC20+ (FR10-90%), and 3 patients (7.0%) as EC20- (FR0%) and 4 patients (9.3%) were classified as EC20?.

There were 209 lesions evaluated in this study. The ^{99m}Tc-etarfolatide status of these lesions was: 110 positive lesions, 29 negative lesions, and 70 non-evaluable. However, for 32 lesions the patient came off therapy prior to the first post-baseline RECIST assessment. Thus only 93 FR positive and 14 FR negative lesions are included.

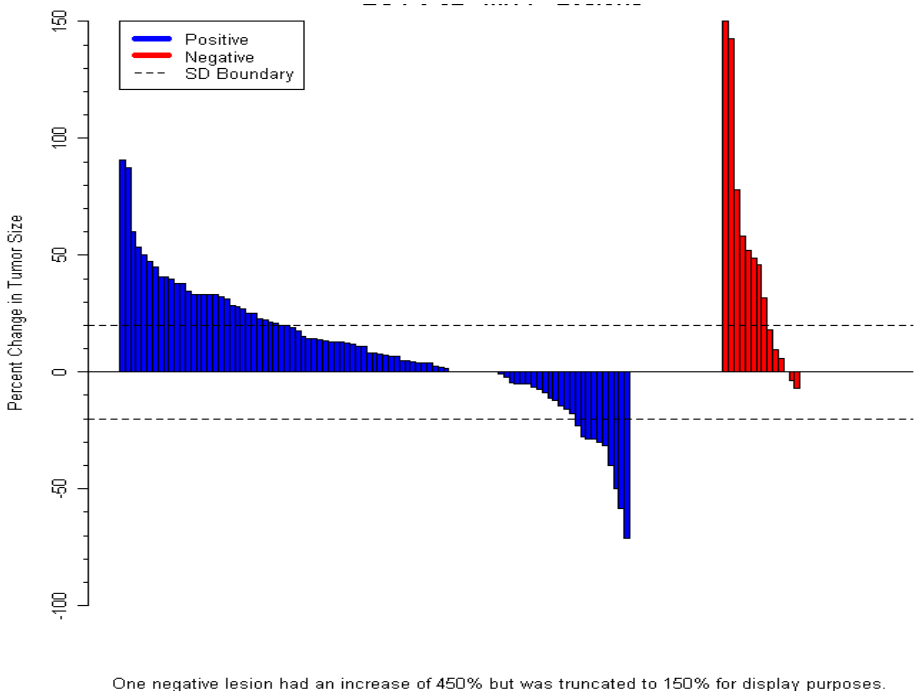


Figure 7: Lesion analysis by FR status - Study EC-FV-02

EC-FV-03

EC-FV-03 was a Phase 2, multicentre, open-label, non-randomised study of vintafolide (EC145) in adult patients with histologically confirmed adenocarcinoma of the lung that had previously been treated with ≥2 cytotoxic-containing chemotherapeutic regimens.

After completion of all screening procedures and confirmation of eligibility, all patients received one intravenous (IV) injection of 0.5 mg of folic acid, followed within 1 to 3 minutes by a 1- to 2-ml injection of 0.1 mg of EC20 labelled with 20 to 25 mCi of technetium-99m. Patients then underwent planar imaging (mid-thigh to head, posterior and anterior images) 1 to 2 hours after injection of ^{99m}Tc-EC20.

Single photon emission computed tomography (SPECT) images of the region(s) known to contain the target lesion(s) were obtained immediately following the acquisition of the planar images. Target lesions were selected by the radiologist according to Response Evaluation Criteria in Solid Tumours (RECIST) Version 1.0. Nuclear medicine physicians then visually assessed ^{99m}Tc-EC20 uptake for each target lesion and classified the uptake as either positive (marked or mild uptake) or negative (no uptake). Target lesions < 15 mm in size were considered non-evaluable unless the reader marked them as having ^{99m}Tc-EC20 uptake. Target lesions that were located in organs of high background uptake (e.g., liver, spleen, bladder, and kidney) were also considered non-evaluable.

A patient score was calculated by dividing the total number of EC20-positive target lesions by the total number of target lesions. Based on EC20 scan results, patients were placed into 1 of 3 groups, as defined below:

Group	Patient Score	Description
EC20(++)	100%	All target lesions EC20 positive
EC20(+)	1%-99%	At least one EC20-positive target lesion but not all target lesions EC20 positive
EC20(-)	0%	No EC20-positive target lesions with at least one evaluable target lesion

Only patients that had EC20 positive target lesions, i.e., at least EC20(+) according to above description, were to be treated with EC145.

Of the 29 patients who were included in the mITT analysis set, 14 patients (48.3%) were classified as EC20++/FR(100%), 14 patients (48.3%) were classified as EC20+/FR(10-90%), and 1 patient (3.4%) was classified as EC20-/FR(0%).

There were 115 lesions evaluated in this study. The ^{99m}Tc-etarfolatide status of these lesions was: 73 positive lesions, 28 negative lesions, and 14 non-evaluable. However, for 31 lesions the patient came of study prior to the first RECIST assessment. Therefore, the waterfall plot includes 70 lesions (53 FR-positive and 17 FR-negative).

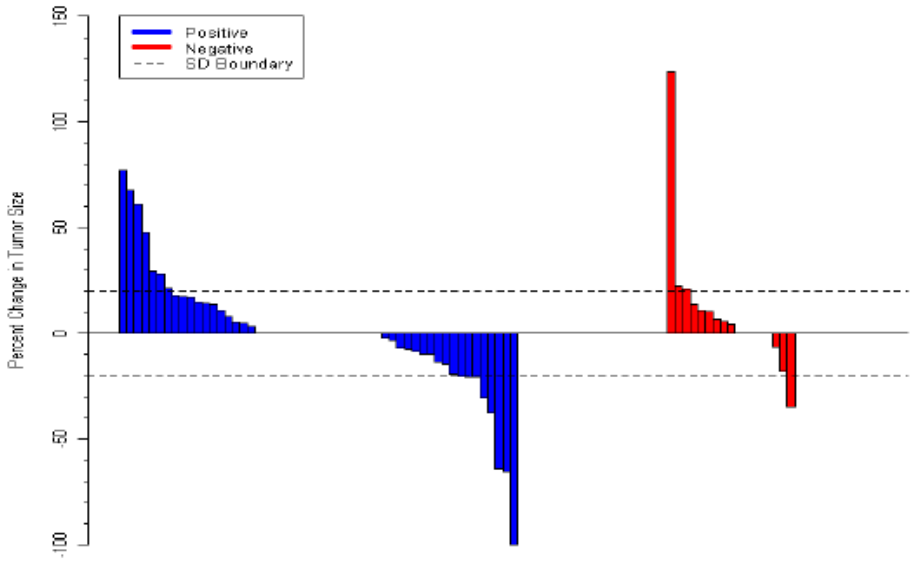


Figure 8: Lesion analysis by FR status - Study EC-FV-03

2.5.4. Discussion on clinical efficacy

Design and conduct of clinical studies

The main challenge when developing a new diagnostic is to identify the standard of truth. In the early studies of the development program IHC was used as truth standard. Attempts to correlate IHC with ^{99m}Tc-etarfolatide outcome were unsuccessful in the exploratory study EC 20.2, especially with respect to specificity of FR scanning using IHC as truth standard, but available data are very limited. The applicant also argued that the use of tissue-based diagnostic tests such as IHC as "truth standard" was problematic as ^{99m}Tc-etarfolatide and IHC measure different entities.
^{99m}Tc-etarfolatide measures the percentage of target lesions that show radiotracer uptake and is a

whole body assessment of FR expression, whereas IHC measures receptor density on a single tissue sample. Also, ^{99m}Tc-etarfolatide and vintafolide bind to functional or active FR expressed on membranes accessible to blood, whereas IHC detects functional and non-functional FR expressed on all membranes.

Therefore, the studies program was turned towards predictions of clinical efficacy of vintafolide, i.e. the clinical utility for ^{99m}Tc-etarfolatide in detecting patients that would benefit from treatment with vintafolide. This approach was considered acceptable.

As such, all of the studies submitted in support of the clinical utility of Folcepri contained data relevant to the ^{99m}Tc-etarfolatide and vintafolide. Supportive studies (EC-FV-02 and EC-FV-03) were non-comparative studies with regards to the therapeutic agent vintafolide. Comparative data relevant to the diagnostic agent, ^{99m}Tc-etarfolatide, are available from one phase 2 study (EC-FV-04) which evaluated treatment with vintafolide and pegylated liposomal doxorubicin (PLD) in combination versus treatment with PLD alone.

An issue common to all the studies was the limited numbers of patients in each arm for subgroups based on the levels of scan positivity/negativity.

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 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 analysis

Dose finding and optimal time for imaging

Results from the formulation study provided showed the efficiency of different doses of etarfolatide in binding to the radioisotope ^{99m}Tc. The chelation of etarfolatide with ^{99m}Tc during the labelling procedure resulted in less than 5% of the total injected dose consisting of ^{99m}Tc-etarfolatide (data not shown). Therefore a large percentage of the injected dose is unlabelled or “cold” etarfolatide. This cold etarfolatide may also bind to the folate receptor, essentially competing with ^{99m}Tc-etarfolatide for folate receptor binding sites on tumour tissues, thereby reducing the activity in the tumour. Therefore, the objective in selecting an etarfolatide dose was to identify the lowest dose of etarfolatide that meets specification of radiochemical purity or percentage of technetium-99m chelated to etarfolatide. According to the formulation study, 0.1 mg was the minimum amount of etarfolatide that provides the proper ratio for efficient radiolabeling with technetium-99m. Higher amounts of etarfolatide did not significantly improve radiochemical purity.

Although the formulation study does not provide direct evidence of the dose of etarfolatide needed for adequate imaging of all tumours in patients with any particular diagnosis, the approach followed is supported by a non-clinical study evaluating the impact of adding cold etarfolatide to a 0.5 mg dose of ^{99m}Tc-etarfolatide on radioactivity in the tumour (data not shown) where increasing

amounts of cold etarfolatide resulted in a reduction in the percentage of the injected dose of ^{99m}Tc-etarfolatide bound to a folate receptor positive tumour xenograft. Therefore, the applicant approach to keep the dose of etarfolatide as low as possible was endorsed by the CHMP.

The optimal time for imaging was studied as part of the clinical study EC20.2. The on-site nuclear medicine physician designated the 1-hour planar imaging time point as the optimal imaging time point for 8 (66.7%) of the 12 patients based on both the fully blinded and blinded-to-outcome evaluations. Study EC20.2 did not include a specific comparison of SPECT (rather than planar) images at 1 and 2-4 hours, since SPECT images were not obtained at 1 hour. The SPECT images at 2-4 hours had higher sensitivity compared with planar imaging at 1 and 2-4 hours. This greater ^{99m}Tc-etarfolatide positivity was considered to be related to the imaging modality since SPECT in general has greater sensitivity than planar imaging. Overall, the evidence to support the SPECT imaging at 1 hour is partly based on the conducted study EC20.2 but also based on multiple considerations including reasonable clinical practicality for patients as well as parameters such as radioactivity half-life and pharmacokinetics. This approach was considered acceptable by the CHMP.

An improvement in tumour to non-tumour (i.e. background) ratios was observed in preclinical studies following pre-injection of a small amount of folic acid before administering ^{99m}Tc-etarfolatide (see non-clinical section). The claim that pre-injection of folic acid improves imaging ^{99m}Tc-etarfolatide was evaluated in studies EC20.1 and EC20.11 in which some of the participants received an injection of 0.5 to 2.0 mg of folic acid 1 to 3 minutes before the injection of ^{99m}Tc-etarfolatide. In study EC20.1, although the data were based on four images each in 4 different subjects and therefore limited, folic acid at a dose of 0.5 mg prior administration of ^{99m}Tc-etarfolatide has been shown qualitatively to improve imaging by suppressing background uptake. The provided data from study EC20.11 showed a decreased uptake of ^{99m}Tc-etarfolatide in the normal abdominal organs with pre-injection of folic acid, and thereby supports the claim that intravenous folic acid could enhance the SPECT image quality, by reducing background uptake. Oral folic acid and folinic acid are not substitutes for intravenous folic acid due to receptor affinity and bioavailability differences relative to intravenously administered folic acid.

Clinical utility

A FR score was used to create three groups of patients, FR(100%) meaning that all lesions are FR positive, FR(0%) meaning that all lesions are FR negative and FR(10–90%) falling in between. This approach was considered reasonable.

Results from the supportive clinical studies EC-FV-02 and EC-FV-03 suggested a clinical utility for ^{99m}Tc-etarfolatide in detecting patients that may benefit from treatment with vintafolide. In these studies, it was shown on lesion level, especially in study EC-FV-02, that ^{99m}Tc-etarfolatide negative lesions are unlikely to respond by tumour shrinkage to vintafolide. Thus, the sensitivity of ^{99m}Tc-etarfolatide scan appears acceptably demonstrated from a clinical perspective whilst specificity cannot be assessed as absence of tumour shrinkage might be a consequence of absence of FR expression as well as resistance to vintafolide.

In the randomised comparative study (EC-FV-04) the clinical utility of ^{99m}Tc-etarfolatide scanning was further investigated. Even though only 94 patients contributed with scan and efficacy data it is considered sufficiently well demonstrated that patients with negative FR scans do not benefit from treatment with vintafolide whilst a benefit was observed in terms of improvement in progression free survival in the patients with 100% scan positive target lesions.

As study EC-FV-04 was a randomised study, it was possible to differentiate between the prognostic and predictive value of ^{99m}Tc-etarfolatide. The outcome in the PLD alone arm was poor in the FR(100%) subgroup compared with FR(0%). This was expected based on external data. FR expression as measured by ^{99m}Tc-etarfolatide thus has a prognostic value in ovarian cancer. If only median PFS is considered, add-on vintafolide seems to overcome the negative prognostic value of FR(100%) expression.

PFS in relation to the scan status showed an improving hazard ratio with increasing levels of scan positivity. Further, the population with 10-90% FR/scan positivity encompassed a wide range of scan positivity and a more precise threshold determination would have been useful. The "negative" effect on PFS in the FR (0%) is, in the absence of treatment related deaths, highly likely to be caused by baseline imbalances in this very small subgroup. With respect to the intermediate outcome in the FR(10-90%) subgroup, further analyses will be undertaken in the ongoing confirmatory study (EC-FV-06) with the aim to provide physicians with further data to guide the use of vintafolide. Currently, however, the indication should be restricted to patients with all lesions being FR positive.

Altogether, even though cut-offs were rather arbitrary (but for 100% and 0%), data from study EC-FV-04 confirm the clinical utility of ^{99m}Tc-etarfolatide scanning.

Read procedure of the scans

In the EC-FV-04 study, FR positivity was determined following the etarfolatide read procedure based on visual assessment. Readers assessed target lesions as FR positive (marked or mild uptake) or FR negative (no uptake). The applicant argued that quantitative tumour to background scoring was explored and compared with visual binary assessment of lesions and no relationship between tumour/background ratio and response was observed whilst visual yes/no scoring correlated to response. In order to ensure consistency in the etarfolatide scan assessment the applicant proposed a detailed training program that includes numerous examples and test cases. The CHMP agreed that the attempt to construct a "signal to noise" score for the identification of positive lesions was unsuccessful and that a detailed training programme was needed to further optimise sensitivity and specificity of the test.

The reliability and reproducibility of assessing folate-receptor positive patients was assessed in study 203119, a sub-study of the pivotal study EC-FV-04 evaluating inter- and intra-reader variability. In the selected subgroup of scans of good quality and with specifically trained readers, the inter-reader agreement was moderate to substantial both on a patient and lesion level. As expected the intra-reader agreement rates were better (substantial to almost perfect) than the inter-reader rates. It was noted that kappa statistics might be regarded as too conservative in case of distribution imbalances as in the FR(+) vs. FR(-) cut-off.

The applicant also provided an analysis of the clinical implications for potential misread. Misclassification in the order of 15% was considered high with the most frequent consequence being that a misread patient will not receive vintafolide, which may be a loss of chance. However, there are currently no alternative methods at hand providing more accurate data as regards FR expression suitable to guide FR targeting therapy with vintafolide. In the absence of objective criteria defining lesion positivity, and in order to reduce inter-reader variability, the CHMP requested that detailed guidance is provided to the readers of scans in the SmPC (see section 4.4 Special warnings and precautions) in addition to the readers training programme.

Regarding alternative means to reduce reader variability, an evaluation of the use of SPECT/CT cameras for image acquisition is included in the phase 3 study, EC-FV-06. These images could potentially increase the reliability of the read by providing integrated CT and SPECT images.

Due to high background activity, hepatic lesions are non-evaluable with ^{99m}Tc-etarfolatide. Based on preclinical data indicative of activity of vintafolide also in known FR negative lesions these lesions were by default considered FR positive. Circumstantial data support the notion that liver lesions should be regarded as FR positive. In Study EC-FV-02, patients were treated with single agent vintafolide and tumour shrinkage was compared between positive lesions, negative lesions, and liver lesions. The analysis showed that the liver lesions responded similarly to FR-positive lesions in that these lesions showed tumour shrinkage. In the sensitivity analysis performed in study EC-FV-04, a minor shift in the HRs followed as a consequence of liver lesions considered non-evaluable or FR positive. In this study, patients with liver lesions were assigned to the FR(100%) subgroup if all extra-hepatic lesions were FR positive. The CHMP considered that this should be reflected in the SmPC to adequately informed scan readers (see SmPC section 4.4, Special warnings and precautions). Further information on this aspect in the FR(10-90%) group might also be available from the confirmatory study, if a sufficient number of patients were included based only on liver lesion by default regarded as FR positive.

Image acquisition

The spatial resolution of the SPECT method used in the trial (15 mm) was considered poor and an imaging procedure with higher resolution would have been desirable, but was not generally available at time of initiation of the study. It is not self-evident how a higher resolution would impact on clinical utility of ^{99m}Tc-etarfolatide, i.e. the benefit/risk of vintafolide. The CHMP recommended that alternative methods with higher resolution should be considered in the future. In addition, the CHMP considered that ^{99m}Tc-etarfolatide should only be used for the assessment of functional folate receptor expression on tumour lesions and not for staging and restaging of ovarian cancer (see SmPC section 4.4, Special warnings and precautions).

Use of ^{99m}Tc-etarfolatide with other therapeutic agents

Etarfolatide and vintafolide share structural and functional characteristics which enable their use as a companion diagnostic imaging agent and therapeutic combination. Based on these shared structural and functional mechanisms, it is reasonable to assume that etarfolatide would be effective in predicting response to other FR-targeted agents, as long as these same criteria are met. The benefit of using etarfolatide might be possible to extrapolate to a new medicinal product provided that folic acid is the targeting part of the medicinal product. As for any new therapeutic medicinal product, a favourable benefit/risk balance for the new product in association with etarfolatide must be demonstrated. Other products such as those using monoclonal antibodies for targeting, however, are unlikely to have similar benefit from the etarfolatide diagnostic procedure as etarfolatide targets active alpha and beta FR, whilst monoclonal antibodies based product might be of benefit also in case of non-active FR and might in addition be designed to target only alpha or beta receptors. Considering the above, the CHMP recommended that the indication should at this stage be restricted as a predictive marker to vintafolide.

Additional efficacy data needed in the context of a conditional MA

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.

The clinical utility of Folcepri in detecting patients suitable for treatment with vintafolide is based on efficacy data 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 in order to confirm the benefit of vintafolide in combination with PLD in the intended indication, and thus the clinical utility of Folcepri.

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.

2.5.5. Conclusions on the clinical efficacy

Clinical utility of Folcepri as a companion diagnostic to vintafolide is considered well documented based on available clinical efficacy data with vintafolide. Based on the fact that there are missing efficacy data for vintafolide, the CHMP considers the following measures necessary to address the missing clinical utility data of Folcepri in the context of a conditional MA:

- 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, to further support the clinical utility of ^{99m}Tc-etarfolatide scan for selection of patients for treatment with vintafolide in combination with PLD
    - Final clinical study report: March 2017

2.6. Clinical safety

Safety data were provided from 13 completed studies in which 563 patients were administered at least one dose of ^{99m}Tc-etarfolatide.

Table 34: Summary of Clinical Studies and Tabulation of Subjects Contributing to the Safety Analysis of ^{99m}Tc-etarfolatide

Study Number	Study Phase	Cancer Type(s)	AE reporting window	No of Pts[2]
EC20.1	Phase 1 – Safety and Dosimetry	Ovarian	24 hrs	8
EC20.2	Phase 1 – Safety	Ovarian, Endometrial	12-72 hrs	12
EC20.3	Phase 2 – Exploratory	Renal cell carcinoma	30 days	43
EC20.4		Ovarian, Endometrial	30 days	16
EC20.7		Pituitary tumour	7 days	7
EC20.8		Solid tumours	30 days	15
EC20.9		Renal cell carcinoma	7 days	74
EC-FV-04	Phase 2 – Diagnostic/Therapeutic Pivotal Combination Study	Ovarian, fallopian tube, primary peritoneal	7 days[1]	115
EC-FV-02	Phase 2 – Diagnostic/Therapeutic Combination Study	Ovarian, endometrial, primary peritoneal, endometrium	7 days[1]	64
EC-FV-03	Phase 2 – Diagnostic/Therapeutic Combination Study	Lung	7 days[1]	60
EC-0225-01	Phase 1 – Diagnostic/Therapeutic Combination Studies	Solid tumors	7 days[1]	74
EC-0489-01	Phase 1 – Diagnostic/Therapeutic Combination Study	Solid tumors	7 days[1]	57
EC-FI-004	Phase 2 – Diagnostic/Therapeutic Combination Study	Renal cell carcinoma	7 days[1]	18

[1] Seven days or until administration of the first dose of therapeutic, whichever came first. Adverse events reported as "possibly" "probably" or "definitely" related to ^{99m}Tc-etarfolatide that occurred beyond the 7 day reporting window but occurred before administration of the first dose of therapeutic drug were also included.
[2] Number of patients who received at least one dose of ^{99m}Tc-etarfolatide

Patient exposure

Table 35: Patient Exposure (Data Cut-Off 1 September 2011)

	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	0	0	0	0
Open studies	637	563	469	0
Post marketing	0	0	0	0
Compassionate use	0	0	0	0

[1] Number of patients that signed informed consent at sites participating in etarfolatide imaging
[2] Number of patients that received at least one dose of etarfolatide
[3] Number of patients that received at least one dose of 20-25 mCi etarfolatide
* In general this refers to 6 months and 12 months continuous exposure data, or intermittent exposure.

A total of 563 study participants received 581 doses (18 patients received an additional dose). The mean injection volume was 1.4 ml, and the mean injected decay corrected ^{99m}Tc-etarfolatide activity was 23 mCi, indicating good compliance with the ^{99m}Tc-etarfolatide dosing instructions which state that patients should receive 20-25 mCi. No patients received unlabelled Folcepri.

The patients ranged from 28 to 88 years with a median age of 61 years. The vast majority of the patients were white (501 subjects, 89.0%) while a total of 45 patients (8%) were black or African American. As regards gender, the majority was female (60.2%).

Table 36: Demographic characteristics – ^{99m}Tc-etarfolatide safety population

Category	**Subjects**	
Age Group and Gender	Male	Female
Age group 1 ≥ 65 years	82	116
Age group 2 < 65 years	142	223
Ethnicity		
Hispanic or Latino	15	
Not Hispanic or Latino	527	
Missing	21	
Race		
White	501	
Black or African American	45	
Asian	10	
Native Hawaiian/Other Pacific Islander	1	
Other	2	
Missing	4	
Special Populations		

Pregnant women	0
Lactating women	0
Renal impairment	0
Hepatic impairment	0
Cardiac impairment	0

Adverse events

Safety assessments included summaries of treatment emergent adverse events (TEAEs) regardless of drug causality, drug-related TEAEs, other serious TEAEs, withdrawals due to AEs, laboratory findings, and vital signs. The analyses based on the safety population included all events according to the following adverse event reporting periods.

The adverse event reporting period for the studies with ^{99m}Tc-etarfolatide alone was protocol specific, ranging from 12 hours to 30 days following ^{99m}Tc-etarfolatide administration.

The diagnostic/therapeutic combination studies were designed to gather data regarding the use of ^{99m}Tc-etarfolatide to identify patients likely to respond to FR targeted therapies. The ^{99m}Tc-etarfolatide adverse event reporting period for these studies was 7 days following ^{99m}Tc-etarfolatide administration or until administration of the first dose of therapeutic whichever occurred first. Adverse events related to ^{99m}Tc-etarfolatide that occurred beyond the 7 day reporting window but occurred before administration of the first dose of therapeutic drug were included in all ^{99m}Tc-etarfolatide safety reporting analyses. Unrelated adverse events were not included. For patients who underwent an optional second ^{99m}Tc-etarfolatide imaging scan, any ^{99m}Tc-etarfolatide adverse events with an onset > 30 days after the last dose of therapeutic and within 7 days after the second administration of ^{99m}Tc-etarfolatide were also included in all safety reporting analyses.

Table 37: Overall summary of TEAE - ^{99m}Tc-etarfolatide Safety Population (n=563)

	^{99m} Tc-etarfolatide (N=563) n (%)
Number of Participants Reporting at Least One TEAE	115 (20.4%)
Number of Participants Reporting at Least One Treatment-Emergent Serious Adverse Event	24 (4.3%)
Number of Participants Reporting at Least One Treatment-Emergent Drug-Related Adverse Event	19 (3.4%)
Number of Participants Reporting at Least One Treatment-Emergent Drug-Related Adverse Event Resulting in Withdrawal of ^{99m}Tc-etarfolatide	1 (0.2%)
Number of Participants Reporting at Least One Treatment-Emergent Drug-Related Serious Adverse Event	1 (0.2%)
Number of Participants Reporting at Least One Treatment-Emergent Drug-Related Serious Adverse Event Resulting in Withdrawal of ^{99m}Tc-etarfolatide	0 (0.0%)
Number of Participants Reporting at Least One Treatment-Emergent	2 (0.4%)

Drug-Related Adverse Event of Grade 3 or 4	
Number of Participants Reporting at Least One Treatment-Emergent Drug-Related Serious Adverse Event of Grade 3 or 4	1 (0.2%)
Number of Deaths (Grade 5)	2 (0.4%)
Number of Deaths (Grade 5) within 7 days post ^{99m}Tc-etarfolatide	1 (0.2%)[1]
Number of Drug-Related Deaths	0 (0.0%)

Adverse events are coded in accordance with MedDRA Dictionary (Versions 6.0-12.0). Grades are based on CTCAE V3.0. Drug-related adverse events include those with a definite, probable, or possible drug-relationship.
[1]Patient EC20.2 001-009 died 27 days after receiving ^{99m}Tc-etarfolatide; since the AE started within 7 days post ^{99m}Tc-etarfolatide it is included as a serious non-drug related AE (exacerbation of her ovarian cancer) resulting in Death (Grade 5) within 7 days post ^{99m}Tc-etarfolatide

Treatment-emergent adverse events by grade regardless of causality

A total of 115 subjects (20.4%) had at least one TEAE, regardless of drug relationship. The most common TEAEs (≥1%) were nausea (2.3%), abdominal pain (1.6%), anorexia (1.4%), dyspnoea (1.4%), vomiting (1.6%), constipation (1.2%), anaemia (1.1%), and fatigue (1.1%). No single TEAE occurred at a frequency higher than 2.3% and most TEAEs were Grade 1 (9.1%) or Grade 2 (6.0%). TEAE Grade 3 were reported in 25 subjects (4.4%) and consisted mainly of gastrointestinal disorders (1.8%) with abdominal pain (0.5%), ascites (0.7%) and nausea (0.5%). In addition, dyspnoea and pleural effusion were reported in 0.5% and 0.4% respectively.

A total of three (0.5%) subjects with Grade 4 were reported (pulmonary embolism, cerebrovascular accident and abdominal pain). Two Grade 5 fatal TEAEs were reported, however neither was considered related to ^{99m}Tc-etarfolatide.

In patients who received ^{99m}Tc-etarfolatide alone (N=175), a total of 23 patients (13.1%) who received ^{99m}Tc-etarfolatide had at least one TEAE, regardless of drug relationship. The most common TEAEs (≥1%) were protein urine present (2.3%), neutrophil percentage increased (1.7%), nausea (1.1%), lymphocyte percentage decreased (1.1%), and headache (1.1%). No TEAE occurred at a frequency higher than 2.3% and most TEAEs were Grade 1 or 2, with few Grade 3 or 4 events reported. Two Grade 5 fatal TEAEs were reported.

Drug-related treatment emergent AEs

TEAEs considered by the investigator to be drug-related were reported for 19 patients (3.4%). The majority being of Grade 1 or 2 whereas only a few Grade 3 events were reported (nausea, vomiting, and abdominal discomfort). Nausea and vomiting were the only drug-related TEAEs that occurred in more than one patient, with both occurring in three patients. No Grade 4 or Grade 5 drug-related TEAEs were reported. Pruritus was reported in 0.2% of patients.

Adverse events were further reviewed by the sponsor for causal relationship using the following criteria:

1. Clinical importance

2. Association with disease state or prior therapeutic treatment

The majority of adverse reactions attributed by the investigator had most likely an etiology due to the nature of the disease status of the patient.

Table 38: Etarfolatide adverse drug reactions associated with disease state

MedDRA Preferred Term	Time from etarfolatide Admin. to AE Onset (days)	Disease
Pain	3	Other (Endocervical)
Abdominal discomfort	1	Colorectal
Nausea	1	Head and Neck
Anorexia	5	Ovarian
Nausea	5	Ovarian
Vomiting	5	Ovarian
Constipation	0	Lung
Vomiting	3	Ovarian
Pruritus	0	Ovarian
Oedema peripheral	10	Primary Peritoneal
Nausea	0	Renal cell carcinoma
Vomiting	0	Renal cell carcinoma
Abdominal pain lower	6	Renal cell carcinoma
Dry skin	3	Ovarian
Skin exfoliation	3	Ovarian

Serious adverse event / deaths / other significant events

Deaths

Two patients (0.4%) experienced a fatal TEAE. Both were considered unrelated to ^{99m}Tc-etarfolatide.

A 56-year old male in Study EC20.3 with metastatic clear cell renal carcinoma received one dose of ^{99m}Tc-etarfolatide and completed his scan on Day 1 without incident. On Study Day 29, the patient was hospitalized due to significant deterioration in his performance status. A CT scan confirmed disease progression and the patient died on Study Day 30. The investigator assessed the death as due to disease progression and unrelated to ^{99m}Tc-etarfolatide.

A 51-year old woman in Study EC20.2 with ovarian cancer received one dose of ^{99m}Tc-etarfolatide and completed her scan on Day 1 without incident. The patient was reported to have an exacerbation of her ovarian cancer beginning on Study Day 1. She was hospitalized and died of disease progression on Study Day 27. The death was judged to be not related to ^{99m}Tc-etarfolatide.

Serious Adverse Events

At least one treatment-emergent SAE was reported in 24 (4.3%) of the 563 ^{99m}Tc-etarfolatide treated patients. Serious adverse events were most commonly related to the MedDRA Gastrointestinal Disorders (1.8%), with abdominal pain (0.7%), and ascites (0.7%) the most commonly reported SAEs. Serious adverse events (nausea and vomiting) were considered to be related to ^{99m}Tc-etarfolatide in one of the 563 patients (0.2%).

System Organ Class Preferred Term	^{99m} Tc-etarfolatide (N=563) n (%)
Number of Participants Reporting at Least One Serious TEAE	24 (4.3%)
Cardiac disorders	1 (0.2%)
Atrial flutter	1 (0.2%)
Gastrointestinal disorders	10 (1.8%)
Abdominal pain	4 (0.7%)
Ascites	4 (0.7%)
Constipation	1 (0.2%)
Intestinal obstruction	1 (0.2%)
Nausea	2 (0.4%)
Small intestinal obstruction	1 (0.2%)
Vomiting	2 (0.4%)
General disorders and administration site conditions	2 (0.4%)
Death	1 (0.2%)
Ulcer haemorrhage	1 (0.2%)
Hepatobiliary disorders	1 (0.2%)
Cholangitis	1 (0.2%)
Infections and infestations	1 (0.2%)
Pneumonia	1 (0.2%)
Musculoskeletal and connective tissue disorders	1 (0.2%)
Musculoskeletal chest pain	1 (0.2%)
Neoplasms benign, malignant and unspecified (incl cysts and	1 (0.2%)
Neoplasm progression	1 (0.2%)
Nervous system disorders	2 (0.4%)
Cerebrovascular accident	1 (0.2%)
Convulsion	1 (0.2%)
Renal and urinary disorders	1 (0.2%)
Hydronephrosis	1 (0.2%)
Respiratory, thoracic and mediastinal disorders	6 (1.1%)
Dyspnoea	2 (0.4%)
Pleural effusion	2 (0.4%)
Pneumothorax	1 (0.2%)
Pulmonary embolism	1 (0.2%)
Vascular disorders	1 (0.2%)
Vascular pseudoaneurysm	1 (0.2%)

Patients are counted once for each system organ class and for each preferred term. Adverse events are coded in accordance with MedDRA Dictionary (Versions 6.0-12.0). Grades are based on CTCAE V3.0.

Laboratory findings

Haematology, Clinical Chemistry and urine were assessed at baseline and 1-7 days post ^{99m}Tc-etarfolatide administration. Since they were not done centrally, no pooled analysis is

available. Results from individual studies showed no clinically important effects of ^{99m}Tc-etarfolatide on haematology, serum chemistry or urinalysis.

Vital signs

In the seven imaging studies where ^{99m}Tc-etarfolatide was administered alone (i.e. not followed by a therapeutic agent) prior to SPECT scanning, vital signs such as heart rate, respiratory rate, systolic / diastolic blood pressure and body temperature were collected and documented pre and post-injection of ^{99m}Tc-etarfolatide. There was no clinically significant change in any of the vital sign parameters.

Safety in special populations

Age

Table 39: Adverse Event Summary by Age

	Age <65 yrs (N=365) n (%)	65-74 yrs (N=154) n (%)	75-84 yrs (N=41) n (%)	85 + (N=3) n (%)
Total	78 (21.4%)	32 (20.8%)	5 (12.2%)	0 (0.0%)
Fatal	2 (0.5%)	0 (0.0%)	0 (0.0%)	0 (0.0%)
Serious	17 (4.7%)	7 (4.5%)	0 (0.0%)	0 (0.0%)
Withdrawal	1 (0.3%)	0 (0.0%)	0 (0.0%)	0 (0.0%)
CNS (confusion/extrapyramidal)[1]	1 (0.3%)	1 (0.6%)	0 (0.0%)	0 (0.0%)
AE related to falling [2]	1 (0.3%)	1 (0.6%)	0 (0.0%)	0 (0.0%)
CV events [3]	4 (1.1%)	2 (1.3%)	0 (0.0%)	0 (0.0%)
Cerebrovascular events[4]	3 (0.8%)	2 (1.3%)	0 (0.0%)	0 (0.0%)
Infections [5]	10 (2.7%)	4 (2.6%)	1 (2.4%)	0 (0.0%)

[1] CNS-Related includes Preferred Terms (if present): Confusional State, Extrapyramidal disorder, Memory impairment, Cognitive disorder, Tremor, Delirium
[2] Falling includes Preferred Term (if present): Fall, Gait disturbance, Ataxia, Balance disorder
[3] Cardiovascular-Related includes Preferred Term (if present): Palpitations, Myocardial infarction, Tachycardia, Arrhythmia supraventricular, Chest pain, Sinus tachycardia, Atrial flutter
[4] Cerebrovascular-Related includes Preferred Terms (if present): Cerebrovascular accident, Convulsion, Hemiparesis, Pulmonary embolism, Thrombosis, Venous thrombosis, Deep vein thrombosis, Iliac artery thrombosis
[5] Infection-Related includes SOC: Infections and infestations and Preferred Terms (if present): Leukocytosis, Febrile neutropenia, Pyrexia, Cholangitis acute, Cholecystitis acute, Leukocyturia, Productive cough

Overall, there was no clinically significant difference in the TEAEs that occurred in patients ≥ 65 years of age (n=198) compared with patients < 65 years of age (n=365), both regardless of drug causality or drug relatedness. Most TEAEs occurred in < 1% of patients and were of Grade 1 and 2.

For the age group < 65 a total of 78 (21.4 %) TEAEs were reported and for the ≥ 65 age group, 37 (18.7%). Similarly in both age groups the majority were of Grade 1 and Grade 2 and mostly related to the MedDRA Gastrointestinal disorders (25 subjects (6.8%) and 12 subjects (6.1%) respectively).

Two deaths occurred in the <65 age group while no deaths occurred in the ≥ 65 age group.

Treatment-Emergent drug-related AEs were few in both age groups (14 (3.8%) and 5 (2.5%) respectively) and mainly due to constipation, nausea and vomiting.

Gender

Overall, there was no clinically significant difference in the TEAEs that occurred in male patients (n=224) compared to female patients (n=339), regardless of drug causality or drug relatedness. Most TEAEs occurred in < 1% of patients and were of Grade 1 and 2. TEAEs for males were reported in 38 (17.0%) subjects and for females 77 (22.7%) subjects. Similar in both gender groups were MedDRA Gastrointestinal disorders as the most frequently reported; 12 (5.4%) and 25 (7.4%) subjects respectively.

Discontinuation due to adverse events

One of the 563 patients (0.2%) experienced one TEAE of tremor that was considered by the investigator to be related to ^{99m}Tc-etarfolatide and led to withdrawal of ^{99m}Tc-etarfolatide. The patient was a 62-year-old woman in Study EC-FV-02 with advanced ovarian cancer and a history of constipation, pulmonary coccidioidomycosis, chronic obstructive pulmonary disease (COPD), and hypertension who received a single bolus intravenous (IV) dose of 0.1 mg of ^{99m}Tc-etarfolatide. Three days later, the patient experienced moderate tremor, which the investigator considered as possibly related to ^{99m}Tc-etarfolatide. The event resolved and the patient began treatment with vintafolide.

^{99m}Tc-etarfolatide dosimetry

Technetium (^{99m}Tc) is produced by means of a (^{99}Mo/^{99m}Tc) generator and decays with the emission of gamma radiation with a mean energy of 140 keV and a half-life of 6.02 hours to technetium (^{99}Tc) which, in view of its long half-life of 2.13 x 10^5 years can be regarded as quasi stable.

In study EC20.1, the radiation dosimetry was determined from a combination of 4 participants who did not receive folic acid and 4 participants who received 0.5-2.0 mg folic acid.

Injected doses of ^{99m}Tc-etarfolatide contained an average of 18.2 ± 1.6 mCi (range, 16-19.9 mCi) radioactivity and 87.5 ± 5.3 µg (range, 80.0 – 95.0 µg) of etarfolatide. Radiochemical purity averaged 96.3 ± 2.1% (range, 91.7 – 98.9%).

The largest estimated absorbed dose average in this study was to the kidneys (at 340 mrem/mCi), and the second largest was to the urinary bladder wall (at 94 mrem/mCi). The average effective dose equivalent was 55 mrem/mCi. For a 20 mCi administration, this yields an effective dose equivalent of 1100 mrem. This compares to the following average effective dose equivalents for other nuclear medicine scans: 1.0 mrem for 10 mCi of Kr-81 Gas, 16 mrem for 0.001 mCi of Co-57 Cyanocobalamin, 300 mrem for 10 mCi of ^{99m}Tc diethylenetriaminepentaacetic acid (DTPA), 1200 mrem for 0.5 mCi of In-111 white blood cells (WBC), and 2100 mrem for 5 mCi of Ga-67 Citrate.

In study EC20.11, whole body conjugate planar image data obtained at 5 min, 1, 4, 6-8, and 20-24 hours post injection were analysed.

Dosimetry estimates for ^{99m}Tc-etarfolatide based on data from participants who received 0.5 mg folic acid pre-injection are presented in the table below (N=11).

Table 40: Average dose absorbed per activity administered in study EC20.11

Organ / tissue	**Average dose absorbed per activity administered (µGy/MBq)**
Adrenals	6.0
Brain	1.7
Breasts	1.6
Gallbladder Wall	8.8
LLI Wall	5.7
Small Intestine	11
Stomach Wall	3.8
ULI Wall	10
Heart Wall	3.3
Kidneys	25
Liver	14
Lungs	3.4
Muscle	2.8
Ovaries	6.1
Pancreas	6.7
Red Marrow	12
Osteogenic Cells	10
Salivary Glands	2.8
Skin	1.5
Spleen	11
Testes	6.4
Thymus	2.2
Thyroid	6.9
Urinary Bladder Wall	23
Uterus	9.0
Total Body	3.7
Effective Dose [µSv/MBq]	7.7

The effective dose resulting from the administration of a (maximal recommended) activity of 925 MBq of technetium (^{99m}Tc) Folcepri for an adult weighing 70 kg is approximately 7.1 mSv.

For an administered activity of 925 MBq the typical radiation dose to the critical organs (kidneys, bladder, liver and red marrow) are 23.1, 21.3, 13.0, 11.1 mGy, respectively.

Post marketing experience

There is no marketing experience of ^{99m}Tc-etarfolatide at this time.

2.6.1. Discussion on clinical safety

^{99m}Tc-etarfolatide has been developed as a companion diagnostic test for the selection of adult patients for whom treatment with vintafolide, a folate receptor (FR) targeted therapeutic, is being considered. ^{99m}Tc-etarfolatide is intended to be used for single photon emission computed tomography (SPECT) imaging, in combination with CT or MRI. Hence, ^{99m}Tc-etarfolatide is for diagnostic use only and as such meant to be administered as a single injection. ^{99m}Tc-etarfolatide is for intravenous use only and must not be given by other routes of administration. In addition, unlabelled etarfolatide must not be administered directly to the patient.

The safety database consists of the pooling of thirteen completed studies comprising 563 subjects whereof 175 subjects received ^{99m}Tc-etarfolatide only while 388 subjects received ^{99m}Tc-etarfolatide followed by a therapeutic agent. In three of the studies, subjects were given the option to undergo a second ^{99m}Tc-etarfolatide scan which 18 subjects opted to and subsequently 581 doses of ^{99m}Tc-etarfolatide have been administered in total. The magnitude of the safety population is considered adequate.

Overall, the incidence of TEAEs considered drug-related by the investigator was low (3.4%) with the vast majority being of Grade 1 or 2, with the exception for a few Grade 3 events (nausea, vomiting, and abdominal discomfort at 0.2% each). No Grade 4 or Grade 5 drug-related TEAEs were reported.

In one subject a drug-related TEAE was reported as resulting in withdrawal of ^{99m}Tc-etarfolatide which is believed to be a reporting error. This patient was enrolled in Study EC-FV-02 and three days after receiving ^{99m}Tc-etarfolatide the patient experienced moderate tremor which the investigator considered possibly related to ^{99m}Tc-etarfolatide. The event resolved and the patient was started on treatment with vintafolide.

Two patients died while on study, due to disease progression. The incidence of serious adverse events was low (4.3%), with only one (0.2%) considered as drug-related by the investigator (nausea and vomiting in a single patient).

A further review based on clinical importance and association with disease state or prior therapeutic treatment revealed that the majority of adverse reactions attributed by the investigator to the drug had an etiology due to the nature of the disease status of the patients. Overall, only pruritus was considered potentially related to etarfolatide (see SmPC section 4.8, Undesirable effects). Gastrointestinal disorders were not considered related to etarfolatide based on the absence of any mechanistic explanation for the gastrointestinal effects of etarfolatide and absence of non-clinical signals at dosages much higher than used clinically. The majority of gastrointestinal TEAEs were reported in subjects with abdominal malignancies and therefore the CHMP considered that the cause was the underlying disease rather than related to ^{99m}Tc-etarfolatide.

The immunogenic potential for etarfolatide is considered low owing to the small molecular weight of 746 Da. In addition, the antigenicity study conducted in mice demonstrated no immunogenicity on repeated dosing.

As regards the subjects that received the second dose of ^{99m}Tc-etarfolatide, no drug related AEs were reported.

No clinically significant difference in the safety profile between patients < 65 and ≥ 65 years of age were observed and neither between male and female subjects.

There were no clinically significant changes in vital signs or laboratory results before and after treatment in healthy volunteers and patients.

There is no experience with overdose of ^{99m}Tc-etarfolatide. In the event of administration of a radiation overdose (i.e. > 925 MBq), the absorbed dose to the patient should be reduced where possible by increasing the elimination of the radionuclide from the body by frequent micturition, by forced diuresis and frequent bladder voiding. In general, since ^{99m}Tc-etarfolatide is a radiopharmaceutical, patients must be well hydrated before the start of the examination and urged to void as often as possible during the first hours after the examination in order to reduce radiation.

Reproductive and developmental toxicity studies have not been conducted with ^{99m}Tc-etarfolatide. Radionuclide procedures carried out on pregnant women also involve radiation doses to the fetus and ^{99m}Tc-etarfolatide is therefore contraindicated in pregnancy. It is not known whether ^{99m}Tc-etarfolatide can cause fetal harm when administered to a pregnant woman or can affect reproduction capacity, nor is it known whether ^{99m}Tc-etarfolatide is excreted in human milk. Consistent with clinical practice for diagnostic radiopharmaceuticals administered to a mother who is breastfeeding, consideration should be given to the possibility of delaying the administration of the radionuclide until the mother has ceased breastfeeding. If administration is considered necessary, breastfeeding must be interrupted for 48 hours after receiving ^{99m}Tc-etarfolatide and the expressed feeds discarded.

Consistent with other radiopharmaceuticals products, close contact with infants and pregnant women should also be restricted for 24 hours following the injection of ^{99m}Tc-etarfolatide.

No studies were done in patients with renal, hepatic or cardiac impairment. Dosimetry estimates from study EC20.11, conducted in healthy volunteers receiving folic acid pre-injection, were used to estimate renal radiation exposure for a patient with complete renal impairment and radiation exposure to the liver for a patient with complete hepatic impairment. The results suggested that the potential risk of increased renal toxicity in patients with renal impairment is limited. However, the CHMP considered that that care should be exercised in patients with impaired renal function, due to lower renal excretion and a likely increase in exposure to radioactivity in these patients (see SmPC section 4.4 Special warnings and precautions for use). To minimise the dose of radiation absorbed by the bladder, the patient should be well hydrated before the injection of ^{99m}Tc-etarfolatide and should be encouraged to remain well hydrated and to void frequently during the first 24 hours after injection.

In terms of liver exposure, the effective dose was estimated to increase from approximately 7.1 mSv to roughly 7.4 mSv in the worst case scenario, an estimate within the established hepatic threshold limit specified by the German Radiation Protection Ordinance for occupationally exposed persons (<150 mGy per year). Per the dosimetry estimates from study EC20.11, the maximum clinically indicated dose of 925 MBq ^{99m}Tc-etarfolatide results in a cardiac exposure of 3.1 mGy which is considerably less than the cardiac exposure of a standard of care myocardial perfusion study such as ^{99m}Tc-sestamibi. Based on the data provided, the CHMP agreed that the potential risk appears limited.

Hepatic, cardiac and renal impairment are included as missing information in the Risk Management Plan and will be monitored through routine pharmacovigilance.

Exposure to ionising radiation is potentially linked with cancer induction and a potential for development of hereditary defects. As the effective dose is 7.1 mSv when the maximal recommended activity of 925 MBq (25 mCi) is administered, these adverse reactions are expected to occur with a low probability.

Overall, ^{99m}Tc-etarfolatide as a single injection appears well tolerated. However, in clinical practice it is not inconceivable that there may be circumstances where the treating physician considers re-testing of the FR status indicated. In the event of iterated administration, this may affect the safety profile of ^{99m}Tc-etarfolatide even if this is unlikely. The safety profile of ^{99m}Tc-etarfolatide will be continuously monitored according to the Risk Management Plan.

2.6.2. Conclusions on the clinical safety

The safety assessment of ^{99m}Tc-etarfolatide is based on 563 patients. The incidence of treatment emergent adverse events was low according to each system organ class and a single administration of ^{99m}Tc-etarfolatide appears well-tolerated. Pruritus was the only adverse reaction reported and was uncommon in occurrence. The dose of radiation is similar to that of common studies in nuclear medicine or CT scans.

Overall, there are no major concerns in relation to the safety of a single administration of ^{99m}Tc-etarfolatide.

2.7. Pharmacovigilance

Detailed description of the pharmacovigilance system

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

2.8. Risk Management Plan

PRAC Advice

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

Based on the PRAC review of the Risk Management Plan version 3.0, the PRAC considers by consensus that the risk management system for etarfolatide (Folcepri) for the following indication "*This medicinal product is for diagnostic use only. After radiolabelling with sodium pertechnetate (^{99m}Tc) solution, Folcepri is indicated, after intravenously administered folic acid, for single photon emission computed tomography (SPECT) imaging, in combination with Computed Tomography (CT) or Magnetic Resonance Imaging (MRI), for the selection of adult patients for treatment with vintafolide, a folate receptor (FR) targeted therapeutic for use in ovarian cancer"* is acceptable.

This advice is based on the following content of the Risk Management Plan:

- **Safety concerns**

Table 41: Summary of the Safety Concerns

Summary of safety concerns	
Important identified risks	Exposure to ionizing radiation
Important potential risks	Drug interaction(s) with antifolate therapies Drug interaction(s) with folic acid supplements Risk of misdiagnosis – false positive
Missing information	Paediatric patients Pregnant and lactating women Patients with hepatic or renal impairment Patients with cardiac impairment

The PRAC agreed.

- **Pharmacovigilance plans**

The PRAC, having considered the data submitted, was of the opinion that routine pharmacovigilance is sufficient to identify and characterise the risks of the product.

The PRAC also considered that routine pharmacovigilance is sufficient to monitor the effectiveness of the risk minimisation measures.

- **Risk minimisation measures**

Table 42: Summary table of Risk Minimisation Measures

Safety concern	**Routine risk minimisation activities**	**Additional risk minimisation activities**
Exposure to ionizing radiation	Close monitoring through routine pharmacovigilance Section 4.8 Undesirable Effects Exposure to ionising radiation is potentially linked with cancer induction and a potential for development of hereditary defects. As the effective dose is 7.1 mSv when the maximal recommended activity of 925 MBq (25 mCi) is administered, these adverse reactions are expected to occur with a low probability. Section 4.9 Overdose In the event of administration of a radiation overdose (i.e. > 925 MBq), the absorbed dose to the patient should be reduced where possible by increasing the elimination of the radionuclide from the body through hydration with frequent bladder voiding. Section 6.6 Special Precautions for disposal and other handling Radiopharmaceuticals should be prepared in a manner that satisfies both radiation safety and pharmaceutical quality requirements. Section 11 Dosimetry [Entire section]	None

Drug interactions With antifolate therapies	Close monitoring through routine pharmacovigilance Section 4.4 Special warnings and precautions for use Patients should avoid folate supplements, vitamins enriched with folic acid, and anti-folate therapy for 24 hours prior to receiving Folcepri as they may compromise Folcepri imaging quality Section 4.5 Interaction with other medicinal products and other forms of interaction Patients should avoid folate supplements, vitamins enriched with folic acid, and antifolate therapy for 24 hours prior to receiving Folcepri as they may compromise Folcepri imaging quality	None
Drug interactions with folic acid supplements	Close monitoring through routine pharmacovigilance Section 4.4 Special warnings and precautions for use Oral folic acid and folinic acid are not substitutes for intravenous Neocepri (folic acid) due to receptor affinity and bioavailability differences relative to intravenously administered folic acid. Section 4.5 Interaction with other medicinal products and other forms of interaction Patients should avoid folate supplements, vitamins enriched with folic acid, and anti-folate therapy for 24 hours prior to receiving Folcepri as they may compromise Folcepri imaging quality	None
Risk of misdiagnosis – false positive	Close monitoring through routine pharmacovigilance Section 4.4 Special warnings and precautions for use [The ^{99m}Tc-etarfolatide imaging read procedure is described and illustrations provided in section 4.4 of the SmPC.]	Online and live Folcepri training program Shipping both Neocepri and Folcepri together to the pharmacy.
Paediatric patients	Close monitoring through routine pharmacovigilance Section 4.2 Posology and method of administration The safety and efficacy of Folcepri in patients below the age of 18 years have not been studied. No data are available. Section 5.1 Pharmacodynamic properties The European Medicines Agency has waived the obligation to submit the results of studies with Folcepri in all subsets of the paediatric population for the diagnosis of folate receptor status in malignant tissues (see section 4.2 for information on paediatric use).	None

Pregnant and lactating women	Close monitoring through routine pharmacovigilance Section 4.3 Contraindications Folcepri is contraindicated in pregnant women. Breast-feeding women should discard expressed breast milk for 48 hours after receiving Folcepri (see section 4.6). Section 4.6 Pregnancy There are no data with the use of Folcepri in pregnant women. The use of Folcepri is contraindicated in pregnant women due to potential radiation exposure to the foetus which may cause foetal harm (see section 4.3). Section 4.6 Breast-feeding It is not known whether Folcepri is excreted in breast milk. A risk to a breast-fed child cannot be excluded. Before administering radiopharmaceuticals to a mother who is breastfeeding, consideration should be given to the possibility of delaying the administration of the radionuclide until the mother has ceased breastfeeding. If administration is considered necessary, breastfeeding must be interrupted for 48 hours after receiving Folcepri and the expressed feeds discarded (see section 4.3). Breast feeding can resume after this 48 hour period.	None
Patients with hepatic or renal impairment	Close monitoring through routine pharmacovigilance Section 4.2 Posology and method of administration The safety and efficacy of Folcepri have not been studied in patients with renal, cardiac or hepatic impairment. Careful consideration of the activity to be administered is required since an increased radiation exposure is possible in patients with renal or hepatic impairment. See section 4.4 for additional information on patients with renal impairment. Section 4.4 Special warnings and precautions for use Renal impairment Care must be exercised in patients with impaired renal function, due to lower renal excretion and a likely increase in exposure to radioactivity in these patients. To minimise the dose of radiation absorbed by the bladder, the patient must be well hydrated before the injection of Folcepri and must be encouraged to remain well hydrated and to void frequently during the first 24 hours after injection.	None

Patients with cardiac impairment	Close monitoring through routine pharmacovigilance Section 4.2 Posology and method of administration The safety and efficacy of Folcepri have not been studied in patients with renal, cardiac or hepatic impairment.	None

The PRAC, having considered the data submitted, was of the opinion that the proposed risk minimisation measures are sufficient to minimise the risks of the product in the proposed indication.

The CHMP endorsed this advice without changes.

2.9. 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

Ovarian cancer expressing folate receptors represents an aggressive form of the disease, with poor prognosis and with faster progression of disease and shorter overall survival (Toffoli, 1997; Toffoli, 1998; Chen, 2012). Having a therapy that is targeted to these folate receptors could have the potential to treat patients with this aggressive form of disease. Etarfolatide has been developed as a diagnostic radiopharmaceutical to target folate receptors and as such detect patients with disease suitable for treatment with vintafolide, a folate receptor targeted therapeutic agent for patients with platinum resistant ovarian cancer (PROC).

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. A benefit was observed in terms of improvement in progression free survival in the patients with 100% scan positive target lesions in which the median PFS was 24.0 weeks in the vintafolide+PLD combination arm versus 6.6 weeks in the PLD alone arm with a HR of 0.381. In this study, although only 94 patients contributed with scans, it is considered sufficiently well demonstrated that patients with negative FR scans do not benefit from treatment with vintafolide whilst patients with FR(100%) do benefit, supporting the clinical utility of ^{99m}Tc-etarfolatide.

Results from the supportive clinical studies also suggested a clinical utility for ^{99m}Tc-etarfolatide in detecting patients that may benefit from treatment with vintafolide with some correlation between degree of scan positivity and responses.

The sensitivity of ^{99m}Tc-etarfolatide as a diagnostic agent appears acceptably demonstrated from a clinical perspective based on results from the single-arm supportive studies in which it was

observed that ^{99m}Tc-etarfolatide negative lesions are unlikely to respond by tumour shrinkage to vintafolide.

It has not been demonstrated that a good correlation exists between scan positivity following injection of ^{99m}Tc-etarfolatide, and folate receptor positivity on immunohistochemistry. Therefore ^{99m}Tc-etarfolatide cannot be considered as a diagnostic agent for detection of folate receptor positive cancer and the indication sought rather relates to the clinical utility for ^{99m}Tc-etarfolatide, in detecting patients suitable for treatment with vintafolide.

To decrease the potential risk of misdiagnosis and ensure consistency in the etarfolatide scan assessment, detailed guidance on the read procedure is provided to the readers of scans in the SmPC and a training programme will be put in place.

Uncertainty in the knowledge about the beneficial effects

Clinical data on the efficacy of vintafolide and as such clinical utility data on ^{99m}Tc-etarfolatide are currently available mainly from one phase II 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 and as such confirm the clinical utility of ^{99m}Tc-etarfolatide in detecting patients who would benefit from the treatment. Further clinical efficacy data are expected from the ongoing study EC-FV-06.

The FR also appears to be heterogeneously expressed within a single patient. In 25 out of 61 patients 10 to 90% of the lesions were FR positive. This implies that in patients with all tumour lesions being FR positive, it is likely that there will be a degree of heterogeneity on a lesion level of putative importance for treatment results. Probably due to the small sample size, it was not possible to clarify whether vintafolide as add-on to PLD influenced the pattern of progression, FR+ versus FR- lesions. Overall, the results of EC-FV-04 have not provided a conclusive assessment for which thresholds or degree of scan positivity other than FR(100%) derive maximum benefit from the addition of vintafolide to PLD therapy. As such the indication of vintafolide covers only patients who express the FR on all target lesions. The efficacy results in the FR(10-90%) population is expected to be more fully explored in the ongoing phase 3 trial, Study EC-FV-06.

Risks

Unfavourable effects

Overall, there are no major concerns raised in relation to the safety of etarfolatide. The incidence of drug-related TEAEs was low with the vast majority being of Grade 1 or 2, with the exception for a few Grade 3 events (nausea, vomiting, and abdominal discomfort). No Grade 4 drug-related TEAEs were reported and no drug-related deaths occurred. The incidence of SAEs was low (4.3%), with only one considered as drug-related by the investigator (nausea and vomiting in a single patient). The majority of drug-related TEAEs attributed by the investigator had most likely an etiology due to the nature of the disease of the patient. Overall, only pruritus was considered as an adverse reaction with the frequency uncommon.

Withdrawal from study drug due to AE was not expected and the one existing report is considered a reporting error.

No clinically significant difference in the safety profile between patients < 65 and ≥ 65 years of age were observed and neither between male and female subjects.

There were no clinically significant changes in vital signs.

As regards the subjects that received the second dose of ^{99m}Tc-etarfolatide, no drug related AEs were reported.

The dose of radiation from a ^{99m}Tc-etarfolatide study is similar to that of common studies in nuclear medicine or CT scans.

Uncertainty in the knowledge about the unfavourable effects

There is limited data available on the pharmacokinetics of etarfolatide, and no *in vitro* or *in vivo* drug-drug interaction studies have been performed. In addition there is no data available in patients with impaired organ function. This is adequately addressed in the RMP and in the product information.

It is unclear whether ^{99m}Tc-etarfolatide in clinical practice will be administered only once or will be repeated as to follow the FR status in an individual patient. In the event of iterated administration of ^{99m}Tc-etarfolatide, this may affect the safety profile of the product, even if unlikely. The safety of ^{99m}Tc-etarfolatide will be monitored post-authorisation and reported in periodic safety update reports.

Benefit-risk balance

Importance of favourable and unfavourable effects

The proper use of ^{99m}Tc-etarfolatide would be to enable selection of patients with increased likelihood of response to vintafolide (Vynfinit). The clinical utility of ^{99m}Tc-etarfolatide is supported by the results of two phase 1 and one phase 2 comparative study which have shown that patients with FR(100%) benefit from treatment with vintafolide.

From a safety perspective, a single ^{99m}Tc-etarfolatide appears safe and well-tolerated leaving no outstanding issues or safety signals.

Benefit-risk balance

The benefit-risk balance of ^{99m}Tc-etarfolatide in the proposed indication for the selection of patients for treatment with vintafolide is considered positive.

Discussion on the benefit-risk balance

Additional efficacy and safety 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 and as such confirm the clinical utility of ^{99m}Tc-etarfolatide in detecting patients who would benefit from the treatment. Further clinical efficacy data are expected from the ongoing study EC-FV-06.

The CHMP considered that etarfolatide 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 etarfolatide on the basis of the following reasons:

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

Study EC-FV-04 showed that patients with negative FR scans do not benefit from treatment with vintafolide whilst patients with FR(100%) do benefit, supporting the clinical utility of ^{99m}Tc-etarfolatide. The safety profile of etarfolatide, which was evaluated in more than 550 cancer patients, was manageable with most adverse events reported transient and of mild intensity. Therefore, the benefit-risk balance is positive.

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

Additional comprehensive clinical efficacy 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, which will allow to further define the clinical utility of ^{99m}Tc-etarfolatide scan in a larger subset of patients.

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 etarfolatide 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

Outcome

Based on the CHMP review of data on quality, safety and efficacy, the CHMP considers by consensus that the risk-benefit balance of Folcepri in the following indication, “This medicinal product is for diagnostic use only. After radiolabelling with sodium pertechnetate (^{99m}Tc) solution, Folcepri is indicated, after intravenously administered folic acid, for single photon emission computed tomography (SPECT) imaging, in combination with Computed Tomography (CT) or Magnetic Resonance Imaging (MRI), for the selection of adult patients for treatment with vintafolide, a folate receptor (FR) targeted therapeutic for use in ovarian cancer”, 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.

- **Additional risk minimisation measures**

Prior to launch in each Member State the Marketing Authorisation Holder (MAH) shall agree the final educational programme with the National Competent Authority.

The MAH shall make certain that, following discussions and agreement with the National Competent Authorities in each Member State where Folcepri is marketed, at launch and after launch, all physicians who are expected to use Folcepri are provided with a training course in order to ensure

accurate and reliable interpretation of the SPECT images with respect to the tumour folate receptor (FR) status when using Folcepri as a diagnostic agent.

The physician training course should contain the following key elements:

- information on FR-targeted therapy in cancers with evidence of over-expression of FR, including the fact FR-negative patients are unlikely to respond to such therapy;
- information regarding all the requirements for acquiring accurate assessment of patients' FR status, including guidelines for accurate image acquisition, dosing and administration, image assessment and the appropriate method of calculating patients' FR score;
- a comprehensive set of guided case studies that demonstrate and reinforce key aspects of accurate tumour FR status assessment;
- and a set of self-assessment cases for self-directed assessment of target lesions' FR status.

Expertise and qualification of trainers in on-line, written and in-person training should be ensured.

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 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, to further support the clinical utility of ^{99m}Tc-etarfolatide scan for selection of patients for treatment with vintafolide in combination with PLD 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 etarfolatide is qualified as a new active substance.