Vintafolide: a novel targeted therapy for the treatment of folate receptor expressing tumors

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***Abstract*:** Despite advances in the development of molecularly targeted therapies, limited improvements in overall survival have been noted among many cancer patients with solid tumors, primarily due to development of drug resistance. Accordingly, there is an unmet need for new targeted therapies and treatment approaches for cancer, especially for overcoming resistance. Expression of the folate receptor is upregulated in many tumor types and thus represents an ideal target for cancer treatment. Several folate receptor targeted therapies are in development, including the small molecule drug conjugate vintafolide, the monoclonal antibody farletuzumab, and the antibody-drug conjugate IMGN853. The role of the folate receptor as a target in cancer progression and resistance as well as emerging preclinical and clinical data from studies on those folate receptor targeted agents that are in development with a focus on vintafolide are reviewed. The folate receptor has several unique properties, such as high expression in several tumor types, that make it a rational target for cancer treatment, and allow for selective delivery of folate receptor targeted agents. Early-stage clinical data in lung and ovarian cancer suggest that vintafolide has the potential for combination with other standard approved agents.

Keywords: folate receptor, lung neoplasms, neoplasms, ovarian neoplasms, small molecule drug conjugate, vintafolide

Introduction

There is an ongoing need for the development of new cancer therapies that can effectively target tumor cells without harming normal cells or tissue [Miller *et al.* 2013]. Recent treatment advances include the use of combination chemotherapy, which has had a significant impact on the treatment of most cancer types [DeVita and Chu, 2008]. Targeted cancer therapies such as monoclonal antibodies and small molecule tyrosine kinase inhibitors have also had a significant impact on cancer treatment, demonstrating increased efficacy with improvements in progression-free survival (PFS) over conventional chemotherapeutics alone in many tumor types [Bottsford-Miller *et al.* 2012; Feliz and Tsimberidou, 2013; Giuliano and Pages, 2013; Miller *et al.* 2013; Tang *et al.* 2013; Tejpar *et al.* 2012]. These therapies have the potential to achieve durable antitumor effects without overlapping toxicity [Bicknell, 2005; Imai and Takaoka, 2006; Stegmeier *et al.* 2010]. Targeted therapies are associated with a low toxicity profile, though they often have low single-agent responses [Imai and Takaoka, 2006]. However, a key consideration for targeted therapy is to establish predictive biomarkers and/or imaging techniques to determine which patients would benefit most from a particular targeted-therapy combination [Bicknell, 2005; Stegmeier *et al.* 2010]. Furthermore, like traditional chemotherapy, the emergence of resistance to targeted therapies is a major challenge often faced in the clinic, particularly in patients with advanced tumors [Miller *et al.* 2013]. Thus, there is a clear need for new strategies and targeted approaches to cancer treatment, particularly when combating resistance. Two major categories of currently used targeted therapies include monoclonal antibodies (e.g. trastuzumab, bevacizumab) and small molecule therapies (e.g. tyrosine kinase inhibitors, bortezomib) [Miller *et al.* 2013]. Drug

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conjugates are another major group of targeted therapies that involve a promising approach whereby targeted agents are created by linking a drug or a prodrug to a tissue-targeting molecule or carrier; this group can be further separated into antibody-drug conjugates (ADCs) and small molecule-drug conjugates (SMDCs). The folate receptor (FR) is overexpressed in many epithelial tumors and has been established as a tumor cellular-surface marker for targeted drug delivery [Teng *et al.* 2012]. This has led to the development of a number of FR-targeted agents, including anti-FR monoclonal antibodies, FR-binding ADCs, and folic acid (FA)-based SMDC (FA-SMDC). The aim of this paper is to review the role of the FR as a target in cancer progression and resistance and to consider agents in development that target the FR with a focus on the SMDC vintafolide.

The FR and its role in cancer progression and resistance

The FR and folate metabolism

Folate is essential for DNA replication and the synthesis of nucleotide precursors [Gonen and Assaraf, 2012]. Folates can be found in an oxidized form, FA, or as naturally occurring reduced folates [Gonen and Assaraf, 2012]. However, the major circulating form of folate is 5-methyltetrahydrofolate (5-MeTHF), which is found at low, yet sufficient, physiological concentrations of 5–30 nM in sera [Gonen and Assaraf, 2012; Ifergan and Assaraf, 2008].

Folates can be taken up into cells first by carrier proteins, such as the transmembrane-reduced folate carrier, which is ubiquitously expressed in most normal tissues and malignant tumors, or by the proton-coupled folate transporter in low pH environments, such as the intestine [Zhao *et al.* 2009], and second, through membrane-bound FRs [Gonen and Assaraf, 2012]. FRs are high-affinity folate-binding glycoproteins, of which there are three principal isoforms (α, β, and γ) [Gonen and Assaraf, 2012]. A fourth isoform, FRδ, has also been identified, but it has been difficult to detect in human tissues; therefore, it is suggestive of a highly restricted expression pattern, a splice variant, or a pseudogene [Spiegelstein *et al.* 2000; Tian *et al.* 2012]. FRα and FRβ bind FA as well as 5-MeTHF with high affinity, whereas FRγ is a secreted protein that is not involved in cellular uptake [Antony, 1996; Dosio *et al.* 2010; Gonen and Assaraf, 2012; Kamen and Capdevila, 1986; Tian *et al.* 2012]. After binding to the FR, folate uptake occurs through receptor-mediated endocytosis [Kamen and Capdevila, 1986; Vlahov *et al.* 2006]. It is important to note that FRα plays a critical role in the uptake of serum folates by cells expressing the receptor by binding 5-MeTHF with high affinity and FA with even higher affinity [Antony, 1996; Kamen and Capdevila, 1986; Kamen and Smith, 2012; Tian *et al.* 2012; Westerhof *et al.* 1995].

FRβ is expressed in placenta, colon, thymus, spleen, and various leukemic myelomonocytic cells [Elnakat and Ratnam, 2004; Ratnam *et al.* 1989; Ross *et al.* 1994; Shen *et al.* 1994; Weitman *et al.* 1992a]. In contrast, FRα is expressed mostly in epithelial cells of the uterus, placenta, choroid plexus, retina, and kidney [Gonen and Assaraf, 2012], and it is expressed at very high levels in several tumor types, including ovarian, lung, kidney, and breast cancer, making it a prime candidate for targeted anticancer therapy [Christoph *et al.* 2013; Crane *et al.* 2012; Elnakat and Ratnam, 2006; Nunez *et al.* 2012; Parker *et al.* 2005; Toffoli *et al.* 1997; Weitman *et al.* 1992a]. FRα expression is notably restricted to the apical surfaces of polarized epithelial cells in the kidney (facing the lumen of the tubule) and therefore is not exposed to the bloodstream [Parker *et al.* 2005]. Similarly, lung alveolar lining cells (type I and II pneumocytes) and epithelial cells of the bronchi stain intensely for FRα on the apical membranes facing the airway, which are not accessible to blood-borne folates [Parker *et al.* 2005; Salazar and Ratnam, 2007; Weitman *et al.* 1992b]. It is important to note that FRα expression in normal cells of the uterus, choroid plexus, retina, and kidney is considerably lower than FRα expression at sites not exposed to the bloodstream and in cancer [Parker *et al.* 2005; Ross *et al.* 1994; Weitman *et al.* 1992a, 1992b]. Based on these observations, targeting the FRα may be an effective therapeutic option for the treatment of cancer [Salazar and Ratnam, 2007].

The FR and cancer progression and resistance

The FR appears to play a critical role in cancer progression and resistance. For instance, FRα expression appears to be a negative prognostic factor in patients with ovarian cancer [Kalli *et al.* 2008; Toffoli *et al.* 1998] and may represent a marker for resistance to conventional chemotherapy. In addition, FRα expression is a negative-prognostic factor in breast [Hartmann *et al.* 2007], endometrial [Brown *et al.* 2008], uterine

[Allard *et al.* 2007], and colorectal cancer [Shia *et al.* 2008]. It is important to note that FRα expression does not appear to be influenced by chemotherapy in ovarian and endometrial cancer [Despierre *et al.* 2013]. Taken together, these studies further support the rationale for targeting the FRα in cancer treatment.

Targeting the FRα in tumors

Three general strategies have been used to target therapeutics to FR-expressing tumors: an anti-FR antibody approach, a humanized FRα-binding–ADC approach, and a FA-SMDC approach [Beck *et al.* 2012; Teng *et al.* 2012; Vlahov and Leamon, 2012].

Several anti-FRα antibodies have been developed for targeting of FRα antibodies, including the monoclonal antibody farletuzumab [Armstrong *et al.* 2013; Konner *et al.* 2010; Teng *et al.* 2012], which has a mechanism of action distinct from that of drug conjugates. Farletuzumab is a fully humanized antibody derived from the murine antibody LK26, which binds FRα to promote cell lysis by both complement-dependent cytotoxicity and antibody-dependent cell-mediated cytotoxicity [Farrell *et al.* 2012; Jelovac and Armstrong, 2012; Teng *et al.* 2012]. In addition, the binding of farletuzumab to FRα also suppresses the proliferation and growth of FRα-expressing cells and tumors by preventing the phosphorylation of substrates specific for Lyn kinase [Jelovac and Armstrong, 2012]. Farletuzumab is being investigated as a single agent or in combination with chemotherapy in epithelial-ovarian carcinoma [Armstrong *et al.* 2013; Jelovac and Armstrong, 2012; Konner *et al.* 2010] and in combination with chemotherapy in lung cancer [Thomas *et al.* 2013]. Farletuzumab was evaluated as a single agent and in combination with carboplatin and a taxane in a phase II study in patients with platinum-sensitive ovarian cancer [Armstrong *et al.* 2013]. As a single agent, farletuzumab was well tolerated without additive toxicity when administered with chemotherapy. Further, when combined with chemotherapy, 75% of patients achieved complete response (CR) or partial response (PR). Results from the phase III FAR-121 study in patients with platinum-sensitive ovarian cancer in first relapse showed that farletuzumab in combination with carboplatin and a taxane (paclitaxel or docetaxel) unfortunately did not meet the study's primary endpoint of PFS [Vergote *et al.* 2013].

IMGN853 is an FRα-targeting ADC that is composed of three parts: an anti-FRα antibody that targets the compound to FRα-expressing cancer cells; DM4, a potent cell-killing agent that inhibits tubulin polymerization; and a disulfide-based linker [Ab *et al.* 2011]. IMGN853 is in phase I clinical development for patients with ovarian cancer and other FOLR1 + solid tumors [ClinicalTrials.gov identifier: NCT01609556] [Ab *et al.* 2011]. Preliminary results recently reported indicate that IMGN853 was well tolerated at doses up to 3.3 mg/kg and provided evidence of antitumor activity [Kirkjian *et al.* 2013; Moore *et al.* 2014].

FA-SMDCs were developed because they are smaller than monoclonal antibodies and have a higher affinity for FRs on cancer cells; therefore, they may potentially improve blood clearance and tumor penetration of the attached drug [Pribble and Edelman, 2012; Vlahov and Leamon, 2012]. The proposed mechanism of action of FA-SMDCs is shown in Figure 1 [Vlahov and Leamon, 2012]. First, the FA-based SMDC extravasates from the circulation and binds the high-affinity FR on the tumor cell. The FR and conjugate then enter the cell by endocytosis. The FA-SMDC is sequestered in an endosome, where the pH is lower (because of the presence of proton pumps), causing the SMDC to be released from the FR. Reductive activity inside the endosome then cleaves the disulfide-based linker of the FA-SMDC to release the active drug. Finally, the drug escapes from the endosome to enter the cytosol while the FR recycles back to the cell surface. The whole process is then repeated. Several folate-conjugated cytotoxic agents have been, or are being, evaluated as anticancer therapy, including folate-conjugated 5-fluro-2′deoxyuridine-5′-O-monophosphate, folate-conjugated carboplatin, and folate-conjugated microtubule poisons [Teng *et al.* 2012]. These include EC0225 [Leamon *et al.* 2007a], folate conjugated to both a vinca alkaloid and mitomycin; BMS-753493 (epofolate) [Gokhale *et al.* 2012] [ClinicalTrials.gov identifiers: NCT00546247 and NCT00550017] folate conjugated to epothilone; and EC0489 [ClinicalTrials.gov identifier: NCT00852189], an analogue of vintafolide (*vide infra*).

Patient selection and biomarker analyses

The noninvasive, folate-targeted, single-photon emission computed tomography based companion imaging agent, ^{99m}Tc-etarfolatide (EC20), offers the potential to rationally select patients for

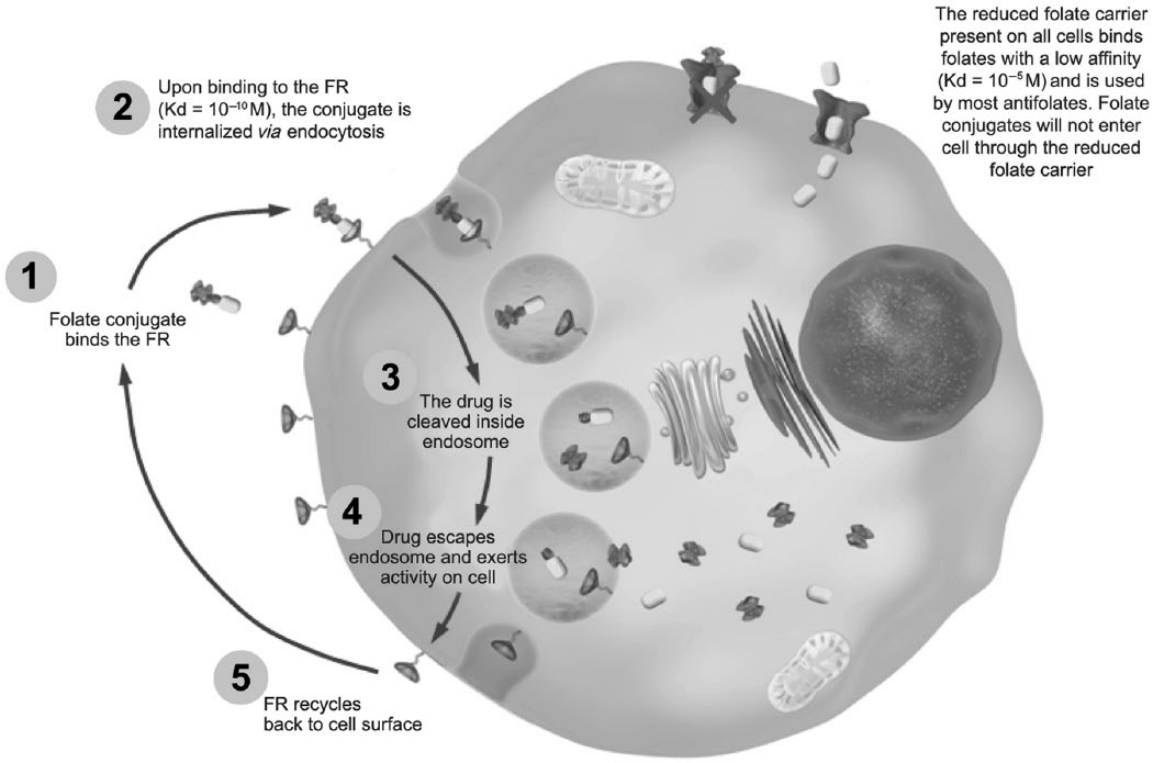

Figure 1. Schematic presentation of tumor cellular uptake of a FA–based, small-molecule drug conjugate. FA, folic acid; FR, folate receptor.

treatment with vintafolide without the need for biopsy [Morris *et al.* 2014; Pribble and Edelman, 2012]. The use of imaging with etarfolatide allows identification of patients whose disease expresses functionally active FR and, therefore, has the potential to respond to FR-targeted treatment [Morris *et al.* 2014]. Using this technique, patients are generally categorized as FR++ (100%; all lesions positive), FR+ (10–90%; at least one lesion positive, but not all positive), and FR– (0%; no lesions positive) (Figure 2) [Naumann *et al.* 2013]. Because EC20 is a noninvasive, real-time assessment of functionally active and anatomically accessible FRs, it has been evaluated as a biomarker of response to FA-SMDCs, such as vintafolide [Leamon *et al.* 2012].

Vintafolide

Chemistry and mechanism of action

Vintafolide is a water-soluble derivative of FA and the vinca alkaloid desacetylvinblastine hydrazide (DAVLBH) (Figure 3) [Dosio *et al.* 2010; Pribble and Edelman, 2012; Vlahov *et al.* 2006]. DAVLBH is a vinca alkaloid that disrupts the formation of the mitotic spindle, thereby inhibiting cell division and inducing cell death [Pribble and Edelman, 2012]. FA and DAVLBH are connected through a peptide spacer and a reducible, self-immolative disulfide-linker system to form vintafolide [Dosio *et al.* 2010; Pribble and Edelman, 2012]. The disulfide linker enables the release of DAVLBH inside the cancer cell after receptor-mediated endocytosis [Pribble and Edelman, 2012]. This is an important feature because the high affinity of FA for the FR can result in ligands remaining attached to the FR for long periods of time, which can reduce the potency of folate-targeted chemotherapeutic agents [Dosio *et al.* 2010]. Vintafolide was designed specifically to bind to high-affinity FR present on the surfaces of cancer cells and to release its active component, DAVLBH, once it enters the endosome of the target cell (Figure 3).

Preclinical studies

Preclinical studies have shown that vintafolide binds to the FRα with high affinity [Leamon *et al.* 2007b]; accordingly, it has highly potent

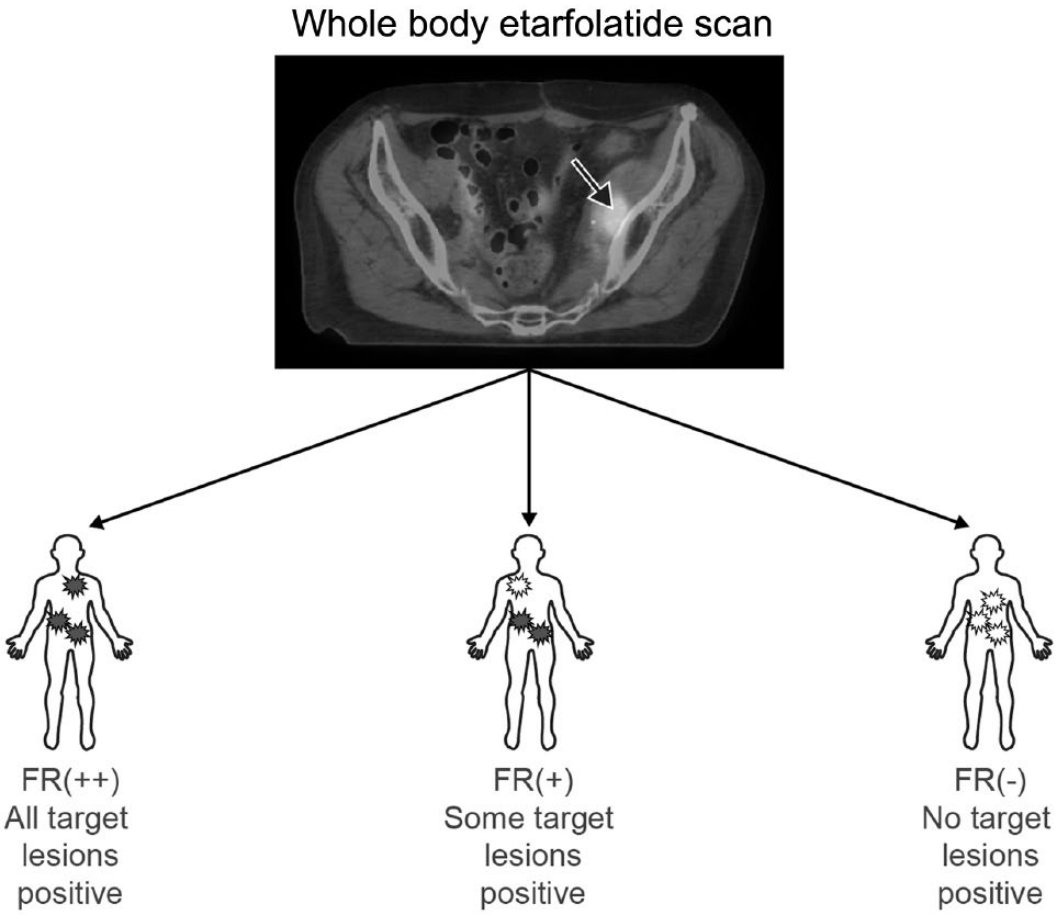

Figure 2. Etarfolatide imaging. Etarfolatide imaging can be used to divide patients into three categories: FR++ (100%; all lesions positive), FR+ (10–90%; at least one lesion positive but not all positive), or FR– (0%; no lesions positive). FR, folate receptor.

and specific antitumor activity against FRα+ tumors [Dosio *et al.* 2010; Leamon *et al.* 2007b, 2012; Reddy *et al.* 2007]. FRα expression is critical for vintafolide activity, and cytotoxicity is dose dependent with an IC_{50} (concentration that inhibits 50% of activity) in the single-digit nanomolar range [Leamon *et al.* 2007b]. Preclinical studies conducted to optimize the dosing regimen have shown that vintafolide is most efficacious when administered on a more frequent schedule and at low-dose levels [Reddy *et al.* 2007], exploiting the natural recycling mechanism of the FR to keep greater pressure on the tumor cell. Specificity for FR-containing cells has been demonstrated by the fact that the binding affinity of vintafolide for the FR is slightly less than the affinity of folate for the FR (0.47 relative to the binding affinity of FA for the FR) [Leamon *et al.* 2007b]. Furthermore, excess free FA has been found to completely block the activity of vintafolide, and FR– cells are resistant to vintafolide. Specificity for FR-containing cells has also been demonstrated in FRα-expressing tumors in M109 tumor-bearing BALB/C mice [Leamon *et al.* 2012].

Pharmacodynamic and pharmacokinetic studies

In a nonrandomized, open-label, dose-comparison, phase I study in patients with refractory or metastatic solid tumors, vintafolide was administered as an intravenous bolus injection or as a 1 h infusion on days 1, 3, and 5 (week 1) and days 15, 17, and 19 (week 3) of a 28-day cycle [Li *et al.* 2009; LoRusso *et al.* 2012]. The maximum tolerated dose (MTD) of vintafolide was 2.5 mg, and the maximum concentration and area under the curve for vintafolide increased in a dose-proportional manner across the dose range studied

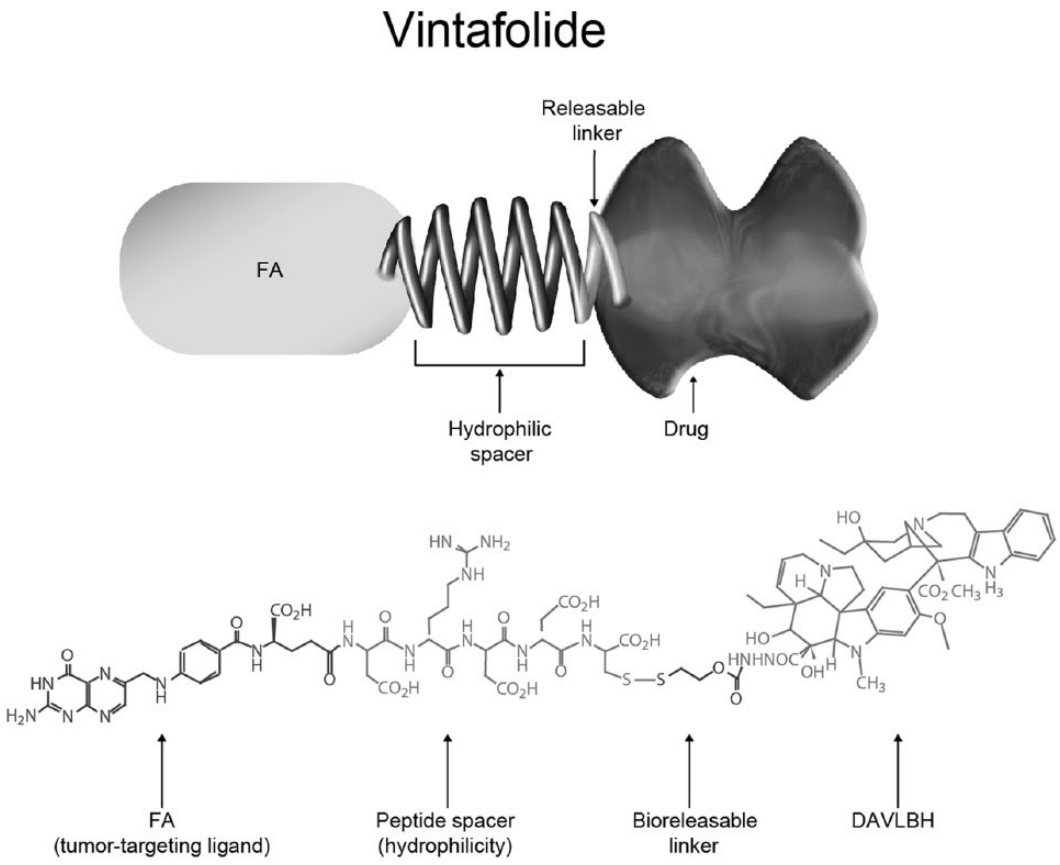

Figure 3. Molecular design and structure of vintafolide. Each molecule of vintafolide contains one FA moiety, which serves as a stable high-affinity binding ligand for the FR (Kd ~0.1 nM), and one vinca alkaloid unit (DAVLBH). Unlike the untargeted DAVLBH molecule, vintafolide is readily soluble in aqueous solutions because of the hydrophilic peptide spacer unit that is placed between the folic acid and DAVLBH moieties. This physical property enables vintafolide to be dosed intravenously without the aid of coadministered solubilizing or dispersing agents. A self-immolative linker system allows for efficient release of the DAVLBH moiety inside the endosome of the targeted FR-expressing tumor cell. DAVLBH, desacetylvinblastine hydrazide; FA, folic acid; FR, folate receptor.

[Li *et al.* 2009; LoRusso *et al.* 2012]. The pharmacokinetics of vintafolide are characterized by a rapid distribution and elimination phase, which includes a short distribution half life (i.e. uptake of the conjugate by FR-expressing tissues *in vivo* is rapid) [Paulos *et al.* 2004], a volume of distribution that is larger than blood volume, and rapid clearance by the kidney and liver [Li *et al.* 2009; Paulos *et al.* 2004]. On the basis of these results, a dose-dense regimen was proposed for phase II studies, in which the same cumulative dose (2.5 mg × six doses per month) was divided into smaller doses of 1.0 mg/day as an intravenous bolus injection (Monday–Friday) for 3 weeks of a four-week cycle, with a total dose of 15 mg/month (this was an induction-style design; after two cycles, patients returned to the three times a week, every other week, four-week cycle regimen for "maintenance") [Li *et al.* 2009; Morris *et al.* 2014].

In the nonrandomized, open-label, dose-comparison, phase I study in patients with refractory or metastatic solid tumors, the MTD of vintafolide was 2.5 mg administered as an intravenous bolus on days 1, 3, and 5 (week 1) and days 15, 17, and 19 (week 3) of a four-week cycle [LoRusso *et al.* 2012]. Preliminary evidence of antitumor activity was demonstrated in two patients with ovarian cancer; one patient had a PR, and the other patient had disease stabilization for longer than 5 months [LoRusso *et al.* 2012]. Vintafolide demonstrated an acceptable safety profile with no evidence of myelosuppression. Constipation, nausea, fatigue, and vomiting were the most commonly reported adverse events.

Clinical studies: ovarian cancer

The efficacy and safety of vintafolide was first assessed in 2007 in a nonrandomized trial [Morris *et al.* 2014], and a number of phase II and III clinical studies have since been conducted in patients with ovarian and lung cancer [Edelman *et al.* 2012a, 2012b; Naumann *et al.* 2013]. The main characteristics and key findings of these clinical trials are described in the following sections and are summarized in Table 1.

Study EC-FV-02 was an open-label, phase II trial of vintafolide monotherapy in patients with platinum-resistant or -refractory advanced epithelial ovarian, primary peritoneal, or endometrial cancer who underwent scanning with EC20 to assess their FR status [Morris *et al.* 2014]. Vintafolide treatment consisted of an induction phase, followed by a maintenance phase for responders (patients achieving CR or PR) or patients who had stable disease (SD) without unacceptable toxicity (Table 1). The disease control rate (DCR) for all evaluable patients (primary endpoint), regardless of EC20 status, was 40% [95% confidence interval (CI) 25.7–55.7%; CR, 0%; PR, 4%; SD, 36%]. Superior outcomes were demonstrated in patients who demonstrated EC20 uptake with DCRs of 57% in those with all FR+ target tumor lesions (FR++) and 43% in those who had at least one but not all FR+ target lesions (FR+), compared with 25% in patients who were FR–. Two patients experienced PR; both patients were FR+, with one FR++. A similar benefit in median overall survival (OS) was seen when patients were assessed by EC20 status, with FR++ patients demonstrating significant improvement in OS compared with FR– patients [hazard ratio (HR) 0.170; $p = 0.001$]. The most common drug-related vintafolide toxicity (all grades) was constipation, followed by fatigue, nausea, anorexia, and neuropathy. The most common grade 3 vintafolide-related toxicities were constipation and fatigue; no grade 4 vintafolide-related toxicities were reported.

The open-label, randomized, phase II PRECEDENT study (EC-FV-04) evaluated the use of vintafolide in combination with pegylated liposomal doxorubicin (PLD) *versus* PLD alone in 162 women with platinum-resistant recurrent ovarian cancer who had received at least two previous cytotoxic regimens (Table 1); the assessment of FR status using the companion imaging diagnostic, EC20, was optional [Naumann *et al.* 2013]. Vintafolide demonstrated significantly improved clinical activity compared with PLD alone, with a median PFS (primary endpoint) of 5.0 months in the vintafolide plus PLD arm compared with 2.7 months in the PLD-alone arm (HR 0.63; 95% CI 0.41–0.96; $p = 0.031$) (Figure 4, Table 1). Furthermore, this benefit with combination therapy was maintained in FR+ (i.e. 10–100% FR+ target tumor lesions) patients (5.7 *versus* 1.7 months; HR 0.547; 95% CI 0.304–0.983; $p = 0.041$) (Table 1); the greatest benefit was in FR++ patients (HR 0.381; 95% CI 0.172–0.845; $p = 0.013$). No significant differences in the Response Evaluation Criteria in Solid Tumors–confirmed objective response rate (ORR; 18% *versus* 12%; $p = 0.479$) and the secondary endpoint of OS (HR 1.010; 95% CI 0.679–1.503; $p = 0.957$) were noted between the combination therapy and the PLD-alone treatment arms. Overall, the drug combination was well tolerated. No cumulative treatment-emergent adverse events (TEAEs) were reported, except palmar-plantar erythrodysesthesia syndrome, which increased in incidence with subsequent cycles in both treatment arms. Most TEAEs in both treatment arms were grade 1 or 2, with higher incidence rates of leukopenia, abdominal pain, peripheral sensory neuropathy (all $p = 0.026$) and neutropenia ($p = 0.021$) in the vintafolide plus PLD arm than in the PLD-alone arm; the incidence of nausea was higher in the PLD-alone arm ($p = 0.036$). For grade 3 or 4 TEAEs, the difference in the incidence of leukopenia between treatment arms was nominally statistically significant ($p = 0.031$). However, the difference in leukopenia incidence between treatment arms was not clinically significant because no increase in febrile neutropenia or infection was reported.

The recently completed randomized, double-blind, placebo-controlled, phase III PROCEED trial (EC-FV-06) [ClinicalTrials.gov identifier: NCT01170650] evaluated vintafolide in combination with PLD compared with PLD alone in patients with FR+, platinum-resistant ovarian cancer. Patients with primary or secondary platinum-resistant, pathology-confirmed epithelial ovarian, fallopian tube, or primary peritoneal cancer were eligible for the study and had their FR status determined by EC20 scans. The primary endpoint of this study was PFS in patients with FR+ tumors. At a prespecified interim futility analysis, the Data Safety Monitoring Board (DSMB) recommended that the study be stopped because vintafolide in combination with PLD *versus* PLD alone did not meet the prespecified

Table 1. Summary of efficacy and safety clinical trial data for vintafolide [Edelman *et al.* 2012a, 2012b; Morris *et al.* 2014; Naumann *et al.* 2013].

Trial	Study design	Participants	Interventions	Key efficacy and safety results
Ovarian cancer trials				
Study EC-FV-02 [ClinicalTrials.gov identifier: NCT00507741] [Morris *et al.* 2014]	Phase II, single-arm, OL, MC	Patients with platinum-resistant or -refractory advanced epithelial ovarian, primary peritoneal, or endometrial cancer ($N = 45$); patients were scanned with EC20 to assess FR status	Vintafolide monotherapy: induction phase (1 mg intravenously, Monday to Friday for 3 weeks, every 28 days for two cycles) followed by a maintenance phase (2.5 mg intravenously, Monday, Wednesday, and Friday during weeks 1 and 3, every 28 days) in responders (CR or PR) and patients with SD without unacceptable toxicity	**Efficacy** DCR* (primary endpoint): *Overall*: 40% (95% CI 25.7–55.7%) *By FR status*$^{\$}$: FR++ (57%; 95% CI 28.9–82.3%); FR+ (43%; 95% CI 27.1–60.5%); FR– (25%; 95% CI 0.6-80.6%) OS: *By FR status*$^{\$}$: FR++ (14.6 months); FR+ (11.6 months); FR– (2.7 months) FR++ *versus* FR+ (HR 0.708; $p = 0.420$) FR++ *versus* FR– (HR 0.170; $p = 0.001$) **Safety** Common vintafolide-related AE (all grades): nausea (29%), anorexia (22%), and neuropathy (20%)
PRECEDENT (EC-FV-04) [ClinicalTrials.gov identifier: NCT00722592] [Naumann *et al.* 2013]	Phase II, R, OL, MC	Patients with platinum-resistant, recurrent ovarian cancer who received up to two previous cytotoxic regimens ($N = 162$); EC20 scans were optional	Combination therapy: vintafolide (2.5 mg intravenously, three times per week during weeks 1 and 3, every 28 days) + PLD (50 mg/m^2 intravenously on day 1, every 28 days) **or** PLD monotherapy	**Efficacy** Combination therapy *versus* PLD monotherapy Median PFS (primary endpoint): *Overall*: 5.0 *versus* 2.7 months (HR 0.63; 95% CI 0.41–0.96; $p = 0.031$) *FR+ patients*: 5.7 *versus* 1.7 months (HR 0.547; 95% CI 0.304–0.983; $p = 0.041$) RECIST-confirmed ORR: *Overall*: 18% *versus* 12% ($p = 0.479$) DCR*: *Overall*: 73% *versus* 53% ($p = 0.018$) Median OS: *Overall*: HR 1.010 (95% CI 0.679–1.503; $p = 0.957$) **Safety** TEAE more common with combination therapy: leukopenia (23% *versus* 8%), abdominal pain (36% *versus* 18%), and peripheral sensory neuropathy (29% *versus* 12%; all $p = 0.026$); neutropenia (44% *versus* 24%; $p = 0.021$) TEAE more common with PLD monotherapy: nausea (8.0% *versus* 0.9%; $p = 0.036$)
PROCEED (EC-FV-06) [ClinicalTrials.gov identifier: NCT01170650]	Phase III, R, DB, PBC	Patients with FR+, platinum-resistant epithelial ovarian, fallopian tube, or primary peritoneal cancer; all patients were scanned with EC20 to assess FR status	Combination therapy: vintafolide (intravenously, three times per week during weeks 1 and 3, every 28 days) + PLD (50 mg/m^2, every 28 days) **or** PLD monotherapy	Primary endpoint: PFS Secondary endpoints: OS, tolerability Results of this trial are anticipated in 2015

(Continued)

Table 1. (Continued)

Trial	Study design	Participants	Interventions	Key efficacy and safety results
Lung cancer trials				
Study EC-FV-03 [ClinicalTrials.gov identifier: NCT00511485] [Edelman *et al.* 2012a, 2012b]	Phase II, single-arm, OL, MC	Patients with progressive adenocarcinoma of the lung who previously received at least two cytotoxic-containing chemotherapeutic regimens (N = 43); at least one 1 EC20+ tumors	Vintafolide monotherapy: induction phase (1 mg intravenously, daily × 5 days for 3 weeks, every 28 days) followed by a maintenance phase (2.5 mg intravenously, three times per week during weeks 1 and 3, every 28 days)	**Efficacy** Clinical benefit response‡ (primary endpoint): *Overall*: 26% (95% CI 14–41%) *By FR status*$: FR++ *versus* FR+ (50% *versus* 14%; p = 0.10) DCR* at 8 weeks: *Overall*: 35% (95% CI 0–12%) Median PFS: *Overall*: 7.4 weeks *By FR status*$: FR++ *versus* FR+ (31.1 *versus* 7.3 weeks; HR 0.326; p = 0.034) Median OS: *Overall*: 42.9 weeks *By FR status*$: FR++ *versus* FR+ (47.2 *versus* 14.9 weeks; HR 0.539; p = 0.101) **Safety** Common drug-related AEs: fatigue (37.2%), constipation (32.6%), nausea (14.0%), and anemia (14.0%)
TARGET (EC-FV-07) [ClinicalTrials.gov identifier: NCT01577654]	Phase II, R, OL, MC	Patients with NSCLC whose condition failed to respond to one previous chemotherapy regimen; all patients have FR++ tumors as assessed by EC20 scans	Vintafolide monotherapy: 2.5 mg twice per week during weeks 1 and 2, every 21 days **or** Combination therapy: vintafolide + docetaxel (75 mg/m^2 intravenously, day 1, every 21 days) **or** Docetaxel monotherapy	Primary endpoint: PFS Secondary endpoint: ORR, DCR, duration of response, duration of disease control, tolerability The study is ongoing

*DCR is defined as the proportion of patients achieving CR, PR, or SD.
$FR status was determined by EC20-based single-photon emission computed tomography to identify patients with FR++ (all target lesions FR+), FR+ (⩾1FR+ target tumor lesion), or FR– (no FR+ target tumor lesions).
‡Clinical benefit response is defined as the ability to receive more than four cycles of therapy, indicating that patients had responded to therapy (SD or radiographic response) and had tolerated therapy well.
AE, adverse event; CI, confidence interval; CR, complete response; DB, double blind; DCR, disease control rate; EC20, ^{99m}Tc-etarfolatide; FR, folate receptor; HR, hazard ratio; MC, multicenter; NSCLC, non-small cell lung cancer; OL, open label; ORR, objective response rate; OS, overall survival; PBC, placebo controlled; PFS, progression-free survival; PLD, pegylated liposomal doxorubicin; PR, partial response; R, randomly assigned; RECIST, Response Evaluation Criteria in Solid Tumors; SD, stable disease; TEAE, treatment-emergent AE.

criteria for PFS to allow continuation of the study. The DSMB did not identify any safety concerns for the patients enrolled in the PROCEED trial. Final results are anticipated to be available in 2015.

Clinical studies: lung cancer

Study EC-FV-03 was a single-arm, open-label trial that evaluated vintafolide in patients with progressive adenocarcinoma of the lung who had previously received at least two cytotoxic-containing chemotherapeutic regimens and were identified as EC20+ (Table 1) [Edelman *et al.* 2012a, 2012b]. The primary objective, clinical benefit response [the ability to receive more than four cycles of therapy, indicating that patients had responded (SD or radiographic response) and had tolerated therapy well], was not met (26%; 95% CI 14–41%); a PR was reported in one patient (2.3%; 95% CI 0–12%). In an exploratory analysis, FR++ patients demonstrated a superior clinical benefit response of 50% compared with 14% in FR+ patients (p = 0.10). Median PFS was 7.4 weeks and median OS was 42.9 weeks for the whole study population. An exploratory analysis of survival outcomes demonstrated a significant improvement in median PFS

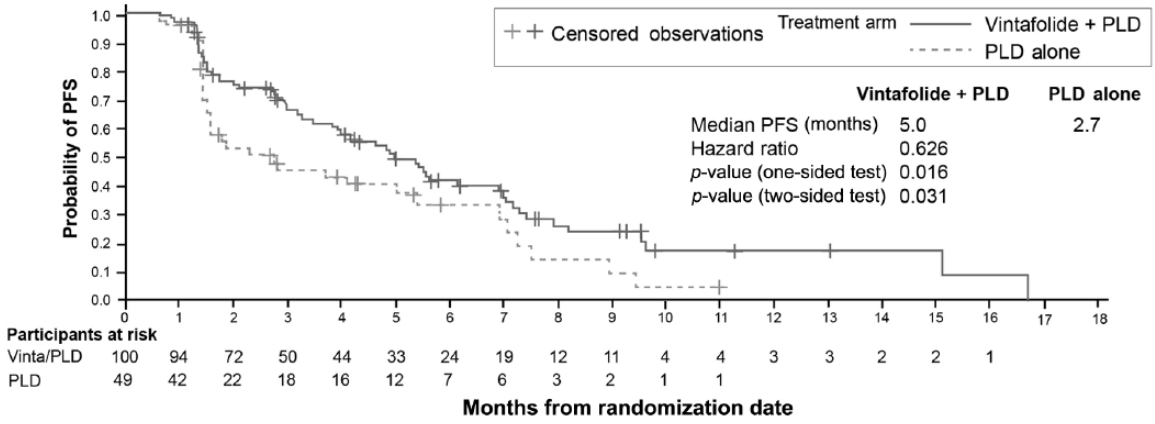

Figure 4. Kaplan–Meier curve of PFS by treatment arm. PFS, progression-free survival; PLD, pegylated liposomal doxorubicin; Vinta, vintafolide. Reprinted with permission from Naumann *et al.* [2013]. © 2013 American Society of Clinical Oncology. All rights reserved.

in patients with FR++ tumors compared with those with FR+ tumors (31.1 *versus* 7.3 weeks; HR 0.326; $p = 0.034$) and a trend toward improvement in median OS (47.2 *versus* 14.9 weeks; HR 0.539; $p = 0.101$) [Edelman *et al.* 2012a].

The randomized, open-label, phase II TARGET trial (EC-FV07) [ClinicalTrials.gov identifier: NCT01577654] is also being conducted to compare vintafolide as second-line treatment with vintafolide plus docetaxel and docetaxel alone in patients with non-small cell lung cancer (NSCLC) who have FR++ tumors. The primary endpoint of this study is PFS, and secondary endpoints include ORR, DCR, duration of response, OS, and safety. Preliminary data for vintafolide in combination with docetaxel showed clinically meaningful improvement across all efficacy endpoints over single-agent docetaxel [Hanna *et al.* 2014]. The median (95% CI) PFS of the vintafolide plus docetaxel and docetaxel groups was 4.2 (2.8–5.4) and 3.3 (1.7–4.2) months, respectively ($p = 0.07$); the median (95% CI) OS was 11.5 (7.3–13.4) and 8.8 (5.4–12.6) months, respectively ($p = 0.29$). The best improvement was observed in the predefined adenocarcinoma patient subgroup with a PFS HR of 0.68 (95% CI 0.41–1.14) and an OS HR of 0.51 (95% CI 0.28–0.94). The safety profile was manageable and consistent with the AEs observed with both therapies.

Safety across clinical studies

In general, the TEAEs observed across clinical studies to date are consistent with the TEAEs associated with vinca alkaloid therapy, with no new or unique TEAE reported [Dosio *et al.* 2010; Pribble and Edelman, 2012]. Although FRα is highly expressed in the kidney [Parker *et al.* 2005], no renal toxicities have been observed [Dosio *et al.* 2010].

Conclusion

Vintafolide has a unique target, the FR, which has several unique properties that make it a rational target for cancer treatment, including high expression in cancer cells of several tumor types and a restricted pattern of tissue-specific expression allowing for selective delivery of FR-targeted agents. Use of the companion diagnostic (etarfolatide; EC20) allows the selection of patients who are most likely to benefit from vintafolide treatment. Vintafolide has been investigated in ongoing phase II and phase III studies in NSCLC and ovarian cancer, respectively, and its favorable toxicity profile provides the potential for combination with other standard approved agents [Reddy *et al.* 2014]. For example, a study comparing vintafolide in combination with PLD and PLD alone indicated that combination therapy may be an effective and safe treatment option for FR+, platinum-resistant disease [Naumann *et al.* 2013], further supporting the investigation of vintafolide in combination with other agents. Additional agents have been investigated, such as the FRα-targeted monoclonal antibody farletuzumab, which has provided disappointing results to date, as well as the ADC IMG853, which is being investigated in phase I studies.

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Conflict of interest statement

Ignace Vergote has no conflicts of interest to disclose. Christopher P. Leamon is an employee of Endocyte, Inc.

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