EXPERT OPINION

Clinical translation of folate receptor-targeted therapeutics

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Introduction: Folate receptor-α (FR-α) has been established as a membrane marker for ovarian cancer. In addition, it is frequently overexpressed in other major types of epithelial tumors. FR-α-based tumor-targeted therapy and drug carriers have been an active area of laboratory research for more than 20 years. Recently, there has been a great increase in the effort to finally translate this promising technology into the clinic and bring FR-targeted therapeutics into the market.
Areas covered: Two FR-targeted therapeutic agents have moved into Phase III clinical trials, the monoclonal antibody farletuzumab and the low molecular weight vintafolide, combined with etarfolatide as a companion imaging agent, representing two alternative strategies for targeting the FR.
Expert opinion: Each of the two strategies has advantages and disadvantages. Identification of the best target patient population is likely critical to the ultimate success of FR-targeted agents in the clinic. A successful clinical strategy may require the integration between FR expression analysis and an optimal combination of FR-targeted therapy and standard chemotherapy. Advancement into Phase III trials and the ongoing clinical development of several additional folate conjugates are likely to usher in a new era of clinical translation and validation of FR-targeted imaging and therapeutic agents.

Keywords: cancer, drug delivery, drug targeting, folate receptor, leukemia

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1. Introduction

Folate is a water soluble vitamin. Tetrahydrofolate, which is a reduced form of folic acid, serves as a coenzyme in one-carbon transfer reactions that are critical for processes, such as nucleotide *de novo* biosynthesis [1]. Membrane-bound isoforms of folate receptors (FRs), including FR–α (encoded by FolR1 gene) and FR–β (encoded by FolR2 gene), are 38 kDa glycosylphosphatidylinositol (GPI)-anchored glycoproteins, both having low nM to sub-nM affinities for folic acid [2]. In contrast, the reduced folate carrier (RFC), an anion transporter primarily responsible for the transmembrane transport of reduced folates, has K_t values in the µM range [3]. Reduced folates and antifolates, such as methotrexate, pralatrexate, and pemetrexed, are transported into cells through the RFC and are subsequently retained in the cytoplasm through polyglutamylation by the folylpoly(γ-glutamate) synthetase [4]. RFC, however, has relatively low affinity for folic acid relative to the FRs and is not expected to transport folate derivatives across the cellular membrane [3]. In contrast, FR-α binds folate derivatives with high affinity and mediates their internalization by endocytosis via a pathway that is associated with caveolae and receptor recycling [5]. FR–α has been shown to be absent in most normal tissues and overexpressed in various types of human cancers [2,6]. Therefore, it has been studied extensively as a tumor cellular surface marker for targeted drug delivery. FR-targeted drug delivery has been discussed in a number of recent review articles [7-10]. This paper will attempt to summarize important developments in the clinical translation of FR-targeted agents.

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Article highlights.

- There are two basic strategies for therapeutic targeting of FRs: anti-FRantibody and folate conjugation.
- Both strategies have led to products in Phase III clinical trials in ovarian cancer, exemplified by farletuzumab and vintafolide/etafolatide.
- Identification of target patient population, based on clinical stage and FR expression status of patients, is highly critical to the success of the clinical development.

This box summarized key points contained in the article.

2. FRs as cellular markers

Expression of either isoform of FRs is highly restricted among human tissues [2,6]. Among normal tissues, FR-α expression is mostly limited to the apical plasma membrane of the polarized kidney and certain epithelial cells [2,6]. This may render them inaccessible to FR-targeting agents in the blood [2]. Meanwhile, the kidney epithelium is accessible to agents that undergo glomerular filtration (molecular weight below 40 kDa) [2]. Furthermore, loss of epithelial cell polarity, which frequently occurs in the tumor, would render FR-α accessible from the circulation [9]. FR-α has been identified as a marker for non-mucinous ovarian carcinomas, of which over 90% show overexpression [11]. Other cancer types, such as endometrial, lung, colorectal, pediatric ependymomas, mesotheliomas, and renal cell carcinomas, all show FR-α expression [12-17]. Interestingly, elevated FR-α expression has been shown to be a negative prognostic factor for at least breast, ovarian, and endometrial cancers [18-20]. High FR-α expressing tumors have been found to have a more rapid proliferation and to be more resistant to therapy [18-20]. Paradoxically, FR-α expression has been found to be a positive prognostic factor in non–small-cell lung cancers [21,22]. FR–β, which shares ~70% sequence homology with FR–α, is most frequently found in a non–folate-binding isoform on normal granulocytes, possibly due to an alternative post-translational modification [2]. Meanwhile, functional FR-β is found in myeloid leukemia and in activated macrophages associated with inflammation and malignant tumor [23-25]. Therefore, FR-β is potentially useful as a marker for myeloid leukemia, for chronic inflammatory diseases, such as rheumatoid arthritis, and for tumor-associated macrophages [23-28].

There are two general strategies for targeting therapeutics to the FR. The first is based on anti-FR antibody and the second is based on folic acid as a high affinity receptor ligand. Significant progress has been made following both these strategies.

3. Immunotherapy based on FR–α specific antibody

Several anti-FR-α antibodies have been developed for therapeutic targeting of FR-α positive tumors. MOv18 and MOv19 are murine monoclonal antibodies recognizing two non-competing epitopes of FR-α, which have been developed by Miotti *et al.* [29] A chimeric version of MOv18 (c-MOv18) has been studied in ovarian cancer for immunotherapy as a therapeutic antibody [30,31]. Other variants of MOv18 include a bi-specific antibody targeting FR-α and CD3 (a T-cell marker) for T-cell therapy [32,33], and an I-131 radiolabelled antibody for radio-immunotherapy [34]. However, the most significant progress in anti-FR–α antibody therapy field may have been made recently with another monoclonal antibody, farletuzumab (MORab-003), developed by Morphotek, Inc. (Exton, PA, USA), a subsidiary of Eisai, Inc. (Japan) [35,36]. Farletuzumab is a fully humanized antibody derived from the murine antibody LK26, originally developed at the Memorial Sloan-Kettering Cancer Center [37,38]. In preclinical studies, farletuzumab has been shown to elicit robust antibody-dependent cellular cytotoxicity (ADCC) and complement-dependent cytotoxicity (CDC), and to inhibit ovarian tumor xenograft in nude mice [36]. In addition, ^{111}In-labeled MORAb-003 has shown promise as an agent for radio-immunoscintigraphy and radio-immunotherapy [39]. A series of clinical trials have been carried out on farletuzumab, mostly in platinum-sensitive ovarian cancer, with promising results [37].

3.1 Clinical development of farletuzumab

In a Phase I dose-finding study (NCT01004380) of 25 platinum-resistant patients with advanced epithelial ovarian cancer, dosages up to 400 mg/m^2 given by weekly infusions were well tolerated [40]. This was followed by a Phase II study (NCT01218516) in platinum-sensitive patients who experienced first relapse to determine the efficacy of farletuzumab as monotherapy and in combination with carboplatin/taxane [41]. Patients with elevated CA-125 without symptoms received farletuzumab as a single agent at a dose of 100 mg/m^2 weekly, until disease progression [41]. Upon symptomatic relapse, the patients were retreated with carboplatin plus taxol or taxotere in combination with farletuzumab for six cycles. Patients experiencing response were placed on farletuzumab for maintenance [41]. Of the 58 patients enrolled, 54 were found eligible and 28 entered the single-agent arm, while 26 entered the combination arm. Overall, 47 patients ultimately received the combination therapy of which 44 were evaluable. Among these, 39 (88.6%) achieved remission based on normal CA-125 level and 9 (20.5%) achieved a second progression-free interval longer than the first remission [41]. Based on Response Evaluation Criteria in Solid Tumors (RECIST), the objective response rate to the combination therapy was 69.9% with stable disease in an additional 23.2%, for an overall rate of 93% for clinical benefit [41]. In addition, disease stabilization was achieved in 38.5% of the patients treated with farletuzumab alone [41]. These values are significantly better than with platinum (88 versus 59% CA-125 response, 20 versus 3% second remission longer than the first remission), published in a 2004 report [41,42]. The promising finding of this trial has led to the initiation of a multicentered Phase III study (FAR-131) of the farletuzumab-carboplatin/taxane in platinum sensitive ovarian cancer patients suffering relapse within 6 – 24 months, which has just completed enrollment of patients. In addition, there is

an active Phase I trial of farletuzumab combination with carboplatin and pegylated liposomal doxorubicin in platinum-sensitive ovarian cancer patients [43] and a Phase II trial of farletuzumab as a first-line agent in combination with traditional platinum-containing chemotherapies in lung adenocarcinoma (NCT01218516). Meanwhile, it is worth noting that a Phase III trial (FAR-122, NCT00738699) of farletuzumab in advanced platinum-resistant ovarian cancer has been discontinued due to its limited survival benefit to the patients. Furthermore, Morphotek has announced that it is actively developing a companion diagnostic assay to identify patients with high FR–α expression in collaboration with Fox Chase Cancer Center. This might suggest the possibility that future trial designs may incorporate stratification of patients based on their tumor FR expression status. It is possible that patients with high FR expression may benefit more from farletuzumab therapy than those with low FR expression. Patient selection based on receptor expression is currently applied to trastuzumab, which targets HER2/neu and has shown superior efficacy in breast cancer patients with high HER/neu expression [44].

An advantage of the anti-FR antibody strategy is that tumor targeting is not affected by the level of FR saturation by folate found in the circulation and that the antibody is presumably FR subtype specific. A potential drawback of this strategy is that in general antibodies are macromolecules characterized by a relatively extended systemic circulation time [35] and presumably slow kinetics of extravasation. This reduces the achievable target-to-non-target tissue ratio. Moreover, a humanized antibody may still elicit a host immune response in the form of a neutralizing anti-idiotypic antibody that may ultimately undercut its therapeutic efficacy. Nevertheless, the successes of other therapeutic monoclonal antibodies, such as trastuzumab and cetuximab, suggest that the overall approach of antibody therapy is potentially viable.

4. Folate conjugates as FR-targeted therapeutics

Folic acid retains high affinity for FRs following derivatization via one of its two carboxylate groups. Folate conjugation, therefore, presents a versatile strategy for FR-targeted drug delivery. This method has been successfully used for the delivery of small molecules [45], macromolecules, and nanocarriers in numerous preclinical studies [7,8]. For nanocarriers, which include liposomes [46], polymeric nanoparticles [47], and various types of nucleic acid vectors [48], a drug carrier typically is conjugated to folate, usually through a PEG linker to overcome steric hindrance surrounding the FR on the cell surface [7]. Among macromolecules, folate has been conjugated to protein toxins [49], antibodies [50-52], polymeric drug carriers [53], and prodrug converting enzyme [54]. A folate-IgG conjugate has been found to function as a therapeutic antibody by inducing ADCC [50]. The folate-IgG was based on polyclonal human γ globulin, which should not be immunogenic. Other than the mechanism of FR binding, this agent has the physical and chemical properties of a therapeutic antibody [50]. It would be interesting to see if this agent will have similar efficacy to anti-FR antibodies, such as MOv18 and farletuzumab.

Clinical translation of FR conjugates, however, has so far been focused on low molecular weight (MW) folate conjugates and been initiated by Endocyte, Inc. (West Lafayette, IN, USA). These agents, including imaging agents [55], haptens (for immunotherapy) [56], and chemotherapy agents [57], are generally characterized by rapid tissue distribution and fast systemic clearance, thus, resulting in very high tumor-to-background tissue targeting ratios. Meanwhile, due to the renal clearance of folate conjugates and the expression of FR-α in the apical membrane of the renal proximal tubules, imaging agents based on low MW folate conjugates have shown persistent accumulation in the kidneys, which appears to be FR-mediated [58]. However, the same has not been shown with therapeutic folate conjugates. Endocyte has developed a series of products based on folate conjugates at various stages of preclinical development or clinical trial for cancer imaging and therapy, as discussed below. It is important to note that, unlike anti-FR antibodies, these folate conjugates target both FR-α and the functional form of FR-β, and FR binding is expected to be competitively inhibited by excess free folate present in the plasma. Low molecular weight folate conjugates have been described as 'small molecule molecular conjugates' or SMDCs.

5. FR-targeted imaging and immunotherapy agents

^{111}In-DTPA-folate was the first FR-targeted low molecular agent to enter clinical trial, for noninvasive imaging of recurrent ovarian carcinomas [58]. Due to the relatively long (2.8 days) half-life and high cost of ^{111}In, a ^{99m}Tc (half-life 6 h)-based imaging agent is much preferred, especially given the rapid clearance kinetics of a low MW folate conjugate. Accordingly, ^{99m}Tc-based etarfolatide (EC20), which was based on a folate derivative of a small peptide, was synthesized and evaluated clinically as a diagnostic imaging agent for solid tumors based on targeting FR–α [59], and for imaging of chronic inflammatory diseases, such as rheumatoid arthritis, based on targeting FR–β [55]. Rather than diagnosis, the primary purpose of etarfolatide currently is as a companion agent to enable pre-selection of patients whose tumors are highly FR$^+$ and thus constitute the best candidates for FR-targeted therapy. Etarfolatide has been a component of more than 13 clinical trials in over 500 patients with ovarian, endometrial, renal, pituitary, and pulmonary cancers, and has been shown to be valuable for predicting response to FR-targeted chemotherapy. It should also be noted that FR expression level has also been identified as a negative prognostic factor for the biological aggressiveness of ovarian and breast cancers [18-20] and a positive prognostic factor for lung cancer [21]. Therefore, etarfolatide imaging may be useful in the clinic for predicting therapeutic response to chemotherapy in general. In addition, a fluorescein isothiocyanate-folate

conjugate (EC17), has been evaluated as an intra-operative fluorescence imaging agent in ovarian cancer patients in a Phase II trial (NCT01511055) [60].

6. FR-targeted chemotherapy agents

The first folate-conjugated cytotoxic agent to be evaluated in tumor therapy was a maytansinoid conjugate [61]. Since then, a series of chemotherapy agents have been conjugated to folate for FR targeting, with varying degree of success. These include folate conjugated 5-fluoro-2′deoxyuridine-5′-O-monophosphate 10-mer [62], carboplatin [63], and several microtubule poisons [57,64,65]. Key design requirements for this approach to work are that the folate conjugate has to be soluble in an aqueous buffer and that the conjugate must be cleaved inside the cell to generate the active drug [57]. This means typically a hydrophilic (typically ionic) and acid-sensitive and/or enzyme cleavable linker would be needed between folate and the drug moieties [57]. In addition, the drug moiety must have very high cytotoxicity, with an IC_{50} value in the low nM to sub-nM range for the FR-targeting strategy to be effective [8]. This is because there are typically only a limited number of copies of FR on the surface of each targeted tumor cell, thereby limiting the number of molecules that can be delivered into each cell via the FR-dependent pathway. Several conjugates have recently been taken into clinical trial by Endocyte.

Vintafolide (EC145) is a folate conjugate of desacetylvinblastine monohydrazide (DAVLBH), which is a derivative of a microtubule destabilizing agent vinblastine [57,66,67]. It has been selected as the first folate-drug conjugate to enter into clinical trials as a therapeutic agent, along with etarfolatide as a companion imaging agent for FR status determination. This drug has so far completed several Phase II clinical trials sponsored by endocyte, with Merck recently joining as a partner, with several trials ongoing. These include trials in patients with advanced ovarian and endometrial cancers (NCT00507741), with progressive adenocarcinoma of the lung (NCT00511485), with recurrent or refractory solid tumors (NCT00308269), and with platinum-resistant ovarian cancer, as a combination with pegylated liposomal doxorubicin (Doxil) (NCT00722592). Of particular interest is NCT00722592, which recently has led to the initiation of a Phase III trial (NCT01170650). In this trial, patients who had received more than two previous chemotherapeutics were randomized to receive vintafolide (2.5 mg i.v. weeks 1 and 3) and Doxil (50 mg/m^2 i.v. q 28d) or Doxil alone at the same dose until progression or death [68]. The results showed an 85% (2.3 month) increase in median progression-free survival (PFS) in the total population and a 260% (4 month) increase in PFS in the subset of patients that had high FR expression based on imaging study using etarfolatide. Encouragingly, this was the first combination therapy to show a significant delay in PFS over standard therapy in platinum-resistant ovarian cancer patients [68,69]. However, this study failed to show a benefit to overall survival (OS). An interesting finding was that FR-negative patients based on etarfolatide imaging did not benefit from vitafolide therapy, whereas patients who were FR positive did, showing a hazard ration (HR) of 0.381 ($p = 0.018$). These findings appeared to validate the strategy of using an imaging agent to identify FR$^+$ patient population for FR-targeted therapy [69]. Vintafolide and etarfolatide are now in a multicentered Phase III trial (NCT01170650). In addition to ovarian cancer, vintafolide is being evaluated in a Phase II trial in non–small-cell lung cancer as a second line agent used in combination with docetaxel (NCT01577654).

Following vintafolide, several additional agents have also been studied in clinical trials recently. EC0225, a folate conjugated to two different agents, a vinca alkaloid and mitomycin, has completed a Phase I trial (NCT00441870). BMS-753493 (Epofolate) is a folate conjugate of epothilone A, a microtubule stabilizing agent. It is a Phase I/II clinical trial sponsored by Bristol-Myers Squibb in advanced cancer (NCT00546247, NCT00550017). Other agents that have reached clinical trial include EC0489, an analog of vintafolide [70] with reduced hepatic clearance. It is in a Phase I trial in patients with refractory or metastatic tumors (NCT00852189).

7. FR-targeted immunotherapy – FolateImmune[85,90]

FolateImmune is a combination of EC17, a folate-fluorescein (as a hapten) conjugate, and EC90, a fluorescein-keyhole limpet hemocyanin (KLH, a carrier protein) conjugate, and an adjuvant GP1-0100 [71,72]. EC17 acts as an adapter molecule that redirects anti-FITC hapten antibody produced by the patient to FR expressing tumor cells [71]. This has resulted in a Phase II trial in patients with progressive metastatic renal cell carcinoma (NCT00485563), which has been terminated. The patients were first vaccinated with EC90 and the adjuvant, and were then treated with EC17, along with low dose interleukin-2 and interferon-α. EC17 is designed to induce anti-hapten antibody-mediated antibody-dependent cellular cytotoxicity and/or phagocytosis [71,72]. Phase I trial demonstrated the safety of this strategy [72]. This approach has certain potential advantages over the farletuzumab strategy since EC17, which 'activates' the therapeutic mechanism is a small molecule and, therefore, is very inexpensive and is rapidly clearing, and thus can potentially achieve high tumor-to-normal targeting ratios. The therapy is only active in the presence of EC17. Therefore, the therapeutic intensity is adjustable.

8. Comparisons of the two FR-targeting strategies

FR-targeting through folate conjugates is associated with several distinctions compared to the antibody-based approach discussed above. Folate conjugates are capable of targeting both FR-α and FR–β. This potentially enables

the targeting of tumors that are low in FR-α but high in FR-β (e.g., tumors that are highly infiltrated by tumor-associated macrophages that are FR–β^+), as well as chronic inflammatory diseases, such as rheumatoid arthritis [26]. On the one hand, this reduces the selectivity for the tumor cells. On the other hand, this could serendipitously expand the range of disease targets and patient population that are responsive to FR-targeted therapy. A low MW folate conjugate is rapidly distributed into tumor tissues and is rapidly cleared from systemic circulation, reducing concentration in the plasma and in non-target tissues. This typically leads to a higher tumor-to-normal tissue ratio [45]. Moreover, in contrast to antibodies, the low MW conjugates are typically not immunogenic and are not subject to denaturation and the associated loss of biological activity. Finally, it is possible to produce these agents by total synthesis as a single pure chemical entity, which is not always possible for macromolecular conjugates.

The potential disadvantages of the strategy of using folate conjugates include interference of FR targeting by circulating folate, which may be influenced by a patient's diet. This may be an issue given the presence of folate supplementation in products, such as enriched flour and uncooked cereal, mandated by the US FDA at 140 μg/100 g and folate as a component of multivitamin supplements [73]. Conversely, it is worth noting that dietary folate has not been a major issue in the clinical studies. In fact, folic acid has been given to the patient in clinical trials to reduce normal tissue uptake of etarfolatide in a clinical trial [58], suggesting that low concentrations of circulating folate may actually be helpful for targeting tumor cells *in vivo*. Another potential issue is the high accumulation in the kidneys due to FR-α expression in the apical membrane of the promixal tubules [59]. The high level accumulation of folate-based radiopharmaceutical suggest that kidney uptake of any low MW folate conjugates is also likely very high since both types of agents similarly bind the FR. Furthermore, the accumulation of these conjugates in the kidneys has been found to be persistent rather than transient. While this has not adversely affected clinical translation of folate conjugates, it may reduce the prospect of clinical application of low MW folate conjugate-based radiopharmaceuticals for targeted radiotherapy. It is worth noting that renal toxicity has not been reported in clinical trials on folate conjugate-based chemotherapies. Finally, the lack of FR subtype specificity of folate conjugates can also be viewed as a negative, if such specificity is desired. Development of subtype-specific conjugates that are based on folate analogs may address this issue in future studies.

Regarding the clinical trial strategies used, there are some important distinctions between the trials for farletuzumab and those for vintafolide. In farletuzumab trials, the drug was used in combination with platinum/taxane and mostly focused on platinum-sensitive early stage ovarian patients. Meanwhile, in vintafolid trials, the drug was combined with Doxil, that is, pegylated liposomal doxorubicin, and focused on platinum-resistant advanced patients, using etarfolatide imaging to identify patients with high FR expression. It is interesting that, in contrast to vintafolide, farletuzumab failed to show benefit in patients that had advanced disease, who presumably are more likely to be FR^+. This may be attributed to the inherent difficulty in treating this patient population. Nevertheless, the developer of farletuzumab, Morphotek, is seeking to develop an FR assay to screen patients for FR expression status. It would be interesting to see the effect of incorporating this assay into a future trial on the clinical efficacy of farletuzumab.

9. Expert opinion

FR-targeted drug delivery is one of the most studied strategies in the field of drug delivery. A large number of drugs and drug carriers have been conjugated to folate for FR targeting. Despite a large number of publications on *in vitro* and *in vivo* studies, relatively few agents have been translated into the clinic. This is because preclinical studies generally involve the use of cell lines and animal tumor models characterized by very high FR expression levels, such as KB cells. The requirement for clinical translation must take into account the more moderate level of FR expression found in clinical situations. The recent movement of fartetuzumab and vitafolitide into Phase III trial suggests that FR targeting is finally reaching a critical point. Meanwhile, continued research is warranted, for example, on developing a serum-based FR assay kit and the issue of FR isoform selectivity. It seems that the strategy of identifying subpopulation of cancer patients with high FR expression will be very important. Given the well-recognized difficulty in finding any effective agents against ovarian cancer in the clinic, identifying the optimum patient population will likely have a large impact on the ultimate success or failure of the Phase III trials. Looking forward, it is reasonable to suggest that more FR-targeted drugs will enter the clinical pipeline given the large volume of preclinical research in this area, either as a therapeutic combination with existing therapy or even as monotherapy. FR-targeting can, therefore, potentially become a prime example in which a combination of strong scientific rationale and sound developmental strategy drives clinical translation.

Declaration of interest

The authors state no conflict of interest and have received no payment in preparation of this manuscript.

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