Targeting the folate receptor: diagnostic and therapeutic approaches to personalize cancer treatments

J. A. Ledermann1, S. Canevari2* & T. Thigpen3

1Department of Medical Oncology, UCL Cancer Institute, London, UK; 2Department of Experimental Oncology and Molecular Medicine, Fondazione IRCCS Istituto Nazionale dei Tumori, Milan, Italy; 3Hematology and Oncology, University of Mississippi Medical School, Jackson, USA

Received 24 December 2014; revised 5 May 2015 and 21 May 2015; accepted 22 May 2015

Background: In cancer therapy, molecularly targeted agents have the potential to maximize antitumor efficacy while minimizing treatment-related toxicity. However, these agents may only be effective in specific tumor subtypes with defined genomic profiles. This emphasizes the importance of developing personalized cancer therapeutic strategies (i.e. through the use of companion diagnostic tests) to appropriately select and treat patients who are likely to benefit from specific targeted therapies, thus leading to improvements in clinical and safety outcomes. A potential biological target is the folate receptor (FR), which has been shown to be overexpressed on the surface of many cancers, including tumors of the lungs and ovaries.

Design: We carried out a literature search to identify how the FR can be a potential target for selected tumors, and how the FR expression can be exploited by targeted therapies.

Results: The two main therapeutic strategies for targeting the FR are based on the use of: (i) an anti-FR antibody (e.g. farletuzumab) and (ii) folate conjugates of folate-targeted chemotherapies and companion radiodiagnostic imaging agents (e.g. vintafolide and ⁹⁹mTc-technetium-etafolatide). Both of these strategies are being assessed in phase III trials.

Conclusions: The important role that the FR plays in cancer development and progression has led to the development of FR-targeted therapeutic approaches. To date, the promising data observed in phase II clinical trials have not been confirmed in phase III studies. Accordingly, there is a need for further research in the refinement of patient selection and identification of new therapeutic combinations. In particular, the development of these targeted therapies requires reliable methods to be developed to detect FR-positive tumors in order to help select patients who may benefit from treatment.

Key words: folate receptor, vintafolide, etarfolatide, ovarian cancer, nonsmall-cell lung cancer

introduction

Molecular-targeted therapies have changed the prognosis of many tumor types; however, balancing the need to destroy cancerous cells with preserving healthy tissue and normal physiologic processes remains a challenge. Furthermore, inhibiting some cancer pathways through targeted therapies may adversely affect signaling pathways in normal cells, resulting in a spectrum of adverse events (AEs) often distinct from cytotoxic chemotherapy [1, 2]. As a paradigmatic example, although the effectiveness of bevacizumab has been seen in ovarian cancer [3–7], predictive biomarkers are urgently needed for the careful selection of patients who are likely to respond to bevacizumab [8] and identification of patients who may be prone to serious toxicities, such as bowel perforation, fistula formation, thrombosis, renal dysfunction, and hypertension [7, 9].

The search for good anticancer targets continues because of the heterogeneous and adaptive nature of many tumor types [1, 2, 10]. One potential target is the alpha isoform of the folate receptor (FR), which is selectively overexpressed in several tumor types, including ovarian cancer and nonsmall-cell lung cancer (NSCLC). In this review, we discuss the concept of personalized cancer therapy, the alpha isoform of the FR as a potential target for selected tumors, its role in both the diagnostic and therapeutic management of specific cancers, and how selectively targeting this receptor may be exploited in the context of personalized medicine.

personalized cancer therapy: the concept

Response targeted therapy depends on variables such as the tumor having the appropriate target and whether the target...
drives cancer growth. It is also affected by mechanisms the tumor uses to bypass the targeted pathway’s oncogenic effects and how the patient processes the drug [10].

Personalized medicine, also known as precision medicine [11], is one of four pillars of systems medicine, which include predictive, preventive, and participatory medicine [12]. Personalized/prediction cancer therapy is based on the concept that patients can be selected for a specific targeted therapy based on molecular characterization of the tumor and its microenvironment, allowing administration of the right treatment to the right patient at the right time [1, 13, 14]. Appropriate patient selection should translate into improved clinical outcomes, measured by response rate, survival, and safety [15].

Biomarkers can be used to measure and evaluate disease prognosis objectively or as a predictor of response to a particular therapeutic intervention. Predictive biomarkers are particularly important in the context of targeted therapy, and a number of biomarker tests have been validated and approved by the US Food and Drug Administration [16–18]. As an example, we will use NSCLC. Patients with advanced NSCLC with specific EGFR mutations have a high likelihood of responding to the EGFR tyrosine kinase inhibitors erlotinib and gefitinib [19, 20]. Similarly, crizotinib has been shown to be active in a small subgroup of patients with ALK rearrangements [21, 22]. For ovarian cancer, validated biomarkers are currently lacking, and research is ongoing to identify biomarkers for use in early detection, defining disease prognosis, predicting response to targeted therapies, and assisting in the planning of individualized treatment [23].

Given that targeted agents often appear to give benefit only to a specific subtype of tumors with a defined genomic profile, it is essential that patients are appropriately selected for treatment. Thus, a need exists to develop and validate appropriate companion diagnostic tests of practical use in the clinical setting. Such tests must demonstrate a clear link between a particular molecular marker and a particular clinical outcome [14].

**role of the FR in cancer development, progression, and treatment**

The folate cycle sustains key metabolic reactions and is essential for rapidly growing cells. Under physiologic conditions, exogenous reduced folates (water-soluble B vitamins) are predominantly transported into cells via the low-affinity, high-capacity, ubiquitously expressed reduced folate carrier (RFC; bidirectional anion-exchange mechanism) [24–26]. Once in the cell, folates play an essential role in the biosynthesis of purines and thymidine, which in turn are required for DNA synthesis, methylation, and repair [24, 25, 27].

Folates are also transported by high-affinity FRs [25]. In humans, there are four isoforms of the FR (FRα, FRβ, FRγ, and FRδ) [28–30]. FRα, FRβ, and FRγ are attached to the cell surface by a glycosylphosphatidylinositol anchor, while FRδ is a secreted protein [27, 31]. A recent study demonstrated that the folate receptor 4 (FOLR4) gene encoded a protein unable to bind folic acid or reduced folate, which led investigators to rename the FRδ in Juno [31]. When folic acid or reduced folate binds to the FR, the cell-surface receptor–ligand complex is transported into the cell via receptor-mediated endocytosis for ligand release [28]. Table 1 shows the distribution of FR isoforms in normal and tumor tissue [27, 30–35]. In the nonmalignant context, FRα tissue distribution is restricted to a limited number of polarized epithelia (uterus, placenta, choroid plexus, lung, and kidney), with expression localized at the apical/luminal surface of polarized cells, precluding contact with the circulation [27–29].

Table 1. Tissue distribution of FR family members

<table>
<thead>
<tr>
<th>Protein namea (gene name)</th>
<th>Normal tissue distribution (cellular expression)</th>
<th>Tumor tissue overexpression</th>
</tr>
</thead>
<tbody>
<tr>
<td>FRα [27, 30, 32, 33] (FOLR1)</td>
<td>Some polarized epithelia: Fallopian tube (timbrated end) Proximal kidney tubules (apical brush border) Type I and II pneumocytes in the lungs (apical) Choroids plexus (apical versus the cerebrospinal fluid) Submandibular salivary glands (luminal) Bronchial glands (luminal) Retinal pigment epithelial cells (basolateral) Trophoblasts in placenta</td>
<td>Epithelial tumors of: Ovary, fallopian tube, and primary peritoneum Kidney Lung Ependymal brain Uterus, breast, colon Malignant pleural mesothelioma</td>
</tr>
<tr>
<td>FRβ [30] (FOLR2)</td>
<td>Hematopoietic tissues: spleen, thymus, peripheral blood (monocytes = low levels; activated macrophages = high levels) Placenta</td>
<td>Hematologic malignancies (AML, CML)</td>
</tr>
<tr>
<td>FRγ [30, 34] (FOLR3)</td>
<td>Hematopoietic tissues: spleen, bone marrow, thymus</td>
<td>Hematologic malignancies Epithelial tumors of ovary, endometrium, cervix Unknown</td>
</tr>
<tr>
<td>FRδ/Juno [31, 35] (FOLR4)</td>
<td>Oocytesb Regulatory T cells</td>
<td></td>
</tr>
</tbody>
</table>

*aFor more details, see also the herein cited references.

bEssential for mammalian fertilization, mouse ortholog fully characterized.

AML, acute myelogenous leukemia; CML, chronic myelogenous leukemia; FOLR1-4, folate receptor 1 (2, 3, or 4) gene; FR, folate receptor.
contrast, FRα is overexpressed in the majority of tumors of the ovary, uterus, or ependymal brain and malignant pleural mesotheliomas, and is also overexpressed in a variable percentage of lung, kidney, breast, and colon carcinomas [27, 30, 32–34]. In the context of malignancy, it is noteworthy that FRα loses its polarized cellular location and instead, the entire cell surface is covered with FRα proteins. The role of FRα expression in tumors is unclear, but may involve modulation of folate uptake from serum [36] or activating signals associated with tumor growth [37]. In vitro and in vivo studies have demonstrated a correlation between human ovarian cancer growth and FR overexpression [38]. Inhibition of FRα expression in naturally expressing FRα-positive tumor cell lines also suppresses cell proliferation [39, 40]. In vivo studies in mouse tumor xenografts expressing human FRα have shown that anti-FRα mAbs suppress tumor growth [41]. Furthermore, FRα expression may also induce drug resistance by enhancing the anti-apoptotic ability of tumor cells [42]. In addition, some evidence suggests that FRα overexpression is not altered after chemotherapy in ovarian and endometrial cancer [43, 44] and may represent a marker for resistance to conventional chemotherapy in ovarian cancer [42, 45].

The prognostic/predictive significance of FRα has been less intensively analyzed and, as a result, remains controversial. Furthermore, the differences in the survival associations of FRα expression that were observed in different histologic/gender subtypes suggest that there are differences in tumor biology [43–47]. Recently, an analysis of tissue microarray of ovarian cancer tumor samples found that FR expression in high-grade serous ovarian carcinoma was associated with increased overall survival (OS) in the first 2 years following diagnosis [48]. However, there is a decreased progression-free survival (PFS) interval associated with FR expression in clear-cell carcinoma [48]. As a result of these data, further analyses should be prospectively investigated in clinical trials.

Because FRα is expressed on the cell surface in a tumor-specific manner, it provides the potential to allow not only tumor localization, but also selected delivery of therapeutic agents to the malignant tissue, minimizing collateral toxic side-effects. There are a number of unique advantages to exploiting FRα as a diagnostic and therapeutic target [30, 49]. First, FRα is located on the luminal surface of epithelial cells in most proliferating nontumor tissues and is inaccessible to circulation. In contrast, FRα is expressed all over the cell in malignant tissue and is accessible via circulation. Second, FR has the ability to bind to folic acid, a relatively innocuous, small molecule that can rapidly penetrate solid tumors and is amenable to chemical conjugation with other molecules. Once a folate conjugate is bound to FR, it is internalized into the cell and the FRα is rapidly recycled to the cell surface via the FR-mediated endocytic pathway, as depicted in Figure 1 [49, 50]. These factors all emphasize the potential role of FRα in the diagnosis and treatment of specific tumor types.

role of FR expression in personalized cancer therapy

Given that FR is expressed in only specific tumor subtypes, it may be a predictive biomarker for FR-directed therapy and useful as part of a personalized approach to treatment. Among the challenges of evaluating and validating cancer biomarkers

Figure 1. Schematic presentation of tumor-cellular uptake of folate and folate conjugate. FR, folate receptor.
is developing appropriate, robust, and practical measurement assays [51]. Various semiquantitative and quantitative methods have been used to assess FRα levels in tumor biopsy specimens or in the circulation; however, there are inherent challenges with many of these techniques, particularly in a clinical setting (Table 2) [42, 46, 47, 52–56].

An attractive option is the in vivo use of a companion imaging agent that allows the whole-body, noninvasive, real-time assessment of FRα expression, facilitating the monitoring of FR status throughout the course of treatment without invasive tissue biopsies. Furthermore, FR expression on tumors can be heterogeneous and the molecular characteristics of tumors may change with time [57–59], suggesting that reliance on historical balances to test for a target or mutation is unsatisfactory. The interaction between folic acid and FRα has been identified as a useful vehicle for allowing imaging probes to be localized on FR-expressing cells. The probe is conjugated to the folic acid molecule, binds with high affinity to the FR and emits an imaging signal. Current methods for detecting probe/folic acid conjugates include fluorescence imaging, magnetic resonance imaging, computed tomography, ultrasound imaging, single-photon emission computed tomography (SPECT), and positron emission tomography. In the context of tumor imaging, a number of folate conjugates have been evaluated [60–67]. One of particular interest is etarfolatide (EC20), an imaging agent composed of 99mTc-technetium (Tc) complexed to a short folate-linked peptide (Figure 2A) [68]. 99mTc is a commonly used radiographic tracer that primarily decays by gamma emission (range, 4.81–19.98 MBq/kg). Between 1 and 2 h after injection, anterior and posterior midthigh to head planar images were acquired with a dual-detector, large-field-of-view gamma-camera equipped with low-energy, high-resolution, parallel-hole collimators. SPECT images of the region that was known to contain the target lesion(s) were obtained after planar imaging. One hundred five patients (68%) had at least one metastatic site that showed uptake of 99mTc-etarfolatide (54% considered to have marked uptake and 46% had mild uptake). There were 17 AEs reported in 8% of patients receiving 99mTc-etarfolatide, but only three events were considered to be ‘possibly related’ to study treatment.

99mTc-etarfolatide has also been used as the companion imaging agent in three phase II studies evaluating the FR-targeted drug conjugate vintafolide in patients with platinum-refractory epithelial ovarian, primary peritoneal, or endometrial cancer [69]; progressive lung adenocarcinoma [70]; and platinum-resistant ovarian cancer [71]. The safety of 99mTc-etarfolatide was

<table>
<thead>
<tr>
<th>Methodologya</th>
<th>Major characteristics</th>
<th>Sensitivityb</th>
</tr>
</thead>
<tbody>
<tr>
<td>Radioligand binding assay [52]</td>
<td>Quantitative assay&lt;br&gt;Applicable to body fluids&lt;br&gt;Need for a long-half-life isotope (3H)&lt;br&gt;Detection of functional FR&lt;br&gt;Does not discriminate between FRα and FRβ</td>
<td>Low (medium after microfiltration) [53]</td>
</tr>
<tr>
<td>Sandwich assay [54]</td>
<td>Quantitative assay&lt;br&gt;Applicable to body fluids&lt;br&gt;Need for highly selected pairs of antibodies&lt;br&gt;Detection of FR-specific isoforms&lt;br&gt;Does not discriminate between active and inactive molecules</td>
<td>Medium</td>
</tr>
<tr>
<td>IHC [46, 47, 55]</td>
<td>Aminable to automation but quantification procedures still in progress&lt;br&gt;Detection of cellular and subcellular location&lt;br&gt;Applicable to archival tissues&lt;br&gt;Low cost</td>
<td>Medium</td>
</tr>
<tr>
<td>Reverse-transcription quantitative PCR [42, 56]</td>
<td>Semiquantitative assay, amenable to automation&lt;br&gt;High dynamic range but amplification needed&lt;br&gt;Does not provide information about the protein expression&lt;br&gt;Still not standardized for FR RNA from archival tissues</td>
<td>High</td>
</tr>
</tbody>
</table>

aReferences are related to assays dedicated to FR detection.
bThe sensitivity is defined on the assay format in general; only in the case of radioligand binding assay is a specific reference for increase in sensitivity available.

FR, folate receptor; IHC, immunohistochemistry; PCR, polymerase chain reaction.
supported in each of these studies. A recent review commented on the use of \(^{99m}\)Tc-etarfolatide \([72]\) as an FR-imaging agent to select for patients who are most likely to respond to FR-targeted therapy. However, these imaging results and their interpretation may be affected by a number of physiological and technical factors, including \(^{99m}\)Tc-etarfolatide uptake by several normal organs, possible false positivity in areas of infection or inflammation, and the use of separate SPECT and CT images \([72]\). In addition, a single-arm, open-label, exploratory study analyzing the correlation between FR expression by immunohistochemistry and \(^{99m}\)Tc-etarfolatide uptake reported an observed agreement between methods of 72% for positive results and 38% for negative results \([61]\).

**FR-targeted therapeutics in personalized cancer therapy**

The selective expression of the FR on cancerous tissues makes it attractive for targeted therapy. At present, two main strategies of FR-targeted therapy are in clinical development: folate conjugates and mAbs \([73–77]\). An alternative or even combinatorial strategy of FR-targeted therapy involves the development of antifolates that selectively target the FR \([25]\). One example is the novel FR\(\alpha\)-targeted thymidylate synthase inhibitor, BGC 945 (ONX-0801), which is selectively transported into FR\(\alpha\)-overexpressing tumors, potentially resulting in minimal toxicity to healthy tissue \([78]\). An ongoing phase I clinical trial is being conducted to assess the safety and pharmacokinetics of BGC 945 in patients with solid tumors \(\text{NCT02360345}\).

**FR-targeted mAbs**

Farletuzumab (MORab003) is a fully humanized FR\(\alpha\)-binding mAb that does not prevent folate from binding to the FR nor does it block FR-mediated transport of folate into the cell \([76,\ 77,\ 79]\). Upon binding to FR\(\alpha\) expressed on tumor cells, farletuzumab exerts its antitumor activity through different modes of action: (i) promotion of tumor cell lysis by antibody-dependent cellular cytotoxicity (ADCC) and complement-dependent cytotoxicity (CDC); (ii) induction of sustained autophagy, resulting in a decrease in tumor cell proliferation; (iii) inhibition of the interaction between FR\(\alpha\) and lyn kinase, thus reducing intracellular growth signaling. In a phase II study carried out in patients with platinum-sensitive recurrent ovarian cancer, farletuzumab in combination with carboplatin/taxane followed by single-agent farletuzumab maintenance demonstrated an improved overall response rate compared with historical controls \([75]\). Toxicity was generally favorable, with infrequent infusion reactions reported. Disappointingly, the efficacy data for farletuzumab in phase III clinical trials \(\text{NCT00849667}\) and \(\text{NCT00738699}\) are conflicting. A large phase III study in patients with platinum-sensitive recurrent ovarian cancer evaluating farletuzumab in combination with carboplatin/taxane compared with carboplatin/taxane alone did not meet the primary end point of improving PFS \([80]\), although a recent post hoc exploratory analysis suggested an improved PFS in some patient subgroups \([81,\ 82]\). Farletuzumab was also investigated in a phase II placebo-controlled study in patients with metastatic adenocarcinoma...
of the lung with FRα-expressing tumors (NCT01218516); enrollment has been completed, but results were not available at the time of this review.

MOv18 immunoglobulin E (IgE) is a novel mouse/human chimeric IgE mAb engineered against FRα. MOv18 IgE was shown to be more efficacious than IgG1 in two ovarian tumor xenograft models [83, 84]. Furthermore, in vitro degranulation assays and the co-incubation of MOv18 IgE with serum samples from 21 patients with metastatic solid tumors suggested that MOv18 IgE may be an efficacious and safe candidate for clinical testing in FRα-expressing solid tumors [85, 86]. As a result, a phase I, proof-of-concept study is planned (CRUKD/14/001).

**Folate cytotoxic drug conjugates**

Covalent linkage of folate to a variety of molecules, including chemotherapy or imaging agents, allows targeted delivery to FR-expressing cells. For drug delivery, the folate-drug conjugate binds with high affinity and specificity to the FR and enters cells via endocytosis, often described as a ‘molecular Trojan horse’ [87]. According to recent unpublished experimental data (by the Matherly research group in collaboration with Endocyte), these conjugates do not appear to be substrates for the ubiquitously expressed RFC and may therefore selectively target tumor tissue (unpublished results). Furthermore, they do not interfere with the folate cycle or inhibit cellular uptake of antifolates such as methotrexate or pemetrexed.

Vintafolide is a water-soluble FR-targeting drug conjugate consisting of a folate moiety covalently linked via a peptide spacer and reducible disulfide linker to desacetylvinblastine monohydrazide (DAVLBH) (Figure 2B) [50, 68, 73, 88–90]. DAVLBH is a vinca alkaloid that prevents cell division through mitotic spindle inhibition, causing apoptosis of mitotically active cells [74]. Once vintafolide is endocytosed by the FR-expressing cell, the pH in the endosome decreases via proton pumps, the disulfide bond linking folate with DAVLBH is cleaved through a reductive process, and the drug is released (Figure 1) [50, 68, 73]. Because vintafolide directly targets FR-expressing cells, non-FR-expressing cells have limited exposure to DAVLBH [88].

**Vintafolide clinical trials**

After antitumor activity was reported in a phase I dose-escalation study of vintafolide in patients with refractory or metastatic cancer [91], a phase II clinical program was initiated evaluating vintafolide in patients with platinum-refractory epithelial ovarian, primary peritoneal, or endometrial cancer [69]; progressive lung adenocarcinoma [70]; and platinum-resistant ovarian cancer (PRECEDENT) [71]. Before treatment, expression of functionally active FR was evaluated by SPECT with 99mTc-etarfolatide. Patients were categorized according to the presence of functionally active FR expression on lesions selected according to Response Evaluation Criteria in Solid Tumors (RECIST): patients with 100% of selected lesions that were FR positive were categorized as FR++, patients with at least one selected lesion that was FR positive (10%–90%) were classified as FR+, and those with no FR-positive lesions (0%) were classified as FR−.

**Lung cancer**

Clinical benefit of vintafolide in patients with recurrent or refractory adenocarcinoma of the lung was evaluated in an open-label study [70]. Patients who had received at least two prior chemotherapy regimens (n = 43) and had at least one lesion with FR expression were treated with induction vintafolide (1.0-mg/day i.v. bolus on days 1–5, 8–12, and 15–19, every 4 weeks for two cycles), then maintenance vintafolide (2.5 mg i.v. three times

---

**Table 3. Comparison of FR-targeting therapeutic approaches in cancer**

<table>
<thead>
<tr>
<th></th>
<th>Vintafolide</th>
<th>Farletuzumab</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Structure</strong></td>
<td>Folate conjugate</td>
<td>Humanized monoclonal antibody</td>
</tr>
<tr>
<td><strong>Target</strong></td>
<td>FRα and FRβ</td>
<td>FRα</td>
</tr>
<tr>
<td><strong>Affinity to FR (K dissociation)</strong></td>
<td>Nanomolar range $10^{-10}$–$10^{-11}$</td>
<td>Micromolar range $10^{-9}$–$10^{-10}$</td>
</tr>
<tr>
<td><strong>Competition with endogenous folates</strong></td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td><strong>Molecular weight</strong></td>
<td>1917</td>
<td>~150 000</td>
</tr>
<tr>
<td><strong>Mechanism of action</strong></td>
<td>Tumor cytotoxicity by [73, 74]: Preventing cell division through mitotic spindle inhibition (experimentally documented)</td>
<td>Tumor cytotoxicity by [75–77]: Antibody-dependent cellular cytotoxicity (experimentally documented)</td>
</tr>
<tr>
<td></td>
<td>Causing apoptosis of mitotically active cells (experimentally documented)</td>
<td>Complement-mediated cytotoxicity (experimentally documented) Induction of cell death associated with autophagy (experimentally documented) Inhibition of association of FRα and lyn kinase (hypothesized)</td>
</tr>
<tr>
<td><strong>Administration route</strong></td>
<td>i.v.</td>
<td>i.v.</td>
</tr>
<tr>
<td><strong>Pharmacokinetics (half-life)</strong></td>
<td>26 min</td>
<td>121–260 h following multiple weekly infusions</td>
</tr>
</tbody>
</table>

FR, folate receptor; i.v., intravenous; $K_{dissociation}$, equilibrium dissociation constant.
weekly on weeks 1 and 3 of a 4-week cycle). In the overall study population, the primary objective of clinical benefit (defined as an ability to receive at least four cycles of therapy) was not met (26%). However, patients with FR++ lesions had a notably higher clinical benefit rate (50%) compared with those with FR+ lesions (14.3%). Furthermore, an overall-survival advantage was noted for the FR++ versus FR+ patients, with a median of 47.2 versus 14.9 weeks [hazard ratio (HR) 0.539; \( P = 0.101 \), one-sided test]. Most common drug-related AEs were fatigue and constipation, with no drug-related grade 4 toxicity.

This phase IIb study (TARGET; NCT01577654), conducted in patients with FR-positive tumors (as determined by imaging with \( 99mTc \)-etarfolatide during the study-screening period), compared the addition of vintafolide to docetaxel with docetaxel alone in patients with advanced NSCLC in whom one prior chemotherapy regimen had failed. The primary end point of the study was PFS [92]. Vintafolide in combination with docetaxel showed clinically meaningful improvement across all efficacy end points over single-agent docetaxel, and the best improvement was seen in the predefined adenocarcinoma patient subgroup: PFS HR of 0.68 (95% confidence interval (CI) 0.41–1.14), OS HR of 0.51 (0.28–0.94). The safety profile was manageable.

**Conclusions**

The FR plays an important role in cancer development and progression, and FR-targeted therapies have led to a need for reliable methods for detecting FR-positive tumors to help select patients who may benefit from treatment.

Results from phase II clinical studies in ovarian and lung cancer demonstrated that both mAb-based and folate-conjugate therapeutic modalities improved outcomes in patients with FR-positive tumor. However, when evaluated in advanced-stage platinum-resistant ovarian cancer in phase III trials, the two modalities failed to reach their primary end point or did not meet the prespecified criteria for PFS, respectively. In particular, the analysis of two phase II studies emphasized the poor prognosis of patients with platinum-resistant recurrent ovarian cancer with FR-expressing tumors compared with those without FR expression when treated with standard chemotherapy. Therefore, the disappointing results seen in phase III studies may be partially attributed to the selection of patients with aggressive disease and a poor prognosis who were unable to respond sufficiently to these therapeutic agents. The disappointing results seen in phase III farletuzumab clinical trials may also be explained by the lack of a priori patient selection for FR expression and farletuzumab’s described mechanisms of action, which require a preserved immune system. On the other hand, the effectiveness of vintafolide may be limited by its ability to recognize both the \( \alpha \) and \( \beta \) isoforms of FR (similar to \( 99mTc \)-etarfolatide) and present a certain degree of toxicity.

In any case, the results of phase II clinical trials indicate that \( 99mTc \)-etarfolatide is a promising biomarker of response to vintafolide and other FR-targeted therapies, which is a much-needed personalized approach to therapy. The agreement between \( 99mTc \)-etarfolatide imaging results and immunohistochemistry was limited for negative cases, and this relatively low specificity of the in vivo methods could be attributed to \( 99mTc \)-etarfolatide imaging.
binding to both the α and β isoforms of FR. In contrast, only the α isoform of FR is detected with immunohistochemistry. Furthermore, the time of tissue sampling and the time of 99mTc-etarfolatide imaging were different. Overall, these data suggest that even if the use of 99mTc-etarfolatide as an imaging agent is likely to provide an accurate assessment of predicted response to folate-conjugate therapy, further immunohistochemical evaluation of tumors that have been infiltrated by activated macrophages expressing FRβ may be required, and other companion diagnostics suitable for either of the two therapeutic approaches (folate conjugates and mAb-based therapy) should be developed. Additional ongoing studies of vintafolide in FR-positive patients selected with 99mTc-etarfolatide and future well-designed studies would help to further define which patients will receive the most benefit from treatment and to confirm the correlation between companion diagnostic results and response to FR-targeted therapy.

Finally, many challenges remain with these agents, which can potentially limit their therapeutic activities (i.e. toxicity and drug resistance) or their predictive value as appropriate, robust, and practical companion diagnostic assays. Future predictive biomarker studies and clinical trials must account for many factors, including study design (i.e. sample size and selection of eligible patients), interpretation of results (i.e. qualitative versus quantitative predictive effects), and factors surrounding the clinical utility of biomarker-based tests and new therapeutic approaches (i.e. toxicity and cost).

acknowledgements

Medical writing assistance was provided by Maxwell Chang, and Matt Grzywacz, PhD, of ApotheCom (Yardley, PA, USA).

funding

This work was supported by Kenilworth, NJ, USA. Grant numbers were not used for the development of this work. On 16 April 2012, MSD/Merck and Endocyte, Inc., entered into an agreement to develop and commercialize vintafolide (EC145). However, Endocyte, Inc., regained global rights to vintafolide on 17 June 2014, after MSD/Merck terminated its collaboration agreement with Endocyte, Inc., for vintafolide.

disclosure

JAL has been an advisory board member (no personal remuneration) for MSD/Merck.

TT has been an advisory board member for Endocyte, Inc.

remaining authors have declared no conflicts of interest.

references

5. Barber EL, Zsizos E, Lurain JR et al. The combination of intravenous bevacizumab and metronomic oral cyclophosphamide is an effective regimen for platinum-resistant recurrent ovarian cancer. J Gynecol Oncol 2013; 24: 258–264.
24. Zwicke GL, Mansoor GA, Jeffery CJ. Utilizing the folate receptor for active targeting of cancer nanotherapeutics. NANO Rev 2012 Dec; 7: (suppl of print), doi: 10.3402/nano.v7i0.x18496.
79. Kamen BA, Smith AK. Farletuzumab, an anti-folate receptor alpha antibody, does not block binding of folate or anti-folates to receptor nor does it alter the potency of anti-folates in vitro. Cancer Chemother Pharmacol 2012; 70: 113–120.