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Therapeutic strategies targeting folate receptor α for ovarian cancer

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Epithelial ovarian cancer (EOC) is the deadliest gynecological cancer, and presents a major clinical challenge due to limited treatment options. Folate receptor alpha (FRα), encoded by the FOLR1 gene, is an attractive therapeutically target due to its prevalent and high expression in EOC cells. Recent basic and translational studies have explored several modalities, such as antibody-drug conjugate (ADC), monoclonal antibodies, small molecules, and folate-drug conjugate, to exploit FRα for EOC treatment. In this review, we summarize the function of FRα, and clinical efficacies of various FRα-based therapeutics. We highlight mirvetuximab soravtansine (MIRV), or Elahere (ImmunoGen), the first FRα-targeting ADC approved by the FDA to treat platinum-resistant ovarian cancer. We discuss potential mechanisms and management of ocular adverse events associated with MIRV administration.

KEYWORDS

ovarian cancer, folate receptor α, FOLR1, mirvetuximab soravtansine, MIRV, Elahere, antibody-drug conjugate, ADC

1 Introduction

Epithelial ovarian cancer (EOC) accounts for approximately 95% of ovarian cancer incidence, and is a leading cause of gynecologic cancer mortality worldwide (1, 2). Current standard-of-care treatment for newly diagnosed patients is cytoreductive debulking surgery plus neoadjuvant or post-operative platinum-based chemotherapy. Most patients initially

respond to chemotherapy, but unfortunately up to 80% will eventually relapse leading to patient demise (3). Thus, platinum resistance presents a major clinical challenge. Angiogenesis inhibitor (bevacizumab) and the poly (ADP-ribose) polymerase inhibitors (olaparib, rucaparib and niraparib) provide some benefits for a subset of patients, but can only delay the relapse of platinum-resistant EOC (4, 5). Notably, recent large-scale clinical trials using immune-checkpoint inhibitors (anti-PD1/L1 monoclonal antibodies) failed to provide clinical benefit in EOC. In the past decades, the 5-year relative survival rates of ovarian cancer have only been moderately improved, from 43% in 1995 to 50% in 2018 in the USA (6, 7). Thus, treatment options for platinum-resistant EOC patients are limited, and present a major unmet clinical need.

Folate receptor alpha (FRα), encoded by the FOLR1 gene, has attracted considerable interest due to its high expression in several cancer types including those of lung and breast. FRα shows restricted tissue expression on the plasma membrane of epithelial cells in kidney, lung, ovary, fallopian tube, uterus, cervix, epididymis and placenta, and is highly expressed in approximately 80% of EOC. Additionally, the ability of FRα to internalize relatively large molecules renders it suitable for developing targeted therapies (8, 9). Despite their anti-tumor effects in preclinical models, folate-cytotoxic drug conjugates and no conjugated humanized antibody have yet to demonstrate clinical efficacies (10). In contrast, mirvetuximab soravtansine (MIRV), or Elahere (ImmunoGen), the first FRα-targeting antibody-drug conjugate (ADC), has recently been approved by the US FDA to treat platinum-resistant ovarian cancer (11). Here, we summarize the biology of folate receptors, review different strategies to target FRα, and discuss potential mechanisms of ocular adverse events associated with MIRV. The approval of MIRV has renewed interest to develop other FRα-targeting therapeutics for treatment beyond EOC.

2 Folate transporter proteins

Humans cannot synthesize folate, an essential vitamin for eukaryotic cell proliferation and differentiation, and must obtain folate from dietary sources (12). The uptake of extracellular folate is achieved mainly through three types of folate transporters, including the reduced folate carrier, RFC (encoded by the SLC19A1 gene), the proton-coupled folate transporter, PCFT (encoded by the SLC46A1 gene), and folate receptors (FRs) (13). Ubiquitously expressed RFC serves as the major route of folate transport into systemic tissues (12), whereas PCFT is a proton-coupled transporter responsible for dietary folate absorption in the small intestine (14). Both RFC and PCFT are low-affinity, high-throughput transporters. In contrast, FRs are high affinity, low-throughput transporters that transfer folate through endocytosis in selected tissues (Figure 1).

Folate trafficking via FRα is considered to proceed via potocytosis, a lipid raft-mediated endocytosis mechanism (15). Folate binds specifically to FRα, forming a receptor-ligand complex, and subsequently intracellular vesicles are generated by invagination and budding off. Once internalized, the vesicles join together to from early endosomes, which acidify and fuse with lysosomes to release folates for the one-carbon metabolic reaction (16, 17).

There are four members in FRs family, including FRα (257aa, 30kDa), FRβ (255aa, 29kDa), FRγ (245aa, 28kDa) and FRδ (250aa, 28.6kDa), encoded by FOLR1 (Gene ID: 2348), FOLR2 (Gene ID: 2350), FOLR3 (Gene ID: 2352) and FOLR4 (Gene ID: 390243), respectively. FRs, also known as the folate binding proteins (FBPs), bind folic acid (FA) and 5-mTHF as well as folate-conjugated compounds with high affinity, and transport them inside cells by receptor-mediated endocytosis. FRα, FRβ and FRδ are all glycophosphatidylinositol (GPI) anchored cell-membrane proteins, whereas FRγ is a secreted protein lack of a GPI anchored region (18). FRα is the most studied family member, and is the focus of this Review. FRβ is mainly expressed in placental and myeloid leukocytes, including activated macrophages, tumor-infiltrating macrophages and acute as well as chronic myelogenous leukemia (19–21). FRβ-null mice are apparently normal, indicating that its function is dispensable to maintain organismal homeostasis (22). FRγ is expressed in neutrophil granulocytes and monocytes. FRδ, also named JUNO, is highly expressed in regulatory T cells and mammalian eggs. FRδ lacks the folate-binding pocket, and is unable to bind folate (23). The interaction between FRδ on the egg surface and IZUMO1 on the sperm surface is critical for mammalian fertilization as FRδ knockout eggs are unable to fuse with sperm (24).

FRα is mainly expressed on the plasma membrane of epithelial cells in several tissues, in particular the apical brush-border membrane of proximal renal tubular cells, retinal pigment epithelium, the choroid plexus (25), type1 and 2 pneumocytes in the lung, ovary, fallopian tube, uterus, cervix, epididymis, submandibular salivary gland, bronchial glands and trophoblasts in the placenta (26). FRα has a high affinity for reduced folates, such as tetrahydrofolate (THF), 5-mTHF and FA.

3 The role of FRα in health

FA is a nutrient essential for embryonic development. Folate deficiency can cause embryonic lethality with neural tube defects and orofacial anomalies (27, 28). FRα and its cargo FA are essential for proper mammalian embryogenesis. Knockout of the Folr1 gene is embryonic lethal in mice around the time of neural tube closure (22). Reduced FRα expression and function is associated with craniofacial anomalies, abnormal heart development, and neural tube defects (29). Consistently, daily maternal folate supplementation, before and

Abbreviations: EOC, epithelial ovarian cancers; ADC, antibody-drug conjugate; FRα, α-folate receptor; MIRV, Mirvetuximab soravtansine; RFC, reduced folate carrier; PCFT, proton-coupled folate transporter; FRs, folate receptors; FBPs, folate binding proteins; THF, tetrahydrofolate; AKI, acute kidney injury; SHR, spontaneously hypertensive rat; sFR, soluble folate receptors; hnRNP E1, heterogeneous nuclear ribonucleoprotein E1; PROC, platinum-resistant epithelial ovarian cancer; AIBW, adjusted ideal body weight; ORR, objective response rate; AEs, adverse events; ADCC, antibody-dependent cellular cytotoxicity; CDC, complement-dependent cytotoxicity; DAVLBH, desacetylvinblastine hydrazide; PBD, pyrrolobenzodiazepine; CAR, chimeric antigen receptor.

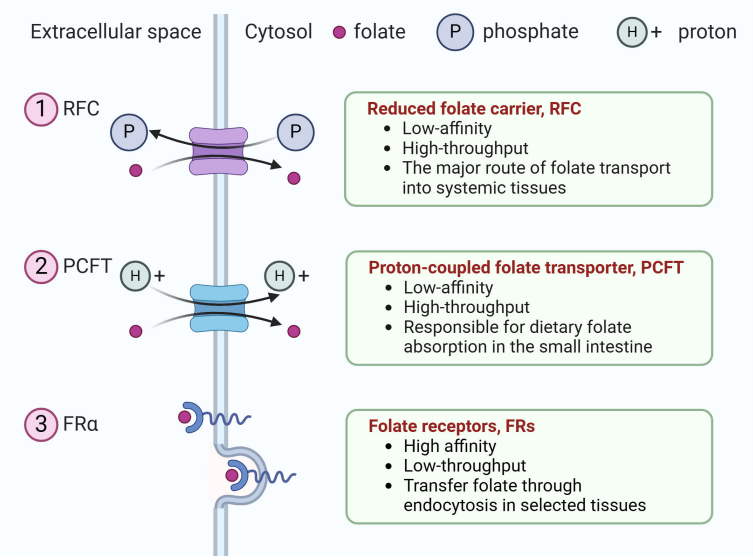

FIGURE 1
The three types of folate transporters. The uptake of extracellular folate is achieved mainly through three types of folate transporters. (1) RFC, an anion antiporter that uses a gradient of higher organic phosphate in the cell to transport folate into the cell while transporting organic phosphate out of the cell, (2) PCFT, a proton-coupled transporter, (3) folate receptor family (only FRα is shown). They transfer folate through endocytosis in selected tissues.

during pregnancy markedly decreased embryonic mortality. Hundreds of genes were differentially expressed at the gestational day 9.5 between Folr1$^{-/-}$ and wild-type embryos. These genes are implicated in the regulation of digestive and cardiovascular system development (27). In the placenta, FRα transports folates from the mother to the fetus (30, 31). Folate deficiency in pregnancy is associated with neural tube defects, restricted fetal growth and fetal programming of diseases later in life (32–34). Importantly, the risk of abnormal pregnancy outcomes is increased in pregnant women taking folate antagonists to treat cancer and other diseases.

FRα is also required to maintains functionalities of several organs in adult animal. Adult mice lacking Folr1 had lower blood folate levels and higher renal folate clearance rate (35). This is because kidneys maintain folate homeostasis in the body through glomerular filtration and tubular reabsorption process. The primary transporter for folate reabsorption in the kidneys is FRα, expressed on the apical surface of proximal tubular cells. FRα transports folate from the tubule lumens into tubular cells via receptor-mediated endocytosis (36). Kidney ischemia-reperfusion injury significantly reduces the expression of FRα and RFC, contributing to low folate level in acute kidney injury (AKI) (37). In spontaneously hypertensive rat (SHR), a deletion variant in the Folr1 promoter region results in impaired folate reabsorption in the renal tubules, and increased risk for diabetes mellitus and cardiovascular disease (38). Within the brain, FRα is selectively expressed in the choroid plexus, and promotes a vesicular transport of 5-mTHF across the choroid plexus (39). It has been reported that mutations in the FOLR1 gene cause cerebral folate transport deficiency resulting in a childhood onset neurodegenerative disease (40–42).

4 FRα in ovarian cancer

FRα is normally expressed in fallopian tube but not the ovary, consistent with EOC originating from the fallopian tube fimbriae rather than from ovary epithelial cells (20, 43). The expression of FRα can be regulated by folate levels. Folate deficiency increases FRα expression *in vivo* and *in vitro* (44). Intracellular folate deficiency is associated with increased homocysteine. Homocysteine can promote the binding of heterogeneous nuclear ribonucleoprotein E1 (hnRNP E1) to the 5' end of FOLR1 mRNA, upregulating FOLR1 expression at the level of translation (45). Folate deficiency also decreases DNA methylation, and global DNA hypomethylation may account for elevated of FRα expression in highly aggressive EOC (46). FRα levels correlate with histological stage and grade (47). A soluble form of FRα, known as soluble folate receptor (sFR), outperforms CA125 as a EOC recurrence marker, even when the CA125 level remains low (18, 48, 49).

4.1 FRα as transporter

It has been proposed that FRα promotes tumorigenesis by increasing folates for one-carbon metabolism (50). However, even when FRα is overexpressed, the main route to transport folate into cells is RFC. RFC accounts for 70% of the uptake of the serum folate 5-mTHF (51). Thus, it is unlikely that increasing folate levels is the primary mechanism of FRα to promote tumorigenesis.

4.2 FRα as transcription factor

Once entering cells by endocytosis, FRα and associated FA can activate several cellular pathways. FRα can translocate into the nucleus and function as a transcription factor to promote the expression of several genes including Oct4, Sox2, Klf4 (52), Hes1 and Fgfr4 (53).

4.3 FRα and cell signaling

In addition, FA, together with FRα, can interact with gp130 to initiate the JAK-STAT3 pathway. Phosphorylated -STAT3 transcriptionally activates its target genes frequently associated with unfavorable patient outcomes (54, 55). The FRα-FA complex also physically interacts with progesterone receptor to promote ERK1/2 phosphorylation (56). FRα can also promote cancer cell metastasis by downregulating the intercellular adhesion molecule E-cadherin (51, 57).

5 Therapeutic strategies targeting FRα

The high expression of FRα in malignant tumors makes it a potential target for anti-tumors drug development. Various strategies have been explored, including monoclonal antibodies, antibody-drug conjugate (ADC), FRα-specific CAR T, vaccines, small molecules, and folate-drug conjugate (Figure 2) (17, 58). Several clinical trials involving FRα-targeted agents are currently ongoing (Table 1). Notably, an ADC drug has recently been approved by US FDA.

5.1 Antibodies

Several FRα-targeting antibodies have been developed, including farletuzumab (IgG1) (74), MOv18 (IgG1) (75), MOv18 (IgE) (76) and MOv19 (IgG2A). Farletuzumab (MORab003; Morphotek, Inc.), the first anti-FRα monoclonal antibody, exhibited anti-tumor activities potentially via inducing antibody-dependent cellular cytotoxicity (ADCC), complement-dependent cytotoxicity (CDC), and persistent tumor cell autophagy leading to reduced cell proliferation and inhibition of the Lyn kinase signaling pathway (77). In a phase I study, farletuzumab showed negligible toxicity in patients with EOC (59). In the phase II study, farletuzumab with carboplatin and taxane enhanced the response rate and duration of response in platinum-sensitive ovarian cancer patients (60). Unfortunately, PFS was not reached in the phase III clinical trial in ovarian cancer patients (61). Nevertheless, farletuzumab was adopted to be the anti-FRα component in ADC drug MORAb-202. MOv18 (IgG1) was not further developed. In a phase I study (NCT02546921), MOv18 (IgE), a chimeric first-in-class IgE antibody, exhibits anti-tumor effectiveness in ovarian cancer patients, with transient urticaria being the most frequent side effect (62). MOv19 (IgG2A) was developed in 1980s. Since

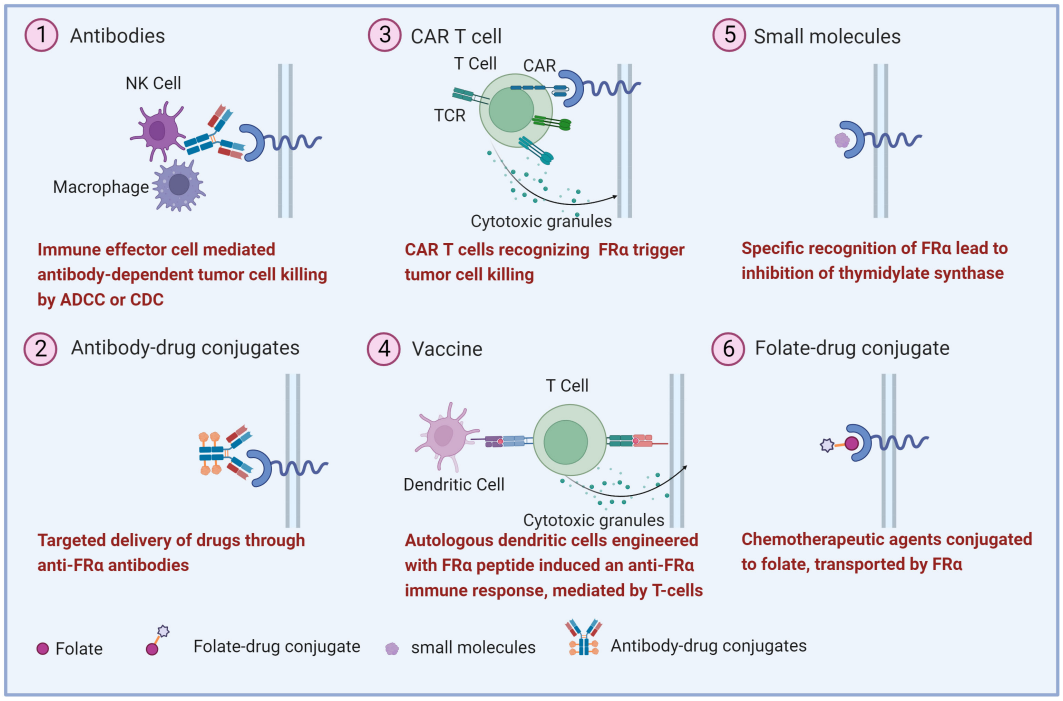

FIGURE 2
Overview of the therapeutic strategies targeting FRα. Various FRα-target strategies in ovarian cancer have been explored including (1) monoclonal antibodies, (2) antibody-drug conjugates, (3) Chimeric antigen receptor (CAR) T cell, (4) vaccine, (5) small molecule and, (6) folate-drug conjugate.

TABLE 1 Key clinical trials using FRα-targeting agents to treat ovarian cancer.

Compound/ Drug	Mechanism	Clinical trial	Outcome	Refs
Monoclonal antibodies				
Farletuzumab/ MORab003	ADCC and CDC	Phase I: Epithelial ovarian, fallopian, or primary peritoneal carcinoma (n=25)	Safe and well tolerated	(59)
		Phase II: relapsed platinum-sensitive ovarian cancer (n=54)	Enhance the response rate and duration of response in recurrent, platinum-sensitive ovarian cancer patients	(60)
		Phase III: ovarian cancer in first platinum-sensitive relapse (n=1100)	Failed to reach PFS endpoints	(61)
MOv18 (IgE)	ADCC and CDC	Phase I: solid tumors expressing FRα (n=26), NCT02546921	Safe and promising antitumor activity in FRα-positive solid tumors	(62)
Antibody-drug conjugate				
MORAB-202	Targeted delivery of drugs through anti- FRα antibodies	Phase I: FRα-positive advanced solid tumors (n=22) NCT03386942	Well-tolerated and promising antitumor activity in FRα-positive solid tumors	(63)
Mirvetuximab Soravtansine/ MIRV/Elahere/ IMGN853	Targeted delivery of drugs through anti-FRα antibodies	Phase I: FRα-positive solid tumors include ovarian cancer (n=44), NCT01609556	Safe and encouraging efficacy	(64)
		Phase Ib: patients with platinum-resistant epithelial ovarian, fallopian tube, or primary peritoneal cancer (n=66), NCT02606305	The combination of MIRV with bevacizumab is well tolerated in patients with platinum-resistant, recurrent ovarian cancer	(65)
		Phase II: platinum-resistant epithelial ovarian cancer (PROC) (n=106), NCT04296890	Favorable tolerability, safety and encouraging efficacy in patients with FRα-high PROC who had received up to three prior therapies	(66)
		Phase III: FRα-positive platinum-resistant ovarian cancer (n=366), NCT02631876	Primary endpoint PFS was not reached	(67)
CAR-T				
Anti- FRα CAR-T +IL-2	CAR-T cells recognizing FRα	Phase I: ovarian cancer (n=14)	Not effective, likely due to short-term survival of CAR-T cells	(68)
Vaccine				
E39+GM-CSF	Cytotoxic T cell reponse elicited by a FRα-dervied peptide	Phase I/IIa: ovarian and endometrial cancer (n=51)	Safe and encouraging efficacy	(69)
Multi-epitope FRα peptide	Cytotoxic T cell response elicited by 5 FRα-derived peptides	Phase I: Ovarian cancer and breast cancer (n=22), NCT01606241	Safe and encouraging efficacy	(70)
Small molecules				
BGC945/CT900/ ONX-0801	Thymidylate synthase inhibitor transported via FRα into cancer cells	Phase I: High-grade serous ovarian cancer (n=109) NCT02360345	Acceptable side effect profiles and significant clinical activity	(71)
Folate-drug conjugate				
EC145/Vintafolide	Chemotherapeutic agents conjugated to folate, transported by FRα	Phase I: refractory solid tumors include ovarian cancer (n=32), NCT00308269	partial response	(72)
		Phase II: recurrent platinum-resistant ovarian cancer who had undergone no more than two prior cytotoxic regimens (n=162), NCT00722592	EC145 plus PLD is superior to the standard therapy	(73)

then, two derivatives of MOv19 have entered the clinical trials. One is M9346A (78), and the other is chimeric antigen receptor (CAR) composed of a MOv19 anti-FRα specific single chain variable fragment (79). M9346A is the anti-FRα antibody component of MIRV (80).

5.2 Anti-FRα ADC

ADC is a drug delivery system, composed of a tumor-targeting monoclonal antibody and a cytotoxic payload joined by a linker (81). Conceptually, this configuration of ADC facilitates the

delivery of cytotoxic drugs specifically to tumor cells, and thus should minimize the damage to normal tissues. However, due to the high affinity of antibody-antigen interaction, ADC could target normal tissues expressing a low level of antigen. Thus, the toxicity profile of ADC may be different from unconjugated cytotoxic payload (1). The innate ability of FRα to internalize large molecules makes it a suitable target for delivering ADC.

5.2.1 MORAb-202

MORAb-202, an ADC that combines the humanized anti-human FRα antibody farletuzumab with the microtubule-targeting drug eribulin, has demonstrated substantial anticancer efficacy in cancer cell lines and in patient-derived xenograft models (63, 82). Of note, eribulin is a license drug to treat metastatic breast cancer in the United States (83, 84). In contrast, payloads in other ADCs are too toxic to be used alone. MORAb-202 is anticipated to cause immunogenic cell death, as has been shown with previous tubulin inhibitor-based ADCs such as T-DM1 (85). The toxicity and pharmacokinetics of MORAb-202 were studied in a cynomolgus monkey model at various dosages (83). The bone marrow was the primary target of MORAb-202 toxicity in monkeys, mostly due to the payload eribulin (86). The efficacy of MORAb-202 depends on the expression level of FRα both *in vitro* and *in vivo* (87). MORAb-202 is now undergoing phase I/II clinical trials to assess its effect in FRα-positive solid tumors (63, 82).

5.2.2 Mirvetuximab soravtansine

MIRV (IMGN853, Elahere) developed by ImmunoGen, is the first ADC to target FRα-expressing tumor cells. It consists of a humanized anti-FRα monoclonal antibody (M9346A) (88), a cleavable linker sulfo-SPDB, and the cytotoxic maytansionoid effector molecule DM4 (88). Once DM4 is accumulated intracellularly, it acts as a potent antimitotic agent by suppressing microtubule dynamics (89). In 2022, MIRV received accelerated approval by US FDA for the treatment of adult patients with FRα-positive, platinum-resistant epithelial ovarian cancer (PROC), fallopian tube cancer or primary peritoneal cancer, previously treated with 1-3 prior systemic anti-cancer regimens (11).

MIRV is taken up by tumor cells through antigen-mediated endocytosis, transported to lysosomes by vesicular trafficking, and degraded to release lysine-Nε-sulfo-SPDB-DM4. The lysine-DM4 is further reduced and S-methylated within the cell, generating hydrophobic maytansinoid derivatives, DM4 and S-methyl-DM4. These three catabolites can inhibit tubulin polymerization and microtubule assembly, leading to cell death. Furthermore, DM4 and S-mehtyl-DM4 can diffuse into intercellular space to kill bystander cells (90). An expansion cohort study of the phase I trial (NCT01609556) found that FRα expression remained stable in biopsy samples following two doses of MIRV, although reductions in post-treatment levels were seen in some patients (91).

The efficacy of MIRV against epithelial ovarian cancer has been investigated in several clinical trials as monotherapy or in combination with other anti-tumor drugs (92). The first-in-human, phase I study (NCT01609556) of MIRV as single agent in patient with EOC and other FRα-positive solid tumors has provided preliminary data on safety and efficacy. A total of 44 patients were enrolled, and the strongest clinical benefit was observed in two EOC patients (64). Thus, additional cohorts were extended as part of the same trial to include individuals with advanced EOC, primary peritoneal or fallopian tube cancers. The objective response rate (ORR) was 22%, and a superior efficacy was observed in the subset of patients with the highest FRα levels (ORR, 31%, PFS 5.4 months) (91). The positive association between FRα expression levels and the efficacy of MIRV prompted another phase I trial, consisting of 46 patients with strong FRα expression (defined as ≥25% of cells with at least 2+ staining intensity by immunohistochemistry). The ORR was 26% and median PFS was 4.8 months (93). These studies established that MIRV had a manageable safety profile, and was effective to control FRα-positive PROC.

In-depth analysis of the phase I results indicated that the response rate was correlated with the number of prior therapies. Patients received four or more priors had a lower response rate (ORR, 13%; PFS 3.9 months) compared with ones received one to three priors (93). On the basis of this observation, the first randomized, multicenter phase III study, FORWARD I (NCT02631876), enrolled platinum-resistant patients (FRα-positive PROC, primary peritoneal or fallopian tube cancer) who have received one to three prior therapies and with high or medium levels of FRα expression, defined as staining intensity ≥2+ in>75% or 50-74% cells, respectively (94). The purpose of this study was to compare the safety and efficacy of MIRV with chemotherapies of investigator's choice (94). A total of 113 ovarian cancer patients were randomly assigned to receive MIRV or chemotherapies of investigator's choice (36 patients in the MIRV arm). The efficacy of the MIRV arm (ORR, 47%; PFS 6.7 months) was superior to outcomes typically seen with established single-agent chemotherapy, including paclitaxel, pegylated liposomal doxorubicin and topotecan. This encouraging result prompted another phase III FORWARD I trial with an expanded population. 366 platinum-resistant ovarian cancer patients were randomly assigned to receive MIRV or chemotherapies of investigator's choice in a 2:1 ratio. However, MIRV did not result in a significant improvement in PFS compared with standard chemotherapy (67), demonstrating that the efficacy of MIRV as monotherapy is limited.

Subsequent clinical trials explored combinatorial approaches. Preclinical studies indicate that MIRV can synergize with carboplatin, doxorubicin, bevacizumab and pegylated liposomal doxorubicin to kill ovarian cancer cells *in vitro* and *in vivo* (95). In FORWARD II trials (65, 96), patients with FRα positive PROC were treated with MIRV and bevacizumab. The objective response rate (ORR) was 39%, including 5 complete responses and 21 partial responses. The median PFS was 6.9 months (65). Thus, the combination of MIRV plus bevacizumab is effective, with long-lasting responses and a manageable safety profile in patients with PROC. A single-arm, phase II study, SORAY (NCT04296890) enrolled 106 FRα-high PROC patients previously undergone one to three treatments, including bevacizumab (66). ORR was 32.4%, with 5 complete and 29 partial responses. The ORR by investigator was 35.3% in patients with one to two priors and 30.2% in patients with three priors. Interestingly, the ORR by investigator was 38% in

patients with prior PARP inhibitor exposure and 27.5% in those without (66).

5.2.2.1 MIRV treatment-related ocular adverse effects

ADCs are expected to target tumor cells with high specificity, and are less toxicity to normal cells than conventional chemotherapies. However, most ADCs exhibit similar toxicity profiles with their cytotoxic payloads (97). The most common treatment-related adverse effects of MIRV were diarrhea, blurred vision, nausea, and fatigue. Most of these adverse events were mild (grade 1 or 2) and were readily manageable with supportive care (64, 91, 93, 97). Reversible ocular adverse events (AEs), primarily corneal keratopathy and blurred vision, frequently occurred among patients (98). This ocular toxicity is likely caused by DM4, as it has been observed in patients treated with other antibody-DM4 conjugates (99, 100). The underlying cause of ocular toxicity is not clear. FRα expression is negative in the eye based on immunohistochemistry. However, the expression of FRα has not been formally ruled out by more sophisticated techniques such as single-cell sequencing. The preventive use of topical corticosteroid eye drops can reduce but not eliminate ocular AEs (101, 102). Further mechanistic studies will be required to disentangle the underlying causes.

5.3 FRα-specific CAR-T

Preclinical investigations have indicated that FRα-specific chimeric antigen receptor (CAR) T cell therapy has promising antitumor effects (103, 104). A phase I trial of a FRα-specific CAR T cell therapy in patients with ovarian cancer showed no reduction in tumor burden, because these T cells did not survive well (68). The addition of costimulatory signals, including CD27, CD28, CD134 (OX-40) and CD137 (4-1BB) into CARs have been shown to promote T-cell survival (104, 105). An improved strategy engineering FRα-specific CAR with a CD137 costimulatory signaling domain in tandem enhanced T-cell persistence in tumor bed, but antitumor activity was still minimal (106). A novel Tandem-CAR encoding an anti-FRα scFv, an anti-MSLN scFv, and two peptide sequences of IL-12 were designed to improve the efficacy, infiltration, persistence, and proliferation of CAR-T cell in ovarian cancer (107). Furthermore, CAR T cells, composed of MOv19 anti-FRα-specific single chain variable fragment fused to 4-1BB and TCRzeta signaling domains (MOv19-BBZ), is currently evaluated by a phase I clinical trial in recurrent high grade serous ovarian cancer patients (78).

5.4 Vaccines

Peptide-based vaccine is another strategy to stimulate antitumor immunity (108, 109). FRα-derived peptides E39 (amino acid 191-199) and E41 (amino acid 245-253) were shown to be immunogenic (110). In a phase I/IIa trial with 51 patients, E39 plus GM-CSF was safe and might be beneficial in preventing the recurrence of high-risk ovarian and endometrial cancers (69). In another phase I clinical trial, the safety and immunogenicity of five FRα-derived peptides were examined in breast and ovarian cancer patients (70). These studies demonstrate that FRα-derived peptides are safe, but their clinical efficacy awaits further investigation.

5.5 Other approaches

5.5.1 Small molecule

BGC 945 (also known as ONX-0801 or CT900) is a thymidylate synthase inhibitor internalized by FRα (111). In a recent phase I clinical trial, the most common BGC945 treatment-related adverse events were fatigue, nausea, diarrhea, cough, anemia, and pneumonitis. Clinical benefit was seen in high-grade serous ovarian cancer patients with medium to high FRα expression (71).

5.5.2 Folate-drug conjugate

It is reasonable to assume that folate-based drug conjugates can enter FRα-expressing cells via endocytosis. The drug conjugates will subsequently be released from FRα due to acidic environment in endosomes, and accumulate intracellularly.

5.5.2.1 Preclinical reagents

EC131, the first folate-drug conjugate, consists of a potent microtubule-stabilizing agent, DM1, linked to FA by intramolecular disulfide bonds. EC131 has not been tested clinically. EC2629 is a folate conjugate of a DNA crosslinking agent pyrrolobenzodiazepine (PBD) linked by a novel DNA-alkylating moiety. Preclinical studies demonstrate that EC2629 has antitumor activity in ovarian, endometrial, and triple negative breast cancers (112). Notably, most ADCs using PBD as the payload are now halted due to excessive toxicity of PBD. No literature regarding EC2629 had been published since 2020, suggesting that its development may be halted as well. BMS753493 is a folate conjugate of the epothilone analog. The frequency and severity of peripheral neuropathy and neutropenia was less in patients treated with BMS748285 than epothilones. However, little efficacy was observed in solid tumors including ovarian cancer, and further development of BMS753493 was halted (113).

5.5.2.2 Agents in clinical stage

EC145 (vintafolide) is a water-soluble derivative of FA linked to the vinca alkaloid desacetylvinblastine hydrazide (DAVLBH). In a phase I clinical trial, one partial response was observed in a patient with metastatic ovarian cancer (72). In a randomized phase II trial of patients with platinum-resistant ovarian cancer, EC145 plus pegylated liposomal doxorubicin exhibited efficacy superior to the standard therapy (73). Unfortunately, in the phase III clinical trial (NCT01170650), the PFS in ovarian cancer patients was not reached (114).

6 Conclusion and future perspectives

The understanding of the molecular characteristics of EOC have advanced in the past decade. However, platinum resistance remains a

major clinical challenge, and renders EOC the most fatal gynecological malignancy. Angiogenesis inhibitors (bevacizumab) and PARP inhibitors (olaparib, rucaparib, and niraparib) have not significantly increased overall survival in most patients. Innovative and effective therapeutic strategies are urgently needed. In this regard, FRα has emerged as an appealing and clinically verified candidate for the development of targeted therapies. The relatively enriched expression of FRα on the surface of cancer cells and the ability of FRα to transport cytotoxic payloads into cancer cells have inspired the development of various therapeutic modalities including antibodies, ADCs, CAR T, vaccines, small molecules, and folate-drug conjugate. Notably, MIRV, a FRα-targeting ADC, has recently been approved by US FDA to treat adult patients with PROC, fallopian tube cancer or primary peritoneal cancer. Several promising FRα-targeting modalities are under clinical evaluation. It will be of interest to see their efficacy on EOC and other FRα-expressing cancer types.

It is also interesting that ADC is the only FRα-targeting modality that has achieved clinical efficacy so far. We speculate that the inhibition of FRα function via monoclonal antibodies may not be enough to inhibit tumor growth. This is because FRα is not a major survival signaling pathway even in FRα-high tumors. In addition, RFC is the major folate transporter and co-expressed with FRα. Although folate is an essential vitamin, suppressing FRα activity is not sufficient to block folate transport into cells. On the other hand, folate-drug conjugates can act similarly as FRα-targeting ADCs to deliver toxic payload into FRα-high cells. However, considering that RFC and PCFT are major folate transporters in many tissues, folate-drug conjugates likely can enter any cells expressing RFC and PCFT. Thus, folate-drug conjugates likely have less targeting specificity and therapeutical index than FRα-targeting ADCs.

In our opinion, further basic and clinical investigations are warranted to maximize the clinical efficacy of MIRV. MIRV is currently only approved for ovarian cancers with high expression of FRα. Considering that FRα is highly expressed in several cancer types, MIRV may be effective in these contexts. In addition, MIRV is known for its bystander effect. Therefore, MIRV may benefit patients with cancers expressing low to moderate level of FRα, analogous to the situation of HER2-targeting ADC, DS-8201a. Lastly, blurred vision occurs in 50-60% of patients treated with MIRV (115, 116). This peculiar high prevalence of ocular toxicity is uncommon in other ADCs, and can be debilitating for patients in our experience. The exact pathological mechanism is yet to be elucidated to improve the prophylactic treatment. Undoubtedly, the landmark approval of MIRV will fuel the interest to develop novel FRα-targeting diagnostic and therapeutic approaches to treat cancer.

Author contributions

JM collected the related paper and drafted the manuscript. LW and LY created the figures. TS, XL, RY, YJ and JL revised this manuscript. QL conceived the structure of manuscript and revised the manuscript. All authors read and approved the final manuscript. All authors contributed to the article and approved the submitted version.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

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