Cytokine and Growth Factor Reviews 45 (2019) 45–52

Contents lists available at ScienceDirect

Cytokine and Growth Factor Reviews

journal homepage: www.elsevier.com/locate/cytogfr

Folate-targeted immunotherapies: Passive and active strategies for cancer

Batoul Farran[a], Eluri Pavitra[b], Prameswari Kasa[c], Sujatha Peela[d], Ganji Seeta Rama Raju[e], Ganji Purnachandra Nagaraju[a,*]

[a] Department of Hematology and Medical Oncology, Winship Cancer Institute, Emory University, Atlanta, GA, 30322, USA
[b] Department of Biological Engineering, Biohybrid Systems Research Center (BSRC), Inha University, 100, Inha-ro, Incheon, 22212, Republic of Korea
[c] Dr. LV Prasad Diagnostics and Research Laboratory, Khairtabad, Hyderabad, 500004, India
[d] Department of Biotechnology, Dr. B.R. Ambedkar University, Srikakulam, Andhra Pradesh, 532410, India
[e] Department of Energy and Materials Engineering, Dongguk University-Seoul, Seoul, 04620, Republic of Korea

ARTICLE INFO

Keywords:
Folate receptor
Immune therapy
Targeted therapy
Vaccines
Solid cancers

ABSTRACT

The glycoprotein FRα is a membrane-attached transport protein that is shielded from the immune system in healthy cells. However, it is upregulated in various malignancies, involved in cancer development and is also immunogenic. Furthermore, FRα is a tumor-associated antigen endowed with unique properties, thus rendering it a suitable target for immunotherapeutic development in cancer. Various anti- FRα immunotherapeutic strategies are thus currently being developed and clinically assessed for the treatment of various solid tumors. These approaches include passive anti-FRα immunotherapies, such as monoclonal antibodies, or active immunotherapies, such as CART, folate haptens and vaccines. In this review, we will explore the advances in the field of FRα-based immune therapies and discuss both their successes and shortcomings in the clinical setting.

1. Introduction

The human folate receptor (FR) is a glycoprotein with increased affinity for folic acid and circulating folate, (6S)N^5-methyltetrahydrofolate [1]. Three genes, hFRα, hFRβ and hFRγ, can encode functional folate receptors in humans [2]. hFRα is the most common isoform. It is expressed at the luminal surfaces of polarized epithelial cells in adult healthy tissues, such as type I and II pneumocytes in the lungs, proximal kidney tubes, choroid plexus, uterus, epididymis, fallopian tube, ovary and throbphoblasts in the placenta [3]. The FRα consists of a globular structure comprising 4 long α helices, 2 short α-helices, 4 short β-strands linked to multiple loop regions [4]. Although its expression is limited in healthy tissues, it is upregulated in various cancers [5,6], such as non-mucinous adenocarcinomas of the uterus, cervix and ovary, testicular choriocarcinoma and brain tumors, breast, lung, colon and kidney cancers.

The overexpression of FRα in many solid tumors, its involvement in cancer pathogenesis and its immunogenicity have rendered it a potential drug target for solid cancers. In fact, research has unveiled the pivotal role that FRα plays in cancer development. For instance, the upregulation of FRα in tumor tissue correlates with an elevated uptake of folate, a key nutrient for dividing cells. Transfecting FRα into cancer cells thus induces faster replication, resulting in worse prognosis and treatment resistance in various malignancies. Seeing as FRα is immunogenic, it has additionally been studied as a promising target for immunotherapeutic interventions. FRα harbors immunogenic sequences that are recognizable by tumor-associated lymphocytes (TAL). Furthermore, two immunogenic peptides derived from FRα, namely E39 and E41, can improve TAL induction and anti-cancer action [7]. In fact, when TALs are stimulated with these peptides, they can recognize and lyse FRα expressing cancer cells from various tissue lineages [8]. These properties have positioned FRα at the forefront of immunotherapeutic drug design, making it an ideal target for the design of passive and active immuno-strategies, such as monoclonal antibodies,

Abbreviations: ACT, adoptive cell transfer; ADC, antibody-drug conjugates; ADCC, antibody-dependent cell-mediated cytotoxicity; BBB, blood-brain-barrier; CAR, chimeric antigen receptor; CART, chimeric antigen receptor T cell therapy; DNA, deoxyribonucleic acid; EGFR, epithelial growth factor receptor; EOC, epithelial ovarian cancer; FA, folic acid; FR, folate receptor; FRα, folate receptor alpha; hFRα, human folate receptor alpha; FRβ, folate receptor beta; IgE, immunoglobulin E; IgG, immunoglobulin G; mAB, monoclonal antibody; NSCLC, non-small cell lung cancer; NFKB, nuclear factor kappa-light-chain-enhancer of activated B cells; PD-1, programmed cell death protein 1; PTEN, phosphatase and tensin homolog; RCC, renal cell carcinoma; RNA, ribonucleic acid; SEERS, surface-enhanced, resonance Raman scattering; siRNA, silencing RNA; TAA, tumor-associated antigens; TAL, tumor-associated lymphocyte; TCB, T cell bispecific antibody; TLR, toll-like receptors; TNFα, tumor necrosis factor alpha; VEGF, vascular endothelial growth factor

* Corresponding author at: 401 Silverthorn Dr, Marietta, GA, 30064, USA.
E-mail address: pganji@emory.edu (G.P. Nagaraju).

https://doi.org/10.1016/j.cytogfr.2019.02.001
Received 24 January 2019; Accepted 4 February 2019
Available online 07 February 2019
1359-6101/

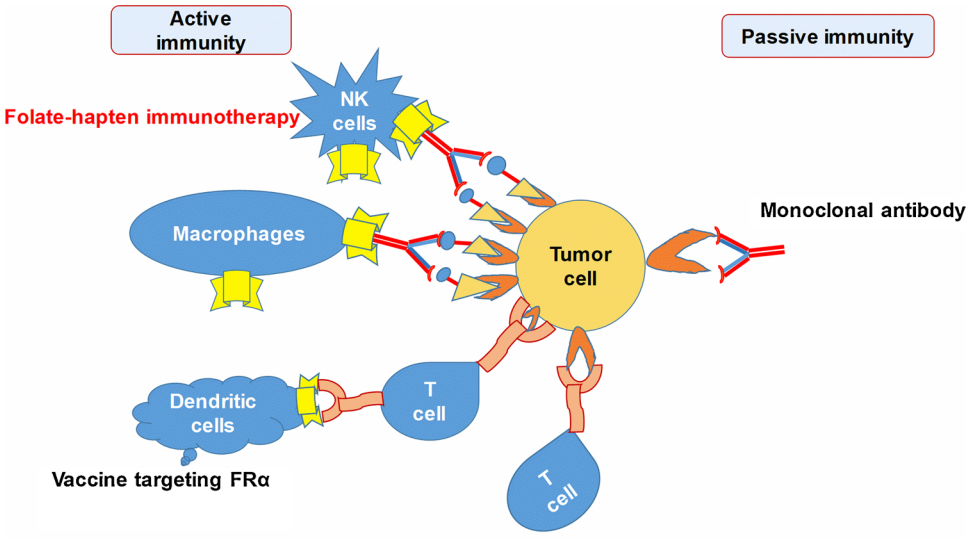

Fig. 1. Different immunotherapy approaches to target FRα. FRα is an ideal target for the design of both passive and active immuno-strategies. Passive immunotherapy includes the injection of monoclonal antibodies that recognize FRα and inhibit its signaling pathways, resulting in malignant cell death. Active strategies include: 1) Chimeric antigen receptor (CAR) T cells, which recognize FRα, resulting in the killing of tumor cells; 2) Vaccines targeting FRα: dendritic cells containing FRα mRNA elicit an anti- FRα immune response carried out by T cells; 3) Folate-hapten immunotherapy: immune effector cells harboring Fc receptors bind to the folate-hapten and FRα complex, leading to antibody-dependent cell mediated cytotoxicity (ADCC) and phagocytosis (ADCP).

CART therapies or folate-based vaccines, aimed at re-instating the body's humoral immune response against tumor cells (Fig. 1). In this review, the various strategies of FRα-based immunotherapies developed to date will be discussed in detail to delineate the advances achieved in this field and the various hurdles that need to be overcome to achieve successful outcomes.

2. FRα in passive immunity

FRα constitutes an attractive immunotherapeutic target to bypass tumor-induced immunosuppression and restore a long-lasting, robust immune response. Consequently, it can be used as a 'docking site' for delivering folate-associated immunotherapeutic agents to FRα-overexpressing tumor cells. Hence, enriching tumor surfaces with foreign haptens through FRα could offer an efficient approach for improving the immunogenicity of tumor cells without provoking an inflammatory response [9]. Various passive and active immuno-strategies have so far been tailored to target immunotherapeutic drugs to tumors that overexpress FRα, including 1) monoclonal anti- FRα antibodies, 2) FRα-targeted localization of T cells, 3) radioimmunotherapy, 4) vaccination against FRα using different approaches, and 5) folate -linked haptens, to name a few [8,9]. A number of these strategies were evaluated in the clinical setting, with differing degrees of success that will be discussed below in greater detail.

2.1. MOv18

The central premise of passive immunity is based on the administration of monoclonal antibody therapy. Accordingly, FRα has been passively targeted with chimeric, mouse and human antibodies, alone or as conjugates for the delivery of T cells, radionuclides and cytokines to cancer tissues [8]. Another approach consists in the autologous transfusion of T cells, which employs T cells that have been genetically altered to localize to FRα-overexpressing cancer cells [8]. Historically, the association between FRα expression and cancer was established following the discovery of 2 mouse monoclonal antibodies, i.e. MOv18 and 19, that can react with OC antigens exclusively without affecting normal tissue [10]. The protein targeted by these antibodies was later isolated and identified as FRα [11]. To reduce their immunogenicity and improve their efficacy, both MOv18 and 19 were modified into chimeric antibodies [12]. A phase I clinical investigation that ensued assessed the effects of increased doses of chimeric MOv18 on patients with recurring OC and reported favorable results that were, however, not corroborated by a subsequent study [13,14]. These findings thus stressed the need for improving these monoclonal antibodies in order to enhance their pharmacokinetic properties. A subsequent investigation evaluated the feasibility of labelling MOv19 with the radioisotope 90Y and found that the antibody retained its binding ability and specific *in vivo* localization capacity to FRα-expressing malignancies, suggesting that the 90Y-MOv18 antibody might be clinically useful [15]. Further optimization of the antibody generated the (177)Lu-labeled MOv18, which displayed greater pharmacokinetic activity and improved therapeutic outcomes as compared to 90Y-MOv18 [16]. Advances have also permitted the development of a monoclonal antibody that reacts against both FRα and FRβ. The rationale behind this development is that a monoclonal antibody that can target both FRα-overexpressing and FRβ-overexpressing tumor-linked macrophages could exert enhanced anti-tumor activity compared to individual anti-FRα and anti-FRβ antibodies [17]. Further development is required to improve this strategy and its clinical outcomes, as results have so far been disappointing.

2.2. MORab-003

MORab-003, also known as farletuzumab, is a humanized formula of the monoclonal murine antibody, LK26, and constitutes a potential passive immunotherapeutic agent for FRα-overexpressing malignancies [8]. The humanized MORab-003 antibody displayed promising cytotoxic activity in animal models, eliciting cancer cell lysis through antibody-induced cytotoxicity [18]. These encouraging findings prompted further investigation of this antibody in the clinical setting. Accordingly, a phase I investigation was performed to test farletuzumab safety in epithelial ovarian cancer (OC). The study revealed that MORab-003 was safely tolerated by pretreated OC patients [19], leading to a phase II study that investigated its clinical activity alone or combined with chemotherapy in refractory platinum-sensitive OC [20]. The trial found that farletuzumab plus carboplatin and taxanes could improve the length of the therapeutic response in tested patients, thus resulting in a phase III study [21]. Unfortunately, the results of this trial were disappointing as farletuzumab failed to meet its PFS end-point. However, close scrutiny of the findings revealed that a specific subgroup of OC patients harboring low baseline CA-125 levels demonstrated increase PFS and OS following farletuzumab treatment [21]. These observations indicate that farletuzumab could be beneficial to a selected subset of the OC population, and that biomarker-based patient selection could improve the outcomes of clinical trials, thus leading to more personalized

targeted therapeutic strategies. Furthermore, as the authors of the study indicate, these findings warrant further investigation to elucidate the contribution of CA125 to natural killer cells activity and the therapeutic efficiency of farletuzumab. For instance, increased CA-125 levels could suppress the immune response of farletuzumab-induced ADCC by inhibiting natural killer cells, thus potentially explaining why patients with depleted CA-125 levels develop a more potent MORab-003-elicited immune response [21]. A subsequent study evaluated the genetic profile of the farletuzumab-responsive population and found that this subset exhibits increased binding affinity to the FCGR3A-158 V receptor. Furthermore, this subset displayed improved clinical outcomes in patients harboring low CA-125 levels coupled to at least one FCGR2A or FCGR3A high-affinity allele [22]. This further highlights the importance of in-depth genotyping and patient screening for enhancing the specificity and outcomes of clinical trials and targeted therapeutic regimens. Additionally, the results of these clinical trials suggest that improving the pharmacokinetic profile of farletuzumab could yield enhanced therapeutic action. In fact, the new-generation MOR-ab-202 antibody, composed of MORab-003 conjugated to eribulin, a microtubule targeting agent, demonstrated improved in vivo specificity and exerted enhanced durable and potent anti-tumor effects in a xenograft model, highlighting its potential as a novel therapeutic strategy [23]. Ultimately, farletuzumab could exert more efficient anti-tumor effects when combined with other targeted agents as part of a therapeutic regimen that suppresses multiple downstream resistance mechanisms.

2.3. IgE

Research indicates that the efficiency of immune therapies in solid tumors can be enhanced by IgE antibodies, particularly FRα-targeted monoclonal IgE, compared to the traditionally used IgG antibodies. This increased efficacy is due to the fact that IgE can bind to Fc receptors on mast cells, macrophages and eosinophils with higher affinity than conventional IgG [8,24], thus highlighting the potential therapeutic applications of IgE. These observations were corroborated by studies revealing that the anti-tumor effects of FRα-targeting MOv18 IgE surpassed those of analogous IgG in xenograft models of ovarian cancer [24], further delineating a strategy for improving immunotherapeutic outcomes in solid cancers. Furthermore, molecular studies have shown that anti- FRαIgE exerts its cytotoxic action by upregulating TNFα, which stimulates MCP-1 synthesis by tumor cells and monocytes, resulting in a chemotactic response. Hence, anti-FR IgE functions by reprogramming macrophages and monocytes in the tumor microenvironment, an observation that argues for the use of IgE antibodies to ameliorate immune surveillance and combat tumors [25,26]. This alternative therapeutic approach could overcome the limitations of conventional IgG antibodies but still requires validation in the clinical setting to ascertain its potency and efficiency. To explore this question, a recent study evaluating the safety of MoV18 IgE in a rodent model confirmed its association with increased TNFα levels and enhanced immune cell infiltration of the tumor [27], further supporting its translation to the clinical setting.

2.4. Cytokine-linked monoclonal antibodies

Another form of passive immunotherapy consists in administering immunomodulating cytokines as monotherapies or combined with other therapeutic regimens to treat malignancies. However, this therapeutic option is poorly tolerated because of its side effects [8] and requires improvements to mitigate its toxicity while enhancing its safety. An effective strategy to diminish these undesired side effects is to target immunomodulatory cytokines to the tumor microenvironment using appropriate tags. This approach was attempted for FRα-overexpressing tumors by interlinking IL-2 to a single chain of MOv19 and was found to exert more potent anti-tumor activity than IL-2 alone in an ovarian cancer model [28]. Recent advances have permitted the design of novel solutions that exploit nanotechnology for enhanced targeted delivery of immunogens and immunocytokines to FRα-overexpressing cells. For instance, an FRα-targeting lipoplex, incorporating plasmid IL-12 was tailored and investigated in an *in vivo* colon cancer model, resulting in enhanced anti-tumor action characterized by increased apoptosis and TNFα secretion and reduced microvessel density [29]. A similar FRα-targeting lipoplex loaded with IL-15 plasmid or pIL15 was also developed and resulted in improved anti-cancer activity in an *in vivo* colon cancer model [30]. These various findings suggest that FRα-targeted delivery of immunogens and immunocytokines could be a potential treatment approach for FRα-positive tumors and warrants additional development and investigation to assess its clinical relevance.

3. Active immunotherapy

In contrast to passive immunotherapy, active immunotherapy is a form of immune therapy aimed at enlisting and activating the host's immune system and instating lasting immune protection against tumor development and progression. Although active immunotherapy has not yet achieved the breakthroughs of its passive counterpart, it remains an attractive option supported by promising outcomes in animal models, dispersed clinical successes [8] and technological advances. Recent years have witnessed increasing efforts to tailor active immunotherapies against FRα-overexpressing malignancies using various strategies such as genetically altered autologous T cells, folate-associated haptens to induce immunogenicity against tumors cells, as well vaccine approaches incorporating single and multi-epitope peptide vaccines [8]. Although this flurry of technological advances has not yet met resounding clinical success, it remains a promising endeavor due to the absence of auto-immune occurrences in active anti-FR immune therapies.

3.1. T cell activating approaches

One of the most attractive immunotherapeutic approaches that have emerged recently is "Adoptive Cell Transfer" or ACT, an innovative strategy based on harnessing and exploiting the patient's own immune cells to combat their tumor. While several forms of ACT have been developed, the most clinically enticing and studied strategy is CAR T-cell therapy, described as "giving patients a living drug" by Renier J. Brentjens from Memorial Sloane Kettering Cancer Center. This therapeutic strategy revolves around T cells, as its name suggests, and consists in drawing blood from patients, isolating their T cells and genetically re-engineer them to produce surface receptors known as chimeric antigen receptors or CARs. The T cells expressing CAR, endowed with a selective tag that allows them to recognize specific molecules expressed on cancer cell surfaces, are then expanded in the laboratory and infused into the patients. This technological advance is specifically pertinent to FR-overexpressing tumors and has led to the engineering of T cells armed with FR tags or bispecific antibodies that have been tested in the clinic.

The first phase I trial testing genetically modified T cells targeted against FR in ovarian cancer had disappointing outcomes. The autologous T cells were harvested and genetically altered to express an Fc/MoV18 fusion protein following which they were reinfused into patients [31]. The T cell transfusion was administered in combination with IL-2 adjuvant in one instance, while the T cells harbored dual-specificity in the other. Unfortunately, the cells failed to persist in the long term, highlighting the necessity of developing solutions to improve T cell persistence. The results of this trial and other clinical trials are listed in Table 1. In contrast, immunotherapy of ovarian cancers using bi-specific monoclonal antibody to retarget T cells lead to tumor regression with mild toxicity although greater improvements in systemic antitumor responses are required to enhance this approach [32]. Protein engineering advances have allowed the development of new-

Table 1
Clinical trials investigating folate immunotherapies in solid cancers.

Treatment	Cancer	Number of patients	Dose	Outcome	Reference
c-MOv18	Ovarian Carcinoma	15	5–75mg	Good binding to antigen	[13]
c-MOv18	Ovarian Carcinoma	5	50mg	No response	[14]
Farletuzumab	Epithelial ovarian carcinoma	25	12.5–400 ng/m2	Safe and well-tolerated	[19]
Farletuzumab	Epithelial ovarian carcinoma	5	100 mg/m2 single or combined with carboplatin and taxane	Significant clinical activity and enhance reponse duration	[20]
Farletuzumab	Ovarian Carcinoma	1,100	Farletuzumab (1.25 mg/kg or 2.5 ml/Kg) with carboplatin and docetaxel or paclitaxel	Failed to achieve endpoints	[21]
Autologous gene modified T cells	14	14	T cells (720,000 IU/kg) and IL2 or allogeneic PBMC	No response observed	[31]
Folate immune	Renal Carcinoma	28	0.2mg	Well tolerated	[44]
Folate immune	Renal Cell Carcinoma	24	1.2mg	Moderate clinical activity	[46]
E-39 + GM-CSF	Ovarian and Endometrial Cancer	51	250mcg	Encouraging efficacy	[50]

generation BsABs and CARs with improved persistence in circulation, targeted localization to the tumor and enhanced tumor toxicity. One such design consisted in FRα-retargeted CAR- T cells, incorporating the CD137 (4-1BB) domain [33,34]. Another group reported the development of an anti-CD3 Fab-folate conjugate that can target T cells to FRα-overexpressing tumors, leading to enhanced T-cell associated elimination of FRα-positive cancer cells *in vitro* [17]. Another solution aimed at modulating the expansion and improving the safety of T cells therapies consisting in tailoring a bi-functional switch composed of folate linked to folate-FITC that can retarget FITC-associated T cell action toward FRα-positive tumors with increased specificity [35]. This construct exerted high cytotoxicity against FRα-positive cells, indicating that this innovative solution could enhance CAR- T cell immunotherapies safety and warrants further development and pre-clinical testing (Fig. 2).

Another paradigm shift consisted in developing a completely human CAR composed of C4 human FRα-specific single chain antibody variable fragments conjugated to T cell signalling intracellular regions [36]. These fully human CARs produced proinflammatory cytokines and displayed cytolytic action against in vitro FRα-overexpressing tumors and resulted in ovarian cancer regression in a xenograft model. Compared to mouse MOv19 based FRα CAR, the human CARs exerted similar cytotoxic effects but demonstrated diminished recognition of normal cells that express low FRα levels [36]. These findings indicate that T cells harboring fully human CARs can effectively eliminate FRα-expressing tumors and could overcome the “on-target and off-tumor” toxicities associated with CARs composed of mouse derived variable antibody fragments, thus establishing new pre-clinical optimization paradigms. Lentiviral technologies were also exploited to design a novel RNA CART T cell strategy. In fact, an RNA platform was employed to re-engineer T cells to express FRα-associated CD27 CARs formed from human components. These RNA CARs elicited potent cytolytic action against FRα-positive tumors and promoted the regression of human ovarian cancer xenograft models [37]. Hence, these findings support the clinical investigation of RNA CARs to test their safety and efficiency in patients and delineate a road map for the translation of this immunotherapeutic approach to the clinic.

Technological developments have also allowed to the design of an innovative therapeutic tool consisting of T cell bispecific antibodies (TCBs) that target FRα and CD3, recruit the T cells to tumor sites and stimulate them. The novel FolR1-TCB lead to the activation of intratumoral T-cells in various malignancies but its efficiency was hindered by the abundance of PD-1 infiltrating T cells [38]. These findings suggest that combining TCBs with other drugs that target T-cell dysfunction could yield enhanced cytotoxic activity and improve immunotherapeutic outcomes. An alternative strategy to ameliorate the efficacy of CART therapies in solid cancers consisted in resorting to oncolytic viruses, which can act synergistically with immunotherapies as a result of their oncolytic immunogenic characteristics and their ability to incorporate transgenes in their genome [39]. For this purpose, an oncolytic adenovirus comprising an EGFR-targeted bispecific T cell engager (OAd-BiTE) was developed and tested in combination with anti- FRαCART cells. BiTE-elicited oncolysis improved CART-cell recruitment and proliferation and enhanced anti-cancer activity in animal models. These findings indicate that combining these two therapeutic approaches can bypass the shortcomings of each alone and warrants further development and clinical testing to assess its efficiency.

Adoptive FR CART immunotherapy has been tested in a number of FRα-overexpressing tumors and has demonstrated promising pre-clinical potential. For instance, Song et al. [40] studied the efficiency of anti-FRαCAR treatment on triple negative BC cells and the anti-tumor action of engineered T cells, both in vitro and in mice. The investigation demonstrated that FRα CAR T cells exerted anti-tumor action against TNBC tumors, specifically those that express increased levels of FRα. These findings indicate that pre-selecting patients that express increased antigen levels could enhance the outcomes of CAR-based approaches in clinical trials and lead to more personalized treatment options. Another study evaluated the anti-tumor activity of CIK cells altered with third generation FRα CARs in

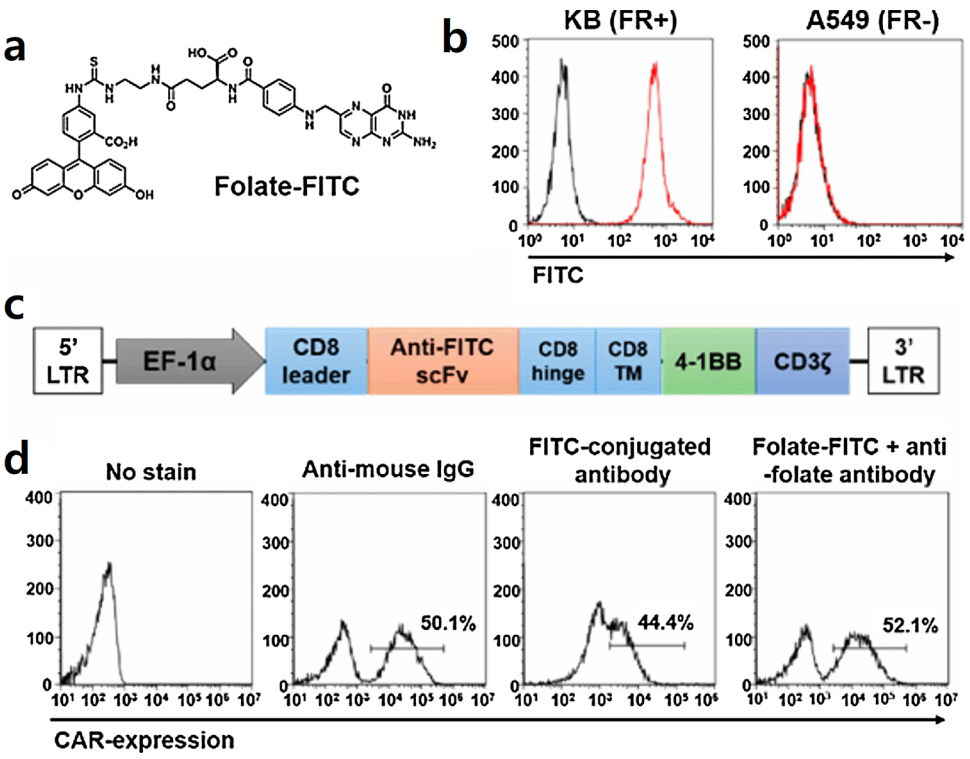

Fig. 2. (a). Structure of folate-FITC. (b). Assessment of folate-FITC binding to KB (FR +) and A549 (FR-) cell lines by flow cytometry. The cells were labeled with (red line) or without (black line) 50 nM folate-FITC and analyzed by flow cytometry in the FITC channel. (c). Anti-FITC CAR lentiviral construct. (d). Anti-FITC CAR expression on human T cells was assessed by direct staining with antimouse IgG F(ab")2 antibody (Jackson Immuno Research) or a FITC-conjugated isotype antibody (eBiosciences). Expression was also detected by primary staining with folateFITC and secondary staining with an antifolate antibody (Novus Biologicals) conjugated with Alexa Fluor 647 dye [35].

ovarian cancer cells and detected enhanced anti-cancer immunity using this approach [41]. Adopting another innovative technical strategy, a third study aimed at tailoring a switch-modulated approach in order to allow increased control of reactivity of CART in NSCLC. The group generated anti-FITC CAR T cells that can target cells expressing both FRα and FRβ [42]. The bi-specific adaptor-modulated CART therapy attacked both tumor cells and tumor-linked macrophages in NSCLC, thus warranting further development and pre-clinical investigation. FRα CART therapy was also tested in gastric cancer and demonstrated favorable results in FR-positive gastric cancer cells [43], thus supporting further investigation.

3.2. Folate-hapten immunotherapy

Folate-hapten immunotherapy is a multi-step targeted strategy aimed at 1) eradicating FRα-overexpressing tumors and 2) re-educating the immune system to identify then eliminate cancer cells, independent of their FRα status [9]. For this purpose, the tumor-expressing animal is vaccinated against foreign antigenic haptens, thus leading to a potent humoral anti-hapten immune response (step 1) [9]. While the immune response of a cancer patient to his/her own tumorigenic antigens might be silenced during tumorigenesis, this step harnesses his/her uncompromised ability to launch an immune attack targeting foreign antigens. Following the induction of an adequate anti-hapten titer, the folate-hapten conjugate is systematically administered as part of step 2 [9]. The tumor specificity of the ligand ensures the selective tagging of cancer cells and avoids the marking of normal cells for destruction. For example, folate-fluorescein (folate-FITC) decorates FRα-expressing tumor cells specifically while sparing normal tissues. Next, step 3 unfolds spontaneously, as the anti-haptens induced in step 1 attach to the exposed haptens on cancer cell surfaces, leading to step 4, which implicates the recognition of the antibody-decorated cancer cells by the immune cells bearing the Fc receptors, including macrophages, dendritic cells and natural T killers [9]. Additionally, cytokines are dispensed during this step to improve the anti-tumor action of the infiltrating immunogenic cells. Finally, step 5 consists in the extermination of tagged cancer cells by antibody-elicited mechanisms, including ADCC and antibody-induced phagocytosis, thus ensuring that the immune system is effectively scrutinizing destroyed cancer cells [9]. This immunotherapeutic strategy provides a rounded plan to reactivate the immune system and harness its natural defenses to eradicate malignant cells, while preventing toxicity to healthy cells, and has been tested in a number of cancers, with varying levels of success (Fig. 3).

The first study to investigate the efficiency of folate-hapten immunotherapy was conducted by Lu and Low [44]. It investigated the ability of folate and fluorescein conjugates in marking the cellular surface of murine lung tumor cells using large hapten numbers, against which a potent antibody titer was previously elicited. IL-2 and IFN-α were used as stimulatory cytokines. The study revealed that a significant enhancement of life span and even complete cures coupled to long-term tumor-targeting immunity were achieved using folate-fluorescein conjugates in combination with lower concentrations of IL-2 and INF-α in this pre-clinical model [44]. This report indicated that the synergy between the immunotherapy and enhancing cytokines is required to boost the immune system and that further optimization of this strategy is necessary to ameliorate the efficiency of folate-targeting immunotherapies. These results prompted a first clinical study investigating folate-hapten immunotherapy in human patients, namely the safety and efficiency of an immunotherapeutic treatment termed Folate Immune in FR-positive renal cell carcinoma patients [45]. This regimen combines a vaccine, i.e. EC90 plus GPI-0100, as well as a folate-hapten conjugate (EC17), which can target the FRα-overexpressing tumor cells. It consists of a sequence of vaccinations directed against (EC90) hapten fluorescein combined to GPI-0100 to induce the generation of FITC-selective antibodies, followed by inoculation with EC17. The hapten causes bridge formation between the anti-hapten antibodies and the tumor cells, thus tagging cancer cells for antibody-elicited eradication. The major side-effected reported in this trial was hypersensitivity to EC17, particularly in underimmunized patients due to anti-FITC IgE antibody formation as well as the occurrence of undetectable levels of bisfluorescein in EC17, which prompted the development of an early desensitization strategy. The overall results of this trial indicated that this regimen was tolerated at clinically suitable doses, leading to a phase II investigation further evaluating the efficiency of this treatment. The phase II study examined whether the folate immunotherapy could be enhanced by combination with IL2 and IFN-α in RCC [46]. The regimen was well-tolerated but displayed limited cytotoxic activity. These results suggested that combining folate immune therapy with alternative companion therapeutic strategies could improve its anti-tumor immunogenic activity.

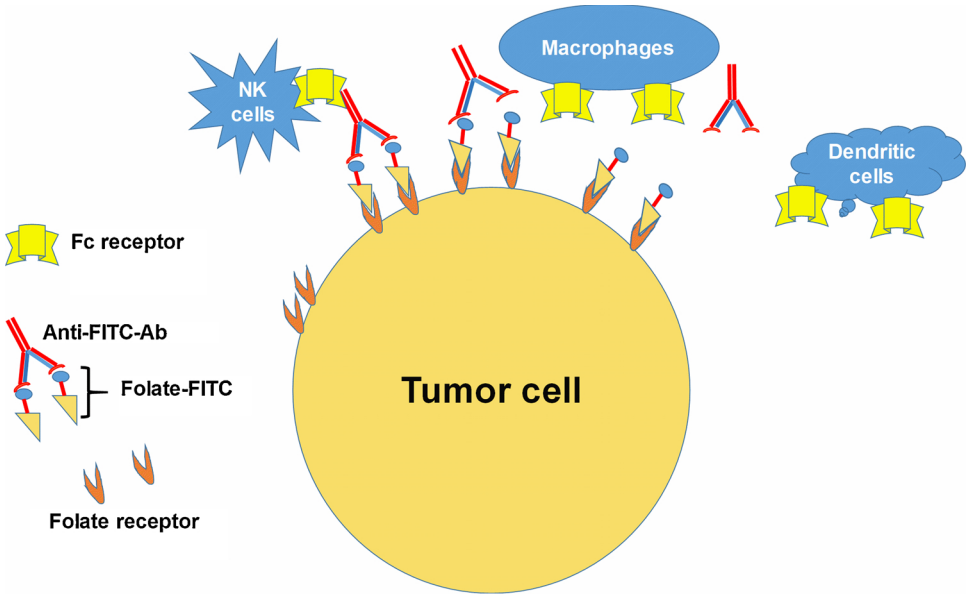

Fig. 3. Effector cells such as macrophages, dendritic cells or natural killer (NK) cells recognize the antibody conjugated to the folate-hapten (folate-FITC) complex on the surface of folate receptor (FR)-positive tumor cells through their Fc receptors. This activates the immune system, eventually leading to the eradication of the tumor cells [9].

For instance, published reports have indicated that combining folate targeted hapten immunotherapy using low-dose radiation therapy in a mouse model seemed to improve anti-tumor activity [47]. Another potential companion therapy consists in the co-administration of folate-fluorescein immunotherapy with VEGFR inhibitors (sunitinib and axitinib). The study revealed synergistic effects between VEGFR inhibitors and folate targeting immunotherapy that enhanced their ability to recruit CD4+ and CD8 + T cells, inhibit tumor development, suppress angiogenesis through immune-cell induced eradication FRα-positive tumor cells and FRα-positive tumor-related MDSCs and macrophages [48]. These findings suggest that this particular combination regimen, incorporating folate immunotherapy and targeted VEGFR therapy, might be a useful strategy against various solid cancers and warrants further investigation in pre-clinical and clinical studies.

3.3. Peptide-based vaccines

Another strategy that harnesses the properties of active immunity consists in the development and administration of peptide-based vaccines. In fact, a peptide-based vaccine incorporates immunogenic peptides, isolated from tumor-associated antigens (TAAs) in conjunction with immune-adjuvants, in order to instate TAA-specific immunogenecity. The use of single peptides bears various advantages. For instance, delivery of individual peptides devoid of non-immunogenic molecules present in common vaccination techniques induces a potent antigen-specific immune response that is crucial to bypass tumor immune escape. Furthermore, vaccine peptides are inexpensive, well-tolerated and generate well-characterized and easily monitored immunological responses (Fig. 4). One major setback of individual peptides, however, is that they are frequently HLA-restricted and only elicit immune responses to epitopes comprised in a vaccine. Hence, the use of multiple epitopes at once could hamper the dosage and effectiveness of individual peptides.

One of the first FRα peptides to be developed were: two HLA-A2-restricted, and MHC class I, FRα peptides, namely E39 or FRα191–199 and E41 or FRα 245–253, that were evaluated for their use in vaccines. Studies showed that TAL elicited with either E39 and E41 can recognize and lyse FRα-positive cancer cells, that lymphocytes presented by dendritic cells with E39 elicit the activity of CTL, and that these lymphocytes were frequent in OC and BC patients at varying stages [49]. A phase I/IIa study of E39 plus granulocyte macrophages and colony stimulating factor (GM-CSF) was conducted in the aim of reducing reappearance in OC and EC patients (n = 51) at increased recurrence risk after conventional therapies [50]. The investigation showed that E39 + GM-CSF was tolerated and induced a potent dose-dependent immunogenic response characterized by encouraging efficacy, specifically within the 1000 mg dose ovarian cancer subgroup, which displayed the highest decrease in recurrence. Based on these preliminary findings, an extended phase II trial will be conducted using a 1000 mcg E39 + GM-CSF together with booster vaccinations in patients harboring disease free ovarian and endometrial cancer patients [50]. These results highlight the importance of carefully selecting patients enrolled in large trials and of fine-tuning doses and companion treatments to ensure the clinical efficacy of tested regimens.

In addition to single peptides, two FRα targeting vaccines have been tailored and investigated in clinical trials. These anti- FRα vaccines target various classes of epitopes to elicit immunogenic responses against each and to stimulate immune memory to help prevent cancer recurrence. For instance, the first multipeptide vaccine combined five immunogenic FRα peptides and was investigated by a small phase I study (n = 22) in breast and ovarian cancer patients [51]. The multi-peptide was safely tolerated with moderate toxicity only and induced FRα-selective T cell responses that persisted following the completion of the vaccine therapy in most patients [51]. These findings imply that further investigations are required to assess the clinical efficacy of this vaccine and whether it constitute a useful strategy for preventing recurrence or combined with the blockade of immune checkpoints. The other multi-epitope FRα vaccine incorporated five distinct HLA-A1/2/3 peptides, including E39, originating from different antigens observed in OC cells. It was evaluated in a small phase I investigation (n = 9), in stage III/IV OC. The vaccine displayed a low toxicity profile and was immunogenic, leading to the production of peptide specific lymphocytes [52]. These various clinical studies highlight the promising prospects of anti- FRα peptide vaccines, revealing their safety and immunogenicity and stressing the need for future trials to evaluate their clinical efficacy and potential as combination/companion treatments alongside other therapeutic regimens.

4. Conclusion

In this review, we have examined the various FRα-based immunotherapeutic strategies developed to target FRα-overexpressing cancers. These strategies comprise approaches that rely on both passive and active immunity. Passive immunity-based formulations focus on the development of monoclonal antibodies such as Mov18, MORab-003 or cytokine-linked monoclonal antibodies that can selectively target FRα-positive cancers. Another attractive translational tactic consists in

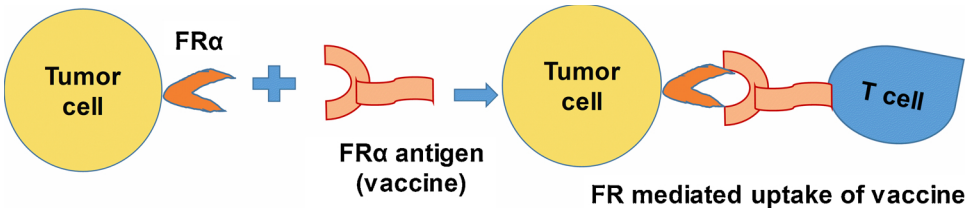

Fig. 4. Peptide-based vaccines: Cytotoxic T cells elicited with the folate receptor alpha (FRα) peptide vaccine elicits an immunogenic response leading to the killing of FRα positive tumor cells.

harnessing the power of active immunity to tailor complex solutions for FRα-positive tumors. These approaches include FRα-CART immunotherapies, folate-hapten therapies or peptide-based vaccines. While these various strategies have been tested in clinical trials, yielding promising results, they require further optimization and additional evaluation on a larger scale to determine their actual therapeutic efficacy against FRα-overexpressing tumors.

Funding

This work did not receive any specific grant from funding agencies.

Conflict of interest

We report no conflicts of interest.

References

[1] H. Matsue, K.G. Rothberg, A. Takashima, B.A. Kamen, R.G. Anderson, S.W. Lacey, Folate receptor allows cells to grow in low concentrations of 5-methyltetrahydrofolate, Proc. Natl. Acad. Sci. U. S. A. 89 (13) (1992) 6006–6009.
[2] A.S. Wibowo, M. Singh, K.M. Reeder, J.J. Carter, A.R. Kovach, W. Meng, M. Ratnam, F. Zhang, C.E. Dann 3rd, Structures of human folate receptors reveal biological trafficking states and diversity in folate and antifolate recognition, Proc. Natl. Acad. Sci. U. S. A. 110 (38) (2013) 15180–15188.
[3] H. Elnakat, M. Ratnam, Distribution, functionality and gene regulation of folate receptor isoforms: implications in targeted therapy, Adv. Drug Deliv. Rev. 56 (8) (2004) 1067–1084.
[4] C. Chen, J. Ke, X.E. Zhou, W. Yi, J.S. Brunzelle, J. Li, E.L. Yong, H.E. Xu, K. Melcher, Structural basis for molecular recognition of folic acid by folate receptors, Nature 500 (7463) (2013) 486–489.
[5] N. Parker, M.J. Turk, E. Westrick, J.D. Lewis, P.S. Low, C.P. Leamon, Folate receptor expression in carcinomas and normal tissues determined by a quantitative radioligand binding assay, Anal. Biochem. 338 (2) (2005) 284–293.
[6] Y.G. Assaraf, C.P. Leamon, J.A. Reddy, The folate receptor as a rational therapeutic target for personalized cancer treatment, Drug Resist. Updat. 17 (4–6) (2014) 89–95.
[7] G.E. Peoples, B.W. Anderson, B. Fisk, A.P. Kudelka, J.T. Wharton, C.G. Ioannides, Ovarian cancer-associated lymphocyte recognition of folate binding protein peptides, Ann. Surg. Oncol. 5 (8) (1998) 743–750.
[8] G.T. Clifton, A.K. Sears, K.S. Clive, J.P. Holmes, E.A. Mittendorf, C.G. Ioannides, S. Ponniah, G.E. Peoples, Folate receptor α: a storied past and promising future in immunotherapy, Hum. Vaccin. 7 (2) (2011) 183–190.
[9] Y. Lu, E. Sega, C.P. Leamon, P.S. Low, Folate receptor-targeted immunotherapy of cancer: mechanism and therapeutic potential, Adv. Drug Deliv. Rev. 56 (8) (2004) 1161–1176.
[10] S. Miotti, S. Canevari, S. Menard, D. Mezzanzanica, G. Porro, S. Pupa, M. Regazzoni, E. Tagliabue, M. Colnaghi, Characterization of human ovarian carcinoma-associated antigens defined by novel monoclonal antibodies with tumor-restricted specificity, Int. J. Cancer 39 (3) (1987) 297–303.
[11] L.R. Coney, A. Tomassetti, L. Carayannopoulos, V. Frasca, B.A. Kamen, M.I. Colnaghi, V.R. Zurawski, Cloning of a tumor-associated antigen: MOv18 and MOv19 antibodies recognize a folate-binding protein, Cancer Res. 51 (22) (1991) 6125–6132.
[12] F. Crippa, G. Buraggi, E. Di Re, M. Gasparini, E. Seregni, S. Canevari, M. Gadina, M. Presti, A. Marini, E. Seccamani, Radioimmunoscintigraphy of ovarian cancer with the MOv18 monoclonal antibody, Eur. J. Cancer 27 (6) (1991) 724–729.
[13] C.F. Moltoff, H.M. Prinssen, P. Kenemans, A.Cv. Hof, Wd. Hollander, R.H. Verheijen, Escalating protein doses of chimeric monoclonal antibody MOv18 immunoglobulin G in ovarian carcinoma patients: a phase I study, Cancer: Interdisc. Int. J. Am. Cancer Soc. 80 (S12) (1997) 2712–2720.
[14] I. van Zanten-Przybysz, C. Molthoff, J. Gebbinck, S. von Mensdorff-Pouilly, R. Verstraeten, P. Kenemans, R. Verheijen, Cellular and humoral responses after multiple injections of unconjugated chimeric monoclonal antibody MOv18 in ovarian cancer patients: a pilot study, J. Cancer Res. Clin. Oncol. 128 (9) (2002) 484–492.
[15] A. Coliva, A. Zacchetti, E. Luison, A. Tomassetti, I. Bongarzone, E. Seregni, E. Bombardieri, F. Martin, A. Giussani, M. Figini, 90 Y Labeling of monoclonal antibody MOv18 and preclinical validation for radioimmunotherapy of human ovarian carcinomas, Cancer Immunology, Immunotherapy 54 (12) (2005) 1200–1213.
[16] A. Zacchetti, A. Coliva, E. Luison, E. Seregni, E. Bombardieri, A. Giussani, M. Figini, S. Canevari, 177Lu-labeled MOv18 as compared to 131I-or 90Y-labeled MOv18 has the better therapeutic effect in eradication of alpha folate receptor-expressing tumor xenografts, Nucl. Med. Biol. 36 (7) (2009) 759–770.
[17] T. Nagai, Y. Furusho, H. Li, K. Hasui, S. Matsukita, K. Sueyoshi, M. Yanagi, M. Hatae, S. Takao, T. Matsuyama, Production of a high-affinity monoclonal antibody reactive with folate receptors alpha and beta, Monoclon. Antib. Immunodiagn. Immunother. 34 (3) (2015) 181–190.
[18] W. Ebel, E.L. Routhier, B. Foley, S. Jacob, J.M. McDonough, R.K. Patel, H.A. Turchin, Q. Chao, J.B. Kline, L.J. Old, Preclinical evaluation of MORAb-003, a humanized monoclonal antibody antagonizing folate receptor-alpha, Cancer Immunity Archive 7 (1) (2007) 6.
[19] J. Konner, K. Bell-McGuinn, P.J. Sabbatini, M.L. Hensley, W. Tew, N. Pandit-Taskar, N. Vander Els, M.D. Phillips, C. Schweizer, S.C. Weil, Farletuzumab, a humanized monoclonal antibody against folate receptor alpha, in epithelial ovarian cancer: a phase I study, Clin. Cancer Res. 16 (21) (2010) 5288–5295.
[20] D.K. Armstrong, A.J. White, S.C. Weil, M. Phillips, R.L. Coleman, Farletuzumab (a monoclonal antibody against folate receptor alpha) in relapsed platinum-sensitive ovarian cancer, Gynecol. Oncol. 129 (3) (2013) 452–458.
[21] I. Vergote, D. Armstrong, G. Scambia, M. Teneriello, J. Sehouli, C. Schweizer, S.C. Weil, A. Bamias, K. Fujiwara, K. Ochiai, A randomized, double-blind, placebo-controlled, phase III study to assess efficacy and safety of weekly farletuzumab in combination with carboplatin and taxane in patients with ovarian cancer in first platinum-sensitive relapse, J. Clin. Oncol. 34 (19) (2016) 2271–2278.
[22] W. Wang, E.B. Somers, E.N. Ross, J.B. Kline, D.J. O'Shannessy, C. Schweizer, S. Weil, L. Grasso, N.C. Nicolaides, FCGR2A and FCGR3A genotypes correlate with farletuzumab response in patients with first-relapsed ovarian cancer exhibiting low CA125, Cytogenet. Genome Res. 152 (4) (2017) 169–179.
[23] X. Cheng, J. Li, K. Tanaka, U. Majumder, A.Z. Milinichik, A.C. Verdi, C.J. Maddage, K.A. Rybinski, S. Fernando, D. Fernando, MORAb-202, an antibody drug conjugate utilizing humanized anti-human FRa farletuzumab and the microtubule-targeting agent eribulin, has potent anti-tumor activity, Mol. Cancer Ther. 17 (12) (2018) 2665–2675.
[24] S.N. Karagiannis, M.G. Bracher, J. Hunt, N. McCloskey, R.L. Beavil, A.J. Beavil, D.J. Fear, R.G. Thompson, N. East, F. Burke, IgE-antibody-dependent immunotherapy of solid tumors: cytotoxic and phagocytic mechanisms of eradication of ovarian cancer cells, J. Immunol. 179 (5) (2007) 2832–2843.
[25] D.H. Josephs, H.J. Bax, T. Dodev, M. Georgouli, M. Nakamura, G. Pellizzari, L. Saul, P. Karagiannis, A. Cheung, C. Herraiz, Anti-folate receptor-α IgE but not IgG recruits macrophages to attack tumors via TNFα/MCP-1 signaling, Cancer Res. (2017).
[26] S.N. Karagiannis, D.H. Josephs, H.J. Bax, J.F. Spicer, Therapeutic IgE antibodies: harnessing a macrophage-mediated immune surveillance mechanism against cancer, Cancer Res. 77 (11) (2017) 2779–2783.
[27] D.H. Josephs, M. Nakamura, H.J. Bax, T.S. Dodev, G. Muirhead, L. Saul, P. Karagiannis, K.M. Ilieva, S. Crescioli, P. Gazinska, An immunologically relevant rodent model demonstrates safety of therapy using a tumour-specific IgE, Allergy 73 (12) (2018) 2328–2341.
[28] C. Melani, M. Figini, D. Nicosia, E. Luison, V. Ramakrishna, G. Casorati, G. Parmiani, Z. Eshhar, S. Canevari, M.P. Colombo, Targeting of interleukin 2 to human ovarian carcinoma by fusion with a single-chain Fv of antifolate receptor antibody, Cancer Res. 58 (18) (1998) 4146–4154.
[29] M. Luo, X. Liang, S.-T. Luo, X.-W. Wei, T. Liu, J. Ren, C.-C. Ma, Y.-H. Yang, B.-L. Wang, L. Liu, Folate-modified lipoplexes delivering the interleukin-12 gene for targeting colon cancer immunogene therapy, J. Biomed. Nanotechnol. 11 (11) (2015) 2011–2023.
[30] X. Liang, M. Luo, X.-W. Wei, C.-C. Ma, Y.-H. Yang, B. Shao, Y.-T. Liu, T. Liu, J. Ren, L. Liu, A folate receptor-targeted lipoplex delivering interleukin-15 gene for colon cancer immunotherapy, Oncotarget 7 (32) (2016) 52207.
[31] M.H. Kershaw, J.A. Westwood, L.L. Parker, G. Wang, Z. Eshhar, S.A. Mavroukakis, D.E. White, J.R. Wunderlich, S. Canevari, L. Rogers-Freezer, A phase I study on adoptive immunotherapy using gene-modified T cells for ovarian cancer, Clin. Cancer Res. 12 (20) (2006) 6106–6115.
[32] S. Canevari, G. Stoter, F. Arienti, G. Bolis, M.I. Colnaghi, E.M. Di Re, A.M. Eggermont, S.H. Goey, J.W. Gratama, C.H. Lamers, Regression of advanced ovarian carcinoma by Intraperitoneal treatment with autologous TLymphocytes retargeted by a bispecific monoclonal antibody, JNCI: J. Natl. Cancer Inst. 87 (19) (1995) 1463–1469.
[33] L.E. Kandalaft, D.J. Powell, G. Coukos, A phase I clinical trial of adoptive transfer of folate receptor-alpha redirected autologous T cells for recurrent ovarian cancer, J. Transl. Med. 10 (1) (2012) 157.

[34] D.-G. Song, Q. Ye, C. Carpenito, M. Poussin, L.-P. Wang, C. Ji, M. Figini, C.H. June, G. Coukos, D.J. Powell, In vivo persistence, tumor localization, and antitumor activity of CAR-engineered T cells is enhanced by costimulatory signaling through CD137 (4-1BB), Cancer Res. 71 (13) (2011) 4617–4627.
[35] M.S. Kim, J.S. Ma, H. Yun, Y. Cao, J.Y. Kim, V. Chi, D. Wang, A. Woods, L. Sherwood, D. Caballero, Redirection of genetically engineered CAR-T cells using bifunctional small molecules, J. Am. Chem. Soc. 137 (8) (2015) 2832–2835.
[36] D.-G. Song, Q. Ye, M. Poussin, L. Liu, M. Figini, D.J. Powell Jr., A fully human chimeric antigen receptor with potent activity against cancer cells but reduced risk for off-tumor toxicity, Oncotarget 6 (25) (2015) 21533.
[37] K. Schutsky, D.-G. Song, R. Lynn, J.B. Smith, M. Poussin, M. Figini, Y. Zhao, D.J. Powell, Rigorous optimization and validation of potent RNA CAR T cell therapy for the treatment of common epithelial cancers expressing folate receptor, Oncotarget 6 (30) (2015) 28911.
[38] J. Schreiner, D.S. Thommen, P. Herzig, M. Bacac, C. Klein, A. Roller, A. Belousov, V. Levitsky, S. Savic, W. Moersig, Expression of inhibitory receptors on intratumoral T cells modulates the activity of a T cell-bispecific antibody targeting folate receptor, Oncoimmunology 5 (2) (2016) e1062969.
[39] A. Wing, C.A. Fajardo, A.D. Posey, C. Shaw, T. Da, R.M. Young, R. Alemany, C.H. June, S. Guedan, Improving CART-cell therapy of solid tumors with oncolytic virus–driven production of a bispecific T-cell engager, Cancer Immunol. Res. 6 (5) (2018) 605–616.
[40] D.-G. Song, Q. Ye, M. Poussin, J.A. Chacon, M. Figini, D.J. Powell, Effective adoptive immunotherapy of triple-negative breast cancer by folate receptor-alpha redirected CAR T cells is influenced by surface antigen expression level, J. Hematol. Oncol. 9 (1) (2016) 56.
[41] S. Zuo, Y. Wen, H. Panha, G. Dai, L. Wang, X. Ren, K. Fu, Modification of cytokine-induced killer cells with folate receptor alpha (FRα)-specific chimeric antigen receptors enhances their antitumor immunity toward FRα-positive ovarian cancers, Mol. Immunol. 85 (2017) 293–304.
[42] W. Chu, Y. Zhou, Q. Tang, M. Wang, Y. Ji, J. Yan, D. Yin, S. Zhang, H. Lu, J. Shen, Bi-specific ligand-controlled chimeric antigen receptor T-cell therapy for non-small cell lung cancer, Biosci. Trends 12 (3) (2018) 298–308.
[43] M. Kim, S. Pyo, C.H. Kang, C.O. Lee, H.K. Lee, S.U. Choi, C.H. Park, Folate receptor 1 (FOLR1) targeted chimeric antigen receptor (CAR) T cells for the treatment of gastric cancer, PLoS One 13 (6) (2018) e0198347.
[44] Y. Lu, P.S. Low, Folate targeting of haptens to cancer cell surfaces mediates immunotherapy of syngeneic murine tumors, Cancer Immunology, Immunotherapy 51 (3) (2002) 153–162.
[45] R.J. Amato, A. Shetty, Y. Lu, R. Ellis, P.S. Low, A phase I study of folate immune therapy (EC90 vaccine administered with GPI-0100 adjuvant followed by EC17) in patients with renal cell carcinoma, J. Immunother. 36 (4) (2013) 268–275.
[46] R.J. Amato, A. Shetty, Y. Lu, P.R. Ellis, V. Mohlere, N. Carnahan, P.S. Low, A phase I/Ib study of folate immune (EC90 vaccine administered with GPI-0100 adjuvant followed by EC17) with interferon-α and interleukin-2 in patients with renal cell carcinoma, J. Immunother. 37 (4) (2014) 237–244.
[47] E.I. Sega, Y. Lu, M. Ringor, C.P. Leamon, P.S. Low, Low-Dose Radiation Potentiates the Therapeutic Efficacy of Folate Receptor–Targeted Hapten Therapy, Int. J. Radiat. Oncol. Biol. Phys. 71 (2) (2008) 559–566.
[48] N.A. Bandara, C.D. Bates, Y. Lu, E.K. Hoylman, P.S. Low, Folate-hapten–mediated immunotherapy synergizes with vascular endothelial growth factor receptor inhibitors in treating murine models of cancer, Mol. Cancer Ther. 16 (3) (2017) 461–468.
[49] D.-K. Kim, T. Lee, A. Castilleja, B. Anderson, G. Peoples, A. Kudelka, J. Murray, T. Sittisomwong, J. Wharton, J. Kim, Folate binding protein peptide 191-199 presented on dendritic cells can stimulate CTL from ovarian and breast cancer patients, Anticancer Res. 19 (4B) (1999) 2907–2916.
[50] D.O. Jackson, K. Byrd, T.J. Vreeland, D.F. Hale, G.S. Herbert, J.M. Greene, E.J. Schneble, J.S. Berry, A.F. Trappey, G.T. Clifton, Interim analysis of a phase I/IIa trial assessing E39+ GM-CSF, a folate binding protein vaccine, to prevent recurrence in ovarian and endometrial cancer patients, Oncotarget 8 (9) (2017) 15912.
[51] K.R. Kalli, M.S. Block, P.M. Kasi, C.L. Erskine, T.J. Hobday, A. Dietz, D. Padley, M.P. Gustafson, B. Shreeder, D. Puglisi-Knutson, Folate receptor alpha peptide vaccine generates immunity in breast and ovarian Cancer patients, Clin. Cancer Res. 24 (13) (2018) 3014–3025.
[52] K.A. Chianese-Bullock, W.P. Irvin Jr., G.R. Petroni, C. Murphy, M. Smolkin, W.C. Olson, E. Coleman, S.A. Boerner, C.J. Nail, P.Y. Neese, A multipeptide vaccine is safe and elicits T-cell responses in participants with advanced stage ovarian cancer, J. Immunother. 31 (4) (2008) 420–430.

Dr. Batoul Farran is a scientist in the Department of Hematology and Medical Oncology at Winship Cancer Institute, Emory University School of Medicine, USA. She obtained her MSc from the American University of Beirut and her PhD in Structural and Molecular Biology from University College London. Her work focuses on translational projects related to targeted and immune therapies in several malignancies. She has authored several publications in highly reputed International journals.

Dr. Eluri Pavitra (Dr. E. Pavitra) is a Postdoc in the Department of Bioengineering at Inha University, Incheon, South Korea. She received her PhD in Optoelectronics from Kyung Hee University in Suwon, South Korea. Her research focuses on development of micro/nano-structured luminescent materials for biological, environmental and solid-state lighting applications. She has published over 35 research papers in highly reputed International journals.

Dr. Prameswari Kasa obtained her PhD in Biotechnology from Sri Padmavati Mahila Visvavidyalayam (Women's University) in Tirupati, Andhra Pradesh, India. Her research focuses on synthesis and characterization of development of micro/nano-structured materials from biological, environmental applications. She has published over 10 research papers in several prestigious peer-reviewed journals and has presented more than 20 abstracts at various national and international conferences. She is life member of the Indian Science Congress Association and the American Association of Cancer Research (AACR).

Dr. Sujatha Peela is working as a Professor in the Department of Biotechnology, Dr.B.R.Ambedkar University, Srikakulam, Andhra Pradesh, INDIA. Dr. Peela received her Ph.D in Biotechnology from Andhra University, Visakhapatnam, INDIA and further pursued her post-doctoral fellowship from University of Chicago, USA on Colon Cancer in the Department of Medicine. Her research focuses on Marine antibiotics and anticancer compounds from actinomycetes. She has published 31 research papers in various highly reputed International journals and attended 68 National and International conferences.

Dr. Ganji Seeta Rama Raju (Dr. G. Seeta Rama Raju)is an Assistant Professor in the Department of Energy and Materials Engineering at Dongguk University Seoul. He obtained his MPhil and his PhD, both in physics, from Sri Venkateswara University in Tirupati, Andhra Pradesh, India. Dr. Seeta Rama Raju research focuses on nanomaterials, imaging and drug delivery in different malignancies. He has published over 80 research papers in highly reputed International journals.

Dr. Ganji Purnachan dra Nagaraju is a faculty member in the Department of Hematology and Medical Oncology at Winship Cancer Institute, Emory University School of Medicine, USA. He obtained his MSc and PhD, both in biotechnology, from Sri Venkateswara University in Tirupati, India. He also received a DSc in Biotechnology from Berhampur University, India. His research focuses on translational projects related to gastrointestinal malignancies. He has published over 70 research papers in highly reputed International journals.