

Folic Acid, Folate Conjugates and Folate Receptors: Novel Applications in Imaging of Cancer and Inflammation-Related Conditions

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Abstract: Selective delivery of imaging agents to target cells remains a major challenge for molecular imaging and targeted therapy. Folate-based targeting leverages the differential expression of folate receptors (FRs), which are frequently overexpressed in various malignancies and in activated macrophages compared with most normal tissues, to mediate selective cellular uptake. Folate and folate-conjugates offer several advantages for targeted delivery: 1) restricted normal-tissue distribution of FRs, 2) high affinity binding to FRs, and 3) straightforward conjugation chemistry that enables linkage to both therapeutic and imaging moieties. In this narrative review, we summarize recent advances in FR-targeted imaging across multiple modalities (PET, SPECT, MRI, and optical imaging), discuss strategies for probe design and pharmacokinetic optimization, and highlight translational progress from preclinical studies to early clinical applications. We also review emerging applications of folate-mediated delivery for gene therapy and immune modulation, and we identify remaining challenges including probe specificity, background uptake, and clinical validation and outline directions for future research and clinical translation.

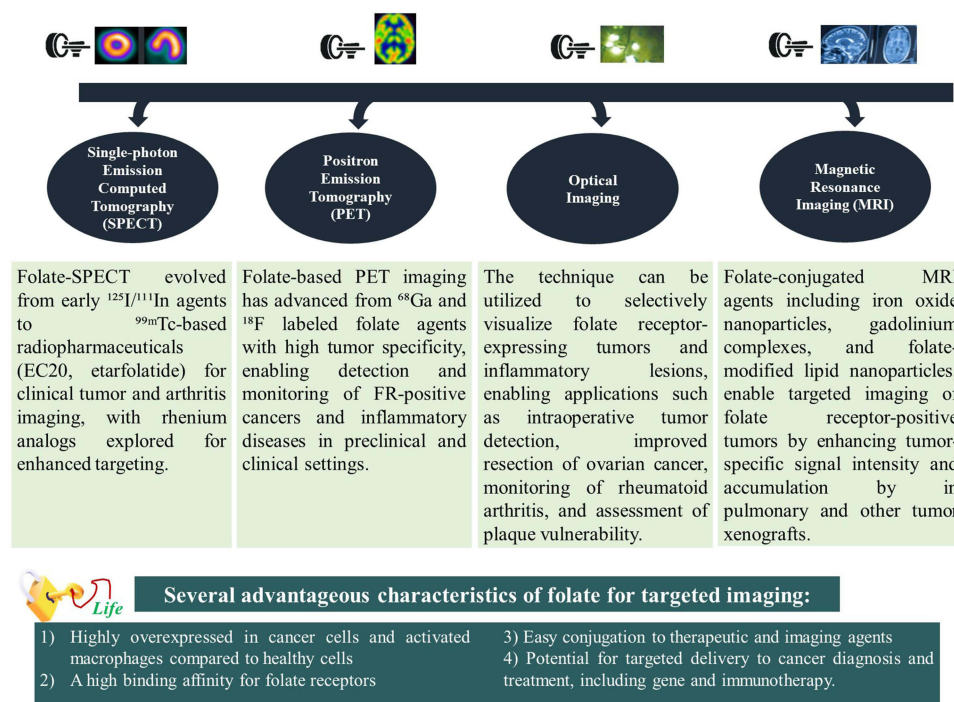
Keywords: folate receptor-targeted imaging, cancer diagnostics, inflammatory disease imaging, PET/SPECT Imaging, MRI contrast agents, optical imaging

Introduction

Cellular targeting for diagnostic and therapeutic agents remains a major challenge in molecular imaging and targeted therapy. Selective uptake depends on specific ligand–receptor interactions; when a ligand cannot bind its receptor, delivery and downstream biological effects are compromised. Early conceptualizations of targeted therapy highlighted the potential to direct agents selectively to diseased cells (for example, Paul Ehrlich’s “magic bullet” concept).¹ One promising candidate for such a targeting ligand is folate (an umbrella term for folic acid and its vitamers), which has a vital role in one-carbon metabolism. While its metabolic function is not the primary focus in this context, its ability to act as a vehicle for linked therapeutic or imaging agents to enter cells is of significant interest. Indeed, when therapeutic agents such as doxorubicin (DOX) or imaging agents can be specifically targeted to abnormal cells, the precision of medical diagnostics and treatment planning is greatly improved. For example, Wu et al employed this strategy, demonstrating that conjugating DOX with folate increased its accumulation in tumors.² Furthermore, folic acid is well-known for mitigating the toxicity of certain drugs, such as methotrexate (MTX). Moreover, studies have shown that combining folate with anticancer or anti-arthritic drugs allows for the use of lower medication doses, thereby reducing side effects while improving potency against tumor cells.^{3,4}

The strategy of conjugating compounds with vitamins for targeted therapy and imaging emerged from foundational studies on endocytosis in plant cells. These investigations explored the transport of various molecules such as biotin, elicitors, and fluorescent streptavidin across plant cell membranes.⁵ Elicitors bind to specific receptors on the plasma membrane, inducing defense signaling against disease, pests, or environmental stress. Streptavidin exhibits a high chemical affinity for biotin, and conjugating this complex with fluorescein allows researchers to visualize its transport. Although

Graphical Abstract



fluorescent streptavidin is a macromolecule that cannot normally cross the cell membrane, these studies observed its accumulation within the major vacuole or tonoplast of the cell.⁵⁻⁷ This observation raised the question of whether a similar mechanism could be used to transport specific compounds into animal cells using vitamins as carriers. Subsequent studies confirmed this hypothesis, demonstrating that riboflavin, for instance, could transport attached proteins into animal cells.⁸ Furthermore, Leamon et al revealed that folate possesses the potential to deliver macromolecules into animal cells, enabling the entry of compounds such as ribonuclease, horseradish peroxidase, serum albumin, IgG, and ferritin.⁹

Clinically, targeted folate-based imaging may help address important unmet needs. Conventional imaging modalities often lack the specificity required to distinguish tumor tissue from inflammatory lesions and may be insensitive to early, treatment-induced changes in cellular phenotype. Targeted probes have the potential to improve lesion characterization, enable more accurate patient stratification, and facilitate earlier assessment of therapeutic response.¹⁰⁻¹³ In addition, folate conjugates support theranostic strategies in which the same targeting vector is used for both imaging and selective therapeutic delivery.¹⁴

Folate is an excellent candidate for receptor-mediated targeting due to several key properties: 1) its receptor is sparingly distributed in normal tissues but is significantly upregulated on cancer cells (up to 500 times more than on healthy cells) and activated macrophages; 2) it has a high binding affinity for folate receptors (FRs); and 3) it can be readily conjugated to both therapeutic and imaging agents.^{3,15} Examples of cancers with high FR expression suitable for this approach include those of the kidney, brain, ovary, lung, breast, endometrium, and colon, as well as hematopoietic malignancies.^{3,5,16-22} Similarly, activated macrophages present in inflammatory and autoimmune diseases, such as osteoarthritis, rheumatoid arthritis, and Crohn's disease, also overexpress FRs, making them targets for folate-based diagnosis and treatment. In addition to imaging, folate-targeting shows promise in other areas, including gene therapy, immunotherapy, and the delivery of antisense oligodeoxyribonucleotides (ODN) and small interfering RNAs (siRNA).^{3,5,19,21,23}

In this review, we focus on the diagnostic applications of folate conjugates. We will begin by providing a brief introduction to the scientific basis for this approach before discussing its application in various imaging modalities,



highlighting key studies in the field. The therapeutic applications of folate-targeting, while also significant, have been reviewed extensively elsewhere and fall outside the scope of this article.^{24,25}

Search Strategy

This narrative review studies comprehensive literature search was conducted across PubMed, Scopus, Web of Science, and Google Scholar to identify relevant studies on folate receptor-targeted imaging published until March 2025 and was restricted to English language publications. The search strategy included keywords and Boolean operators such as “Folate receptor imaging” OR “Folic acid-based imaging” OR “Folate-targeted imaging”, OR “Folate PET” OR “Folate SPECT” OR “Folate MRI” OR “Folate optical imaging”, and “Folate-targeted radiopharmaceuticals” OR “Folate-based molecular imaging”. Additionally, reference lists of key articles were manually screened to identify further relevant studies.

Studies were included if they (1) investigated the use of folate receptor-targeted imaging in cancer or inflammatory diseases, (2) provided preclinical or clinical data on imaging modalities such as PET, SPECT, MRI, or optical imaging, and (3) were published in English between 1970 and 2024.

Folate and Folate Receptors

Folate, folic acid, vitamin B9 and folacin known as one of the B vitamins are stable over a broad range of temperatures and pH, inexpensive, and non-immunogenic and have vital role in DNA and RNA synthesis, amino acids metabolism, methylation, cell division and important reactions in the body, but cells cannot make their own folate and must gain it from different nutrients.^{3,26} There is a small difference between folate and folic acid; folic acid is an artificial form of folate which has a faster digestive absorption than folate.³

The most important transporters for folate uptake are 1) the reduced folate carrier (RFC), 2) the proton-coupled folate transporter and 3) folate receptor (FR) which the third type, a cell surface glycosylphosphatidylinositol (GPI)-anchored glycopolyptid, is the subject our work.^{15,20,27}

Generally, there are three types of receptors for folate: 1) FR1 (FOLR1, alpha), 2) FR2 (FOLR2, beta), 3) FR3 (FOLR3, gamma) and 4) FR4 (FOLR4, delta, Juno).^{5,15,20,28} Structurally, folate and its receptors are similar and this factor result binding them. About 220–260 amino acid polypeptides exist in FR isoforms that 68–79% amino acid sequences have like identity.^{15,27}

FR1: FOLR1 transcript a 38–40 kilodaltons glycosylphosphatidylinositol (GPI)-anchored cell-surface glycoprotein or in short, FR α , which has been found to be associated with cancers but is downregulated in non-malignant tissues.^{20,29}

FR2: This receptor like FR1 (FR β) is a GPI-linked cell surface receptor that is highly expressed in neutrophils and activated macrophages, among granulocytes and encoded by FOLR2.^{15,30}

FR3: FR γ , in contrast to the three other types, lacks a GPI-anchor region and is a secreted protein and encoded by FOLR3. Polymerase chain reaction (PCR) analysis showed that these receptors are expressed in the spleen, bone marrow and thymus, as well as ovarian, cervical and uterine carcinoma.^{15,20,31}

FR4: This receptor is associated with fertilization sperm and egg in mammalian cells and encoded by FOLR4. Indeed, in egg, FR δ is the receptor of Izumo1, an essential cell-surface protein in sperm, and fusing them results in fertilization.³² However, some studies proposed the FR δ can be found on regulatory T cells.^{33,34} Among these receptors, we study first and second kinds of FR that are associated with numerous cancers and inflammatory diseases.

Delivery Imaging Agents

In biology terms, cargo is a set of multi-component connections such as different agents (imaging and/or therapeutic) and couplers (folate).³⁵ The delivery pathway of folate-targeted complexes, from systemic administration to cellular uptake within tumor tissue, is a critical factor in their efficacy. The system should be designed to maintain stability during circulation while allowing timely dissociation once internalized into target cells. When the complex is not bound to FRs, the linkage between folate and the imaging or therapeutic agent must remain intact. However, after receptor-mediated internalization, the bond between folate and the drug cargo should be cleaved to release the active compound within the cell. Upon binding of the folate-conjugated complex to the FR on the cell membrane, the plasma membrane invaginates to form an endocytic vesicle (endosome).^{26,36} The bond between folate and the therapeutic agent remains stable until the

endosomal environment becomes acidic. During endosomal maturation, the acidic pH facilitates cleavage of the linker, leading to the release of the active agent.²⁶ In other words, drug release occurs only after the pH decreases to levels typical of FR-containing endosomes. Disulfide linkers are often used as pH-sensitive bridges between folate and the drug cargo, as they undergo reduction under acidic conditions, promoting intracellular release.^{3,26} Also, after a series of specific reactions, the FR, disulfides and agents are recycled and exited from cells. Therefore, binding folate with agents from the injecting to the linking with receptors, not be broken and be taken down at the right moment. Figure 1 shows the different mechanisms in delivery imaging agents.

FR-Targeted Imaging

Folic acid, due to its high binding affinity to FRs, has attracted considerable attention as a targeting agent over recent decades.^{37–39} The overexpression of FRs in many tumors and their limited presence in most normal tissues make this receptor an excellent candidate for targeted therapy and diagnosis.^{40–42} Moreover, folate-based radiopharmaceuticals have shown promise for imaging certain inflammatory and autoimmune diseases, such as rheumatoid arthritis, owing to FR overexpression on activated macrophages.^{43–45} FR-targeted imaging not only enables the localization and visualization of FR-positive tumors but also provides insight into tumor aggressiveness, as higher-grade tumors tend to express more FRs.^{46,47} Combining FR-targeted imaging with FR-targeted therapy may facilitate a more personalized and effective treatment strategy.³

A wide range of folate conjugates have been developed and evaluated for use as imaging agents in various modalities, including nuclear medicine, MRI, and optical imaging.^{46,47}

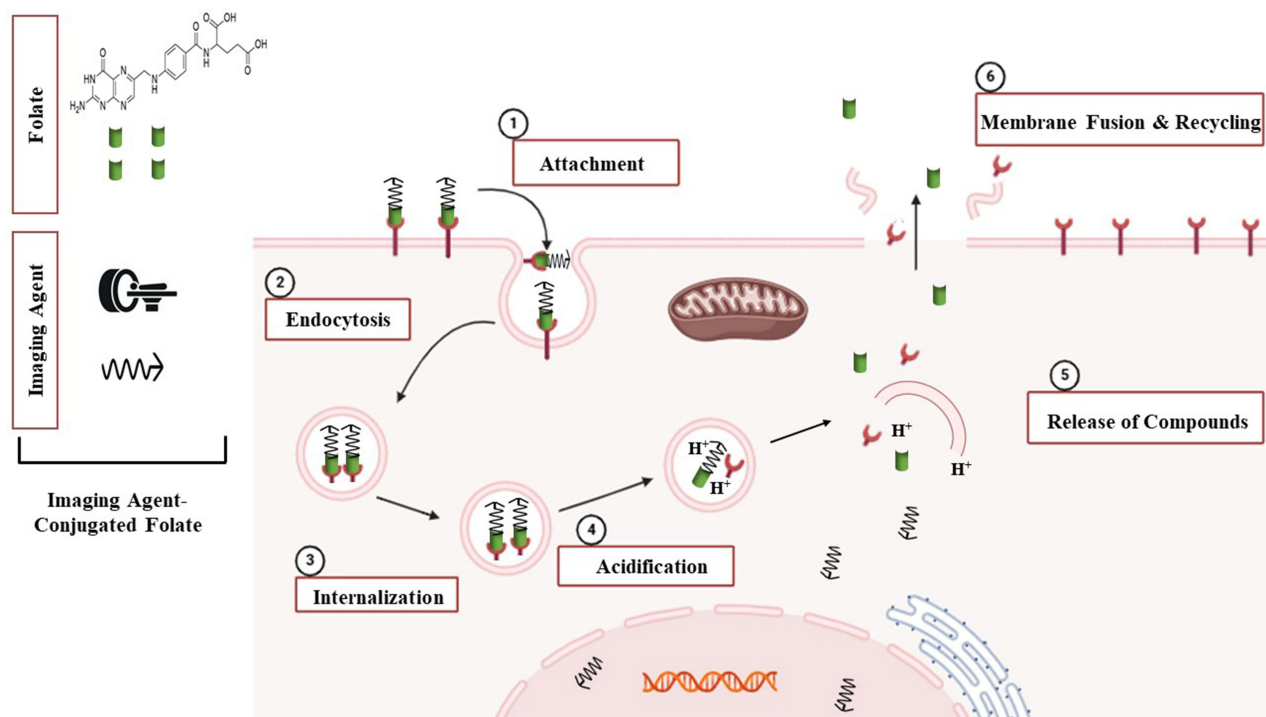


Figure 1 Generally, we can summarize mechanisms of delivery drug agents to six steps: 1) Attachment: binding folate-conjugated imaging factors (complex) to its specific FRs on plasma membrane. 2) Endocytosis: entering complex-receptor to inside the cell and create an endosome 3) Internalization: moving endosome that contain complex-receptor to perform biochemical processes. 4) Acidification: decreasing PH results in reducing affinity between folate and imaging factors. 5) Release of compounds: drug cargo diffuses inside the cell and can alter the action of the organelles or be used for imaging. 6) Membrane fusing and Recycling: eventually, folate and FRs can enter into the recycling processes for the next delivery factors into the cell.

FR-Based SPECT Imaging

Several studies have explored the use of different radionuclides for FR-targeted single-photon emission computed tomography (SPECT). The first folic acid-based radiotracer was labeled with ^{125}I , but its application was soon discontinued due to the isotope's long half-life and suboptimal imaging characteristics.⁴⁸

Subsequently, ^{68}Ga -deferoxamine–folate complexes were evaluated in both in vitro and in vivo studies, demonstrating good binding to FR-positive tumors and favorable tumor-to-blood ratios. However, their clinical translation was limited by high hepatobiliary excretion.^{49,50}

To overcome these issues, ^{111}In -DTPA–folate was developed and tested in vivo, showing encouraging results in mouse models that led to clinical evaluation in patients with ovarian cancer.^{51,52} Because technetium-99m ($^{99\text{m}}\text{Tc}$) is less expensive and easier to produce than ^{111}In , Müller et al labeled histidine–folate with $^{99\text{m}}\text{Tc}$ and evaluated it in nude mice bearing FR-positive tumors. SPECT/CT imaging revealed strong tracer accumulation in tumor tissues as well as physiological uptake in the renal cortex, choroid plexus, and salivary glands.⁵³ Morris et al later used $^{99\text{m}}\text{Tc}$ -etarfolatide for SPECT imaging in women with advanced ovarian cancer undergoing FR-targeted therapy. The study confirmed that this radiotracer was well tolerated and provided valuable information for personalized therapeutic planning.⁵⁴ Similar results were obtained with $^{99\text{m}}\text{Tc}$ -EC20 in clinical trials involving patients with various cancers and rheumatoid arthritis.^{37,55,56} Encouraged by these findings, researchers also investigated the heavier congener of technetium, rhenium, which demonstrated promising tumor uptake and acceptable tumor-to-kidney ratios, suggesting potential for diagnostic use.⁵⁷

In 2001, Leamon and Low reported the clinical use of ^{111}In -DTPA–folate, where approximately 2 mg of the compound containing 5 mCi of ^{111}In was administered intravenously, followed by SPECT imaging (Figure 2).⁵⁸

Moreover, folate-linked radiopharmaceuticals have been investigated for targeted imaging of inflammatory joints, showing favorable results in patients with arthritis using scintigraphy (Figure 3).⁴

FR-Based PET Imaging

Positron emission tomography (PET) is a highly sensitive and quantitative imaging modality with superior spatial resolution, leading to growing interest in developing FR-targeted PET radiopharmaceuticals.⁵⁹ The first agent used for this purpose was ^{68}Ga -deferoxamine–folate.⁶⁰ Subsequently, several ^{68}Ga -labeled folate derivatives including ^{68}Ga -NOTA–folate were developed, which exhibited excellent tumor uptake, slow washout, and minimal kidney and liver accumulation.^{61,62} ^{18}F -labeled folate conjugates have also shown great potential for PET imaging of FR-positive tumors, offering high target-to-background contrast and favorable pharmacokinetics.⁶³ These tracers are considered promising candidates for tumor detection, staging, and therapy monitoring and may serve as alternatives to $^{99\text{m}}\text{Tc}$ -based SPECT

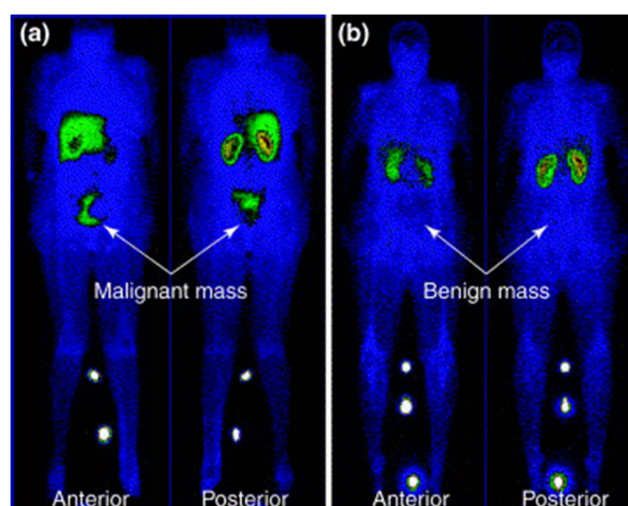


Figure 2 Representative images from two patients suspected of having ovarian cancer. (a) (left) demonstrates a malignant ovarian mass (white arrow). (b) (right) shows a large benign ovarian mass (white arrow). This research was originally published in DDT. Leamon C P. and Low P S. Folate-mediated targeting: from diagnostics to drug and gene delivery. *Drug Discovery Today*. 2001 Jan 1;6(1):44–51. (Permission by customercare@copyright.com).⁵⁸ Full.

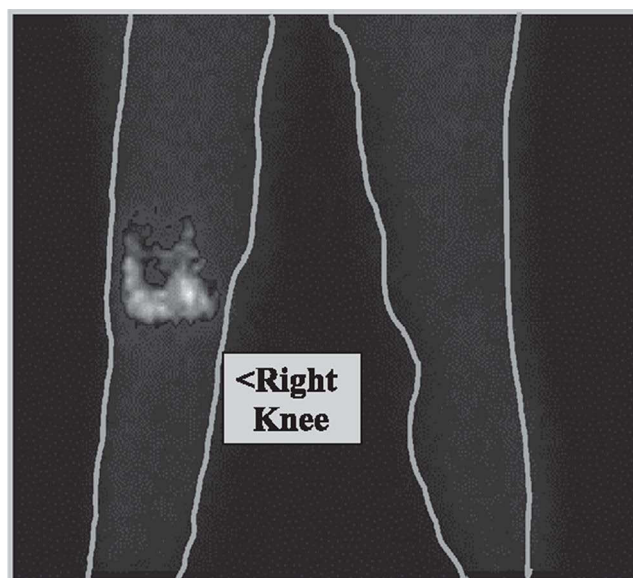


Figure 3 Scintigraphic image of a cancer patient diagnosed with arthritis in the right knee (indicated by the symbol '<'). Administration of 2 mg ^{111}In -DTPA-folate to the patient, intravenously and imaged after 4 hours. This research was originally published in ADDR. Paulos C M, Turk M J, Breur G J, Low P S. Folate receptor-mediated targeting of therapeutic and imaging agents to activated macrophages in rheumatoid arthritis. *Advanced Drug Delivery Reviews*. 2004 Apr 29;56(8):1205–17. (Permission by customercare@copyright.com).⁴

conjugates^{64–66} Müller et al further explored four terbium radionuclides for FR-targeted PET in tumor-bearing mice and reported that ^{152}Tb provided excellent image quality and high tumor visualization.⁶⁷

Recent progress in FR-targeted imaging has expanded its application beyond oncology, particularly for inflammatory disorders. Studies have shown that FR β is selectively expressed on activated macrophages, making it a valuable molecular marker for non-invasive imaging of inflammatory processes.⁶⁸ A first-in-man PET imaging study utilizing [^{18}F] fluoro-PEG-folate has shown remarkable specificity in detecting inflamed joints in rheumatoid arthritis (RA) patients, providing superior target-to-background contrast compared to traditional macrophage tracers like (R)-[^{11}C] PK11195.⁶⁹ Additionally, elevated expression of FR α has been observed in glioma tissues compared with normal brain parenchyma, supporting its use as a diagnostic biomarker. A folate-based PET tracer, [^{18}F] FOL, exhibited a high tumor-to-brain uptake ratio, confirming FR α as a promising target for glioma imaging and potentially for future nanoparticle-based therapeutic approaches.⁷⁰

Furthermore, preclinical evaluations of ^{68}Ga -labeled folate conjugates have demonstrated effective localization in inflammatory lesions, such as in juvenile idiopathic arthritis (JIA), underscoring their potential for monitoring macrophage-driven inflammation.⁷¹ Figure 4 illustrates findings from Bettio et al, highlighting an [^{18}F] folic acid derivative as a promising PET tracer for visualizing FR-positive tumors.⁷²

Optical Imaging

Fluorescence imaging utilizes a variety of probes incorporating polymers, nanomaterials, quantum dots, and silicon-based compounds.⁷³ Among these, folic acid has emerged as an effective targeting ligand for distinguishing cells that express varying levels of folate receptors (FRs). In one notable study, a folate-conjugated compound successfully visualized L1210 metastatic tumor nodules in the liver of DBA mice (Figure 5).⁷⁴

Fluorescence microscopy of breast cancer cells further demonstrated the selective binding of 5-fluorouracil (5-FU) encapsulated within FA-CMC-ZnS:Mn nanoparticles to MCF-7 cells, while negligible binding was observed in control cells. This finding highlights the potential of such nanoconjugates as bioprobes for FR-targeted tumor imaging.⁷⁵

Intraoperative fluorescence imaging offers substantial advantages, including improved detection of primary and metastatic lesions, enhanced staging accuracy, and guidance for complete tumor resection.⁷⁶ Several studies have employed FR-targeted fluorescent contrast agents for intraoperative visualization of FR-positive tumors.⁷⁷ These

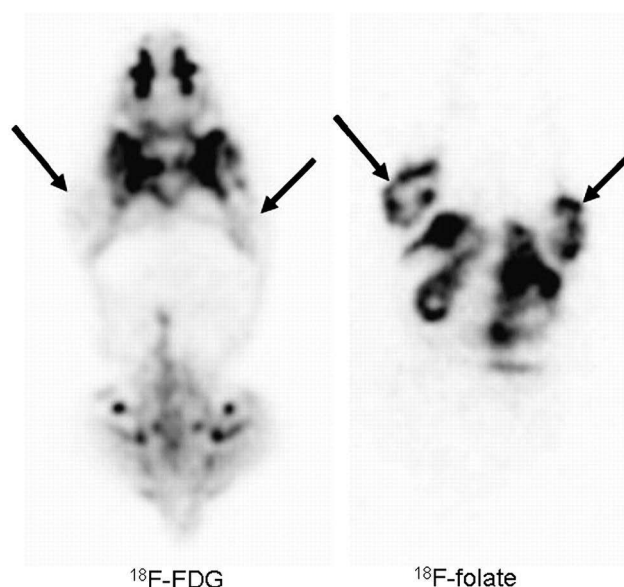


Figure 4 Left: ^{18}F -FDG PET maximum-intensity projection showing tracer accumulation in athymic nude tumor nodules in the upper thorax (right and left), acquired 30 min after injection of 16.9 MBq. Right: coronal ^{18}F -folate PET in the same animal acquired two days later, 75 min after injection of 13.3 MBq. Black arrows indicate the tumor locations. Note the differential biodistribution: ^{18}F -FDG shows uptake in various skeletal muscles, whereas the ^{18}F -folate derivative demonstrates highest accumulation in liver and kidneys. This research was originally published in JNM. Bettio A, Honer M, Müller C, Brühlmeier M, Müller U, Schibli R, Groehn V, Schubiger A, Ametamey S. Synthesis and preclinical evaluation of a folic acid derivative labeled with ^{18}F for PET imaging of folate receptor-positive tumors. J Nucl Med. 2006; 47(7):1153–60. © SNMMI (Permission from the journal).⁷²

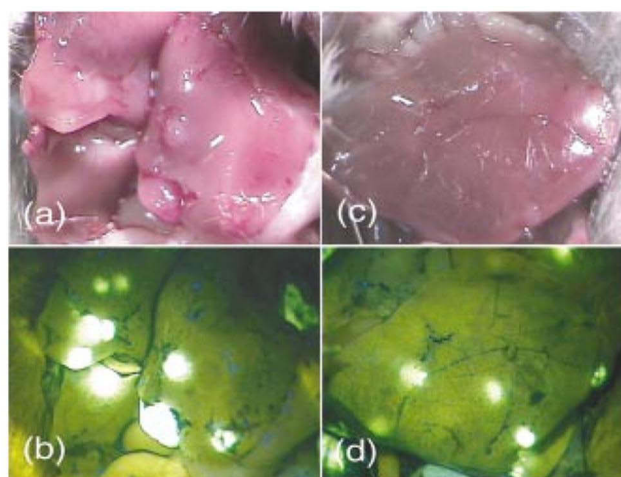


Figure 5 10 nmol of folate-fluorescein was injected to the mouse, intravenously (femoral vein) and imaged 2 h later. Parts (a and b) show the white light and fluorescent light images of the lower lobe of the liver, while parts (c and d) represents the same images of the upper lobe of the liver. This research was originally published in Journal of Biomedical Optics. Kennedy M, Jallad K, Thompson D, Ben-Amotz D, Low P. Optical imaging of metastatic tumors using a folate-targeted fluorescent probe. J Biomed Opt. 2003 Oct;8(4):636–41 (Permission from the journal).⁷⁴

investigations have demonstrated improved detection rates in ovarian cancer, identification of smaller metastatic deposits, and increased completeness of tumor removal.^{74,76,78} Folate-targeted fluorescence probes have also been successfully utilized for non-invasive visualization of rheumatoid arthritis, capitalizing on the overexpression of FR β on activated macrophages.^{43,79,80}

Beyond diagnostic applications, folate-conjugated nanocarriers are increasingly investigated as theranostic platforms, combining targeted drug delivery with molecular imaging capabilities.¹⁴ For instance, folate-functionalized perfluorocarbon nanoemulsions (PFC-NEs) have demonstrated dual functionality in near-infrared fluorescence imaging and cyclooxygenase-2 (COX-2) inhibition, representing a promising approach for imaging and treatment of inflammatory disorders.⁸¹ Similarly,

folate-targeted liposomal methotrexate (FL-MTX) formulations have enhanced drug accumulation in inflamed joints while reducing systemic toxicity and improving therapeutic outcomes in preclinical models of arthritis.⁸² Interestingly, corticosteroid therapy has been shown to upregulate FR β expression in macrophages, suggesting that FR β -targeted imaging may serve as a valuable non-invasive biomarker for monitoring anti-inflammatory treatment responses.⁸³

MRI

Choi et al were the first to report the *in vivo* use of iron oxide nanoparticles (IONPs) functionalized with folate for FR-targeted delivery as magnetic resonance imaging (MRI) contrast agents. Their results demonstrated efficient and rapid endocytosis, high tumor accumulation, and excellent *in vivo* MR contrast enhancement.⁸⁴

Folate-conjugated polyethylene glycol nanoparticles, such as SPIO-PEG-FA, have since been developed as targeted MRI contrast agents, exhibiting effective binding and accumulation in FR-positive tumors in both *in vitro* and *in vivo* models.^{85–87} Studies have further confirmed that FA-SPIOs (folate-conjugated superparamagnetic iron oxide nanoparticles) can serve as multifunctional nanoplateforms for simultaneous cancer diagnosis and therapy.^{88,89} Additionally, folate-functionalized nanoparticles have shown high efficacy as dual-modality contrast agents for MRI/CT imaging.⁹⁰ Folate-targeted iron oxide nanoparticles have also been explored as theranostic agents, enabling both MRI-based tumor imaging and magnetic hyperthermia therapy, with promising preclinical outcomes.⁹¹

Also, Yuan et al have studied on folate-PL-Gd-DTPA (Gd-DTPA-poly-L-lysine) and compared with Gd-DTPA. As illustrated in Figure 6, the T1-weighted MRI images on the right exhibit markedly increased signal enhancement in the pulmonary tumor xenografts following folate administration.⁹²

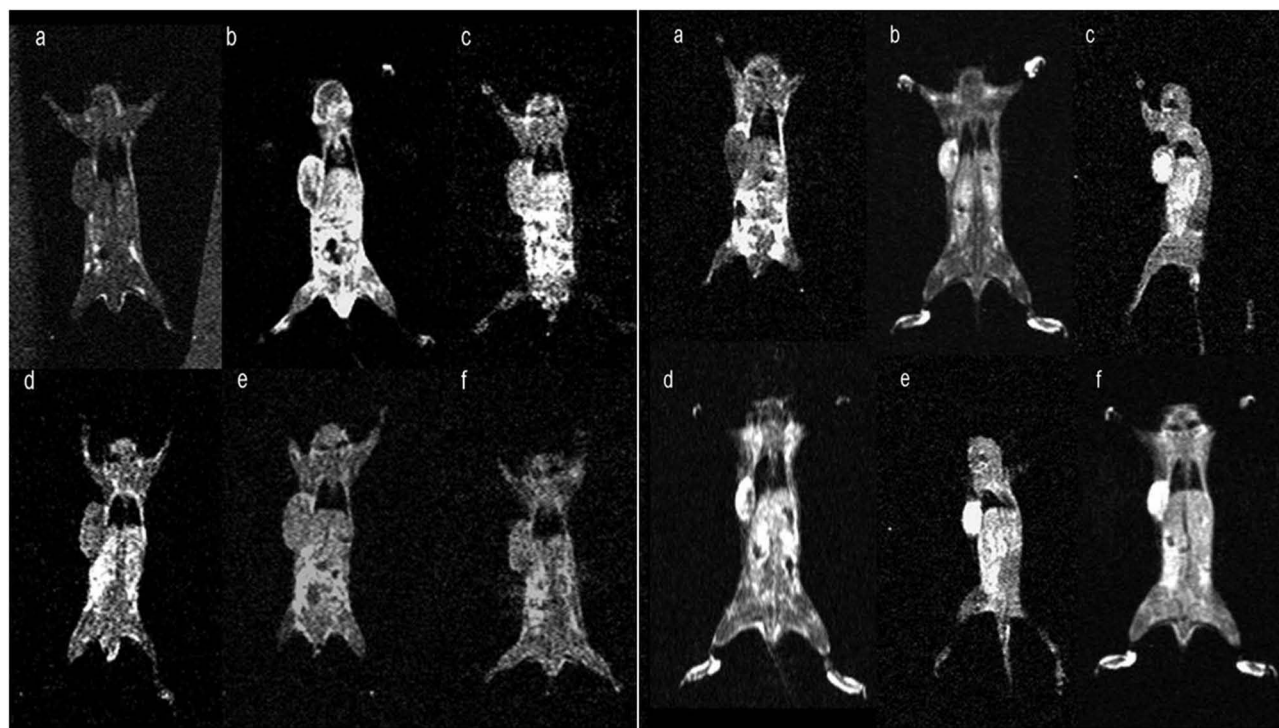


Figure 6 The collection of T1-weighted MRI images in pulmonary tumor xenografts. Left image (Gd-DTPA): (a): pre-injection scanning; (b): post-injection; (c): 12 h post-injection; (d) 24 h time-point post-injection; (e) 48 h time-point post-injection; (f) 72 h time-point post-injection. Right image (folate-PL-Gd-DTPA): (a) pre-injection scanning; (b) post-injection; (c) 12 h post-injection; (d) 24 h time-point post-injection; (e) 48 h time-point post-injection; (f) 72 h time-point post-injection. This research was originally published in Experimental and Therapeutic Medicine journal. Yuan Z, Li W, Ye X, Liu S, Xiao X. Folate receptor-mediated targeted polymeric gadolinium complexes for magnetic resonance imaging in pulmonary tumor xenografts. *Exp Ther Med*. 2012 May;3(5):903–907. (Permission from the journal).⁹²

Clinical Studies

Some studies have investigated overexpression of FRs on malignant cancers. Totally, about 40% of human cancers have folate overexpressed receptors.⁹³ For example, over 90% ovarian cancers numerously express FR α on own cellular membranes.^{3,20,94–96} Furthermore, FR α overexpressed in 35–68% of triple-negative breast cancers, whereas the amount of FR α is 0–20% in non-malignant breast tissue.²⁰ Indeed, in healthy epithelial tissues, it has been shown that FR α overexpressed but because limiting anatomic distribution of FR α on luminal surfaces of normal tissues keeps these regions from FR-targeted agents injected intravenously; otherwise, in kidneys, FR α expressed in the proximal tubules can bind to targeting agents and filtered them from the blood.⁹⁷ Another cancerous parts of human such as ovarian, lung, kidney, endometrium, breast, brain and colon, up-regulated FR α , aggressively.²⁶

Also, it is recognized that a second type of FR β exists on activated macrophages in large numbers. Other experiments and studies confirm this result; in fact, these FRs are cell lock which open occasionally. Abnormal activated macrophages (AAM) are related to inflammation and autoimmune diseases such as osteoarthritis, rheumatoid arthritis, Crohn's disease, Sjogren's disease, lupus, atherosclerosis, diabetes, sarcoidosis, ischemia reperfusion injury, glomerulonephritis, ulcerative colitis, vasculitis and psoriasis.^{3,5,93,98–102} Indeed, about one in seven of leukemias are FR β -positive in acute myeloid leukemia (AML); thus, FR β is observed in M3, M4, M5 and M6 AMLs consistently, but less frequently in M1 and M2 AMLs.^{103,104} Approximately 0.5–1.0% of the world's population suffer from RA, an autoimmune disease with unknown exact etiology that results in destruction, deformity and functional loss of the affected joints.^{105–107}

Therefore, linking special matter to folate in addition of therapy compounds has other applications like medical imaging, and we can conjugate folate with radiotracers or contrast media to improve image quality and attempt to provide useful diagnostic information.

Clinical Trials

Most clinical trials have been done used OTL38, a folate-indole-cyanine green-like conjugate to FR α , with near-infrared (NIR) imaging in during different diagnosing and therapy methods that we express some of them in below.

A Phase II, multicenter, open-label trial, randomized conducted by Randall et al (NCT02317705) had promising results. They investigated the safety and efficacy of OTL38 for intra-operative imaging of FR epithelial ovarian cancer during surgery. OTL38, was injected to 44 patients with ovarian cancer under intraoperative imaging and epithelial surgery. The results showed that OTL38 was safe, efficacious and bound preferentially to ovarian cancer cells in this phase.¹⁰⁸ Also, this study led to Food and Drug Administration (FDA) support of a Phase III trial NCT03180307.

Another trial, NCT02872701, a phase II, multi-center, single dose, open-label, exploratory study, enrolled 110 patients scheduled to undergo endoscopic or thoracic surgery per CT/positron emission tomography (PET) imaging. This study aimed to assess the efficacy of OTL38 and NIR imaging in identifying pulmonary nodules. Due to the overexpression of FR α in lung cancer cells, OTL38 joined to LCC more than other normal tissues.¹⁰⁹

In addition, NCT02645409, a non-randomized and interventional trial, explored the use of fluorescence imaging and OTL38, to detect renal cell carcinoma and lymph nodes during nephrectomy. OTL38 was injected to 14 patients under surgery. In this trial, cancer cells were well visualized.¹¹⁰

One of diagnostic trials about FR β is NCT03938701 which considered patients with inflammatory bowel disease.¹¹¹ Also, the compound used was OTL38 which was injected to 30 patients and fluorescence imaging was performed. The aim of the trial was to evaluate the safety of NIR tracer OTL38 for monitoring disease activity in inflammatory diseases. In fact, the hypothesis was that OTL38 will accumulate in inflamed tissue due to the increased presence of activated macrophages expressing the FR β , enabling better visualization and monitoring of the inflammation, which resulted in better treatment and diagnosis of patients with inflammatory disease. The last clinical trial, NCT03180307, was a Phase III, open-label, multicenter study that enrolled 150 patients with known or suspected ovarian cancer undergoing cytoreductive surgery to evaluate pafolacianine (0.025 mg/kg IV) for intraoperative near-infrared (NIR) imaging. Among 109 patients with folate receptor-positive tumors, pafolacianine identified additional malignant lesions undetected by white light or palpation in 33% of cases ($P < 0.001$), with 83% sensitivity and a 24.8% false-positive rate. Complete (R0) resection was achieved in 62.4% of patients. Treatment was well tolerated, with no serious drug-related adverse events reported.¹¹² Table 1 presents a summary of clinical trials on folate conjugating.

Table 1 A Summary of Clinical Trials on Folate Conjugating

Study	NCT Number	Brief Summary	Status	Number	Period	Intervention	Outcome
OTL38 for Intra-operative Imaging of Folate Receptor-alpha Positive Ovarian Cancer (Phase 2) ¹⁰⁸	NCT02317705 (https://www.clinicaltrials.gov/ct2/show/record/NCT02317705?cond=NCT02872701&draw=2&rank=1)	To test the safety of OTL38 and see if OTL38 helps light up the cancer when viewed with the special camera system test the safety of the special camera system for use along with OTL38 during surgery.	Completed	48	Dec. 2014 to Nov. 2015	Drug: OTL38	OTL38-NIR was safe and efficacious regardless of folate expression levels and merits phase III evaluation.
OTL38 Injection for Intraoperative Imaging of Folate Receptor Positive Lung Nodules [109]	NCT02872701 (https://www.clinicaltrials.gov/ct2/show/record/NCT02872701?cond=NCT02872701&draw=2&rank=1)	A study that its aims are to assess the efficacy of OTL38 and Near Infrared Imaging at identifying pulmonary nodules within the operating theater, and to assess the safety and tolerability of single intravenous doses of OTL38.	Ongoing	110	May 2017 to Nov. 2018	Drug: OTL38 for Injection Folate analog ligand conjugated with an indole cyanine-like green dye as a solution in vials containing 3 mL at 2 mg/mL Device: Near infrared camera imaging system Near infrared camera imaging system	Due to overexpression FR α in with lung cancer cells, OTL38 joined to LCC more than another normal tissues.
Intraoperative Folate Targeted Fluorescence in Renal Cell Carcinoma ¹¹⁰	NCT02645409 (https://www.clinicaltrials.gov/ct2/show/record/NCT02645409?cond=NCT02645409&draw=2&rank=1)	To explore the use of OTL38 and fluorescence imaging to detect renal cell carcinoma in partial nephrectomy at the margins of resection, and in lymph node(s) or other metastases during radical nephrectomy.	Ongoing	14	Dec. 2015 to Apr. 2019	Drug: OTL38 is a folate analog conjugated with a fluorescent dye that emits light in the near infrared spectrum. This longer wavelength allows for deeper penetration of the fluorescent light through tissues with the potential to better image tumors beneath adipose tissue or deeper into organ parenchyma. Device: Intraoperative fluorescence imaging system	In this trial cancer cells were well displayed.
Fluorescence Imaging of Disease Activity in IBD and Rheumatoid Arthritis Using OTL38 ¹¹¹	NCT03938701 (https://www.clinicaltrials.gov/ct2/show/record/NCT03938701?cond=NCT03938701&draw=2&rank=1)	The aim of this study is to evaluate the safety and feasibility of the near infrared tracer OTL38 for monitoring disease activity in inflammatory diseases rheumatoid arthritis and inflammatory bowel disease. The hypothesis is that OTL38 will accumulate in inflamed tissue due to the increased presence of activated macrophages expressing the folate beta receptor, enabling better visualization and monitoring of the inflammation. It is expected that this approach can improve treatment and diagnosis of patients with inflammatory disease.	Active, not recruiting	30	March 2021 to March 2021	Drug: Intravenous administration of 0.0125 mg/kg OTL38 2–3 hours prior to fluorescence imaging. Device: 1) Fluorescence Imaging Rheumatoid Arthritis: Open-air camera - a camera that enables detection of fluorescent signals. 2) Fluorescence Imaging inflammatory bowel disease: Molecular Fluorescence Endoscopy - a flexible fiber-bundle is attached to a fluorescence camera platform to enable the detection of fluorescence signals.	Not provided yet
OTL38 for Intra-operative Imaging of Folate Receptor Positive Ovarian Cancer ¹¹²	NCT03180307 (https://www.clinicaltrials.gov/ct2/show/record/NCT03180307?cond=NCT03180307&draw=2&rank=1)	A Phase 3, randomized, multi-center, single dose, open label, pivotal study in patients diagnosed with, or with high clinical suspicion of, ovarian cancer scheduled to undergo primary surgical cytoreduction, interval debulking, or recurrent ovarian cancer surgery.	Completed	178	Jan. 2018 to Oct. 2020	Drug: OTL38 0.025 mg/kg of OTL38 in 250mL D5W infused intravenously over 60 minutes Other Name: OTL38 for Injection Device: NIR imaging used to excite OTL38 for fluorescence	Using combination OTL38 with fluorescent light was efficient for detecting ovarian cancer in patients.

Anti-Folate Drugs

Several studies have been performed to see the expression of FRs in normal tissues. Results have showed the expression of FRs in kidneys, placenta, lungs and choroid plexus.¹¹³ Most studies that are conducted with Folate-based agents have reported that uptake of these agents can be problematic for these organs specially for the kidney.

Results of using anti-folates such as pemetrexed, methotrexate and raltitrexed opened new perspectives for FR-based imaging and treatment.¹¹⁴ Studies showed that administration of anti-folate with radiofolate, can significantly reduce the kidney retention. Using anti-folate drugs does not affect tumor uptake, so it will led to higher tumor-to-kidney ratio.^{53,54,113,115}

Comparison of Different Imaging Modalities

Folate receptor–targeted imaging has progressed across multiple modalities, each presenting distinct advantages and limitations that must be considered for clinical translation. PET tracers provide the highest sensitivity and quantitative accuracy and are therefore well positioned for lesion detection and treatment monitoring;^{59,70} however, PET is relatively costly, and tracer availability can be limited. SPECT agents, particularly ^{99m}Tc-based conjugates are generally more accessible and economical but often deliver lower spatial resolution than PET and may exhibit non-target uptake in organs such as kidney, choroid plexus, and salivary glands.^{53,54,56} Optical/NIR probes afford real-time intraoperative guidance and excellent sensitivity for superficial targets, but limited tissue penetration restricts their utility primarily to intraoperative or endoscopic applications.^{73,76–78} MRI-based folate probes offer superior soft-tissue contrast and anatomical resolution but demand higher agent concentrations and typically have lower sensitivity than nuclear tracers.^{84–92} Across modalities, common challenges include renal accumulation due to proximal tubule FR expression,^{97,113} heterogeneity in FR expression between FR α (tumor-associated) and FR β (macrophage-associated),^{3,5,11} and unfavorable pharmacokinetics for large conjugates (for example hepatobiliary excretion observed with some deferoxamine–folate constructs).^{49,50} These modality-specific trade-offs argue for modality selection that is driven by the clinical question (detection vs surgical guidance vs therapy monitoring), receptor biology, and tracer pharmacology. The other factor that affects choosing the right modality is the concentration of contrast agent in the tissue which is relevant to the tumor/tissue specificity and receptor capacity. Acceptable MRI and CT images require higher molarities of contrast agents than PET and SPECT. Hence, the specific uptake of tumors or tissues is a factor in considering which imaging modality can best provide the contrast.

FR β -targeted imaging is emerging as a distinct and clinically relevant subfield because FR β is selectively upregulated on activated macrophages in many inflammatory and autoimmune conditions.⁶⁸ Preclinical and early human PET studies (for example with [¹⁸F] fluoro-PEG-folate and several ⁶⁸Ga-labeled conjugates) have demonstrated specific localization to inflammatory lesions and favorable target-to-background contrast, supporting further clinical validation in diseases such as rheumatoid arthritis and inflammatory bowel disease.^{43,69,71,80}

Future Directions

Several challenges remain for clinical translation of FR-targeted imaging. One important consideration is probe size and payload: large or heavily loaded folate conjugates often have unfavorable pharmacokinetics (slower clearance, increased nonspecific uptake) and may exhibit impaired tissue penetration, so optimizing molecular weight and hydrodynamic size is critical to preserve favorable delivery and imaging properties. In some cases, folate conjugates are rapidly cleared from the circulation via renal filtration (on the order of minutes), which reduces systemic residence time but can lead to prominent renal retention and reduced availability for target tissues; strategies to mitigate kidney accumulation (for example, competitive blocker dosing or antifolate pretreatment) should be further explored in clinical settings.^{53,54,96,108,114}

Because each imaging modality has unique strengths and limitations, combining modalities can yield complementary diagnostic information, for example, pairing a high-resolution anatomical modality with a sensitive functional tracer can improve both lesion localization and biological characterization. Further research into tumor biology, receptor heterogeneity, and the pharmacokinetics of folate-conjugated agents will inform probe design and the development of optimized imaging protocols and instrumentation.

A concerted effort is also needed to expand FR β -targeted PET imaging of activated macrophages across in vitro studies, animal models, and well-designed clinical trials to better define diagnostic performance and utility in

autoimmune and inflammatory diseases. In addition to imaging applications, folate conjugation enables therapeutic delivery (theranostics); specific compounds can be linked to folates for treatment of inflammatory diseases and for cancer immunotherapy, and early evidence suggests some of these approaches may reduce systemic toxicity compared with conventional chemotherapy.^{14,113} To advance FR β imaging, we recommend prioritizing well-defined patient cohorts with correlative histology or biomarker endpoints, standardized imaging protocols, and trials designed to assess both diagnostic performance and utility for treatment monitoring. Such targeted efforts will better define the clinical role of FR β tracers and their potential to inform precision management of macrophage-driven diseases.^{3,5,97–99} Future advancements are likely to integrate diagnostic and therapeutic approaches, offering personalized imaging-based monitoring and precision management for inflammatory disorders and oncology. The high specificity of FR β tracers for activated macrophages, combined with their potential for therapeutic intervention, highlights folate-based imaging as a promising frontier for precision medicine; prioritized efforts on renal-sparing strategies, tracer optimization, and multicenter clinical validation will accelerate translation.^{115,116}

Concluding Remarks

The aim of this narrative review was to update the current status of folate-based imaging in oncology and inflammatory diseases. FR-targeted approaches have shown promise across multiple modalities. SPECT agents (notably ^{99m}Tc and ¹¹¹In derivatives) have demonstrated feasibility in preclinical and clinical studies and remain cost-effective options for routine nuclear imaging, although their spatial resolution and non-target uptake (for example, renal and salivary accumulation) can limit performance. PET tracers (⁶⁸Ga, ¹⁸F and related isotopes) offer superior sensitivity and quantitative capability, supporting lesion detection, staging and therapy monitoring, and have been extended to macrophage-driven inflammation and to selected brain tumor models. Optical/NIR folate probes provide high sensitivity for intraoperative guidance and improved detection of small or superficial lesions but are constrained by limited tissue penetration. MRI-based folate constructs, particularly nanoparticle and macromolecular platforms, supply excellent anatomical contrast and potential for dual-modality applications, yet they typically require higher probe concentrations and face sensitivity challenges compared with nuclear tracers.

Key translational challenges discussed above include 1) unacceptable penetration to the tissues of interest, 2) spontaneous accumulation in non-targeted tissues, 3) unsuitable release from endosomes, 4) time of clearance, 5) time of delivery, and 6) toxicity. Addressing these limitations will require standardized tracer production and quantitative imaging protocols, renal-sparing strategies (for example, blocker or antifolate approaches), head-to-head comparative studies across modalities, and larger multicenter clinical trials with harmonized endpoints. In addition, focused validation of FR β -targeted tracers in well-defined inflammatory cohorts is needed to establish diagnostic performance and utility for treatment monitoring.

In conclusion, folate receptor–targeted imaging is a versatile and evolving platform with clear translational potential. Continued optimization of tracer chemistry and pharmacokinetics, rigorous comparative and multicenter validation, and thoughtfully designed theranostic trials will be essential to realize its full potential in precision diagnosis and therapy.

Data Sharing Statement

As this is a review, the manuscript compiles and discusses information from previously published studies. No original data were generated or analyzed for this review.

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Author Contributions

All authors made a significant contribution to the work reported, whether that is in the conception, study design, execution, acquisition of data, analysis and interpretation, or in all these areas; took part in drafting, revising or critically reviewing the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the work.



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