THE POTENTIAL OF FLUORINE¹⁹F IN TARGETED THERAPY

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Abstract: Fluorine-19 (¹⁹F) can provide critical information about drug uptake in cancer tissue when used together with ¹⁹F Magnetic Resonance Imaging (¹⁹F MRI) *in vitro* or *in vivo*. This review is aimed at the current limitations of drugs such as quantitative visualization during treatments of tumor cells. The main rationale for the utility of ¹⁹F MRI is the visualization of fluorinated drug and fluorine conjugates at the cellular *in vitro* and *in vivo* levels.

Keywords: Fluorine-19, MRI, antibody, fluorinated dendrimer, conjugate, adoptive cell therapy

The poor prognoses for many cancers are, in part, due to the biology of cancer but also in part due to the complexity of treatments with a lack of monitoring of drug uptake and action *in vivo*. Magnetic Resonance Imaging (MRI) which is commonly used for the diagnosis and staging of cancer, has not yet been used in its full capacity in the field of drug monitoring. The methodology of incorporating ¹⁹F for tracking antibodies and stem cells, and for assessing tumor oxygenation been explored in pre-clinical and clinical models and there are a few single studies on fluorinated drugs (1-4). MRI is most commonly used for samples containing ¹H nuclei in high concentration in magnetic fields that range between 0.05-14 Tesla. Briefly, MRI evaluates the physical properties of the sample area relative to neighboring areas. MRI *in vivo* is mainly used to control anatomical and morphological changes in the human body. The main applications MRI *in vitro* are monitoring of water and other solvents, controlled release of dosage forms, hydration and diffusion. The use of MRI in pharmacy can provide a platform to transfer knowledge from an *in vitro* study to an *in vivo* study in drug delivery and controlled release of dosage forms. Moreover, drug monitoring *in vivo* is still very limited. There is a diagnostic need to non-invasively visualize drug in

cells. ¹⁹F MRI is a novel preclinical imaging platform for non-invasive cell tracking, quantification of labeled cells that provides image contrast better than paramagnetic agents (iron or gadolinium particles) and conventional ¹H MRI. The low biological abundance of ¹⁹F in tissue provides a highly selective detection of labeled cells and drugs. There has been a growing interest in the medical applications of ¹⁹F MRI; in 1985, there were 3 publications on medical applications of ¹⁹F MRI and 46 publications in 2019 at the time of this writing. In our view, ¹⁹F MRI techniques are needed to provide more efficient early-stage diagnosis, predict disease course, and develop more effective therapy. A qualitative and quantitative study of important agents such as fluorinated antibody or perfluorocarbon tracking agents can be performed using ¹⁹F MRI (5-14).

The potential of antibodies in targeted therapy

The main disadvantage of currently used chemotherapy is nonspecific drug delivery and often reported toxicity. The goal of targeted anticancer therapy is to use antibodies to direct them to target antigen expressed on a tumor (3). The knowledge about localization of the target and antibody binding can characterize the drug efficacy (3). In oncology, the major drugs used in targeted anticancer therapies

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are monoclonal antibodies. By definition, monoclonal antibodies (Mab) are a protein secreted by specific white blood cells of the animal or human origin which have the ability to recognize specific targets called antigens (15). As of 2018, a total of 64 therapeutic antibodies were approved by the FDA; 18 were approved by either the US or the EU in 2017, and 4 have been approved or are under review in 2019 (16, 17). In fact, therapeutic antibody approvals and commercialization are flourishing with 570 antibodies currently in clinical trials (18). To efficiently kill the cancer cell expressing the targeted antigens, Mab often requires additional modification. Modifications of therapeutic Mab include coupling with radioactive isotopes, bacterial toxins and various co-administered drugs. The agent conjugated to the antibody such as an isotope can penetrate the tumor tissue and can be tracked by using a suitable technique (19). Of the 64 approved therapeutic Mab that are now available for targeted anticancer therapy in the clinic, perhaps the most well-known are rituximab (lymphoma, leukemia), trastuzumab (breast cancer), ofatumumab (chronic lymphocytic leukemia), obinutuzumab (follicular lymphoma), cetuximab and panitumumab (colorectal cancer), one of which (trastuzumab) has been conjugated to the cytotoxic drug mertansine to form a clinically approved antibody-drug conjugate (ADC) (20). Antibody-drug conjugates such as the Trastuzumab-dendrimer-fluorine conjugate drug delivery system could be used as alternatives to native antibodies for tumor targeting applications (3). Antibodies are engineered to track a specific tumor antigen and bind themselves to the surface of cancer cells. Due to antibody target specificity, formulations of ADC's that can deliver cytotoxic drugs are and will continue to be of great importance. Clinical benefit has been observed with several antibody-drug conjugates including polatuzumab vedotin-piiq (lymphoma), brentuximab vedotin (Hodgkin's lymphoma), trastuzumab emtansine (breast cancer), inotuzumab ozogamicin (leukemia) (20). Further development of therapeutic antibodies and ADC that may be tracked by incorporation of ^{19}F may also lead to improvements in the clinical utility of these drugs.

The potential of dendrimers in targeted therapy

Up to now, the application of dendrimers in cancer research has been emphasized in several fields including improvement of drug efficacy and diagnosis. Studies have shown that molecules 100 nm in diameter do not effectively diffuse across the vascular endothelium (21, 22). Cationic dendrimers can be cleared from circulation due to electrostatic

binding to tissue vasculature (23). Anionic and neutral dendrimers have longer circulation times (23).

PAMAM dendrimers have unique 3D architecture and nanoscale size (24). Butle and coworkers investigated dysprosium [Dy]-DOTA-PAMAM(G5) conjugates as a possible new class of macromolecular spin-spin contrast agents (24). The applicability of dendrimers as platforms for drug delivery was established for Cisplatin. Cisplatin was encapsulated in dendrimer polymers and increased efficacy with less toxicity (25). PAMAM dendrimers with cationic surface charge which can potentially serve as siRNA condensing agents as well as robust nano-vectors for targeted delivery (26). Several researchers have successfully conjugated venlafaxine, 5-aminosalicylic acid (27), methotrexate, propranolol (28), with PAMAM dendrimers. The results have shown enhanced solubility and controlled release of drugs from these complexes in comparison to the pure drug. Apart from these, many anticancer drugs such as cisplatin (29), doxorubicin (30), penicillin V (31), epirubicin (32), and paclitaxel (33) have also been conjugated and used for drug targeting. Dendrimers are biocompatible (34), non-immunogenic, water-soluble and have been attached to many biological molecules such as proteins, synthetic drugs, and small molecules. Antibodies conjugated to dendrimers have been shown to internalize in cells faster and more efficiently than antibodies alone (34). The cellular uptake of antibody-dendrimer conjugates was studied by confocal laser scanning microscopy, a technique not applicable to *in vivo* immunotherapy. Antibody-dendrimer conjugates suitable for MRI studies have not yet been developed.

One study has demonstrated ^{19}F MRI at 4.0 Tesla imaging after injection of 200 μL of 20 mg/mL PAMAM(G6) dendrimers modified with 15 to 38 heptafluoroacyl substituents in female B6 mice (35). In this paper, images showed that particulates remain in circulation and are efficiently filtered by the liver (35). Another study described a synthesis of PAMAM(G5) dendrimers conjugated to anti-Her-2 Mab (36). Here, the efficiency of the conjugates after administration to MCA207 cells *in vitro* was detected by flow cytometry and confocal microscopy with a fluorescent probe. In addition, 2.4 nmol of the conjugate was injected into mice intravenously and the dendrimer-Mab conjugate was predominantly internalized into Her-2 expressing tumor cells. The advantages of this application are that dendrimer-Mab conjugates selectively targets cancer cells and does not go to the organs such as the spleen. Dendrimer-Her-2 monoclonal antibody conjugates have been shown to target Her-2 overexpressing

cells but in the absence of antibody, PAMAM(G5) dendrimer does not bind to the Her-2 target (36). *In vivo* results in mice obtained in this study using dendrimer-Her-2 monoclonal antibody conjugates showed no hair loss, no weight loss and reduction of tumor size with low concentrations of Her-2 MAb (37). Confocal microscopy also demonstrated that the conjugate is present in the tumors, attached to and internalized by many of the tumor cells (37). In another study, the use of dendrimer-Her-2 MAb conjugated to methotrexate showed 75% tumor reduction (37). The quantification of cancer cell killing was not reported. One more promising study described the application of ^{19}F MRI at 1.5 Tesla to image large fluorinated dendrimers. The lowest detectable concentration for ^{19}F MRI reported in this study was 18.5 μM of fluorinated dendrimer (or 10 mM in ^{19}F concentration) with a scan time of only 150 seconds. Reported spin-lattice relaxation time of the fluorinated dendrimer is 366 ms which is lower than that for trifluoroethanol which is 2379 ms. Similarly, measured spin-spin relaxation time was 122 ms and 267 ms for the fluorinated dendrimer and trifluoroethanol, respectively (38).

The potential of ^{19}F Magnetic Resonance Imaging in targeted therapy

The Magnetic Resonance sensitivity of fluorine ^{19}F is close to that of ^1H (about 90%) and ^{19}F has 100% natural abundance. However, the small amount of fluorine in biological samples has limited its clinical applications. ^{19}F labeled drug delivery systems allow for highly-specific detection due to the absence of background signals in the host, thus ^{19}F labeled compounds can act as proxy biomarkers (39, 40). Due to undetectable ^{19}F concentrations in tissue, there is a near-zero background signal that makes ^{19}F MRI measurements advantageous when compared to standard ^1H MRI. The use of ^{19}F MRI greatly improves drug tracking by providing a signal which varies in a direct relationship with the amount ^{19}F present without any background signal from tissue (40). Therefore, ^{19}F MRI is able to detect ^{19}F compounds delivered only to the tissue of interest since the natural content of ^{19}F (10^{-3} $\mu\text{mol/g}$ wet tissue) are below the detection limit. This is of particular importance for ^{19}F studies as ^{19}F content is low and signal averaging is always needed to provide a high signal-to-noise ratio (SNR). As the SNR increases with the root square of scan time, long scan times are needed. To shorten relaxation time T_1 , a paramagnetic material such as lanthanide ion may be placed in the proximity of ^{19}F . The most common applications of ^{19}F MR in cancer research are imaging and spectroscopy

of ^{19}F labeled anticancer agents when applied to cell cultures as they offer a controlled and systematic way to investigate cellular and molecular properties associated with disease (41). Additional applications of ^{19}F MR are in tissue metabolomics, quantification of drug delivery, cellular tracking (42), tissue pH measurements and detection of ^{19}F labeled cells. ^{19}F MR provides only localized detection and visualization of ^{19}F labeled cells and ^1H MRI image overlays are used in conjunction to give anatomical details (41). Other applications include tissue perfusion, tissue oximetry and visualization of labeled cells (41). In regards to the application of ^{19}F MR for the visualization of labeled cells, it is important to mention that the first clinical trial involving imaging of PFC treated therapeutic dendritic cells in patients with stage 4 colorectal cancer was reported in 2014 (4). Ahrens et al. described this trial as an evaluation of potential adverse effects resulting from injection of ^{19}F labeled dendritic cells (none reported) and the ability to visualize these labeled cells using a clinical scanner. The encouraging imaging data obtained were said to enable “in vivo cytometry” (4). In addition to the clinical study of PFC labeled therapeutic dendritic cells, other preclinical trials have been conducted in imaging adoptive cell therapy by ^{19}F MRI. Adoptive cell therapy is the introduction of immune cells to a patient for counteracting a tumors anti-surveillance mechanism (14). Therapeutic immune cells that have been labeled with PFC and tracked by ^{19}F MRI include lymphocytes, T cells, natural killer cells, and peripheral blood mononuclear cells (14).

The potential of perfluorocarbons in targeted therapy

Perfluorochemicals e.g perfluorooctylbromide (PFOB) or perfluoro-15-crown-5-ether (PFCE) are a class of compounds without hydrogen. All perfluorochemicals are sensitive to changes in oxygen tension. In addition, they have high gas solubility and behave like liquid gas. In particular, PFOB is twice as dense as water. Due to the absence of natural fluorine in the cells, there is no background noise making this method very sensitive. The local oxygenation of a culture can be measured using fluorine T maps. Tumors cells generate lactic acid (43) and this has led to postulate that tumors are acidic (44-46). It is commonly known that cancer cells are hypoxic, therefore the measurements of the tissue oxygenation are important in the evaluation of the tumor microenvironment. ^{19}F MR can be used to measure oxygen content because the T relaxation time of fluorine nuclei is reverse proportional to the dissolved molecular oxygen concentration. Therefore, ^{19}F MR

spectroscopy and MRI have been used extensively to monitor changes in the tumor oxygenation as a function of various therapeutic interventions in animal models.

However, ^{19}F MRI has been used for a variety of research applications including studying tumor metabolism (47-49) and mapping liquid ventilation (49). Many of the studies were performed at high magnetic fields 4.7 Tesla or greater (50-53). Also, many of the studies were acquired with an increased concentration of ^{19}F (54, 55).

To allow clinical studies, *in vivo* concentrations of fluorine-containing metabolites must be larger than about 200 mol/g tissue to be detectable in reasonable measurements times 5-15 min at $B=1-2$ Tesla. For animal studies at 7 Tesla, an amount of 50 nmol/g can be detected within 30 min. In *ex vivo* studies 5-10 nmol/g can be detected within 1-2 h at 11.7 Tesla (47-55). The ability to non-invasively monitor the oxygenation state of individual tumor cells has important implications for planning and evaluation therapy. The presence of hypoxia limits the success of radiotherapy in animal tumors. Residual malignant cells protected from these therapeutic modalities by hypoxia may regroup to cause local recurrence of the disease. Consequently, knowledge of the oxygenation status of cells within solid tumors before and during treatment would be extremely valuable. The spin-lattice relaxation rate ($1/T_1$) of PFOB and PFCE will increase linearly with increasing oxygen concentration. Most of those papers show that metabolomics can also be applied to the discovery of metabolic pathway of a drug which can help to monitor drug. There are few reports of using ^{19}F to measure cancer cell metabolism, which is emerging as a highly critical variable in successful drug delivery.

CONCLUSION

The application of ^{19}F MRI to the quantification of drug delivery, cell tracking, tissue pH measurements, oximetry, and visualization of adoptive cell therapy is gaining in popularity. Currently, the use of ^{19}F MRI is having a significant impact on the field of immunotherapy by providing critical improvements in our ability to visualize MAb uptake by cancer tissue and quantification of the response of tissue to treatment. In 2014, the first clinical use of ^{19}F MRI in dendritic cells containing perfluorocarbon for visualizing adoptive cell therapy was reported. The construction of monoclonal antibody- ^{19}F conjugates that contain a high number of equivalent ^{19}F atoms for imaging and quantifica-

tion is currently an active area of research, and, in particular, PAMAM fluorinated dendrimers are being explored as potential ^{19}F -bearing groups that can be covalently attached to MAb with minimal interference with their ability to target receptors. The ability to visualize and quantify MAb distribution and therapeutic immune cells after administration *in vivo* is extremely desirable and will continue to be the impetus for the development of new methodologies that can be imaged using ^{19}F MR.

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Conflict of interest

The authors declare no conflicts of interest.

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