1536012118792317-1536012118792317; Janssen, *et al., Academic Press:* 2019; Vol. 165, pp 371-399; Werry, *et al., Int J Mol Sci* 2019, 20 (13), 3161; Papadopoulos, *Experimental Neurology* 2009, 219 (1), 53-57).

[005] Other imaging technique include computed tomography (CT) and Magnetic Resonance Imaging (MRI). Magnetic Resonance Imaging (MRI) uses radio-waves in the presence of a strong magnetic field that surrounds the opening of the MRI machine where the patient lies to get tissues to emit radio waves of their own. Different tissues (including tumors) emit a more or less intense signal based on their chemical makeup, so a picture of the body organs can be displayed on a computer screen. Much like CT scans, MRI can produce three-dimensional images of sections of the body, but MRI is sometimes more sensitive than CT scans for distinguishing soft tissues.

[006] Accordingly, in some aspects, the disclosure provides compositions and methods for non-invasive detection of sites of inflammation or neuroinflammation in a subject, and methods of making and use thereof. In some aspects, the disclosure provides compositions for *in vivo* molecular imaging of cancer cells, such as metastatic cancer cells, and methods of making and use thereof. In some aspects, the disclosure provides compositions and methods for selectively targeting radiotherapy to cancer cells.

SUMMARY

[007] The disclosure relate to the discovery that dendrimers (e.g., hydroxyl PAMAM dendrimers) conjugated or complexed with radionuclides, such as ^{18}F (Fluorine-18), ^{89}Zr (Zirconium-89), ^{90}Y (Yttrium-90), and ^{177}Lu (Luthenium-177), can cross the BBB in the presence of reactive microglia, and selectively target these microglia after systemic administration. In some examples, these conjugates are stable Positron emission tomography (PET) imaging probes for the non-invasive and specific imaging of reactive microglia *in vivo* and stable radiotherapy agents for the treatment of tumors. In some examples, the disclosure provides compositions and methods for detecting one or more inflammatory sites in a subject in need thereof. In some examples, the disclosure provides compositions and methods for treating cancer in a subject in need thereof.

[007a] In one aspect, the disclosure provides a compound comprising a dendrimer conjugated to a radionuclide, wherein the dendrimer comprises a polyamidoamine (PAMAM) dendrimer

comprising a plurality of surface hydroxyl groups and at least one surface hydroxyl group forming an ether linkage between the dendrimer and the radionuclide, and wherein the radionuclide is fluorine-18 (18F).

[007b] In another aspect, the disclosure provides a pharmaceutical composition comprising the compound of the disclosure and a pharmaceutically acceptable excipient.

[007c] In yet another aspect, the disclosure provides a method for detecting or imaging one or more inflammatory and/or cancer cells in a subject in need thereof, wherein an effective amount of the compound of the disclosure is to be administered to the subject.

[007d] In yet another aspect, the disclosure provides use of the compound of the disclosure in the manufacture of a medicament for treating cancer in a subject in need thereof, wherein the compound is to be administered to the subject.

[008] In some examples, the disclosure provides compositions of hydroxyl-terminated dendrimers conjugated to one or more imaging agents *via* ether linkages. In some examples, hydroxyl-

terminated dendrimers are conjugated to imaging agents, where imaging agents can include radionuclide or MRI contrast agents. In some examples, the dendrimer conjugates selectively accumulate within reactive microglia at sites of inflammation and in reactive immune cells in tumor as stable imaging probes *in vivo* or radiotherapeutics. In some examples, the dendrimer conjugates also treat the site of inflammation or cancer, for example, by selectively delivering radiotherapeutic agents to the site of inflammation or cancer. In other examples, the dendrimer conjugates include one or more additional active agents. Therefore, in some examples, the dendrimer conjugates deliver one or more additional active agents *in vivo* to sites of inflammation, neuroinflammation, or tumor with enhanced stability. In some examples, the radionuclides and/or the MRI contrast agents are attached to the dendrimer via an ether linkage.

[009] The disclosure provides a composition comprising a compound that comprises a dendrimer conjugated to a radionuclide or an MRI contrast agent through an ester, ether, or amide linkage. In some examples, the dendrimer comprises a high density of surface hydroxyl groups. In some examples, the dendrimer is conjugated to the radionuclide or the MRI contrast agent through an ether or amide linkage. In some examples, the dendrimer is conjugated to the radionuclide or the MRI contrast agent through an ether linkage.

[0010] In some examples, the radionuclide or the MRI contrast agent is conjugated to the ester, ether, or amide linkage through a spacer. In some examples, the spacer comprises alkyl groups, heteroalkyl groups, and/or alkylaryl groups. In some examples, the spacer comprises a peptide. In some examples, the spacer comprises polyethylene glycol.

[0011] In some examples, conjugation of the radionuclide or the MRI contrast agent occurs on less than 50% of total available surface functional groups of the dendrimer prior to the conjugation. In some examples, conjugation of the radionuclide or the MRI contrast agent occurs on less than 5%, less than 10%, less than 20%, less than 30%, or less than 40% of total available surface functional groups of the dendrimer prior to the conjugation.

[0012] In some examples, the radionuclide is selected from the group consisting of ^{18}F, ^{51}Mn, ^{52}Fe, ^{60}Cu, ^{68}Ga, ^{72}As, ^{94m}Tc, ^{110}In, ^{18}F, ^{124}I, ^{125}I, ^{131}I, ^{123}I, ^{77}Br, ^{76}Br, ^{99m}Tc, ^{51}Cr, ^{67}Ga, ^{68}Ga, ^{47}Sc, ^{51}Cr, ^{167}Tm, ^{141}Ce, ^{111}In, ^{168}Yb, ^{175}Yb, ^{140}La, ^{90}Y, ^{88}Y, ^{153}Sm, ^{166}Ho, ^{165}Dy, ^{166}Dy, ^{62}Cu, ^{64}Cu, ^{67}Cu, ^{97}Ru, ^{103}Ru, ^{186}Re, ^{188}Re, ^{203}Pb, ^{211}Bi, ^{212}Bi, ^{213}Bi, ^{214}Bi, ^{105}Rh, ^{109}Pd, ^{117m}Sn, ^{149}Pm, ^{161}Tb, ^{177}Lu, ^{225}Ac, ^{198}Au, ^{199}Au, and ^{89}Zr. In some examples, the radionuclide is ^{18}F,

^{89}Zr, ^{90}Y, or ^{177}Lu. In some examples, the MRI contrast agent is selected from the group consisting of Gd, Mn, $BaSO_4$, iron oxides, and iron platinum. In some examples, the MRI contrast agent is Gd.

[0013] In some examples, the dendrimer is selected from the group consisting of polyamidoamine (PAMAM) dendrimers, polypropylamine (POPAM) dendrimers, polyethylenimine dendrimers, polylysine dendrimers, polyester dendrimers, iptycene dendrimers, aliphatic poly(ether) dendrimers, and aromatic polyether dendrimers.

[0014] In some examples, the zeta potential of the compound is between -25 mV and 25 mV. In some examples, the zeta potential of the compound is between -20 mV and 20 mV, between -10 mV and 10 mV, between -10 mV and 5 mV, between -5 mV and 5 mV, or between -2 mV and 2 mV. In some examples, the surface charge of the compound is neutral or near-neutral.

[0015] In some examples, the disclosure provides methods for detecting one or more inflammatory sites in a subject in need thereof. The disclosure provides methods for imaging one or more inflammatory sites in a subject in need thereof. In some examples, the methods comprise administering to the subject an effective amount of a composition described herein. For example, in some examples, the composition comprises a compound comprising a dendrimer conjugated to a radionuclide or an MRI contrast agent through an ester, ether, or amide linkage, where the dendrimer comprises a high density of surface hydroxyl groups.

[0016] In some examples, the methods selectively deliver one or more radionuclides or magnetic resonance imaging (MRI) contrast agents to the target sites of the recipient. In some examples, the methods include administering to a subject a formulation including hydroxyl-terminated dendrimers conjugated to one or more radionuclides or MRI contrast agents *via* ether linkages. Exemplary radionuclides that can be delivered include ^{18}F, ^{51}Mn, ^{52}Fe, ^{60}Cu, ^{68}Ga, ^{72}As, ^{94}mTc, or ^{110}In, ^{18}F, ^{124}I, ^{125}I, ^{131}I, ^{123}I, ^{77}Br, ^{76}Br, ^{99m}Tc, ^{51}Cr, ^{67}Ga, ^{68}Ga, ^{47}Sc, ^{51}Cr, ^{167}Tm, ^{141}Ce, ^{111}In, ^{168}Yb, ^{175}Yb, ^{140}La, ^{90}Y, ^{88}Y, ^{153}Sm, ^{166}Ho, ^{165}Dy, ^{166}Dy, ^{62}Cu, ^{64}Cu, ^{67}Cu, ^{97}Ru, ^{103}Ru, ^{186}Re, ^{188}Re, ^{203}Pb, ^{211}Bi, ^{212}Bi, ^{213}Bi, ^{214}Bi, ^{105}Rh, ^{109}Pd, ^{117}mSn, ^{149}Pm, ^{161}Tb, ^{177}Lu, ^{225}Ac, ^{198}Au and ^{199}Au, or ^{89}Zr. Exemplary MRI contrast agents that can be delivered include Gd, Mn, $BaSO_4$, iron oxides, and iron platinum.

[0017] In some examples, the methods deliver one or more radionuclides or MRI contrast agents to the subject in an amount effective to achieve a physiological response in the subject.

The dendrimers are optionally complexed to, covalently conjugated to, or having intra-molecularly dispersed or encapsulated therein one or more additional active agents, such as therapeutic or prophylactic agents. In some examples, the formulation is administered systemically to a subject, in an amount effective to detect, diagnose or monitor one or more inflammatory sites or cancer in the recipient, and/or to treat, alleviate or prevent one or more symptoms of the inflammation or cancer in the subject. Exemplary inflammatory sites or cancers that can be detected, diagnosed or monitored and/or treated include sites of autoimmune diseases or disorder, sites of neuroinflammation in the brain, and solid tumors, including brain tumors. Additional exemplary inflammatory sites include those associated with one or more inflammatory diseases, or associated with sepsis or septic shock, or caused by any mechanism of macrophage activation including macrophage activation syndrome. In some examples, the one or more inflammatory sites in the subject include one or more sites of neuroinflammation in the central nervous system. In some examples, the one or more sites of neuroinflammation in the central nervous system in the subject are associated with Alzheimer's disease. In some examples, the one or more inflammatory sites in the subject are associated with amyotrophic lateral sclerosis (ALS). In some examples, the dendrimers are generation 4, generation 5, or generation 6 poly(amidoamine) (PAMAM) hydroxyl-terminated dendrimers.

[0018] In some examples, the disclosure provides compositions and methods for detecting cancer cells in a subject *in vivo*. In some examples, the method involves administering to the subject one or more hydroxyl-terminated dendrimers conjugated or complexed with one or more radionuclides and/or one or more MRI contrast agents, that selectively target a tumor region. In some examples, the subject can then be imaged with a molecular imaging device to detect the hydroxyl-terminated dendrimers conjugated or complexed with one or more radionuclides in the subject. In some examples, the subject can also be imaged with an MRI scanner to detect the hydroxyl-terminated dendrimers conjugated or complexed with one or more MRI contrast agents in the subject. Thus, in some examples, detection of the labeled hydroxyl-terminated dendrimers in an organ or tissue of a subject can be an indication of cancer cells in the organ.

[0019] In some examples, the cancer cells can be primary tumors or metastasized cancer cells. Therefore, in some examples, the methods involve administering dendrimers conjugated or complexed with one or more radionuclides to a subject diagnosed with a primary tumor to identify metastasized cancer cells. In other examples, the method involves

administering dendrimers conjugated or complexed with one or more radionuclides to a subject at risk of cancer to detect primary or occult tumors. Non-limiting examples of cancer cells that can be detected by the disclosed methods include renal cell carcinoma.

[0020] In some examples, the disclosure provides a method for treating a neurodegenerative disorder. In some examples, the method comprises administering to a subject in need thereof an effective amount of a composition described herein. In some examples, the composition comprises a compound comprising a dendrimer conjugated to a radionuclide or an MRI contrast agent through an ester, ether, or amide linkage, where the dendrimer comprises a high density of surface hydroxyl groups. In some examples, the neurodegenerative disorder is Alzheimer's disease. In some examples, the neurodegenerative disorder is ALS.

[0021] Exemplary devices for use in molecular imaging include, for example, devices for positron emission tomography (PET) scanning; devices for computed tomography (CT) and nuclear medicine imaging; devices for magnetic resonance imaging (MRI). In some examples, the molecular imaging devices are a gamma camera suitable for positron emission tomography (PET) scanning and an MRI scanner.

[0022] In some examples, the formulation can be formulated for intravenous administration to the subject or for enteral administration. In some examples, the formulation is administered prior to, in conjunction with, subsequent to, or in alternation with treatment with one or more additional procedures or therapies. Exemplary additional procedures include administering one or more therapeutic, prophylactic and/or diagnostic agents to prevent or treat one or more symptoms of associated diseases or conditions of inflammation, neuroinflammation, and cancers.

[0023] In some examples, the disclosure provides pharmaceutical formulations of hydroxyl dendrimers complexed to, covalently conjugated to, or encapsulated therein one or more radionuclides. In some examples, the disclosure provides kits including the hydroxyl dendrimers complexed to, covalently conjugated to, or encapsulated therein one or more radionuclides or one or more MRI contrast agents.

[0024] In some examples, the disclosure provides methods of making hydroxyl dendrimers complexed to, covalently conjugated to, or encapsulated therein one or more radionuclides.

BRIEF DESCRIPTION OF THE DRAWINGS

CLAIMS

We claim:

1. A compound comprising a dendrimer conjugated to a radionuclide, wherein the dendrimer comprises a polyamidoamine (PAMAM) dendrimer comprising a plurality of surface hydroxyl groups and at least one surface hydroxyl group forming an ether linkage between the dendrimer and the radionuclide, and wherein the radionuclide is fluorine-18 (^{18}F).

2. The compound of claim 1 or 2, wherein the dendrimer comprises a generation 4, generation 5, generation 6, generation 7, or generation 8 PAMAM dendrimer.

3. The compound of claim 1 or 2, wherein the dendrimer comprises a generation 4, generation 5, or generation 6 PAMAM dendrimer.

4. The compound of any one of claims 1-3, wherein the dendrimer comprises a generation 4 PAMAM dendrimer.

5. The compound of any one of claims 1-4, wherein the radionuclide is conjugated to the ether linkage through a spacer.

6. The compound of claim 5, wherein the spacer is covalently attached to the radionuclide.

7. The compound of claim 5 or 6, wherein the spacer comprises alkyl groups, heteroalkyl groups, or alkylaryl groups.

8. The compound of any one of claims 5-7, wherein the spacer comprises a peptide.

9. The compound of any one of claims 5-8, wherein the spacer comprises polyethylene glycol.

10. The compound of any one of claims 1-9, wherein conjugation of the radionuclide occurs on less than 50% of total available surface functional groups of the dendrimer prior to the conjugation.

11. The compound of any one of claims 1-10, wherein conjugation of the radionuclide occurs on less than 5%, less than 10%, less than 20%, less than 30%, or less than 40% of total available surface functional groups of the dendrimer prior to the conjugation.

12. The compound of any one of claims 1-11, wherein the zeta potential of the compound is between -25 mV and 25 mV.

13. The compound of any one of claims 1-12, wherein the zeta potential of the compound is between -20 mV and 20 mV, between -10 mV and 10 mV, between -10 mV and 5 mV, between -5 mV and 5 mV, or between -2 mV and 2 mV.

14. The compound of any one of claims 1-13, wherein the surface charge of the compound is neutral or near-neutral.

15. The compound of any one of claims 1-14, wherein the dendrimer is further conjugated to one or more therapeutic agents.

16. A pharmaceutical composition comprising the compound of any one of claims 1-15 and a pharmaceutically acceptable excipient.

17. The pharmaceutical composition of claim 16, formulated for intraperitoneal, intravenous, intrathecal, intratumoral, or oral administration.

18. A method for detecting or imaging one or more inflammatory and/or cancer cells in a subject in need thereof, wherein an effective amount of the compound of any one of claims 1-15 is to be administered to the subject.

19. The method of claim 18, wherein the compound is to be administered systemically to the subject.

20. The method of claim 18 or claim 19, wherein the compound is to be administered via intraperitoneal, intravenous, intrathecal, intratumoral, or oral route.

21. The method of any one of claims 18-20, wherein the compound is to be administered in an amount effective to detect, diagnose or monitor one or more inflammatory sites or cancer in the subject.

22. The method of any one of claims 18-21, wherein the one or more inflammatory sites in the subject are associated with one or more inflammatory diseases, or associated with sepsis or septic shock, or caused by any mechanism of macrophage activation including macrophage activation syndrome.

23. The method of any one of claims 18-21, wherein the one or more inflammatory sites in the subject are associated with one or more autoimmune diseases or disorders.

24. The method of claim 23, wherein the one or more autoimmune diseases or disorders are selected from the group consisting of rheumatoid arthritis, psoriasis, psoriatic arthritis, multiple sclerosis, Sjögren syndrome, Myasthenia gravis, Graves disease, Addison disease, Celiac disease, Hashimoto thyroiditis, Pernicious anemia, Guillain-Barre syndrome, Chronic inflammatory demyelinating polyneuropathy, vasculitis, systemic lupus erythematosus (SLE), type 1 diabetes, inflammatory bowel disease, and thyroid diseases.

25. The method of any one of claims 18-21, wherein the one or more inflammatory sites in the subject include one or more sites of neuroinflammation in the central nervous system.

26. The method of claim 25, wherein the one or more sites of neuroinflammation in the central nervous system in the subject are associated with Alzheimer’s disease.

27. The method of any one of claims 18-21, wherein the one or more inflammatory sites in the subject are associated with amyotrophic lateral sclerosis (ALS).

28. The method of any one of claims 18-21, wherein the subject has cancer.

29. The method of claim 28, wherein the cancer is solid tumor of any type or brain metastases.

30. The method of any one of claims 18-29, wherein the method further comprises the step of imaging the subject with a molecular imaging device to detect the dendrimers conjugated to one or more radionuclides in the subject, wherein detection of the dendrimers conjugated to one or more radionuclides indicates presence of inflammation or cancer cells.

31. The method of claim 30, wherein the molecular imaging device comprises a gamma camera for positron emission tomography (PET) scanning or a scanner for magnetic resonance imaging.

32. Use of the compound of any one of claims 1-15 in the manufacture of a medicament for treating cancer in a subject in need thereof, wherein the compound is to be administered to the subject.

33. The use of claim 32, wherein the compound is to be administered systemically to the subject.

34. The use of claim 32 or claim 33, wherein the compound is to be administered via intraperitoneal, intravenous, intrathecal, intratumoral or oral route.

35. The use of any one of claims 32-34, wherein the cancer is breast cancer, ovarian cancer, uterine cancer, prostate cancer, testicular germ cell tumor, brain cancer, gastric cancer, esophagus cancer, lung cancer, liver cancer, renal cell cancer, or colon cancer.

36. The use of any one of claims 32-35, wherein the effective amount is effective to reduce tumor size.