Chapter 27

Radiopharmaceuticals for Molecular Imaging and Theranostics of Glioblastoma

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Abstract– The present chapter provides a comprehensive review of the more relevant contributions of nuclear modalities and radiopharmaceuticals for the diagnosis, therapy and follow-up of treatment in glioblastoma. Initially, a general overview of the different nuclear modalities and principal characteristics of radiopharmaceuticals for diagnostic or therapy are presented, anticipating that many readers are not familiar with the field. Special attention is given to the most important aspects involved in the design and preclinical evaluation of radiopharmaceuticals for glioblastoma targeting, as well as to the relevant molecular targets and to the cellular and animal models used to identify and evaluate candidates for further clinical trials. The chapter also contains a summarized description of the radioactive agents tested as radiopharmaceuticals for imaging and radiotheranostics of glioblastoma, either at the preclinical or clinical level, including radiolabeled small molecules, radiopeptides, radiolabeled antibodies and radioactive nanoparticles.

Keywords – Glioblastoma, Nuclear Imaging, Radiopharmaceuticals, Theranostics

Abbreviatures – 2-Dimensional (2D); 3-Dimensional (3D); Auger Electrons (AE); Silver Nanoparticle (AgNP); Alisertib (Ali); Diacetyl-bis(N4-methylthiosemicarbazone) (ATSM); Gold Nanoparticle (AuNP); Blood-Brain Barrier (BBB); Bombesin (BBN); N,N-bis-(2-mercaptoethyl)-N',N'-diethylethylenediamine (BMEDA); Carbonic Anhydrase (CA); Convection Enhanced Delivery (CED); Choline (Cho); Computed Tomography (CT); Chlorotoxin (CTX/ ClTX); Chemokine Receptor-4 (CXCR4); Double Strand Breaks (DBS); Deoxycytidine Kinase (dCK); Deoxyribonucleic acid (DNA); 1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetic acid (DOTA); 1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetic acid-Tyr3 (DOTATATE); 1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetic acid-D-Phe1,Tyr3 (DOTATOC); diethylenetriaminepentaacetic acid (DTPA); Extracellular Matrix (ECM); Epidermal Growth Factor Receptor (EGFR); Enhanced Permeability and Retention (EPR); Extra-Domain B of Fibronectin (EDB-FN); Fibroblast Activation Protein (FAP); Fibroblast Activation Protein Inhibitors (FAPIs); Fluoro-azomycin arabinoside (FAZA); Fluorocholine (FCho); 2-fluoro-2-deoxy-D-glucose (FDG); 2,2-dihydroxymethyl-3-fluoropropyl-2-nitroimidazole (FDiFA); Fluoro-dihydroxyphenylalanine (FDOPA); Fluoroethyltyrosine (FET); Fluoro-erythro-nitroimidazole (FET-NIM); Flortanidazole (F-HX4); Fluorothymidine (FLT); Fluoromisonidazole (FMISO); FluorThanatrace (F-TT); Glioblastoma (GBM); Glucose Transporter 1 (GLUT1); Gastrin-releasing Peptide Receptor (GRPR); Glioma Stem-like Cells (GSCs); Gray (Gy); Hyaluronan (HA); Hexamethylpropyleneamine-oxime (HMPAO); Nimotuzumab (h-R3); Interleukin-13 receptor subunit α-2 (IL13RA2); Linear Energy Transfer (LET); Lipid Nanocapsule (LNC); Monoclonal Antibodies (mAb); Etaracizumab (MEDI-522); Methionine (MET); Methoxyisobutylisonitrile (MIBI); Matrix Metalloproteinase (MMP); Magnetic Resonance Imaging (MRI); Mesoporous Silica Nanoparticle (MSN); Neural Cell Adhesion Molecule (NCAM); Near-Infrared Fluorescent (NIRF); Neurokinin-1 Receptor (NK1R); Nanoparticle (NP); Poly(ADP-ribose) Polymerase 1 (PARP1); Poly(ADP-ribose) Polymerase inhibitor-

fluorescently labelled (PARPi-FL); Patient-derived Orthotopic Xenografts (PDOXs); Polyethylene Glycol (PEG); Positron Emission Tomography (PET); poly(lactic-co-glycolic acid) (PLGA); Peptide Receptor Radionuclide Therapy (PRRT); Quantum Dot (QD); Relative Biological Effectiveness (RBE); Arginylglycylaspartic acid (RGD); Radioimmunotherapy (RIT); Substance P (SP); Single-Photon Emission Computed Tomography (SPECT); Somatostatin Receptor 2 (SSTR2); Tetrofosmin (TF); Temozolomide (TMZ); Tumour Necrosis Treatments (TNT); Targeted Radionuclide Therapy (TRT); Translocator Protein (TSPO); Positron (β^+); Electron (β^-); Gamma (γ); 1-(2-chlorophenyl)-N-methyl-N-(1-methylpropyl)-3-isoquinoline carboxamide ((R)PK11195).

27.1 INTRODUCTION

Glioblastoma (GBM) is the most frequent form of brain tumours, accounting for approximately 15% of all primary brain tumours in adults. Nowadays, the overall survival of GBM patients remains extremely low with an average lifespan of approximately 15 months after diagnosis (1, 2). Classical approaches, like open surgery, chemotherapy and radiotherapy, still remain the most commonly used strategies in GBM therapy (3). This scenario reflects the genetic complexity of GBM whose progression is accompanied by an intricate molecular evolution with increased number of genetic aberrations, which hamper the rise of effective therapies able to control the different biological processes involved in GBM tumour growth (4, 5).

Due to these difficulties, only four compounds have been approved by the Food and Drug Administration for clinical use in GBM patients. One of these compounds is the 5-aminolevulinic acid used for fluorescence laparoscopic imaging in intraoperative procedures. The other three are the therapeutic drugs bevacizumab, carmustine and temozolomide (TMZ), that present moderated results in the treatment of this deadly disease (6). Nonetheless, the recent advances on GBM genomic characterization and on the identification of its signaling pathways should leverage the development of new molecular targeted therapies, namely based on monoclonal antibodies (mAb) for immunotherapy, tyrosine-kinase inhibitors and cellular approaches (e.g., chimeric antigen receptor genetically modified T cells or dendritic cells). Unfortunately, the majority of the clinical trials performed with these innovative molecular and cellular therapies led to suboptimal therapeutic outcomes. These disappointing results partly reflect the prevalence of a population-based approach in the design of clinical trials, instead of a personalized approach with a specific selection of GBM patients accordingly to the "molecular profile" of their disease. Besides other requisites, the rise of the personalized treatment of GBM with a molecular profile-oriented selection of patients requires an increasing crosstalk between molecular biology and *in vivo* molecular imaging. Nuclear medicine and radiopharmaceuticals offer unique features to bridge the gap and promote this crosstalk.

Nuclear medicine modalities involve the administration of radiolabeled drugs, called radiopharmaceuticals, which are used for diagnostic or therapeutic applications depending on the physical properties of the labeling radionuclide. The two fundamental nuclear medicine imaging techniques are the Single-Photon Emission Computed Tomography (SPECT) and the Positron Emission Tomography (PET). The low detection limits of SPECT and PET allow the *in vivo* visualization of biomarkers at low local concentration thus providing biochemical/biological information with clinical relevance in a non-invasive way (7, 8).

Therapeutic nuclear medicine makes use of radiopharmaceuticals carrying radionuclides that emit ionizing particles, such as β^- or α particles. In particular, targeted radionuclide therapy (TRT) is emerging as a promising anti-cancer modality for patient-tailored treatments, including radioimmunotherapy (RIT) that takes advantage from the on-going improvements in immunotherapies. Additionally, the same targeting biomolecule recognizing a particular molecular target can be labeled either with a diagnostic or with a therapeutic radionuclide, allowing the development of a patient-specific treatment. Thus, nuclear medicine modalities offer the unique advantage of easily switching from a diagnostic radionuclide to a therapeutic one, using the same or related chemical entities, giving rise to an increasing number of clinical applications with theranostic radiopharmaceuticals (9). Nuclear medicine modalities play an increasing role in the diagnosis and treatment of cancer in the clinical onset. In the particular case of GBM, several clinical trials were performed in the past decades with diagnostic and/or therapeutic radiopharmaceuticals, essentially based on radiolabeled peptides or antibodies (10-12). Moreover, PET and SPECT imaging can give an invaluable contribution for the "in vivo" characterization of the "molecular profile" of GBM, allowing the selection and follow-up of patients to be treated with experimental treatment options, namely molecular targeted drugs or immunotherapies.

Given the increasing relevance and potential of Nuclear Medicine in the detection and management of disease, this chapter provides a review of contribution of radiopharmaceuticals for the diagnostic and therapy in GBM, with particular focus on their design and preclinical evaluation in cellular and animal models. Furthermore, an up-to-date overview of the different types of radioactive agents is also provided.

27.2 NUCLEAR MEDICINE AND RADIOPHARMACEUTICALS

27.2.1 Nuclear Medicine Modalities

Radioactive decay is the process by which the nucleus of an unstable atom releases energy by spontaneous emission of ionizing radiation, such as photons (γ), alpha particles (α), beta particles (positrons (β^+) or electrons (β^-)). Such process may be internal, happening in the nucleus of the unstable atom or may occasionally involve an inner electron of the atom, through electron capture or internal capture. These unstable atoms are called radionuclides and can be naturally occurring or artificially produced (13). Nuclear medicine is the medical specialty that makes use of artificially produced radionuclides and their properties for diagnostic and/or therapeutic purposes.

Radionuclides used in cancer diagnostic and therapy are generally attached to a carrier molecule, forming a radiopharmaceutical compound, that is able to bind to specific targets expressed in the tumour cells. The pharmaceutical component of the radiopharmaceutical, which targets for instance a specific protein in cells, can be a peptide, a monoclonal antibody, or other types of supramolecular structures like nanoparticles (NPs).

The nuclear imaging modalities SPECT and PET have an important role in the diagnostic of GBM allowing for a non-invasive assessment of the tumour's aggressiveness, differentiation of treatment-induced necrosis from tumour recurrence, prognosis estimation and assessment of response to treatment (14).

SPECT is a tomographic scintigraphy where computer-generated 3 dimensional (3D) images of a radioisotope distribution are produced by detecting single photons from acquired multiple-planar images (15). Radionuclides that decay through the emission of photons are used for this imaging technique (Figure 1). SPECT data is collected based on the recording of the photons detected independently from each other. This detection relies on the use of physical collimation in order to obtain directional information for each incident photon. The detection of the photons is performed by a gamma camera with a single or multiple detector heads, composed of scintillation crystals capable of recording the photons that were not collimated (13, 16). After the administration of the radioactive compound, photons emitted will be detected using the appropriate equipment. These photons may or may not have a variety of interactions with the matter, such as photoelectric effect or Compton scattering, the predominant type of interaction (17). As a consequence, some of the emitted photons will reach the gamma-camera detectors either uninterrupted or deflected from their original direction. Photons that reach the detectors parallel to the collimator holes will reach the scintillation crystals and ionize them. De-excitation of the scintillation crystals will occur through light emission that is detected by photomultiplier tubes that will convert it to an electrical signal.

PET is also a non-invasive imaging modality that provides functional and biochemical information through the injection of a radioactive compound, detection of the radiation, and reconstruction of the biodistribution of the injected compound, being routinely used for the diagnostic, grading, and staging of tumours as well as for the assessment of the efficacy of the therapeutic course (18, 19).

The radioactive compound used for PET imaging must include a neutron-deficient radioisotope that emits a positron when decaying to a stable state. The emitted positron will travel a very short distance, of about 1 to 2 mm, and interacts with an electron, leading to an annihilation reaction and consequent production of two 511 keV photons that will travel in opposite directions along an approximately straight line (Figure 1). These photons are detected by the PET scanner detectors that use scintillator crystals coupled to photomultipliers. When the two photons are detected in a short-coincidence time window (usually 1 to 10 ns), a true-coincidence event occurs. The total number of true-coincidence events detected by the opposed detectors will be proportional to the total amount of the radionuclide present in the tube of response (19). Unlike in the case of SPECT, in PET is not necessary to have physical collimation, since there is an electronic collimation resultant of the detection of the annihilation events, leading to an increased sensitivity.

Combination of SPECT or PET imaging with an anatomical imaging technique, such as computed tomography (CT) or magnetic resonance imaging (MRI) provides a synergistic combination of the functional with the structural information (19). Even though SPECT is less expensive than PET and has a wider availability (14), PET has a better spatial resolution, allowing the detection of very small tumours, as well as a quantitative measurement of physiological parameters (14), being therefore the image modality of choice regarding GBM diagnostic (12).

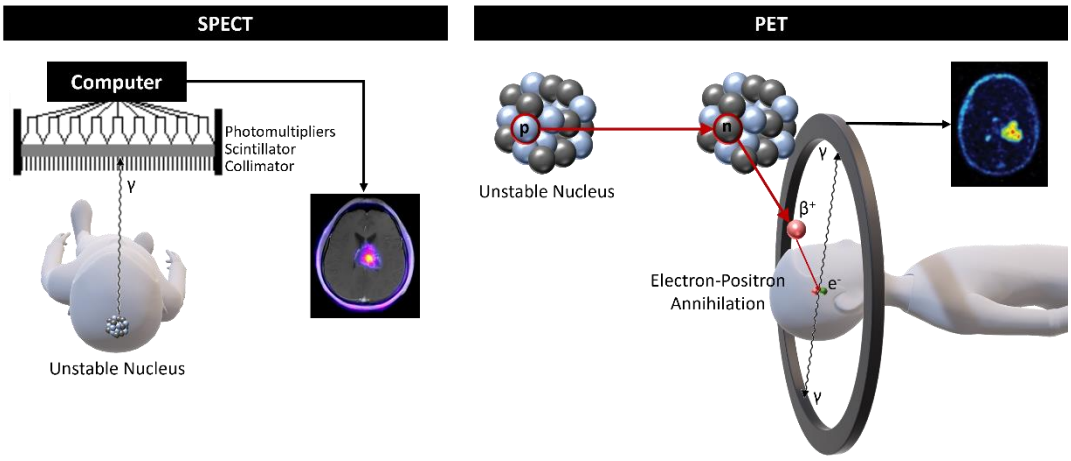

Figure 1. Single-Photon Emission Computed Tomography (SPECT) and Positron Emission Tomography (PET) imaging schemes. Glioblastoma scans with SPECT and PET were obtained from (20) and (21), respectively.

As mentioned above, nuclear medicine can also be applied for therapeutic purposes. Radionuclides suitable for GBM therapy include particle emitters that deliver ionizing radiation such as β^- or α particles or Auger electrons (AE) (22). GBM targeted radionuclide therapy can be achieved through the targeting of molecular biomarkers and pathways on a cellular level, by combining suitable targeting vectors with a therapeutic radionuclide (23). Furthermore, it is possible to pursue a theranostic approach in which there is a combination of both therapeutics and diagnostic, using a radionuclide with simultaneous emission of diagnostic radiation and therapeutic particles or using more than one radionuclide, allowing for image-guided therapy and also for defining the treatment outcome at an early stage (10). When a theranostic approach is being used for GBM, PET or SPECT imaging can confirm the presence of the specific molecular target before applying the targeted radionuclide therapy, or in image-guided therapy (12).

27.2.2 Medical Radionuclides

As mentioned previously, radionuclides are unstable atoms with excess energy that will release particles and/or ionizing radiation to become stable, in the so-called radioactive decay. The radioactive decay can be measured with a time period called half-life, corresponding to the time it takes for the radioactivity of the radioisotope to reduce to one half of the original level, being unique for each radionuclide. The half-life of the clinical isotopes is an important parameter when it comes to choosing the appropriate diagnostic and/or therapeutic radionuclide, since it should match the biological half-life of the respective radiopharmaceuticals to obtain an optimal effective half-life for its purpose.

In Table 1 are described certain physical characteristics (half-life, emission type, maximum energy of emission and soft tissue penetration) of some of the radionuclides used for GBM diagnostic, therapy or for both simultaneously, in the theranostic approach.

Among the radionuclides used for SPECT imaging, ^{201}TI was one of the most widely employed before ^{99m}Tc-labeled tracers emerged, such as [^{99m}Tc]methoxyisobutylisonitrile ([^{99m}Tc]MIBI), [^{99m}Tc]hexamethylpropyleneamine-oxime ([^{99m}Tc]HMPAO) or [^{99m}Tc]tetrofosmin ([^{99m}Tc]TF). ^{99m}Tc has a shorter half-life, allowing the administration of a higher dose, and an optimal emission energy that will undergo less attenuation and scatter (24).

One of the most common radionuclides used in PET imaging is ^{18}F. Several radiotracers are available either based on sugar or amino acid analogues, such as 2-[^{18}F]fluoro-2-deoxy-D-glucose ([^{18}F]FDG), [^{18}F]fluoroethyltyrosine ([^{18}F]FET) and [^{18}F]fluoro-dihydroxyphenylalanine ([^{18}F]FDOPA), nucleosides, as [^{18}F]fluorothymidine ([^{18}F]FLT), or based in choline, a precursor for the biosynthesis of phospholipids, such as [^{18}F]fluorocholine ([^{18}F]FCho) (25). Even though [^{18}F]FDG is the gold standard for PET imaging, the high physiological brain uptake of glucose and the non-specific uptake in cerebral inflammatory processes hinders its application for GBM correct delineation and diagnostic (12).

Table 1. Physical properties of medical radionuclides used in GBM diagnostic, therapy and theranostic.

Radionuclide	$T_{1/2}$ (h)	Emission type	E_{max} (keV)	Range in soft tissue (mm)	Ref.
Diagnostic					
Technetium-99m (^{99m}Tc)	6	γ	140.5	-	(24)
Thallium-201 (^{201}Tl)	73	γ	167	-	(26)
Carbon-11 (^{11}C)	0.34	β^+	968	-	(27)
Nitrogen-13 (^{13}N)	0.17	β^+	1190	-	(27)
Oxygen-15 (^{15}O)	0.03	β^+	1720	-	(27)
Fluorine-18 (^{18}F)	1.83	β^+	635	-	(28)
Scandium-44 (^{44}Sc)	3.94	β^+ /γ	1474 /1157	-	(10, 29)
Copper-62 (^{62}Cu)	0.16	β^+	2925	-	(30)
Gallium-68 (^{68}Ga)	1.14	β^+	1920	-	(10)
Yttrium-86 (^{86}Y)	14.7	β^+ /γ	3153 /1854	-	(10, 31)
Zirconium-89 (^{89}Zr)	78.4	β^+ /γ	897 /909	-	(10, 32)
Indium-124 (^{124}I)	100.3	β^+	2138	-	(10)
Therapy					
Strontium-89 (^{89}Sr)	1 217.7	β^-	1496	8	(33)
Yttrium-90 (^{90}Y)	64	β^-	2280.1	11.0	(34)
Astatine-211 (^{211}At)	7.2	α	7.45	0.080	(22)
Bismuth-213 (^{213}Bi)	0.76	α	8.4	0.1	(22)
Actinium-225 (^{225}Ac)	240	α	8.4	0.1	(22)
Theranostic					
Copper-64 (^{64}Cu)	12.7	γ β^+ β^- AE	1 675 657 579 6.84	- - 1.4 0.005	(35-38)
Copper-67 (^{67}Cu)	61.8	β^- /γ	562185	3 /-	(39, 40)
Indium-111 (^{111}In)	67.2	γ /AE	245 /0.325	- /0.01	(41, 42)
Iodine-125 (^{125}I)	1 426	γ /AE	35/3.19	-/*nm*	(22)
Iodine-131 (^{131}I)	193	β^- /γ	606.3 /364.5	2.2 /-	(33, 34)
Lutetium-177 (^{177}Lu)	162	β^- /γ	498.3 /208	2.0 /-	(22, 34)
Rhenium-186 (^{186}Re)	89.2	β^- /γ	1 069.5 /137	5.0 /-	(22, 33)
Rhenium-188 (^{188}Re)	16.9	β^- / γ	2 120.4 /155	10.8 /-	(22, 43)

Other PET radionuclides, such as ^{15}O, ^{13}N and ^{11}C, are also very useful given their easy incorporation into natural substances as water, carbohydrates, amino acids or lipids (27). Some of the radiotracers that incorporate these radionuclides and were already tested in GBM include, [^{15}O]-labeled water, [^{11}C]methionine ([^{11}C]MET) or [^{11}C]choline ([^{11}C]Cho) (25). Nevertheless, these isotopes have very short half-lives being their use restricted to centres with adjacent cyclotron units, making harder their application in clinical practice (25).

For PET diagnostic radiopharmaceuticals that have slow kinetics it is important to use positron emitters with longer half-lives, such as ^{89}Zr and ^{124}I. On the other hand, for radiopharmaceuticals with faster kinetics, intermediate or short half-life radionuclides are more suitable, such as ^{86}Y, ^{18}F, ^{44}Sc and ^{68}Ga (10). All these radionuclides are produced in cyclotrons, however ^{68}Ga and ^{44}Sc can be obtained using commercially available generators making them more widely accessible (41, 44).

The β^- emitters are among the radionuclides most commonly used and/or evaluated for GBM therapy (e.g., ^{177}Lu, ^{131}I, ^{186}Rh, ^{188}Rh and ^{90}Y). However, some alpha emitters are also under evaluation for GBM targeted therapies, namely ^{211}At, ^{225}Ac and ^{213}Bi (45). One of the factors that influences the choice of radionuclide is the tumour size, and while a large mass requires an emitter with a longer range, minimal residual disease benefits from an emitter with a shorter range (22), as highlighted in Figure 2.

The β^- emitters, like ^{131}I,^{177}Lu and ^{90}Y, are of interest mainly due to their cross-fire effect, allowing them to cross up to 300 cell diameters, in a range from 0.2 to 12 mm, which makes them efficient for the treatment of

bulky, heterogeneous primary and recurrent GBM tumours larger than 0.5 cm (46). These physical properties of the β^- emitters have clear advantages, as the large irradiation of the tumour margins and the "cross fire" effects can surpass the possible heterogeneous distributions of the radiopharmaceutical within the tumour mass and inside its cells and tumour environment. However, these effects of β^- particles also have intrinsic disadvantages such as augmented radiotoxicity in the surrounding healthy tissues due its irradiation (22). Moreover, it is important to consider that the lower linear energy transfer (LET) of these β^- emitting radionuclides, from 0.2 to 2 keV/µm, and their relative biological effectiveness (RBE), make them efficient only in particular cases of adequate tumour oxygenation and proliferation, excluding radioresistant and hypoxic types of GBM (12).

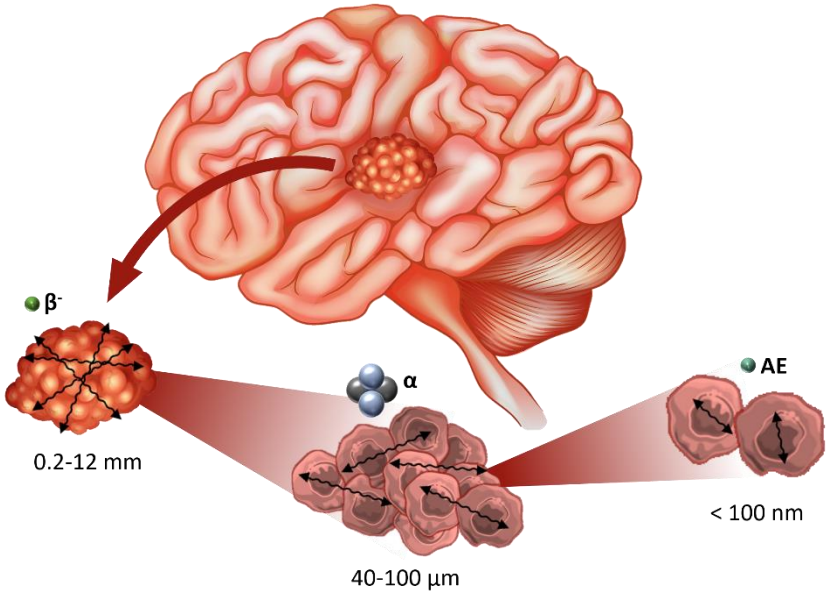

Figure 2. Representation of the different types of emission of therapeutic radionuclides and their tissue penetration.

The α emitters are characterized by a short tissue range, from only 40 to 100 µm (the average cell diameter range) and a high LET, leading to a highly efficient cell killing capacity and consequently a high RBE (47, 48). These characteristics make the α-particles emitters, as ^{211}At, ^{225}Ac and ^{213}Bi, useful to eradicate isolated tumour cells (22), cerebral micro-metastases, minimally recurrent GBM lesions or residual GBM tumours (45, 46, 49). Overall, α-particles emitters can provide a very local irradiation with very low toxicity (50). However, these α emitters have a limited availability, which hinders their widespread clinical use (51). Furthermore, due to their short range it is important to ensure that these radionuclides can infiltrate into the tumour microenvironment to exert a damaging effect.

Auger electrons emitters, such as ^{64}Cu, ^{123}I and ^{125}I, ^{111}In, or ^{161}Tb, emit low-energy electrons that have an even shorter range than α particles, smaller than 100 nm, but also have a high LET and RBE. Furthermore, these emitters are less dependent on the oxygenation state of the tumour microenvironment allowing them to overcome the negative effects of hypoxic and necrotic areas (22, 45, 52-55). Given their small range, the AE emitters radionuclides need an intracellular targeted delivery, close to the tumour cell nucleus or to another radiosensitive organelle (e.g., the mitochondria), where they can cause direct DNA double strand breaks. Therefore, internalization of these emitters into GBM tumour cells nuclei is extremely important (47). This goal can be achieved, for example, by targeting enzymes that are recruited to the cell nucleus, as the case of poly(ADP-ribose) Polymerase 1 (PARP1), as it was showed by (56), or by incorporating the radionuclide into the a nucleoside that is incorporated by all DNA-synthetizing tumour cells (57).

27.3 DESIGN AND PRECLINICAL EVALUATION OF RADIOPHARMACEUTICALS FOR GBM TARGETING

27.3.1 Design of the Radiopharmaceuticals

Generally, radiopharmaceuticals are constituted by three main components: a vector molecule, a radionuclide, and a linker between them. The choice of the radionuclide will determine the intent of the radiopharmaceutical developed, either for a diagnostic, therapeutic or theranostic purpose, while the vector molecule will be determinant for the biological targeting.

A large variety of radionuclides has been employed in the design of radiopharmaceuticals for imaging and theranostics of glioblastoma, as indicated in Table 1. From a chemical and radiochemical point of view, these radionuclides can be divided into two major classes: non-metallic radioisotopes (including radiohalogens) and radiometals. For non-metallic radioisotopes (e.g, ^{11}C, ^{18}F, ^{131}I), their incorporation in the desired molecules is performed through the formation of covalent bonds with aliphatic or aromatic groups of the molecule backbone. Some of these non-metallic radioisotopes correspond to so-called classical PET radionuclides (e.g. ^{11}C and ^{18}F) that, due to their short-life, ranging from a few minutes to a few hours, usually require fast and automated processes to produce the corresponding radiopharmaceuticals. These automated radiosynthesis start from adequate "cold" and radioactive precursors, developed within the so-called "PET chemistry" that emerged in the past few decades as a new branch of medicinal chemistry (58). In the case of radiometals (e.g. ^{99m}Tc, $^{67/68}Ga$, ^{89}Zr, ^{111}In, ^{177}Lu or ^{225}Ac), the synthesis of the radiopharmaceuticals normally involves the formation of a coordination complex, using adequate bifunctional chelators able to bind *d*- and *f*-transition metal ions. Some of the available chelators, like 1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetic acid (DOTA), allow the stable coordination of a large range of radiometals, namely trivalent ones like $^{67/68}Ga$, ^{111}In, or ^{177}Lu. However, there is no universal chelator fitting all radiometals and therefore tailor-made chelators need to be designed for some, as is the case of ^{89}Zr, copper radioisotopes and radioactinides (59, 60).

Several types of chemical forms have been evaluated for the design of radiopharmaceuticals for imaging and theranostics of GBM. As exemplified in Figure 3, the tested compounds span from simple inorganic salts like $^{64}CuCl_2$ to more elaborated molecular structures, such as radiolabeled glucose derivatives or amino acids, radiopeptides, radiolabeled antibodies and radiolabeled NPs (10-12, 22). Independently of its chemical form, the radiopharmaceutical must be stable in physiological conditions to ensure that the radionuclide will reach the GBM tissues, and to avoid its uncontrolled release in the body with reduction of the image´s quality and undue irradiation of non-targeted organs.

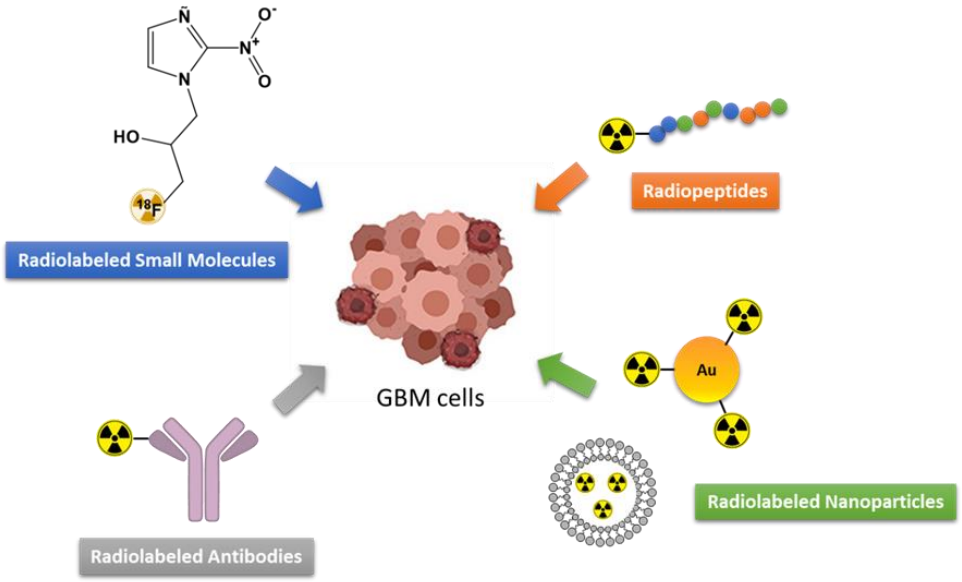

Figure 3. Schematic representation of different types of radiopharmaceuticals evaluated for GBM imaging and therapy.

For GBM therapeutic radiopharmaceuticals, several parameters need to be optimized to achieve a selective irradiation of tumour cells with minimization of the unwanted irradiation of non-target tissues. These are mostly dependent on the selection of the radionuclide (discussed in section 2.2), targeting vector, biodistribution profile of the radiopharmaceutical and administration route.

Concerning the selection of the targeting vector, radiolabeled peptides are a valuable strategy to target tumour receptors. Peptides can favour a more effective infiltration of the radiopharmaceutical into the extracellular space of a tumour cell mass and a faster blood clearance, with minimization of haematological and bone marrow damage, and overall appropriate target-to-non-target ratios (61). However, peptides tend to show a high renal uptake and retention with consequent increase of renal radiotoxicity, which is the major drawback of peptide receptor radionuclide therapy (PRRT) (62). Preclinical and clinical studies have shown that high-molecular-weight compounds like mAbs can also be effective GBM radiopharmaceuticals, namely by bypassing their intrinsic biodistribution limitations upon intratumoural or intracavitary administration (8, 11). Based on well-validated targets, RIT and PRRT of glioblastoma are progressing with encouraging results in the completed and on-going clinical trials (11, 12, 22).

Radionuclide therapy of glioblastoma with radiolabeled nanoparticles is also an emerging field. These nanocarriers can have a variety of core structures, such as organic polymers or metallic material and can also be radiolabeled with the different radionuclides described (51). Radioactive nanocarriers can passively accumulate in the tumour or can be decorated with a biologically active peptide or with a mAb for a specific targeting, in the same way of the molecular radiopharmaceuticals used in RIT and PRRT. In a passive approach, the radiolabeled nanoparticles exhibit the so-called enhanced permeability and retention (EPR) effect, in which large sized particles tend to accumulate more, and have a higher retention, in tumour tissues (63). The disadvantage of this type of approach is the dependency of the existence of a surgical access to reach the delivery point, and the possible leakage of the radiopharmaceutical to systemic circulation and diffusion to the extra-cellular matrix (22). With the active targeting approach, the leakage and diffusion risks are bypassed, existing a greater confinement of the radiopharmaceutical inside the tumour or the intra-resected tumour cavity (22).

Besides the nuclear properties, chemical nature of the radionuclide and selection of targeting vectors, the development of radiopharmaceuticals for diagnostic and therapy of glioblastoma needs to take into consideration the possible administration route. To define the administration route, the ability of a diagnostic or therapeutic radiopharmaceutical to cross the blood–brain barrier (BBB) is a crucial issue (64). For the classical intravenous injection route, the radiopharmaceutical must be ideally a small molecule that is freely diffusible or actively transported through the BBB. However, a radiolabeled molecule fulfilling this requisite will not necessarily perform as an adequate GBM imaging or therapeutic agent. For instance, 2-[^{18}F]fluoro-2-deoxy-D-glucose ([^{18}F]FDG), the most used PET tracer for cancer imaging, crosses the BBB by active transport mediated by the glucose transporter GLUT1 (65). However, the high physiological brain uptake of glucose and the non-specific uptake in cerebral inflammatory processes hampers the application of [^{18}F]FDG PET imaging for brain tumour delineation and diagnosis, as already mentioned above (10). GBM is locally a very invasive tumour that often compromises the BBB integrity and increases its permeability, facilitating the passage of drugs and of the radiopharmaceutical. It is important to have in mind that this increased BBB permeability is dynamic and heterogeneous, and possibly absent in the invasive edges of the GBM tumour (66, 67). Despite its aggressiveness, GBM only rarely undergoes a metastatic spread, in part due to the short survival experienced by the patients. Thus, the intratumoural or intra-resected tumoural cavity injection are options that can be used in GBM therapy with possible benefits for the patient. In particular, the convection enhanced delivery (CED) is an innovative technique, in which a therapeutic radiopharmaceutical is directly administrated into a brain tumour through a surgically implanted cannula, bypassing the BBB (see Figure 4) (68). CED administration of a radiopharmaceutical leads to an increased diffusion and more homogeneous distribution of the radioactive molecule in the GBM tissue, which can be a crucial factor to obtain efficient therapeutic effects, particularly for radionuclides emitting short range particles.

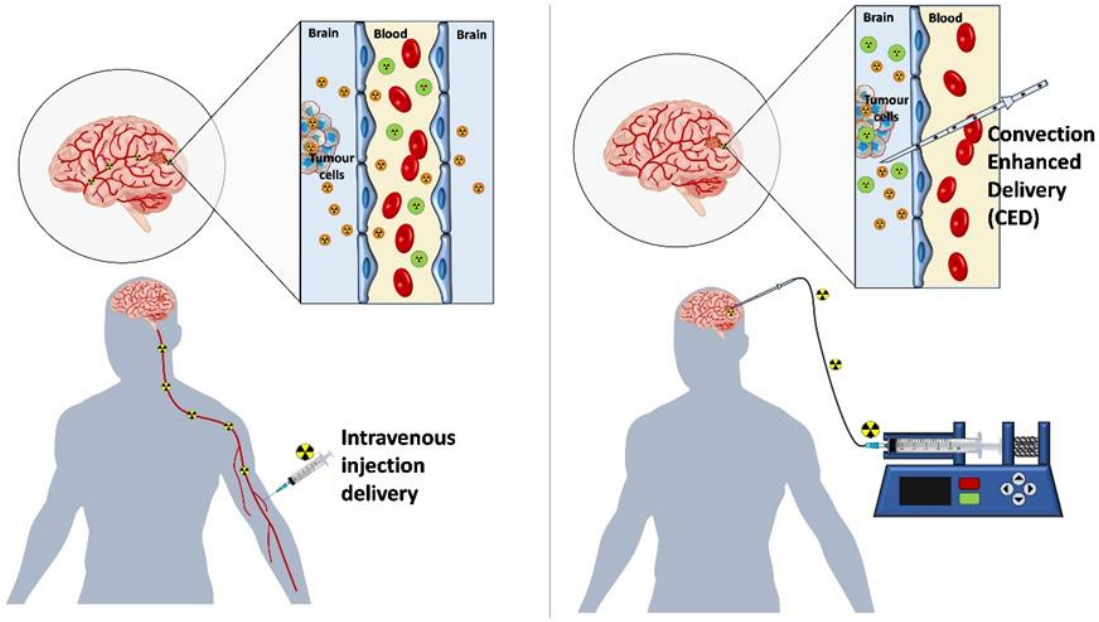

Figure 4. Schematic representation of the administration of a radiopharmaceutical via intravenous injection (left panel) or Convection Enhanced Delivery (CED) (right panel).

27.3.2 Targets for Imaging and Theranostics of GBM

As discussed in the previous sections, the two major determinants of success of a radiopharmaceutical for imaging and targeted therapy are the choice of the radionuclide and the selection of the biological target. In general terms, a target for cancer imaging or therapy should be highly abundant in the tumour, but present negligible or no expression in normal cells. The most explored targets are cell surface antigens, which can be more easily accessible to radiopharmaceuticals present in the bloodstream or extracellular environment. Furthermore, the target density should be uniform in all tumour cells, and it is estimated that for antibody-targeting the antigen expression should be > 100k sites per cell (69). When considering therapeutic and theranostics agents another relevant characteristic is the internalization of the receptor, or cell surface target, upon binding to the radiopharmaceutical, which contributes to retention and accumulation of the radionuclide intracellularly. This specific and efficient targeting of tumoural cells maximizes the dose of therapeutic radiation to the tumoural cells and tissue, while reducing, or ideally sparing, the unwanted effects in normal organs, either the ones surrounding the diseased region or the ones involved in the excretion pathways of the radiopharmaceutical.

Glioblastoma, as one of the most recurrent types of brain tumours presenting a limited range of therapeutic options, has been a priority in the search for anticancer therapeutic strategies. In the effort of understanding the pathophysiology underlying the development of cancer, the United States government-funded initiative, The Cancer Genome Atlas, used GBM as its first studied cancer (70). Since then, an update on the landscape of somatic genomic alterations has been reported, based on the multidimensional and comprehensive characterization of more than 500 GBM tumours (4). More recently, the Ivy Glioblastoma Atlas Project has explored the anatomic and genetic basis of glioblastoma at the cellular and molecular levels (71). The data generated by these large-scale initiatives facilitates the identification of novel biomarkers that can be explored as potential targets for imaging and therapy of GBM (72).

The malignant phenotype of GBM is driven by several mutations in key molecular pathways, such as signal transduction, as well as by alterations in biological contexts such as tumour microenvironment, hypoxia and stem cells. As such, the imaging or theranostic targets explored might not be limited to cancer cells, but can also encompass other tumour components, including vasculature, stromal cells, extracellular matrix (ECM), and infiltrating immune cells. In Table 2 are summarized the most relevant molecular targets for GBM imaging or theranostics, evaluated either clinically or pre-clinically. For the most explored, a brief overview of their biological relevance in the pathophysiology of GBM is given. In section 27.4, the radiopharmaceuticals targeting these proteins will be discussed in further detail.

Table 2- Biological targets explored for imaging and theranostics in Glioblastoma.

Target	Biological Process	Expression in GBM tumours	Selected References
Cadherin 5	Cell adhesion /ECM	Endothelial cells	(73)
Carbonic anhydrase XII (CAXII)	Response to hypoxia	Tumour cells	(74, 75)
Chemokine Receptor-4 (CXCR4)	Cell migration and proliferation	Tumour cells, infiltrating immune cells	(76, 77)
Deoxycytidine kinase (dCK)	Cell metabolism/resistance to therapy	Tumour cells, infiltrating immune cells	(78)
DNA Histone H1 complex	Chromatin structure/ hypoxia	Tumour cells	(79)
Epidermal growth factor receptor (EGFR)	Cell growth/survival	Tumour cells	(4)
Extra-Domain B of Fibronectin (EDB-FN)	Cell adhesion/ECM	Tumour cells, endothelial cells	(80, 81)
Fibroblast activation protein (FAP)	ECM/inflammation	Tumour cells, stromal cells	(82, 83)
Gastrin-releasing peptide receptor (GRPR)	Cell signalling	Tumour cells	(84)
GD2 Disialoganglioside	Cell adhesion	Tumour cells	(85, 86)
Hyaluronan (HA)	Cell adhesion /ECM	Tumour and stromal cells	(87)
Interleukin-13 receptor subunit α-2 (IL13RA2)	Cell signalling	Tumour cells, infiltrating immune cells	(88)
Integrin alpha-V beta-3 ($\alpha_v\beta_3$)	Cell adhesion/angiogenesis	Tumour cells, endothelial cells	(89)
Matrix Metalloproteinases 2 and 9 (MMP2/MMP9)	Cell adhesion/ECM	Tumour cells, infiltrating immune cells	(90, 91)
Mitochondrial translocator protein (TSPO)	Cell growth/Apoptosis	Tumour cells, infiltrating immune cells	(92)
Neural cell adhesion molecule (NCAM)	Cell adhesion	Tumour cells	(93)
Neurokinin-1 receptor (NK1R)	Cell signalling/cell survival	Tumour cells	(94)
Poly (ADP-ribose) polymerase 1 (PARP1)	DNA repair	Tumour cells	(95)
Somatostatin Receptor 2 (SSTR2)	Cell signalling/cell survival	Tumour cells, infiltrating immune cells (?)	(96, 97)
Tenascin-C	Cell adhesion/ECM	Tumour cells/endothelial cells	(98)

The most investigated targets for molecular imaging with radioactive probes or targeted radiotherapy have been the ones related with ECM and cell migration and invasion, such as tenascin-C and fibronectin. The ECM proteins, their receptors, and other molecules such as glycans, contribute to normal cell behaviour but also to brain tumour proliferation, progression and migration with tumour cells presenting an abnormal overexpression of several components. This altered tumour microenvironment is highly relevant in the development of the invasive phenotype of GBM, but also for impaired diffusion of therapeutic drugs, being one of the major issues in GBM resistance to treatment (99).

The second class of relevant targets is composed of cell surface receptors involved in cell signaling pathways, mostly G-protein coupled receptors (GPCR), such as NK1R and GRPR, or receptor tyrosine Kinases, as EGFR. These targets are, in general, also overexpressed in other types of cancer, which can facilitate the development of improved agents for GBM targeting. Interestingly, the Cancer Genome Atlas consortium undertook a comprehensive and integrated molecular analysis of over 11K tumours from more than 30 of the most prevalent forms of cancer and was able to identify molecular relationships across a large diverse set of human cancers (100).

Despite the promising results obtained, several factors might contribute to the complexity of achieving effective imaging and/or therapeutic targeting of GBM. Phenotypic instability is a reason for concern as complex epigenetic factors can upregulate or downregulate target activation. It has been shown in clinical trials that there is a need for a continuous validation of target expression in GBM therapy, as reported for anti-EGFR therapy clinical trial where loss of expression was detected in the recurrence stage in more than 60% of the patients (101). Additionally, the relationship between receptor expression and radiopharmaceutical binding and uptake pathways may cause receptor saturation upon injection of therapeutic doses, as observed for instance for the SSTRs (102).

Moreover, the pathophysiology of most GBM is not based on the mutation or dysregulation of a single pathway and, therefore, a strategy with a multi-targeted therapeutic design might also be of interest, such as combining selected membrane signaling proteins with ECM targets (103).

Lastly, one cannot disregard other potential targets or pathways identified by genetic and phenotypic large scale studies mentioned before, which remain largely unexplored for molecular imaging and theranostics, such as the Ras/phosphoinositide 3-kinase pathway, cell cycle pathways, and immune checkpoints (104). Although targeting of some of these commonly observed alterations has been investigated as potential therapeutic strategies for GBM, several issues related with the design and production of radiopharmaceuticals, namely target specificity and selectivity, as well as radiochemistry difficulties, have hindered their application in this field (12).

27.3.3 Cellular and Animal Models

The development of radiopharmaceuticals has relied on the use of preclinical *in vitro* and *in vivo* models, which have proven to be important platforms for the prediction of the efficacy of novel compounds in the clinical setting. The choice of the appropriate model, however, is not without its challenges. An ideal model should mimic the original histological and molecular features of human GBMs, as well as their heterogenous nature, while also providing a flexible, reproducible and scalable system that can be recurrently used without loss of those desirable characteristics (105, 106). Nevertheless, currently, there is not available a single GBM model that is completely representative of a patient's tumour and its associated microenvironment, which explains why most of the therapeutic strategies that enter human clinical trials fail to be approved (106). Thus, novel therapeutic alternatives should be carefully evaluated using the preclinical model (or combination of models) offering the highest predictive value, in order to maximize the cost-effectiveness of the preclinical research and maximize the chances of succeeding in clinical trials.

Historically, the large majority of the therapeutic options evaluated in GBM have been assessed making use of traditional 2-dimensional (2D) cellular cultures of human GBM-derived cell lines. Those have provided significant contributions to understand the overall biology and specific molecular mechanisms operating in GBM cells (106, 107). Moreover, the development of recent techniques that allow the simultaneous, large-scale analysis of vast panels of cancer cell lines has rekindled the interest in this type of model and on its potential clinical utility in translational research (108). Several GBM cell-lines are commercially available, being easy to handle in a laboratory setting due to their high growth rates, which allows access to a large number of cancer cells in a reproducible way, and to their high engraftment abilities, required for the generation of tumours in appropriate animal models (106, 107). Among the GBM cell lines used for research purposes, U251 and U87 are the two that are most commonly studied. Both were derived from human GBMs, cultured *in vitro*, and xenografted into immunodeficient mice in order to generate rodent models of this disease that are widely used nowadays (106, 107). At least to some extent, the genetic abnormalities of the original GBM tumours were maintained in these cell lines, making them extremely useful in the study of key carcinogenesis-related cell signaling pathways (106, 107). However, both of these cell lines, originally isolated in the 1960s (109, 110), have shown to be lacking the heterogeneity observed in patients' tumours, while their continuous passaging has been proved to lead to the occurrence of genetic mutations and genetic drift, making them less representative of their tumour of origin (111-113).

Due to the limitations inherent to the use of monolayer cultures, there has been an increased awareness in the scientific community of the need to transition from 2D to 3D culture models that better recapitulate the complexity and heterogeneity of GBMs, in order to obtain results with improved clinical translatability (114). Namely, 3D models are expected to allow the recreation of crucial tumoural environmental cues and phenotypes, normally missing in 2D culture, such as: the existence of interactions between tumour cells and the extracellular matrix or cellular elements of the microenvironment (immune cells, endothelial cells, and fibroblasts); the establishment of oxygen, nutrients, and metabolites gradients; and the preservation of the tumour's heterogeneity, for instance, through the maintenance of a significant glioma stem-like cells (GSCs) population, which have been shown to contribute to drug resistance and disease recurrence (114).

The use of 3D models of GBM is currently still expanding, but the first step into their development was the growth of patient-derived neurospheres, through the cultivation of neural stem cells in serum-free medium, which were demonstrated to better recapitulate the genetic and phenotype background of tumours (115). In addition, intracranial implantation of neurosphere-derived GSCs in immunodeficient mouse models allows the formation of a highly invasive and heterogenous tumour, a feature not observed in 2D-derived mouse models of the disease (116, 117). The major disadvantage of the use of the neurospheres model is their lack of representativity in terms of the original tumour's complex structural architecture, which can be overcome through the use of more advanced, 3D organoid cultures (118). This *ex vivo* culture system has been emerging as a much more representative model to study the biology of the tumours, since it allows for the cancer cells to associate in an architecture that retains many of the histological features, intra- and inter- cellular heterogeneity, and genetic profile of the primary tumour from which they were derived (118). To avoid the limitations linked to their propagation in culture, cultivation of organoids derived from patients is performed in the brain of immunodeficient rodents (originating the so-called patient-derived orthotopic xenografts, PDOXs). However, current efforts are being employed on the development of PDOXs propagated in immunocompetent humanized mice, which should be particularly important in the study of immunotherapeutic compounds (118).

Largely due to their closer proximity to human biology, *in vivo* animal models have frequently been explored in preclinical studies. In particular, three types of mouse models have been used for GBM: xenografts, genetically engineered, and syngeneic mouse models (106, 119, 120). Xenograft mouse models are among the ones most commonly used. They result from the transplantation of human GBM cells, through subcutaneous or orthotopic engraftment, into an immunocompromised mouse host (121). The xenografts used can originate from GBM cells, grown in 2D or 3D culture, or be patient-derived xenografts. The later, despite being more costly and less available overall, are clearly preferable as they result from the implantation of patients' tissues

without any transitional cell culture step, being far more representative of the characteristics of the primary tumours (106, 120). Orthotopic engraftment, particularly of material originating directly from the patients, originating PDOXs, are also much more desirable as they preserve the brain microenvironment and blood brain barrier permeability, as well as the tumour's infiltrative behavior (106, 120).

The development of genetically engineered mouse models was based on the induction of modifications in key genes or cellular signaling pathways found to be altered in human tumours, allowing to accurately recapitulate many of the histopathological and biological features of human GBMs in an immune-competent environment (122). As such, it has proven to be a relevant system to study tumour initiation and evolution and the effectiveness of novel therapies, as well as the molecular mechanisms underlying those phenomena (106, 119, 120).

In turn, syngeneic models rely on the implementation of cancer cells into immunocompetent mice and are thus particularly useful in the study of compounds with immunotherapeutic potential, since they allow to assess the interaction and impact of the immune system on the developing tumour (106, 119, 120, 123). Despite their utility, each model has its own disadvantages. Moreover, in addition to their inherent practical limitations, such as the difficulty of following the tumour's growth in the brain, the use of animal models also raises important ethical questions that should be factored in when choosing the appropriate preclinical model to use (114, 119).

27.4 RADIOACTIVE AGENTS FOR IMAGING AND THERANOSTICS OF GBM

27.4.1 Radiolabeled Small Molecules

In general, small-molecule based compounds have the potential to display more attractive pharmacokinetic properties than macromolecular pharmaceuticals, namely allowing for faster targeting and clearance from the body (9, 124). In particular for GBM, small molecules are expected to have a higher ability to cross BBB, which is considered one of the most significant hurdles to be overcome for an efficient management of the disease and guarantee therapeutic effectiveness (64). As such, different small molecule radiopharmaceuticals have been developed, exploiting a variety of relevant molecular targets and biologically relevant processes in GBM.

Among those, detection of hypoxia using PET tracers is of high clinical relevance, since the hypoxic status of a tumour is known to be important for the development of GBM, also being involved in disease aggressiveness, radioresistance and recurrence (53). One large group of compounds available for hypoxia imaging is based on the existence of a nitroimidazole moiety that will be trapped in hypoxic cells, acting as a sensor of oxygen levels. Among those compounds is the first hypoxia tracer ever developed, ^{18}F-labeled fluoromisonidazole ([^{18}F]-FMISO), which has been recognized as one of the most clinically relevant PET tracers for hypoxia in many cancers, including glioma (125, 126). Other related compounds, possessing similar chemical structures that differ mainly on the linker groups used and in the position of the labelled fluorine, include fluoro-azomycin arabinoside ([^{18}F]-FAZA), fluoro-erythro-nitroimidazole ([^{18}F]-FET-NIM), and flortanidazole ([^{18}F]-FHX4) (126). The major advantage of these compounds over [^{18}F]-FMISO is their higher hydrophilicity, which can promote better clearance from non-target tissues (126). The same holds true for a second-generation compound, [^{18}F]-FDiFA, which exhibits rapid clearance from the body, while allowing to achieve PET images of comparable quality to the ones obtained using [^{18}F]FMISO (127). One other hypoxia-sensitive PET tracer that has been explored for GBM imaging is the metal complex [^{62}Cu(II)]- or [^{64}Cu(II]]-diacetyl-bis(N4-methylthiosemicarbazone) ([^{62}Cu]- or [^{64}Cu][CuATSM], which has the advantage of exhibiting a faster clearance kinetics than [^{18}F]-FMISO, allowing to obtain results in a shorter time window after injection (18). In fact, several studies have supported that [^{62}Cu]- or [^{64}Cu][CuATSM] can be used to image brain cancer, while addressing the hypoxic status of tumours in a non-invasive way (128-130) (Figure

5). In addition, it has been reported at the preclinical (131) and patients (35) level that a simpler chemical form of copper, [^{64}Cu]-copper dichloride ([^{64}Cu]$CuCl_2$), with some favourable and unique biological properties, allows the imaging of brain tumours with the potential to simultaneously detect the hypoxic status of the cells (132).

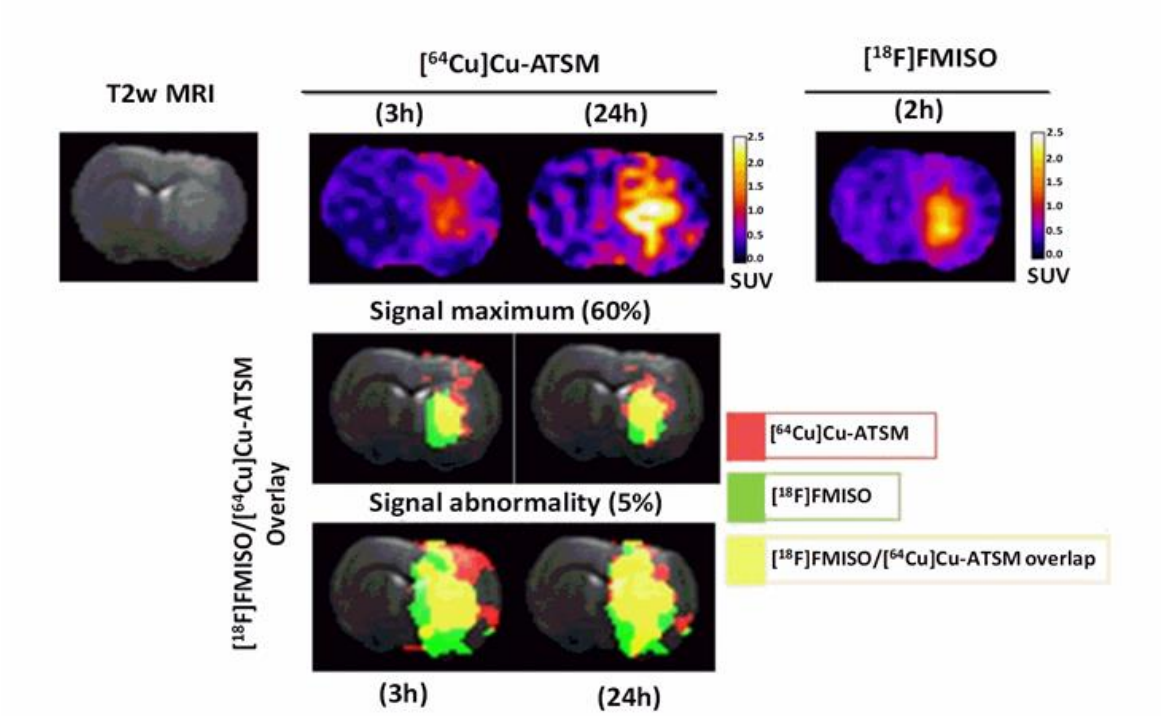

Figure 5. Hypoxia µPET study in a C6 rat GBM model, highlighting the overlay (in yellow) in signal between the [^{18}F]-FMISO (green) and [^{64}Cu][CuATSM] (red) signals. The image was obtained from (132).

Another biological target that has also been exploited for the development of small radioactive molecules is the enzyme poly (ADP-ribose) polymerase 1 (PARP1), involved in cellular repair of DNA (133). This enzyme has been shown to be overexpressed in GBM, exhibiting relatively low expression in non-tumoural brain tissue (134). PARP inhibitors were the first agents targeting the DNA damage response to be approved for cancer therapy, making them interesting candidates to be radiolabeled in order to develop novel radiopharmaceuticals for cancer imaging or therapy (133). In this context, the first inhibitor modified to be used as a PET imaging agent was olaparib radiolabeled with ^{18}F ([^{18}F]-olaparib) (135), which was also later on radiolabeled with ^{123}I ([^{123}I]-olaparib) and preclinically evaluated as a SPECT imaging agent (136), achieving mild success. A second olaparib-based derivative, PARPi, was developed and evaluated preclinically as a bimodal imaging tracer, combining optical and PET imaging in the form of the fluorescently labelled [^{18}F]-PARPi-FL (137), and as a targeted radionuclide therapeutic agent through its radiolabeling with ^{131}I ([^{131}I]-PARPi) (138). The later has exhibited promising results, having led to increased survival of treated animals, although it needs to be further assessed regarding its clinical relevance (138). However, it is the compound FluorThanatrace ([^{18}F]-TT), derived from the inhibitor rucaparib, that has recently attracted the most attention for imaging using PET/CT, due to the encouraging results obtained in several preclinical studies, and also based on the existence of an ongoing clinical trial (NCT04221061) in GBM patients (18, 134, 139).

FAP is a protease abundantly expressed in stroma cells, found on the microenvironment of human GBM, which has a very low expression in healthy tissues (82). Imaging of GBM by targeting FAP has thus been attempted based on the development of radiolabeled FAP inhibitors (FAPIs). Among those, relative success has been achieved through the use of a ^{68}Ga-labeled FAPI, which in glioma patients has shown potential to discriminate between low-grade and high-grade gliomas (140), as well as to aid in the assessment of target volume delineation in combination with MRI (141). In an attempt to expand the range of PET tracers available, a preclinical study has also recently reported the synthesis and evaluation of a ^{18}F-labeled glycosylated FAPI (142).

Stratification of brain tumours, to predict which patients will be responsive to a certain targeted therapy, is an important aspect for GBM management. For that purpose, another biological target that has been explored is the protein TSPO that is highly expressed in cancer cells, being considered a hallmark of GBM (92). In the clinical setting, PET imaging using radiolabeled 1-(2-chlorophenyl)-N-methyl-N-(1-methylpropyl)-3-isoquinoline carboxamide ([^{11}C]-(R)PK11195) allowed to discriminate between high-grade and low-grade glioma and healthy tissues (143), while the development of a novel ^{18}F-radiolabeled TSPO ligand ([^{18}F]-GE-180) exhibited a promisingly high tumour-to-background contrast for GBM imaging (144).

Abnormal cellular proliferation is another cancer hallmark that can be targeted by small radioactive molecules. One of those molecules is [^{18}F]-clofarabine, an analogue of neutral amino acids, which explores the natural role of the kinase dCK, which in cells is involved in phosphorylation of deoxycytidine, a step required for DNA synthesis to occur. [^{18}F]-clofarabine is able to cross the BBB and to outline regions of immune activity in the brain, being very interesting for the localization and quantification of the response of GBM patients to immunotherapeutic regimens (78).

27.4.2 Radiopeptides

As described in previous sections, the development of radiolabeled peptide agonists/antagonists for a specific biological target in tumour sites has led to the emergence of PRRT. In regards to GBM, various biological targets suitable for these approaches have been explored and different radiopeptides evaluated both pre-clinically and in human trials (145, 146).

Somatostatin receptors (SSTR), of which five types of them are known (SSTR1-SSTR5), are some of the most explored in radiopharmaceutical development for GBM treatment, particularly SSTR2, which is overexpressed in neuroendocrine tumours (147, 148). Among the peptides developed for SSTR targeting, octreotide derivatives have seen the most success. Initial clinical trials performed for [^{90}Y]Y-DOTA-lanreotide in 2002, in 154 patients, showed 41% with stable tumour disease and 14% with regressive tumour disease, after treatment with cumulative doses up to 232 mCi (149). With the relative success of [^{90}Y]Y-DOTA-lanreotide, other octreotide derivatives have emerged, including [^{90}Y]Y-DOTATOC (DOTA-D-Phe^{1}-Tyr^{3}-octreotide) and [^{177}Lu]Lu-DOTATATE (DOTA-Tyr^{3}-octreotate), which to date are some of the most used radiopeptides in clinical application for various cancer treatments (150, 151). Additionally, the use of positron or gamma-emitting radionuclides (^{68}Ga, ^{111}In) in place of ^{90}Y or ^{177}Lu provides imaging data through PET or SPECT, which allows the prediction of the biological distribution and therapeutic performance of these radiopharmaceuticals (152, 153). Clinical trials in three patients with recurrent high-grade glioma treated with [^{90}Y]Y-DOTATOC showed complete remission in one of the patients, and another with partial remission (154). There are, however, some limitations to the wide application of these radiolabeled octreotide derivatives, due to their high kidney uptake, which can lead to nephrotoxicity, although this can be attenuated through infusion of amino acids to reduce the renal excretion of the radiopharmaceuticals (155-157).

Neurokinin type 1 (NK1) receptors are another widely explored target for PRRT in GBM treatment. NK1R is mostly distributed in the peripheral and central nervous systems, and is known to be overexpressed in primary

malignant gliomas like GBM (158, 159). The main physiological ligand for NK1R is substance P (SP), a peptide which belongs to the family of tachykinin neurotransmitters (160). Its biological half-life can be of several hours within the tumour site; it can be however rapidly degraded in the blood stream due to serum peptidases, which beckons for SP radiopharmaceuticals to be intratumourally administered (159, 161). A pilot study involving 20 patients with 4 of them bearing GBMs was performed using [^{90}Y]Y-DOTAGA-Substance P, and alternatively with the ^{177}Lu and ^{213}Bi-labeled counterparts to reduce the "cross-fire effect" in critically located tumours. The results showed no toxicity associated with the radiopharmaceutical administration, with high intratumoural dose localization and a median survival of 11 months in patients after initiation of treatment (159). ^{90}Y-DOTAGA-Substance P also demonstrated positive results in neoadjuvant radionuclide therapy in patients with GBM. In a phase I study where 17 patients received intratumoural administration of ^{90}Y-DOTAGA-Substance P, 13 of the patients achieved tumour resection in a range of 96%, with no evidence of acute toxicity or side effects (162).

Clinical trials were also performed using [^{213}Bi]Bi-DOTAGA-Substance P, for the treatment of critically located gliomas (Figure 6). The study demonstrated the potential of ^{213}Bi-DOTAGA-Substance P to be used as an effective radiopharmaceutical for these critically located gliomas, allowing surgical removal of the tumour upon treatment, and improving prognosis (161). Following on these promising results, another clinical trial with 9 patients with recurring GBM injected with [^{213}Bi]Bi-DOTAGA-Substance P, showed that the treatment was well tolerated and the median overall survival time from the first diagnosis was 52.3 months (163). Although these ^{213}Bi-labeled Substance P derivatives showed promising results, the short-half-life of ^{213}Bi (45.6 min) presents serious challenges in radiopharmaceutical preparation and distribution. In order to tackle this issue, studies have explored alternative radionuclides, namely the α emitters ^{225}Ac or ^{211}At, with half-lives of 9.9 d and 7.21 h, respectively, which are under ongoing clinical trials (164, 165).

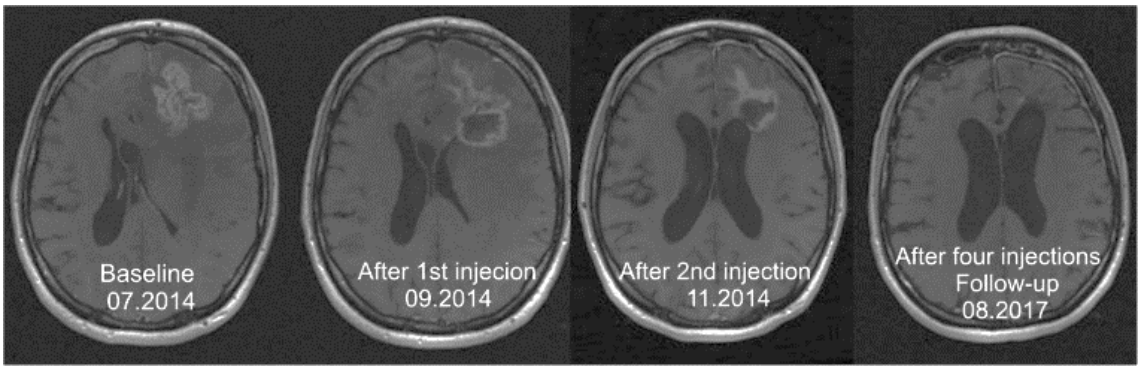

Figure 6. MRI evaluation of a 32-year-old woman suffering from a secondary GBM, following treatment consisting of surgery, radio- and chemotherapy with TMZ and four cycles of [^{213}Bi]Bi-DOTA-SP. Image adapted from reference (163).

Chemokines are molecules responsible for the regulation of the chemotaxis of leukocytes in tissues, and are also involved in promoting mitosis and modulation of apoptosis, survival and angiogenesis, which are related to tumour growth (166, 167). Among the family of chemokines, CXCR4 has been verified to be up-regulated in gliomas, particularly high-grade ones (168). The radiopeptide [^{68}Ga]Pentixafor, based on the cyclic pentapeptide cyclo(D-Tyr^{1}-[NMe]-D-Orn^{2}-Arg^{3}-2-Nal^{4}-Gly^{5}), was evaluated as a PET agent for CXCR4 targeting, demonstrating a high affinity towards the receptor (169). A pilot study performed in GBM patients injected with [^{68}Ga]Pentixafor showed a high retention in the majority of GBM lesions, which proved the

effectiveness of this radiopeptide for non-invasive *in vivo* CXCR4 quantification and tumour diagnosis (170, 171). The relative success of [^{68}Ga]Pentixafor prompted researchers to explore its therapeutic counterpart, [^{177}Lu]Pentixather, which to date is still in preclinical evaluation. However, preliminary studies show interesting results of [^{177}Lu]Pentixather for GBM treatment, with suitable clearance kinetics and tumour retention, leading to a high uptake of therapeutic doses in tumour sites (172).

MMPs are another interesting biological target for GBM treatment. These proteins are present in elevated levels in GBM cell lines and tissues when compared with healthy brain samples (173). In recent years, scorpion toxins have been studied as potential candidates for anticancer drugs (174). Among these, chlorotoxin (CTX), has emerged as one of the most promising (175). CTX is a 36-amino acid peptide, which can bind to GBM cells, particularly to MMP-2 located in the extracellular membrane, and with no cross reactivity to normal brain cells. In a clinical trial, 17 GBM patients received a single dose of ^{131}I-TM-601 (radiopharmaceutical based on a synthetic CTX), upon cytoreductive craniotomy surgery. The administration was tolerated and ^{131}I-TM-601 was found to bound to the tumour periphery with long-term retention, and reduced accumulation in other tissues (176). After 180 days, four of the patients had stable disease and one had partial response, while after 30 months two of these patients showed no evidence of disease.

Other biological targets for radiopeptides that have received attention more recently are the receptors EGFR, integrin $\alpha_v\beta_3$ and the GRPR. EGFR is overexpressed in a variety of tumours, including GBM (177). A recent study used EGFR targeting peptides, EEEEYFELV and DEDEYFELV, labeled with ^{131}I, in order to evaluate the radiochemical and biological properties of these radiopeptides towards EGFr in rat GBM cells (C6). Results showed that the radiolabeled compounds presented a significant binding and internalization capability *in vitro*, as well as a considerable brain uptake in mice, particularly for [^{131}I]I-EEEEYFELV (178). The same research group also explored the efficiency and biological properties of two integrin $\alpha_v\beta_3$ targeting peptides, GRGDYV and GRGDHV, towards GBM cells. Much like EGFR, integrin $\alpha_v\beta_3$ molecules are also overexpressed in a variety of cancer cells, including GBM (179). The integrin $\alpha_v\beta_3$ targeting peptides GRGDYV and GRGDHV were radiolabeled with ^{131}I and ^{99m}Tc, respectively. Studies showed a high binding and internalization capability for both compounds in C6 rat GBM cells, and significant brain uptake in mice (180).

GRPR is a well-known receptor involved in cell signaling cascades, and its presence has been verified in various glioma cell lines (181, 182). Natural bombesin (BBN) is an amphibian homolog for GRP, consisting of 14 amino acids with high affinity towards GRPR. Its affinity for the receptor, however, is mainly attributed to the last 8 amino acids in its sequence, Gln-Trp-Ala-Val-Gly-His-Leu-Met-NH_2 (BBN(7-14) (183). In a clinical study, 4 healthy volunteers and 11 glioma patients (2 with GBM), were injected with the PET imaging neuropeptide ^{68}Ga-NOTA-Aca-BBN(7-14). The radiopharmaceutical was well tolerated in all healthy volunteers, with no adverse side-effects, and showed an overall high signal retention in the glioma patients (184).

27.4.3 Radiolabeled antibodies

Among the various biological targets explored for RIT, the extracellular matrix protein tenascin-C is one of the most studied. Tenascin-C induces pro-angiogenic secretome in GBM cells and is overexpressed in over 90% of GBM cases (185, 186). Clinical studies using an anti-tenascin monoclonal antibody (mAb), BC-2 labeled with ^{131}I, were performed in ten patients, with recurring GBM after surgery. No significant systemic and local toxicity was observed, and 24 h post-injection, 4.9%/g of dose was accumulated in the tumour. Upon multiple injections, 4 of the patients had stable disease, while two of them showed partial disease and one complete remission (187). Following up on the relative success of BC-2, another anti-tenascin mAb (BC-4) also radiolabeled with ^{131}I, was studied for the treatment of 30 patients with GBM. Upon treatment, 7 patients had stable disease, 4 of them had partial remission and 4 complete remission (188). A few years later, the same

research group performed a major phase I and II clinical trial, using ^{131}I-labeled BC-2 and BC-4, to treat 111 patients (91 with GBM). Results showed that in the group of 74 phase II GBM patients, 10 had stable disease, 9 partial remission, 23 no evidence of disease and one complete remission (189).

^{90}Y has also been used to label BC-4 as an alternative to ^{131}I. In a study involving 73 GBM patients, 35 of them were submitted to a combined treatment of RIT with ^{90}Y-labeled BC-4 and the chemotherapeutic agent TMZ, and the other 38 were only submitted to RIT. The results confirmed the higher effectiveness of the combined therapeutic treatment, with a median overall survival of 25 months, as opposed to 17.5 months for the patients that received only RIT (190).

81C6 is another anti-tenascin mAb that has seen relative success for GBM treatment. In a phase II clinical trial, ^{131}I-labeled 81C6 was injected into 33 patients with previously untreated gliomas (27 of them being GBM), follow by conventional external-beam radiotherapy and alkylator-based chemotherapy. The treatment led to a median survival of 79.4 weeks for the patients with GBM (191). In later years, novel clinical trials with ^{131}I-86C6 were performed in order to further evaluate the efficacy and related toxicity of this radiopharmaceutical in GBM patients. The overall treatments increased the median survival to 20.6 months for newly diagnosed tumours, and 14.5 months for recurrent disease (192, 193). However, in some cases, acute hematologic toxicity and acute reversible neurotoxicity were reported in patients. In an interesting study, 18 patients were treated with the α-emitting ^{211}At-labeled 81C6, and the median survival time was 54 weeks upon treatment. Additionally, no dose-limiting toxicity was observed; however, 6 of the patients still developed reversible neurotoxicity (194).

EGFR has also been explored as a biological target for RIT of GBM. The ^{125}I-labeled anti-EGFR-425 was one of the first mAbs to be studied in clinical trials. A phase II trial was performed in 180 patients diagnosed with astrocytoma and GBM, which had received prior surgery and radiation therapy, and some chemotherapy. Upon treatment, the overall median survival for patients with GBM was 13.4 months, which demonstrated a remarkable improvement (195). Following up on these positive results, the same ^{125}I-labeled mAb was used in another phase II clinical trial to test its efficacy in adjuvant RIT in patients with GBM. From the 192 patients involved in this trial, following surgery and radiation therapy, some also received TMZ in combination with the RIT treatment. The overall median survival was 14.5 and 20.2 months for patients that received just the [^{125}I]I-anti-EGFR-425 and for those that were treated with a combination of the radiolabeled mAb and TMZ, respectively (196).

Another EGFR targeted mAb for RIT is the ^{188}Re-labeled Nimotuzumab (h-R3). This mAb underwent phase I clinical trials with 11 patients, 8 of them with GBM. The treatment consisted of a single dose administration of [^{188}Re]Re-Nimotuzumab, and the maximal tolerated dose was established at 10 mCi. The 6 patients treated with 10 mCi showed transitory worsening of pre-existing neurological symptoms, and one developed radionecrosis. Two of these patients presented a complete response after 3 years of treatment, and one presented a partial response for more than a year.(43)

DNA histone H1 complex is an intracellular antigen that is present in necrotic regions of tumours (197). Treatments with this type of targeting are designated tumour necrosis treatments (TNT). The commercial compound ^{131}I-chTNT-1/B mAb (Cotara®), was the first to undergo phase I and II clinical trials based on TNT. In this study, 51 patients were treated with Cotara to evaluate and define the dosing regimens for the radiopharmaceutical. It was also observed for patients with GBM an overall survival of 41 weeks (198, 199).

With the steady emergence of RIT, other biological targets have been explored for GBM targeting with mAbs, including CAs, cadherin 5 and integrin $\alpha_v\beta_3$. Although these targets present interesting biological features for GBM RIT, to date there are no reported clinical trials with radiolabeled mAbs specific for these antigens and the reported works describe uniquely preclinical studies. The CAs enzymes are a prognostic biomarker that correlates with tumour progression, which means that they are absent in normal healthy tissues (200, 201). Preliminary studies of a ^{177}Lu-labeled DTPA-6A10 Fab, a CA targeting antigen, performed in human GBM xenograft mice, demonstrated the specific binding of the radiolabeled Fab to CA in tumour cells,

and significant tumour uptake 6 h post-injection (34). Integrin $\alpha_v\beta_3$ is another interesting target for RIT. Etaracizumab (MEDI-522), commercially known as Abegrin®, is a mAb known to bind and block integrin $\alpha_v\beta_3$-mediated angiogenesis (202). In a preliminary study, Abegrin was radiolabeled with ^{90}Y and injected into human GBM xenograft mice. The treated mice showed partial regression of tumour volume, and reduced cancerous cell proliferation (203).

Cadherins are calcium-binding proteins that are involved in cancer cell adhesion and migration, particularly cadherin 5, which is an endothelial cell marker in GBM (73, 204). [^{225}Ac]Ac-E4G10, a cadherin 5 targeting mAb, was administered in GBM xenograft mice with the intent to evaluate its efficacy in the remodelling of the tumour microenvironment. Results confirmed the remodelling of the GBM vascular microenvironment, relief of edema and depletion of regulatory T and endothelial progenitor cells (205).

27.4.4 Radioactive Nanoparticles

Radioactive NPs have emerged as interesting tools for the development of novel platforms for the delivery of medically relevant radioisotopes to tumour sites (206, 207), although to date and to our best knowledge, there were no clinical trials performed on radiolabeled NPs for GBM treatment. However, various studies have been reported on the evaluation of these nanocarriers as potential radiopharmaceuticals towards GBM. These studies include a wide range of NP types, from organic to metallic, and various sizes, with tumour targeting based on passive or active approaches.

Liposomes can be conveniently loaded with different types of drugs and/or radiopharmaceuticals, by taking advantage of their hydrophilic inner core to encapsulate these molecules. ^{99m}Tc/^{188}Re-BMEDA loaded liposomes were injected into human U87 GBM xenograft mice, with doses of up to 1850 Gy (for ^{188}Re), without evidence of toxicity. Additionally, the treated mice had a median survival of 126 days, compared with 49 days for control mice (208).

Lipid nanocapsules (LNCs) are very similar by design to liposomes, with only slight differences in composition and function (209). LNCs loaded with the lipophilic compound ^{188}Re-SSS, were injected in rat orthotopic glioma models by CED administration. The radiolabeled LNCs showed significant survival benefits for the rat glioma models, with cure rates of 83% (210). In recent years, LNCs functionalized with 12G5, a CXCR4-targeting antibody, and loaded with lipophilic thiobenzoate complexes of ^{188}Re, were developed as targeted nanocarriers for GBM. The LNCs were administered by convection-enhanced delivery to CXCR4-positive GBM orthotopic and xenogenic mice. Results showed a significant improvement in median survival, indicating that these targeting nanocarriers may provide optimal benefits for clinical application in GBM treatment (211).

^{125}I-labeled polymeric NPs of polyethylene glycol (PEG), functionalized with a RGD peptide derivative and a near-infrared (NIRF) dye (croconaine, CR780), were studied as a $\alpha_v\beta_3$ integrin targeting platform for multimodal theranostics of GBM. The radiolabeled polymeric NPs, [^{125}I]RGD-CR780-PEG5k, showed highly integrated properties, suitable for nuclear, optical and thermal therapy modalities; human GBM tumour-bearing mice treated with these nanocarriers had an increased median survival. The authors also considered the possibility of loading these platforms with anticancer drugs, for chemo- and radio-thermal combined therapy (212).

Silver NPs (AgNPs) are known to have toxicity issues, which can limit their use in biomedical applications, but can also be used advantageously in anticancer drug development (213). Interestingly, polymeric NPs have been explored to deliver AgNPs and the anticancer drug alisertib (Ali) to GBM tumours. The polymer used was PLGA-*b*-PEG-COOH functionalized with Cltx, a peptide that binds specifically to MMP-2. The AgNPs and Ali-loaded NPs were also labeled directly with ^{99m}Tc for SPECT imaging. Results showed induced cytotoxicity against GBM cells, and tumour shrinkage in GBM xenograft mice (214).

Self-assembling peptides are biological materials with particular structures that are formed in response to certain thermodynamic and kinetic conditions (215). Recently, a study on self-assembled peptide NPs was reported, using the cyclic heptapeptide Arg-Gly-Asp-Lys-Leu-Ala-Lys with the RGD sequence for targeting of the $\alpha_v\beta_3$ integrin receptor and the KLAK pro-apoptotic motif for mitochondria targeting, and carrying a DTPA moiety conjugated to the terminal Lys and a NIRF dye (Cy5.5) attached to the middle Lys. These NPs, C5.5@SAPD, were radiolabeled with ^{99m}Tc for SPECT imaging, and *in vitro* studies showed that they have high affinity for the $\alpha_v\beta_3$ integrin receptor, being also capable of inducing apoptosis in human GBM cells. These results also translated to *in vivo* studies in GBM subcutaneously induced xenografts, in which a high tumour accumulation of ^{99m}Tc-labeled C5.5@SAPD was observed, as well as a remarkable therapeutic response after one week of treatment (216).

Inorganic NPs offer alternative functionalities to organic NPs, particularly in regards to optical properties, as seen in the case of gold nanoparticles (AuNPs) or quantum dots (QDs) (217), while mesoporous silica nanoparticles (MSNs) exhibit great capacity for drug loading and controlled bio-active drug release (218). MSNs functionalized with a DOTA derivative were radiolabeled with ^{111}In and evaluated on orthotopic GBM mice models. These NPs showed a very low toxicity and high stability, and were capable of actively migrating towards the glioma xenografts after both intracranial and systemic administrations (219) (Figure 7).

AuNPs have gained much interest in the latest decades in radiopharmaceutical development, not only because of their optical properties, but also due to their biocompatibility, low toxicity, and ease of functionalization (8, 220). Ferro-Flores et al. studied ^{177}Lu-labeled AuNPs decorated with a cyclic Arg-Gly-Asp (RGD) peptide, recognizing the $\alpha_v\beta_3$ integrin receptor, which is not specific for GBM but is involved in tumour angiogenesis (221). Following the intratumoural administration of the AuNPs in a murine C6-glioma xenograft model, the authors verified highest tumour retention and enhanced reduction in tumour growth for [^{177}Lu]Lu-AuNP-RGD when compared with the same ^{177}Lu-labeled AuNPs without the RGD peptide. These results pointed out that the target-specific approach can lead to improved GBM treatments based on intratumoural application of radiolabeled nanoseeds. In a more recent study, AuNPs functionalized with a DOTA derivative and decorated with a Substance P peptide for NK1R targeting, were developed as potential nanoseeds for GBM treatment, and successfully labeled with different medically relevant radioisotopes, including ^{67}Ga, ^{125}I and ^{177}Lu. Cellular studies showed that the substance P peptide plays a crucial role in the accumulation of the NPs in NK1R-positive cells, and that the target-specific ^{177}Lu-labeled nanocarriers displayed more pronounced radiobiological effects (51). In a similar way, Bilewicz et al. have designed AuNPs carrying a Substance P derivative (SP(5–11)) and proceeded with their labeling with ^{211}At. The resulting ^{211}At-labeled AuNPs showed high *in vitro* radiocytotoxicity in T98G glioblastoma cells, moderately larger than that showed in the same cell line by the radiolabeled AuNPs without the SP peptide (222).

One of the main advantages of QDs is their inherent optical properties, including high quantum yield, high photostability and tunable fluorescence emission (223). Intrinsically radioactive QDs have been developed by direct incorporation of ^{64}Cu into the nanostructure, affording the radiochemically stable NPs [^{64}Cu]CuInS/ZnS. These QDs have shown high tumour uptake in human GBM xenografted mice, and imaging studies showed promising *in vivo* PET/self-illuminating luminescence capabilities (224).

Metallofullerenes are another interesting type of NPs, which exhibit combined properties of the carbon cages and the metallic moieties. For instance, metallofullerenes with Gd in their composition have magnetic properties that allow them to be explored for MRI (225). In a preclinical trial, a functionalized metallofullerene (f-$Gd_3N@C_{80}$), decorated with a DOTA derivative for ^{177}Lu-labeling, was administered in human GBM tumour-bearing mice, via CED injection. The MR images obtained showed high tumour retention, which also led to an effective brachytherapy treatment, as verified by the extended survival time of the treated animals, up to 2.5 higher than the untreated group (226).

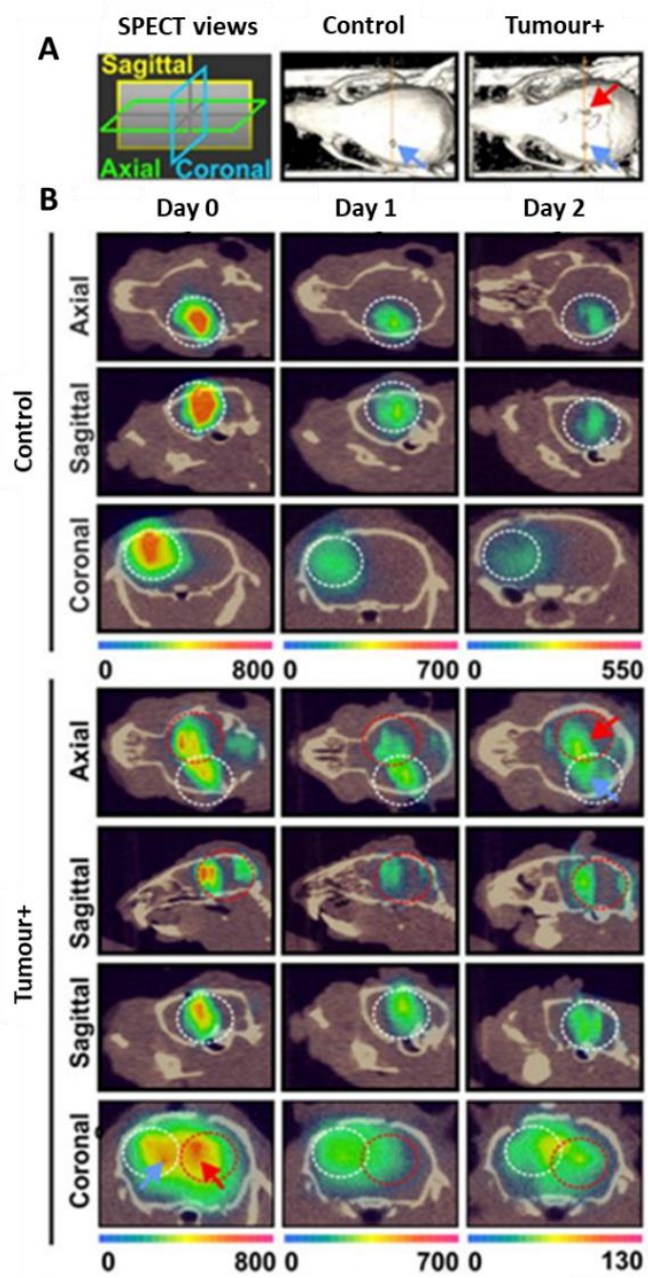

Figure 7. SPECT imaging of mouse brain after intracerebral delivery of NSCs loaded with ^{111}In-MSN. (A) Three-dimensional reconstruction of cranial CT scan shows NSC and glioma cell injection sites. (B) SPECT scans corresponding to radiolabeled NSCs at injection site at days 0, 1, and 2. Image adapted from reference (219).

27.5 CONCLUSIONS

In the past decades, nuclear medicine modalities had an increasing contribution in the development of new tools for the diagnostic and/or treatment of glioblastoma, with encouraging results at preclinical and clinical levels. These advancements have been fuelled by the favourable features of nuclear imaging techniques (PET and SPECT) and targeted radionuclide therapy, such as their high sensitivity, non-invasiveness and intrinsically low toxicity, that favour the translation to the clinical setting. In addition, a large variety of medical radionuclides is already available and well-established radiochemical strategies can be applied to obtain radiopharmaceuticals in diverse chemical forms, for specific recognition of different GBM molecular targets and application by different administration routes depending on the tumour invasiveness and its cerebral localization.

Most relevantly, PET or SPECT scans can image the tumour masses and metastases by targeting GBM cancer cells with the appropriate specific diagnostic radiopharmaceutical. Thereafter, guided by the imaging data and almost in real time, it is possible to select the most appropriate therapeutic radiopharmaceutical for a given patient, better adjusted to the molecular fingerprint of the target GBM tumours, and tune the administrated radioactive doses on an individual basis. Thus, nuclear modalities can offer unique possibilities to leverage precision and molecular medicine approaches for the personalized theranostic of GBM.

Despite these progresses, the survival rate of GBM patients remains low and significant improvements are needed to reverse this scenario. Towards this goal, the improved molecular characterization of the disease will allow to explore with radiopharmaceuticals new clinically relevant targets for GBM theranostics, such as microenvironment immune checkpoint proteins. Nevertheless, it is important to have in mind that a unique treatment modality will hardly circumvent the current difficulties due to genetic complexity of GBM. Therefore, the combination of radiopharmaceuticals with other therapies might open new avenues for the improvement of treatment and management of the disease. This may include the combination with radiosensitizers, immunotherapy drugs and even external radiotherapy. For the development of these multimodality treatment approaches the versatility of nuclear medicine modalities and radiopharmaceuticals, ongoing progresses in the production of innovative theranostic radionuclides (227) and emerging nanotechnologies, among other scientific and technological developments, will be of paramount importance.

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