ACCSCIENCE PUBLISHING

Advances in Radiotherapy & Nuclear Medicine

REVIEW ARTICLE

Exploring tumor heterogeneity: The role of PET/CT with various radiopharmaceuticals in diagnosis and treatment guidance

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(This article belongs to the *Special Issue: Recent Developments in Radiopharmaceuticals*)

Abstract

Cancer development is a multi-step process that undergoes multiple alterations over time. The tumor microenvironment (TME) contains tumor cells and stroma, including blood cells, fibroblasts, and immune cells, which undergo spatial and temporal changes. These changes contribute to tumor heterogeneity, leading to treatment failure and poor prognosis. As we move toward personalized medicine with the approval of targeted and other therapies, identifying tumor heterogeneity is becoming crucial to management. Positron emission tomography/computed tomography (PET/CT) with various radiopharmaceuticals plays an important role in diagnosing and highlighting heterogeneity non-invasively, guiding treatment decisions, and assessing treatment response. Variability in tracer distribution of ^{18}F-fluorodeoxyglucose (FDG) and various radiopharmaceuticals when coupled together can target various tumor characteristics and, therefore, play an important role in diagnosing heterogeneity. Some of the commonly paired radiopharmaceuticals include ^{18}F-FDG with ^{68}Gallium DOTA (1,4,7,10-tetraazacyclododecane-tetraacetic acid) peptide for neuroendocrine tumors, ^{18}F-FDG with ^{68}Ga-prostate specific membrane antigen for prostate cancers, ^{18}F-metafluorobenzylguanidine with ^{18}F-FDG and ^{68}Ga-DOTA peptide for neural crest tumors, and ^{18}F-fluoroestradiol with ^{18}F-FDG for breast cancers. Many other tracers, including ^{68}Ga-fibroblast activation protein inhibitor and labeled integrins, attach to various components on tumor cells and TME and have displayed significantly positive effects in certain tumors. However, their potential role as a biomarker to evaluate tumor heterogeneity and its clinical relevance remains largely uninvestigated.

Keywords: Cancer; Heterogeneity; ^{18}F- FDG; PET/CT

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Citation: Jain A, Kheruka S, Usmani S, *et al*., Exploring tumor heterogeneity: The role of PET/CT with various radiopharmaceuticals in diagnosis and treatment guidance. *Adv Radiother Nucl Med.* 2025;3(2):1-15.
doi: 10.36922/ARNM025040005

Received: January 21, 2025

Revised: February 19, 2025

Accepted: March 5, 2025

Published online: March 20, 2025

1. Introduction

Cancer development is a multi-step process that undergoes multiple alterations spatially and temporally. During its evolution process, heterogeneity develops between various cancer cells, displaying distinct characteristics, either within the primary tumor or among its metastases.[1,2] Tumor heterogeneity plays a crucial role in cancer management as it directly impacts treatment outcomes and patient prognosis. As we move from

traditional treatment methods (e.g., chemotherapy and radiotherapy) to more personalized (precise) medicine – incorporating targeted drugs and individualized treatments based on each patient's unique genes, environment, and lifestyle – assessing tumor heterogeneity becomes increasingly important. In recent years, several anticancer drugs have been approved and specifically developed to target the unique morphological, molecular, and genetic characteristics of cancer cells. Accurate diagnosis and quantification of tumor heterogeneity is crucial to predict disease progression, as heterogeneity can be highly variable even within a single individual.[3] Diagnosing tumor heterogeneity is quite challenging, and the role of pathology, radiological imaging, radiomics, and artificial intelligence (AI) has been described in various literature.[4] Radiological images, including ultrasound, computed tomography (CT), and magnetic resonance imaging (MRI), can identify heterogeneity to a certain extent by texture analysis and the shape and volume of lesions.[5] However, radiological imaging requires special software and expertise and cannot provide comprehensive information on total body lesions in a single scan, as positron emission tomography/CT (PET/CT) can. This review delves into various aspects of PET/CT in describing heterogeneity and its impact on clinical management. Radiomics and AI techniques are further enhancing our understanding of tumor complexity. This short review also describes the role of different PET radiopharmaceuticals (tracers) in imaging various characteristics of tumor tissue that contribute to heterogeneity in various cancers.

2. Tumor heterogeneity: Concepts and mechanisms

Cancer and its metastases arise from a process of uncontrolled and unchecked cell proliferation, which invades healthy tissues and spreads throughout the body. Tumor clonality represents a key characteristic of malignancy and is defined as the expansion of transformed cells derived from a founding cell that initially acquired deregulated growth capability. In contrast, cancer development is a multi-step process in which cells undergo several genetic or environmental alterations, transforming into cell types different from their original progenitor cells and contributing to heterogeneity.[1]

Solid cancer lesions consist of tumor cells and the tumor microenvironment (TME). The TME is made of cells and extracellular components (blood and lymphatic vessels, cytokines, mediators, etc.). The cellular components included in this complex are tumor cells, immune cells, and stromal cells (fibroblasts, mesenchymal stem cells, adipocytes, endothelial cells, and pericytes). Within the TME, stromal components secrete growth factors and angiogenic factors (e.g., transforming growth factor β1, vascular endothelial growth factor, interleukin 6, and tumor necrosis alpha [TNF-α]). This dynamic environment provides nutritional and mechanical support that enhances cancer metabolism, promoting tumor growth through local invasion and resistance to cell death signals. The characteristics of the TME vary greatly between tumor types – it can be fibrotic, rich in blood vessels, or even necrotic. Tumor cells also exhibit various phenotypic characteristics, such as cell surface receptors, rate of antigenicity, metabolic activity, hormone receptor status, and indicators of tumor aggressiveness or metastatic potential. These characteristics provide crucial insights into the behavior and biology of tumors, aiding in diagnosis, prognosis, and the development of targeted therapies. In addition, various factors, such as genetic mutations, cellular heterogeneity, and microenvironment interactions, also play significant roles in determining tumor behavior and treatment response. As tumors transition from primary to invasive disease, the tumor and its microenvironment evolve, resulting in the spatial and temporal emergence of tumor heterogeneity.[2,3]

Spatial heterogeneity refers to the variation in tumor characteristics, such as cell morphology and genetic makeup, within the primary tumor or between its metastases at a single time point. Conversely, temporal heterogeneity describes changes in these tumor characteristics that develop over time as the disease progresses. Both forms of heterogeneity play a critical role in understanding tumor behavior, treatment resistance, and disease evolution.[4] Detection and quantification of spatial and temporal tumor heterogeneity can provide critical insights for cancer treatment. Jonsson *et al.*[5] studied spatial heterogeneity on PET/CT scans in three types of cancers using an image registration-based framework. Their findings demonstrated that spatial tumor heterogeneity can be effectively detected and quantified based on lesion frequency, volume, and metabolic activity. This approach highlights the potential of advanced imaging techniques to better understand tumor behavior and guide personalized treatment strategies.[5] Hughes *et al.*,[6] in their study on lung cancer using PET/CT scans, demonstrated that quantification of spatial fluorodeoxyglucose (FDG) uptake heterogeneity provides prognostic value beyond standardized uptake value (SUV) and the clinical stage of cancer. Various studies have demonstrated that tumors and their metastases consist of different clones of cells, each with distinct genetic, epigenetic, proteomic, and transcriptomic profiles, yet descending from a common ancestor. These diverse clones may exhibit varying responses to treatment, with some clones resisting therapy and continuing to proliferate,

ultimately leading to treatment failure. This dynamic process contributes to the development of visible heterogeneity over time, which is referred to as temporal heterogeneity.[7] In a study on neuroblastoma, the authors noted that PET/CT-based diagnosis of intratumor heterogeneity in high-risk patients served as a strong prognostic indicator. This highlights the importance of serial advanced imaging techniques in understanding temporal tumor evolution and predicting clinical outcomes.[8]

Heterogeneity can manifest between different cells within a single tumor (intra-tumor) or across different patients harboring the same tumor type and can even be observed when comparing primary tumors to their metastases (inter-lesion). The diverse nature of tumor heterogeneity presents a significant challenge in cancer management, highlighting the need for more sophisticated diagnostic and therapeutic approaches.[9]

3. Challenges in diagnosing tumor heterogeneity

Diagnosing and precisely characterizing tumor heterogeneity can be highly challenging. Cancers and their molecular subtypes are typically identified using small biopsy samples taken from the primary site or a metastatic site. However, these biopsy samples are often limited in size and may not fully represent the entire tumor's heterogeneity. In addition, performing biopsies on all metastatic sites involved in cancer is impractical. In a study by one of the authors on medulloblastoma, high-grade glioma, and renal cell carcinoma, it was demonstrated that, in terms of spatial heterogeneity, at least five biopsies are required to detect 80% of somatic variants.[10]

Similarly, other authors, like Savas *et al.*,[11] have demonstrated the presence of spatial heterogeneity between primary tumors and their metastases in biopsy samples from breast cancer patients.

As biopsy samples are small and may not fully capture tumor type and heterogeneity, this approach may result in inadequate treatment. This can lead to the administration of one or more therapies that are not beneficial for a given patient's cancer, with potential harm/morbidity through lack of an effective drug regime. Heterogeneity also appears to be the primary cause of drug resistance. While patients may initially react to a given therapy, new and resistant cancer cell clones regularly emerge within the tumor, leading to disease progression or relapse. This innate variability within tumors makes developing a one-size-fits-all treatment approach exceedingly difficult.[12]

Medical imaging techniques possess the ability to capture tumor characteristics at the cellular and molecular levels. Advanced radiological imaging modalities (e.g., CT and MRI) have demonstrated some capability in visualizing tumor heterogeneity; however, these techniques require the integration of advanced software radionics and AI.[13] The transformative development of PET/CT imaging has opened new avenues for investigating cancer characteristics and biology at the cellular and molecular levels. To date, ^{18}F-FDG has been the most widely used radiotracer for staging, restaging, and monitoring therapy response in different tumors. ^{18}F-FDG has also been used for prognostic assessments. Its role in diagnosing tumor heterogeneity appears promising. In addition, the broad range of radiopharmaceuticals (tracers) utilized for PET/CT, coupled with whole-body imaging, has made PET a potential option for non-invasively revealing tumor heterogeneity.

4. Tumor heterogeneity highlighted by various PET radiopharmaceuticals

In recent years, advances in PET/CT technology have transformed the field of oncology. With a deeper understanding of the intricate TME, scientists have developed an array of innovative PET tracers focusing on improving cancer staging, restaging, and management. PET radiopharmaceuticals target a variety of mediators that localize in tumor cells and the cancer microenvironment through various mechanisms, providing different information on cell biology and growth. The application of these specialized PET tracers has allowed physicians and researchers to gain a more nuanced view of tumor heterogeneity.

There are two potential methods for assessing tumor heterogeneity using PET scans: (i) assessing heterogeneity in tracer distribution and quantifying metabolic parameters in ^{18}F-FDG PET scans, and (ii) by utilizing multiple radiopharmaceuticals that target different characteristics of tumor cells and microenvironment.

5. ^{18}F-FDG PET/CT: The workhorse of oncological imaging

^{18}F-FDG PET/CT remains the most widely used radiopharmaceutical for staging and response assessment of various malignancies. Increased glucose metabolism and uptake are observed in various cancer cells due to the upregulation of GLUT transporters and hexokinase expression. ^{18}F-FDG enters the cells through glucose transporters and is phosphorylated by hexokinase to produce ^{18}F-FDG-6-phosphate, which remains trapped in the cell and can be detected by PET scan. FDG uptake in tumor cells depends on tumor histopathology, differentiation, aggressiveness, proliferative activity, and

other biological processes.[14] ^{18}F-FDG PET scans can quantify voxel-based glucose metabolism within tumors; FDG uptake and distribution in a tumor are associated with the expression of glucose transporters and hexokinase within a given lesion, as well as between different sites of that single tumor.

^{18}F-FDG PET/CT scans can quantify variations in tracer uptake by assessing metabolic parameters, such as SUV (SUV_{max}; SUV_{mean}), metabolic tumor volume (MTV), and total lesion glycolysis (TLG). These metrics can be combined with other advanced texture features (e.g., entropy and uniformity) to capture tumor variability and heterogeneity.[14]

The potential of ^{18}F-FDG PET/CT scans in evaluating the heterogeneity at the tumor level has been explored by several authors.[15] It has been described that tumor heterogeneity may lead to heterogeneous or sparse ^{18}F-FDG distribution. High SUV regions on ^{18}F-FDG PET scans are indicative of aggressive clones. Cancers of lung, head and neck, and oligodendroglioma have revealed a clear relationship between the variability in FDG distribution with pathological variability. Increased heterogeneity of FDG uptake is associated with reduced survival, highlighting the prognostic importance of PET imaging.[16-18] Similarly, in sarcoma and cervical cancers, ^{18}F-FDG PET uptake and its heterogeneous distribution are also known to correlate with prognosis and patient outcomes. Variations in FDG uptake within these tumors can provide valuable insights into aggressiveness and potential behavior with higher variation in SUV uptake, indicating more aggressive tumor biology and poorer prognosis. Therefore, the future focus of ^{18}F-FDG PET scanning is to utilize this information on tumor heterogeneity to enhance precise treatment planning and diagnosis.[19-21]

Furthermore, this heterogeneity information is instrumental in radiotherapy planning. By identifying regions within the tumor that exhibit higher metabolic activity, clinicians can tailor radiotherapy treatments to target these areas more effectively, potentially improving treatment efficacy and minimizing damage to surrounding healthy tissue. This tailored approach aims to enhance treatment effectiveness and reduce harm to neighboring healthy tissue, ultimately contributing to better patient outcomes.[22,23]

6. Beyond ^{18}F-FDG: Commonly used paired specialized PET tracers in different cancers

In addition to ^{18}F-FDG, various PET tracers have been developed over time to target different tumor components. The distribution and uptake of these tracers provide a comprehensive, non-invasive map of both inter- and intratumoral heterogeneity. For example, endocrine-related cancers, such as thyroid and neuroendocrine tumors (NETs), exhibit a wide range of clinical behaviors. Well-differentiated tumors tend to be indolent and retain their endocrine function, while poorly differentiated tumors are more aggressive. ^{18}F-FDG PET is particularly effective in identifying this aggressive phenotype. Similarly, different molecular subtypes of breast cancer can be targeted with specific tracers, offering a complete picture of the tumor and its heterogeneity. Prostate cancers tend to be differentiated or poorly differentiated, with diagnosis performed using ^{68}Ga-PSMA and ^{18}F-FDG PET scans. In addition, some may even exhibit neuroendocrine differentiation, which can be identified with ^{68}Ga-DOTA peptide PET scans.[24] This approach captures complementary biological features of the tumor and its microenvironment, enabling a more comprehensive assessment of heterogeneity. Combining these methods can improve the characterization of tumor behavior, predict treatment resistance, and guide personalized therapy by mapping both metabolic and molecular heterogeneity.

Various PET radiopharmaceuticals used clinically and in research are shown in Table 1.[24,25] Herein, we describe some of these tracers, their known combinations, and their potential applications in various tumors.

6.1. ^{68}Ga-DOTA peptides PET/CT for NET

Tumor heterogeneity is seen commonly in NET. NET overexpresses somatostatin receptors (SSTRs); PET/CT with ^{68}Ga-DOTA peptide (DOTATATE/DOTANOC/DOTATOC) reflects somatostatin receptor expression in the tumor and identifies cell differentiation and grade of the tumor. High ^{68}Ga-labeled peptide binding is correlated with well-differentiated, low-grade lesions, while reduced binding implies poorly differentiated tumors or higher-grade tumors. ^{18}F-FDG PET is sometimes coupled with ^{68}Ga-labeled peptide scans, as FDG accumulation is related to the metabolic activity of the tumor, displaying higher sensitivity for poorly differentiated, aggressive tumors with poor outcomes.[26] The phenomenon observed between the two tracers reveals substantial metabolic and receptor heterogeneity. These methodologies enable doctors to evaluate inter-lesion variability, with differences in uptake between primary and metastatic locations, directly influencing therapy eligibility, such as patient selection for somatostatin receptor therapy (SSRT).[27] Peptide receptor radionuclide therapy (PRRT) is radionuclide therapy for NET, which is positive in ^{68}Ga- DOTA peptide PET/CT scans and displays minimal or no uptake in ^{18}F-FDG PET/CT scans. Therefore, combining DOTA peptide PET/CT scans with other tracers, most commonly FDG PET scans, can diagnose heterogeneous clones of tumors

Table 1. Mechanism of action and indications of various positron emission tomography (PET) radiopharmaceuticals[24,25]

PET radiopharmaceutical	Mechanism of localization	Oncological indication
^{18}F-fluorodeoxyglucose (FDG)	Enters the cell through GLUT 1 and 3 transporter, phosphorylated and trapped inside the cell	Staging, restaging, and response assessment of a wide range of tumors, including breast, lung, GI tumors, melanoma, etc.
^{68}Ga-DOTA -peptides	Binds to somatostatin receptors on tumor cells	Staging and restaging of neuroendocrine tumors
^{18}F-fluoro-L DOPA (^{18}F-DOPA)	Transported into the cell by large neutral amino acid transporters L-type (LAT) and stored in secretory vesicles	Diagnosis, staging, and follow-up of neural crest tumors, for example, neuroblastoma, paraganglioma, brain tumors, and congenital hyperinsulinemia
^{18}F-metafluorobenzylguanidine (mFBG)	Norepinephrine analog transported by norepinephrine transporters overexpressed on tumor cells	Detection and staging of neural crest tumors
^{68}Ga/^{18}F/^{64}Cu-prostate-specific membrane antigen (PSMA)	Binds to PSMA (type II transmembrane protein) on prostate cancer cells	Staging and detection of recurrence in prostate cancer
^{68}Ga-fibroblast activation protein inhibitor (FAPI)	Binds to FAP in the stroma of tumor microenvironment	Diagnosis and staging of various tumors, for example, pancreas, cholangiocarcinoma, breast, etc.
^{18}F-fluoroestradiol (FES)	Binds to estrogen receptors in the nucleus of ER-expressing cells	Diagnosis and follow-up of hormone-positive breast cancer
Various ^{89}Zirconium labeled tracers		
^{89}Zr-trastuzumab	Labeled antibodies (immune-PET); monoclonal antibody that binds to HER2; targets EGFR; binds to VEGFA; antibodies act against PD-L1; targets CD38 antigen expressed on myeloma cells	Diagnosis and staging of HER2-positive breast cancers
^{89}Zr-cituximab		Diagnosis of cancers with overexpression of EGFR
^{89}Zr-bevacizumab		Diagnosis of cancers with overexpression of VEGF
^{89}Zr-atezolizumab		Detection of PD-L1-positive tumors
^{89}Zr-DFO-daratumumab		Diagnosis of multiple myeloma
^{68}Ga-RGD/trivehexin	Binds with integrins expressed on tumor cells or angiogenic blood vessels	Diagnosis of cancers, for example, pancreas, head and neck, etc.
^{18}F-fluoroethyl-l-tyrosine (^{18}F-FET); ^{11}C-methyl-l-methionine (^{11}C-MET)	Amino acid analogs transported in cells by overexpressed transmembrane LAT on tumor cells	Diagnosis and grading of brain tumors
^{18}F-fluciclovine	Amino acid analog enters the cell by neutral amino acid transporter	Diagnosis of biochemically recurrent prostate cancer and other tumors
^{11}C-acetate/palmitate	Converted to acetyl CoA in cells for cholesterol and fatty acid synthesis, which are integrated into the cell membrane or oxidized in mitochondria	Diagnosis of prostate, renal, brain, and HCC cancers
^{18}F/^{11}C-choline	Phosphorylated to phosphorylcholine within cells and integrated into phospholipids in the cell membrane	Diagnosis of the brain, prostate, lung, bladder cancers, etc.
^{18}F-FLT (thymidine)	Enters cell by facilitated diffusion through nucleoside transporters and is trapped in the cell	Diagnosis and response assessment of cancers, for example, lung, breast, colon, lymphoma
^{18} F-misonidazole	Nitroimidazole compound that enters the cell through blood flow and gets trapped in hypoxic cells	Detects hypoxia in tumors, for example, lung, head and neck, and brain tumors

(Cont'd...)

Table 1. ***(Continued)***

PET radiopharmaceutical	Mechanism of localization	Oncological indication
^{18} F-ICMT-11	Binds in caspase 3-specific cells undergoing apoptosis	Identification of treatment-induced apoptosis and response to treatment in malignant tumors
^{124}I -annexin V	Binds to phosphatidyl serine on the surface of apoptotic cells	
^{18}F-sodium fluoride (NaF)	Deposits in bone through chemisorption, converting hydroxyapatite to fluorapatite	Detection of bony metastases
^{68}Ga-pentaxifor	Targets cells exhibiting CXCR 4 expression	Diagnosis of multiple myeloma and other tumors
^{68}Ga-DOTA exendin-4	Amino acid peptides, like GLP 1, bind to GLP receptors on pancreatic beta cells	Diagnosis of insulinoma
^{18}F-fludarabine	Purine analog that inhibits DNA synthesis	Diagnosis of hematological malignancies
^{68}Ga-glypican 3	Oncofetal acetyl heparan sulfate glycoprotein expressed on HCC	Diagnosis of HCC

Abbreviations: CXCR4: Chemokine receptor type 4; DNA; Deoxyribonucleic acid; DOPA: 1,4,7,10-Tetraazacyclododecane-tetraacetic acid; DOTA: 1,4,7,10-Tetraazacyclododecane-tetraacetic acid; 18 F-ICMT -11: (18F-(S)-1-((1-(2-fluoroethyl)-1H-[1,2,3]-triazol-4-yl) methyl)-5-(2 (2,4-difluorophenoxymethyl)-pyrrolidine-1-sulfonyl) isatin); ER: Estrogen receptors; EGFR: Epidermal growth factor receptor; FAP: Fibroblast activation protein; GLP-1: Glucagon-like peptide 1; GLUT: Facilitated diffusion Glucose Transporters; HCC: Hepatocellular carcinoma; HER2: Human epidermal growth factor receptor 2; PD-L1: Programmed cell death ligand-1; PSMA: Prostate specific membrane antigen; RGD: Peptide Arg-Gly-Asp; VEGFA; Vascular endothelial growth factor A.

and guide treatment strategies.[26] Kaewput *et al.*[28] have demonstrated that ^{68}Ga-DOTA peptide and ^{18}F-FDG PET scans serve complementary roles in patients with gastroenteropancreatic (GEP) NET, simultaneously enhancing diagnostic accuracy due to the heterogeneity of lesions. Similarly, many authors, including Zhou *et al.*,[29] have revealed that dual tracer PET with ^{68}Ga-DOTA peptide and ^{18}F-FDG PET should be performed in all patients with an initial diagnosis of NET due to the heterogeneous nature of these tumors. Nogareda Seoane *et al.*[30] demonstrated that ^{68}Ga-DOTA peptide and ^{18}F-FDG PET scans collectively reveal the molecular heterogeneity of metastatic lesions in G2 and G3 NET, facilitating the appropriate selection of patients for radionuclide therapy (PRRT). Dual tracer PET imaging with ^{18}F-FDG and ^{68}Ga-DOTA peptide demonstrating tumor heterogeneity is displayed in Figure 1.

6.2. PET/CT in neural crest tumors

PET/CT with various radiopharmaceuticals is remarkably effective for imaging a group of tumors arising from the neural crest, including medullary thyroid cancers, neuroblastoma, paraganglioma, pheochromocytoma, and ganglioneuroma. These tumors express different amines and peptides that can be targeted by various PET tracers, such as ^{18}F-dihydroxyphenylalanine (^{18}F-DOPA), ^{68}Ga-DOTA peptides, ^{18}F-FDG, and other tracers (norepinephrine analog ^{18}F- metafluorobenzylguanidine [MFBG], resembling single photon emission computed tomography [SPECT] tracer ^{123}Iodine-Meta-Iodobenzylguanidine [1231-MIBG]). These tracers exhibit variable uptake capacities in tumors, aiding in diagnosis and therapeutic management. These tumors accumulate amino acids and amine precursors. ^{18}F-DOPA is a radiolabeled amino acid that enters the tumor cell through the L-amino acid transporter (LAT1 and LAT2). In the cells, ^{18}F-DOPA is decarboxylated to dopamine and transported and stored inside secretory vesicles. Cancer cells have increased synthesis and storage of amines, like dopamine; therefore, PET imaging with DOPA enables the diagnosis of these tumor cells. This renders PET imaging with ^{18}F-DOPA remarkably helpful for diagnosing neuroblastoma and other neural crest tumors, as well as congenital hyperinsulinemia. Neural crest tumors frequently overexpress norepinephrine transporters, that is, proteins that facilitate the uptake of norepinephrine, epinephrine, and dopamine across cell membranes. ^{18}F-MFBG is a norepinephrine analog that can be targeted to diagnose neural crest tumors. These PET tracers have varied uptake capacities in tumor sites depending on their biological characteristics, demonstrating heterogeneity that helps refine oncology diagnosis and treatment strategies.[13,31]In a study, involving the evaluation of recurrent and metastatic paraganglioma with different PET tracers, authors found that ^{68}Ga-DOTA-1-Nal3-octreotide (DOTANOC) PET/CT displayed more bone lesions, while ^{18}F-3,4- dihydroxy-6-[^{18}F]fluoro (FDOPA) PET was superior in detecting liver lesions, demonstrating the heterogeneous nature of tumor lesions being detected variably by different PET tracers.[32] Similarly, in a study on

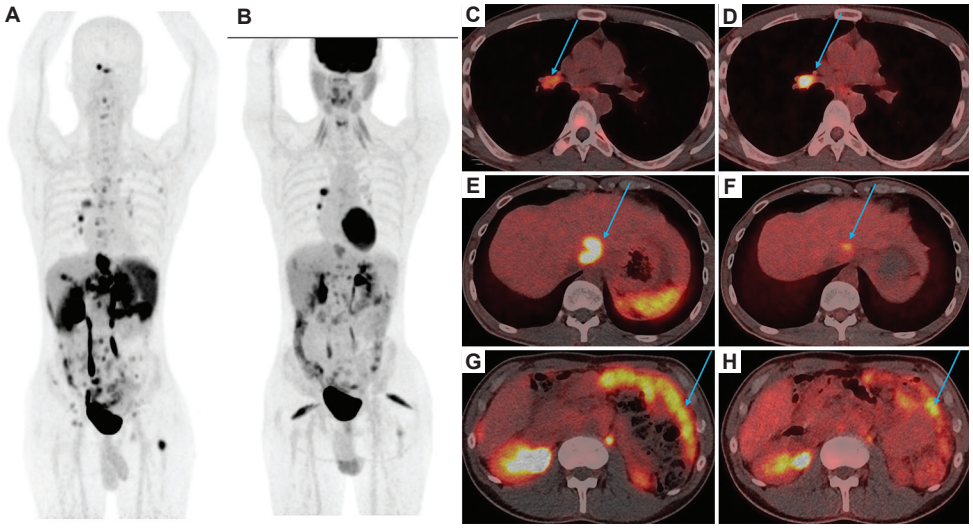

Figure 1. Positron emission tomography/computed tomography (PET/CT) imaging of a 33-year-old male with metastatic pancreatic cancer demonstrates heterogeneous lesions on the PET/CT scan. (A and B) maximum intensity projection (MIP) images of ^{68}Ga-DOTATOC-PET (A) and ^{18}F-FDG-PET (B) display multiple lung, liver, bone, lymph node, and peritoneal metastases. (C and D) Fused PET/CT of ^{68}Ga-DOTATOC (C) and ^{18}F-FDG PET (D) display higher uptake of ^{18}F-FDG in the right hilar node. (E–H) Fused ^{68}Ga-DOTATOC images of the abdomen (E and G) display higher uptake in liver and peritoneal metastases compared to the ^{18}F-FDG images (F and H). Blue arrows indicate metastatic lesions in the right hilar node, liver and peritoneum.
Abbreviations: DOTATOC: Gallium-68 (DOTA0-Phe1-Tyr3) octreotide; FDG: ^{18}F-fluorodeoxyglucose.

paraganglioma and pheochromocytoma, 68Ga-DOTA-DPhe1, Tyr3-octreotate (DOTATATE) is recommended for initial imaging, and depending on the findings, FDG and MIBG may be added in select cases for complete staging and guide treatment decisions.[33]

6.3. ^{68}Ga-PSMA PET/CT for prostate cancer

Prostate-specific membrane antigen (PSMA) is a type II transmembrane protein that is overexpressed on the surface of prostate cancer cells. ^{68}Ga-PSMA PET/CT is outstanding in its ability to bind prostate cancer cells and their metastases, making it highly effective for staging and biochemical recurrence in patients with prostate cancer, with high diagnostic accuracy. Despite PSMA PET being highly sensitive to well-differentiated tumor cells and having a significant diagnostic impact, some lesions may not be observed due to poor differentiation. Wang *et al.*[34] have demonstrated that the addition of FDG PET to PSMA PET scans increased lesion detection rates in high-risk prostate cancer compared to reliance on a single agent alone. In their study, a total of 114 lesions were diagnosed in 37 patients. Out of a total 114 lesions, 81 lesions were PSMA+FDG+/-, while 33 were PSMA-FDG+. Similarly, neuroendocrine differentiation of prostate cancer could be detected using ^{18}F- or ^{68}Ga-labeled DOTA peptides, therefore demonstrating tumor heterogeneity.[35]

Dual tracer PET/CT with ^{18}F-FDG and ^{68}Ga-PSMA also determines the eligibility and outcome of ^{177}lutetium (Lu)-PSMA therapy in metastatic castration-resistant prostate cancers. This radionuclide therapy leads to increased progression-free survival and overall survival.[36] Pabst *et al.*,[36] in their study on castration-resistant prostate carcinoma, identified significant tumor heterogeneity using PET/CT with three tracers, including ^{18}F-FDG, ^{68}Ga-fibroblast activation protein inhibitor (FAPI), and ^{68}Ga/^{18}F-PSMA. Patients selected for ^{177}Lu-PSMA with a PSMA-dominant phenotype experienced improved overall survival.[36]

6.4. Other novel radiopharmaceuticals

6.4.1. ^{68}Ga-FAPI PET/CT for imaging fibroblasts in the stroma of the TME

Fibroblast activation protein (FAP) is a cell surface type II serine protease overexpressed in the stromal tissues of certain tumors to which ^{68}Ga-FAPI can bind. ^{68}Ga-FAPI is a tracer capable of imaging highly fibrosis-rich tumors, such as pancreatic, cholangiocarcinoma, and breast cancers. FAPI PET scans have displayed higher sensitivity for certain low-grade or well-differentiated tumors, which have low uptake in ^{18}F-FDG PET scans.[37] Liu *et al.*,[38] in their review on diagnosing primary and metastatic lesions in various abdominal and pelvic malignancies, indicated that ^{18}F-FDG together with ^{68}Ga-FAPI have higher overall diagnostic performance, denoting the utility of two different tracers in detecting various malignant lesions. Similarly, Yue *et al.*,[39] in their study on recurrent colorectal

cancers, demonstrated that ^{68}Ga-FAPI PET/CT had higher sensitivity in detecting lymph node and peritoneal metastases, while ^{18}F-FDG PET/CT had higher sensitivity in detecting bone metastases; therefore, they proposed a combination of two tracers for PET/CT imaging for better patient management. Patients with tumor lesions positive on ^{68}Ga-FAPI PET/CT scans can be considered for ^{177}Lu-FAPI therapy; however, clear indications and benefits are still under research.[37]

6.4.2. ^{18}F- FES PET/CT for breast cancer

^{18}F-FDG PET/CT scans are routinely conducted for staging and restaging of all types of breast cancer, including its various molecular subtypes. A large number of patients are hormone-positive, that is, estrogen receptor (ER)-positive and progesterone receptor (PR)-positive. Hormone-positive patients can be treated with targeted therapies. The diagnosis of hormone receptor status is performed using immunohistochemical straining of biopsy samples, but this approach has certain challenges and does not fully reflect hormone status and heterogeneity of the entire tumor burden. PET radiopharmaceutical ^{18}F-fluoroestradiol (FES) provides a noninvasive alternative by measuring ER expression in tumors. FES is a lipophilic molecule that is similar to estradiol and binds to ERs in the nucleus of ER-expressing cells. ^{18}F-FES PET scans can detect more lesions with mild or no uptake on FDG PET scans in receptor-positive breast cancer. Studies have demonstrated the superiority of FES PET over other modalities, such as bone scans or ^{18}F-FDG PET scans, in the staging and restaging of hormone-positive breast cancer.[40,41] Matushita *et al.*[40] demonstrated that FES PET has a pooled sensitivity of 82% and a pooled specificity of 94%, highlighting good diagnostic accuracy, with a pooled area under the curve (AUC) of 0.8899 for ER-positive lesions in breast cancer patients. Another study by Liu *et al.*[41] studied a total of 238 lesions in newly diagnosed ER-positive breast cancer. They reported a higher number of lesions detected with FES compared to FDG, with a higher sensitivity of 90.8% for ^{18}F-FES compared to 82.8% for ^{18}F-FDG PET.[41] Recent advancements in PET imaging have also enabled the use of ^{89}Zr-labeled trastuzumab, a monoclonal antibody that binds to human epidermal growth factor receptor 2 (HER2), to visualize HER2-positive breast cancer sites *in vivo*. These developments underscore the growing role of PET radiopharmaceuticals in personalizing breast cancer treatment.[42,43]

Tumor cells and their metastases may contain subclones of cells with variations in molecular subtypes; therefore, single tracers targeting a particular hormone subtype may be inadequate to identify all the tumor lesions. Combining ^{18}F-FES with ^{18}F-FDG and other PET tracers allows clinicians to better differentiate between tumors with varying receptor expressions, providing a clearer picture of tumor heterogeneity and enabling more effective patient management strategies. Several studies have suggested that combining two PET tracers (including metabolic tracer and HER2-targeting agent) provides a clear benefit in evaluating heterogeneity in breast cancer patients and planning treatment strategies.[44,45]

6.5. Various evolving tracers

6.5.1. ^{68}Ga-RGD PET/CT

Tumor cells and angiogenic blood vessels express integrins on their surface. These integrins, along with vascular endothelial growth factor receptors (VEGFRs), regulate the process of angiogenesis. As the peptide Arg-Gly-Asp (RGD) binds with $\alpha v\beta 3$ integrins, RGD labeled with ^{68}Ga or ^{18}F has been used for the diagnosis and staging of a wide range of cancers, including breast cancer, glioma, lung cancer, head-and-neck cancer, melanoma, renal cell cancer, etc. The sensitivity of ^{18}F-galacto-RGD or ^{18}F-fluciclatide was found to be 88–94% for all lesions, with a lower sensitivity of 71–88% for metastatic lymph nodes and distant metastases.[46] In addition, other integrins (e.g., $\alpha v\beta 6$), which are expressed in epithelial cells, have been targeted using ^{68}Ga-trivehexin PET. This imaging modality is particularly useful for diagnosing pancreatic cancer and head-and-neck squamous cell carcinoma.[47]

6.5.2. Other evolving tracers

Other PET tracers, such as ^{18}F-fluorothymidine (FLT), target cell proliferation, ^{11}C-methionine, and ^{18}F-fluoroethyltyrosine (FET), are amino acid analogs that exhibit uptake in enhanced protein synthesis. Tumor hypoxia can be imaged using ^{18}F-fluoromesonidazole (FMISO) and PET with ^{11}C-acetate as the marker for cell membrane lipid synthesis. These tracers have limited global availability and are used in only a few centers, primarily for research purposes. New tracers, such as ^{68}Ga-pentixafor for multiple myeloma and labeled antibodies, have demonstrated promising results and are currently undergoing various research and clinical trials. Chemokine receptor CXCR4 is overexpressed in certain tumors, especially lymphoproliferative diseases (e.g., lymphoma and multiple myeloma), and various agents, including ^{68}Ga-pentixafor, are used to target them.[48] Exendin-4 is similar to glucagon-like peptide-1 (GLP-1), which binds to pancreatic β-cells, and ^{68}Ga-DOTA-exendin-4 PET is utilized for imaging insulinoma.[49] Glypican 3 (GPC3) is a glycoprotein labeled with ^{68}Ga, displaying a high uptake in hepatocellular carcinoma on PET scans, with very low expression in normal tissue and hepatitis.[50] These tracers have proven to be beneficial in various tumors as described

above; however, they are still under investigation, and their role in tumor heterogeneity remains undefined.

7. Clinical applications of PET/CT in tumor heterogeneity

7.1. Diagnosis and initial staging and treatment planning

Diagnosing and staging cancer can be complex and challenging due to the considerable biological, cellular, and tissue variation within tumors. This diversity occurs not only between cancer cells in an individual patient but also between tumors in different patients. Such heterogeneity complicates choosing appropriate treatment plans, as certain therapies may not be effective against different clones of cancer cells. The use of multiple PET radiotracers offers a solution by enabling visualization of the various parts of tumor cells and their microenvironments. In addition, PET scans provide full-body, non-invasive imaging in a solitary session, which is highly beneficial for detecting tumor heterogeneity. ^{18}F-FDG PET scans can diagnose heterogeneity by analyzing non-homogenous tracer distribution in the tumor sites with areas of high and low uptake. Similarly, applying multiple PET tracers can help identify tumor diversity, as different lesions may absorb various tracers differently, depending on the underlying biology of the tumor cells.

Various quantitative parameters (e.g., SUV, TLG, and MTV) further assist in diagnosing heterogeneity and assessing disease prognosis. Tracer uptake patterns may reveal the presence of different cancer cell clones, each with varying levels of aggressiveness, which can influence the overall prognosis of the patient. In addition, due to significant heterogeneity, the same treatment regime may not be equally effective on different clones of cancer cells and can lead to treatment failure or resistance. It has been reported that low-grade tumors exhibit lower SUV values on ^{18}F-FDG PET scans, while higher-grade tumors have high SUV values.[51] In addition, some lesions with low FDG uptake may exhibit high uptake when imaged with different tracers. Research conducted by Marusyk *et al.*[9] and Zhou *et al.*[52] has demonstrated that the integration of several tracers, including ^{18}F-FDG and ^{68}Ga-FAPI, enhances the visualization of tumor heterogeneity, uncovering metabolic and stromal variations that influence diagnostic and staging precision.[9,13,51,52] Therefore, combining multiple tracers in PET scans provides a more comprehensive view of all lesions and enhances the detection of tumor heterogeneity.

7.2. Monitoring treatment response

Heterogeneity in cancer leads to significant challenges in treatment response and its assessment and influences outcomes. Heterogeneity can be spatial (as described above) or temporal, which develops over time. Since the development of heterogeneity is a dynamic process and may develop temporally, serial PET/CT scans during the treatment process may provide diagnostic insights. As the disease progresses, the treatment-resistant clones of cancer cells continue growing, leading to disease progression; consequently necessitating a change in therapy regime. For example, breast cancer patients display significant intratumoral and interpatient heterogeneity due to the presence of various molecular subtypes. The tumor cells and their metastases express dynamic changes in their molecular subtypes over time, which affects treatment response to neoadjuvant therapy. These variations can be identified on PET/CT scans, thereby providing critical insights into patient management.[53]

Spatiotemporal heterogeneity is often seen in gastrointestinal tumors, as cells change their characteristics during metastases to different anatomical sites over time. The RECIST and PERCIST guidelines designed to assess response on radiological and PET imaging are not suitable for assessing response with targeted drugs, as the response is lesion- or organ-specific. Zhou *et al.*,[52] in their study on colorectal tumors, reported a better response in hepatic metastases compared to lungs and lymph nodes when treated with targeted therapies. In another study by Schmid *et al.*[53] on lung cancer patients treated with immunotherapy, an organ-specific response was observed, with better response in lymph nodes compared to liver, bone, and adrenal glands. Heterogeneity is associated with prognosis, overall progression-free survival, and overall survival, thus it is related to drug efficacy. Therefore, better strategies for diagnosis and response assessment to address heterogeneity are needed for cancer management.

7.3. Tumor heterogeneity as a cause of mixed response in patients on systemic therapies

Tumor heterogeneity frequently leads to difficulties in treatment and poor outcomes. In numerous cases, follow-up PET/CT scans after systemic therapy reveal a mixed response, where both responding and progressing cells (i.e., resistant to treatment) coexist inside the patient. This combined response reflects the heterogeneous characteristics of different tumor lesions. This heterogeneity is either present from the beginning or develops temporally during treatment, where some cancer cells become resistant to treatment. During therapy, the treatment-sensitive subclones of heterogenous tumors respond, while the resistant subclones continue growing and form tumors, leading to the detection of a mixed response on PET/CT scans. In a study on lung cancer, Zhong *et al.*[54] highlighted the high incidence of mixed response in lung

cancer treated with targeted therapies. They concluded that mixed response is a poor prognostic factor and occurs due to inter-/intratumor heterogeneity.[54] Other studies have also demonstrated heterogeneity as a reason for poor prognosis and treatment failure.[3,55,56] ^{18}F-FDG PET/CT is also becoming a reliable tool for evaluating the response of tumors to immunotherapy. Takao *et al.*[57] evaluated lesion characteristics in patients with mixed response to immunotherapy and found a significant difference between clonality and tumor-infiltrating lymphocytes between primary lesions and lymph node metastases. This heterogeneity leads to mixed response and progression in lymph nodes with an overall poor prognosis.[57,58] A mixed response demonstrating the dynamic nature of tumor heterogeneity is presented in Figure 2.

8. Advanced techniques and future directions

8.1. Radiomics and texture analysis

Radiomics has recently become an area of substantial focus in medicine due to its potential to extract vast amounts of quantitative data from medical images that are difficult to reveal or quantify with the naked human eye. Radiomics features, such as texture, shape, and intensity, are strongly correlated with tissue heterogeneity and cancer aggressiveness, making it a powerful tool for diagnosing and characterizing tumors. In the domain of nuclear medicine, radiomics has been extensively applied to PET and SPECT scans, providing crucial insights into a patient's prognosis, treatment response, and tumor biology.[14,59,60]

Radiomics analysis of PET/CT images focuses on several key parameters, including:

(i) SUV: Reflects the metabolic activity of tumors and is widely used to assess tumor aggressiveness.
(ii) Total lesion glycolysis (TLG): Combines metabolic activity and tumor volume, providing a more comprehensive measure of tumor burden.
(iii) Metabolically active tumor volume (MTV): Quantifies the volume of tumor tissue with active glucose metabolism, which is useful for assessing tumor heterogeneity and treatment response.[14,59]

Texture analysis is a critical component of radiomics, enabling the quantification of spatial variations in tracer uptake within tumors. By analyzing various features, such as shape, size, volume, compactness, and sphericity, radiomics can extract information at the cellular level. These features can reveal subtle patterns indicative of tumor aggressiveness, treatment resistance, or response to therapy. For example, heterogeneous texture patterns on PET/CT scans have been associated with poorer prognosis and higher tumor aggressiveness in various cancers, including lung, breast, and head and neck cancers.[59]

8.2. Radiomics workflow

The radiomics workflow begins with the computerized extraction of features from the region of interest

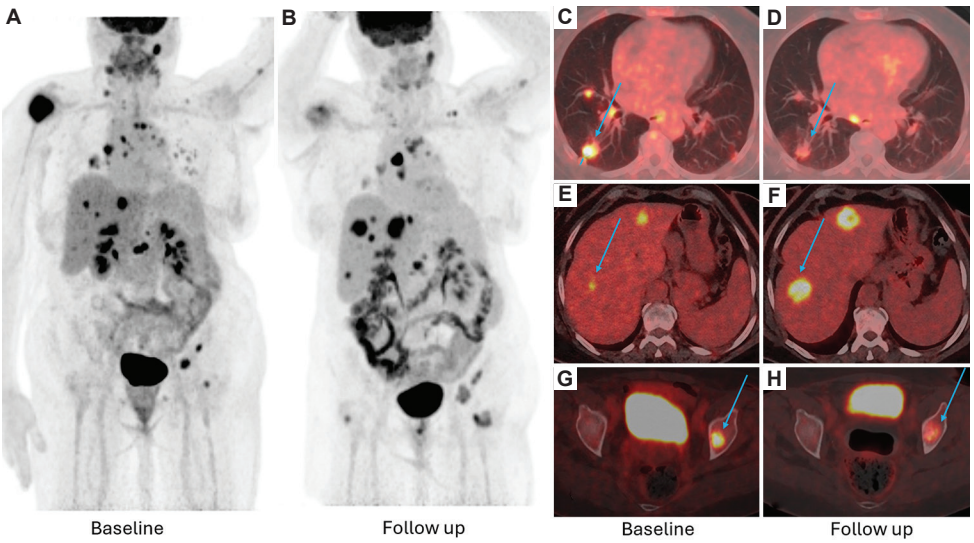

Figure 2. Positron emission tomography/computed tomography (PET/CT) imaging of a 60-year-old woman with lung cancer with multiple metastases. (A and B) Maximum Intensity Projection (MIP) images of ^{18}F-FDG-PET/CT at baseline (A) and ^{18}F-FDG-PET/CT at follow-up after three cycles of chemotherapy (B) display mixed responses in multiple metastases. (C–H) Fused PET/CT images at baseline (C, E, and G) and after chemotherapy (D, F, and H) display responses in lung and bone metastases and progression in liver metastases. Blue arrows indicate metastatic lesions in the lung, liver, and bone.
Abbreviation: FDG: ^{18}F-fluorodeoxyglucose.

(ROI) in medical images. Ensuring the robustness and reproducibility of these features is paramount, as variations in imaging protocols or segmentation methods can affect the results. After feature extraction, optimal features are selected, and AI-based models are incorporated to analyze the data. These models can predict clinical outcomes, such as treatment response or survival, based on the extracted radiomic features. For instance, AI-driven radiomics models have demonstrated promise in predicting responses to immunotherapy and chemotherapy in patients with non-small cell lung cancer (NSCLC) and breast cancer.[60]

8.3. Applications in tumor heterogeneity

Radiomics plays a significant role in characterizing tumor heterogeneity, which is a major challenge in cancer management. By analyzing spatial and temporal variations in tracer uptake, radiomics can identify subregions within tumors that exhibit different biological behaviors. This information is crucial for personalized treatment planning, as it allows clinicians to target aggressive tumor regions more effectively. For example, studies have demonstrated that radiomic features derived from ^{18}F-FDG PET/CT scans can predict intratumoral heterogeneity and guide radiotherapy planning by identifying regions with high metabolic activity.[14,59]

8.4. Future directions of radiomics

The integration of radiomics with AI and machine learning is expected to further enhance the diagnostic and predictive capabilities of PET/CT imaging. Advanced algorithms, such as convolutional neural networks (CNNs), can automatically segment tumors and classify them based on molecular subtypes with high accuracy. In addition, radiomics combined with multi-tracer PET imaging (e.g., ^{18}F-FDG and ^{68}Ga-PSMA) can provide a more comprehensive understanding of tumor heterogeneity, enabling more precise and personalized cancer management.[59]

9. AI and machine learning applications

The field of PET/CT imaging is rapidly evolving, with radiomics and AI emerging as powerful tools for enhancing tumor characterization and treatment planning. Incorporating radiomics with AI can develop models that will lead to detailed analysis of medical images. Radiomics offers a means to capture tumor heterogeneity beyond what is visually apparent by the high-throughput extraction of quantitative features from medical images. Texture analysis, a key component of radiomics, can quantify spatial variations in tracer uptake, potentially revealing subtle patterns indicative of tumor aggressiveness or treatment resistance.

Machine learning algorithms and AI, when applied to these radiomic features, can identify complex patterns and associations that may predict treatment outcomes or guide personalized therapy decisions. For example, CNNs have demonstrated the ability to automatically segment tumors and classify them based on molecular subtypes with high accuracy. In addition, AI-driven evaluation of PET/CT scans combined with clinical information can generate predictive analytics forecasting treatment outcomes and patient prognosis.

These advanced techniques are paving the way for more precise and personalized cancer management, potentially allowing for early identification of treatment resistance and enabling adaptive treatment strategies. However, it is important to note that while these methods are promising, they still require extensive validation in large, multi-center studies before widespread clinical implementation. The integration of AI and deep learning with conventional PET/CT analysis represents a major step toward unlocking the full diagnostic and predictive power of molecular imaging in oncology.[61-63]

10. Challenges and limitations of PET/CT

Future developments in PET/CT technology and the expanding knowledge of tumor heterogeneity will undoubtedly improve the diagnosis, restaging, and response prediction of new radiopharmaceuticals. However, the application of PET/CT with new radiopharmaceuticals in everyday clinical practice is also constrained by many challenges:

(i) Cost and accessibility: PET/CT scans are expensive, and the cost can be prohibitive for many patients, especially in low-resource settings. The high cost of radiopharmaceuticals, coupled with the need for specialized equipment and facilities, limits the widespread availability of this technology. Addressing disparities in access to advanced PET imaging technologies is crucial to ensure equitable healthcare delivery.[13,15]

(ii) Time and workflow: PET/CT scans require significant preparation, imaging, and interpretation time. The synthesis of radiopharmaceuticals, patient preparation, and the scanning process itself can be time-consuming, which may delay treatment decisions. In addition, the need for multiple scans to assess tumor heterogeneity further complicates the workflow.[13,17]

(iii) Radiation exposure: PET/CT scans involve exposure to ionizing radiation, which raises concerns, especially for patients requiring frequent follow-up scans. While the radiation dose is generally considered safe, cumulative exposure over time can increase the risk

of secondary malignancies, particularly in younger patients.[13,15]

(iv) Availability of radiopharmaceuticals and trained personnel: The production and distribution of radiopharmaceuticals are complex and require specialized facilities. Many regions lack the infrastructure to produce these tracers, thereby limiting availability. In addition, the interpretation of PET/CT scans requires highly trained personnel, including nuclear medicine physicians and radiologists, which may not be available in all healthcare settings.[13,15]

(v) False positives and specificity: PET tracers can display false positivity in inflammatory and benign conditions, leading to potential misinterpretation and overdiagnosis. For example, ^{18}F-FDG, the most commonly used tracer, can accumulate in areas of infection or inflammation, mimicking malignant lesions. This lack of specificity can complicate treatment decisions and lead to unnecessary interventions.[13,17]

(vi) Ethical considerations and over-reliance on imaging: There is a growing concern about the over-reliance on imaging in cancer management, which may lead to overtreatment or unnecessary interventions. Ethical considerations, such as the balance between the benefits of early detection and the risks of overdiagnosis, need to be carefully weighed. In addition, the psychological impact of frequent imaging and the potential for false positives should be considered in patient management.[13,17]

(vii) PET/CT images have limited spatial resolution, and other factors, including scatter and attenuation artifacts, may lead to noisy images that adversely affect image analysis, even with AI. PET tracer uptake variability, due to various factors, including breathing artifacts, misregistrations, and truncation artifacts, may also be added to the challenges.[13]

11. Future directions

To overcome these limitations, ongoing research is focused on developing more cost-effective and widely available radiopharmaceuticals, reducing radiation exposure, and improving the specificity of PET tracers. Advances in AI and radiomics may also help streamline the interpretation process, reducing the reliance on highly specialized personnel. Furthermore, efforts to standardize protocols and improve access to PET/CT technology in underserved regions are essential to ensure equitable cancer care.[62]

12. Conclusion

Tumor heterogeneity is a complex and dynamic phenomenon that poses significant challenges for cancer diagnosis and treatment. PET/CT imaging, with its ability to provide metabolic and molecular insights, plays a crucial role in assessing tumor heterogeneity and guiding personalized treatment strategies. However, multiple PET/CT scans are coupled with multiple challenges which must be carefully considered. Despite these challenges, PET/CT remains a powerful tool in diagnosing heterogeneity and cancer management. Future developments in radiomics, AI, and novel tracers hold great promise for overcoming these challenges and improving patient outcomes.

Acknowledgments

None.

Funding

None.

Conflict of interest

The authors declare that they have no competing interests.

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Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Availability of data

Not applicable.

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