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Radiolabeled EGFR TKI as predictive imaging biomarkers in NSCLC patients – an overview

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Non-small cell lung cancer (NSCLC) has one of the highest cancer-related mortality rates worldwide. In a subgroup of NSCLC, tumor growth is driven by epidermal growth factor receptors (EGFR) that harbor an activating mutation. These patients are best treated with EGFR tyrosine kinase inhibitors (EGFR TKI). Identifying the EGFR mutational status on a tumor biopsy or a liquid biopsy using tumor DNA sequencing techniques is the current approach to predict tumor response on EGFR TKI therapy. However, due to difficulty in reaching tumor sites, and varying inter- and intralesional tumor heterogeneity, biopsies are not always possible or representative of all tumor lesions, highlighting the need for alternative biomarkers that predict tumor response. Positron emission tomography (PET) studies using EGFR TKI-based tracers have shown that EGFR mutational status could be identified, and that tracer uptake could potentially be used as a biomarker for tumor response. However, despite their likely predictive and monitoring value, the EGFR TKI-PET biomarkers are not yet qualified to be used in the routine clinical practice. In this review, we will discuss the currently investigated EGFR-directed PET biomarkers, elaborate on the typical biomarker development process, and describe how the advances, challenges, and opportunities of EGFR PET biomarkers relate to this process on their way to qualification for routine clinical practice.

KEYWORDS
NSCLC, EGFR TKI, PET/CT, radiolabeled EGFR TKI, molecular imaging

1 Introduction

Lung cancer is one of the most prevalent cancer types worldwide (1). Lung cancer accounts for approximately 22% of all cancer-related mortality, emphasizing that lung cancer is not only a highly prevalent cancer type, but also one of the deadliest (1). For decades, the standard of care treatment for advanced stage non-small cell lung cancer

(NSCLC) was only chemotherapy (2–5). The introduction of tyrosine kinase inhibitors (TKI) directed against the epidermal growth factor receptor (EGFR), an oncogenic driver pathway promoting cell growth and division, led to a shift in the treatment paradigm of EGFR mutation positive NSCLC, and, ultimately to an acceleration of the development of targeted therapies against other oncogenic driver mutation targets (2–5). Wild type EGFR activation is ligand-dependent, i.e., the EGFR kinase function only activates if an EGF ligand is bound at the extracellular binding site of the receptor (6). However, with activating mutations in the EGFR kinase domain, activation occurs in the absence of a ligand, leading to tumor cell proliferation and growth (6). EGFR TKIs bind with high affinity at the kinase domain of the mutated EGFR and block its function (6, 7). As a result, patients harboring activating EGFR mutations achieve higher tumor responses on EGFR TKI than on conventional chemotherapy (2–4, 8).

The iPASS trial was the first trial that clearly showed the superior clinical efficacy of EGFR TKI as compared to conventional chemotherapy. In this study, Mok et al. demonstrated that the first-generation EGFR TKI gefitinib achieved a higher progression-free survival (PFS) in the intention-to-treat population (HR 0.74; 95%CI 0.65 to 0.85; P<0.001) (3). Many other first-line phase 3 clinical studies using the first-generation EGFR TKI gefitinib or erlotinib, showed comparable results (2, 4, 9, 10). In contrast to the first-generation TKIs, the second-generation TKIs afatinib and dacomitinib were characterized by an irreversible binding of the TKI to the EGFR kinase domain and by multi-kinase targeting (5, 10–15). These second-generation TKIs had possibly a superior efficacy as compared to first-generation TKI at the cost of slightly higher toxicities (10, 16). The third-generation TKI osimertinib was primarily designed to target the secondary resistance mutation T790M (17–21). In the AURA3 trial, patients with T790M secondary mutations, occurring as resistance mutations on an initial treatment with gefitinib or erlotinib, were randomized between osimertinib versus conventional chemotherapy (17). Osimertinib showed superior PFS (10.1m vs. 4.4m; HR 0.30; 95%CI 0.23 to 0.41; P<0.001). The objective response rate was also significantly better with osimertinib (71%; 95% CI, 65 to 76) than with chemotherapy (31%; 95% CI, 24 to 40) (OR 5.39; 95%CI 3.47 to 8.48; P<0.001) (17). Surprisingly, osimertinib also performed above expectations as a first-line therapy. In the FLAURA study, treatment-naïve EGFR mutation positive patients were randomized to osimertinib versus a first-generation EGFR TKI (22). Osimertinib showed superior PFS (18.9m vs. 10.2m; HR 0.46; 95%CI 0.37 to 0.57; P<0.001). In a recent update of the study results, osimertinib also showed OS superiority as compared to the first-generation TKI (38.6m vs. 31.8m; HR 0.80; 95%CI 0.64 to 1.00; P=0.046) (23). These developments illustrate that over the course of approximately a decade, significant advances have been made in the treatment of EGFR mutation positive NSCLC, and that the identification of these patients is of paramount importance.

Diagnosis through next-generation sequencing of tumor DNA, obtained through a histological biopsy, is the gold standard for identifying tumor EGFR mutations (24). Unfortunately, taking biopsies is invasive, at risk for complications and not always possible due to difficult to reach tumor sites. Also, biopsies may not always be representative for all the tumor lesions due to varying intra- and interlesional heterogeneity, this may especially be of importance when resistance occurs and mapping the residual sensitivity for TKI treatment is needed (24). To overcome these limitations new biomarkers have been investigated. Liquid biopsies are ever more used in situations when representative tumor biopsies cannot be obtained. Even though the current sensitivity of liquid biopsies is approximately 70% with specificities above 90%, not all patients can be diagnosed using liquid biopsies alone (25, 26). Also, liquid biopsies do not address the limitation of tumor heterogeneity. Alternatively, in recent years, imaging studies using radiolabeled EGFR TKI have shown that PET could potentially be of value for identifying EGFR mutation positive patients and predicting tumor sensitivity to EGFR TKI (27–31).

In this review, we will discuss the current EGFR-directed PET tracers that have been investigated in EGFR mutated NSCLC. The special focus will lie with radiolabeled EGFR TKI: inertly labeled EGFR TKI used as a PET tracer in NSCLC patients. In addition, we will discuss the framework of the PET biomarker development process, highlighting the different contexts of use to better elucidate the stage in which these EGFR TKI PET biomarkers are at. We will describe the challenges, but also the recent advances and opportunities that could help EGFR PET on its path to generating qualified predictive biomarkers for clinical use.

2 Current EGFR PET biomarkers

2.1 PET biomarker background

PET is a molecular imaging technique, widely in use in the staging and treatment monitoring schedules in cancer patients. A radioactively labeled compound used as a tracer, which is expected to accumulate at the site of a specific target in the tumor lesion, is injected into the body and its distribution is then imaged. When using a validated tracer, its accumulation in the tumor and other sites is expected to be sensitive and quantifiable. The tracer accumulation or the so-called tracer uptake can be measured using different metrics, which can serve as biomarkers.

In general, a biomarker is a measurable indicator of a biological process and in case of PET imaging, this can be a

measure derived from the tracer uptake in tumors or in healthy tissues, e.g., the Standardized Uptake Value (SUV) or the Distribution Volume (V_T).

Also, depending on their aims, biomarkers will have different 'contexts of use'. The evidence that is necessary to support qualification towards clinical practice is dependent on the specific context of use. The FDA Qualification Framework recommends categorizing biomarkers using the BEST biomarker categories according to their aims, as described in Figure 1 (32).

Considering EGFR, PET should provide a *predictive biomarker*, which is most relevant for the clinical practice. The presence of common EGFR mutations (i.e., exon19 deletions, exon21 L858R) are highly predictive for response to TKI therapy; however, in case of uncommon mutations, less is known regarding their clinical relevance and tumor TKI responses may vary greatly between different uncommon mutations. A predictive PET biomarker would therefore be most interesting.

EGFR directed PET biomarkers will *de facto* never be able to diagnose an activating EGFR mutation, as this requires tumor DNA sequencing on tumor tissue or liquid biopsies. Therefore, a PET imaging biomarker could never be a *diagnostic biomarker* that replaces DNA sequencing. On the other hand, PET imaging biomarkers could very well become qualified as predictive biomarkers to predict tumor sensitivity to EGFR TKI as mentioned before.

A *monitoring biomarker* is also of interest, as all tumors eventually develop resistance to EGFR TKI, in which case it could be of clinical importance to know whether lesions or parts of lesions remain TKI sensitive to decide whether TKI should be continued beyond progression.

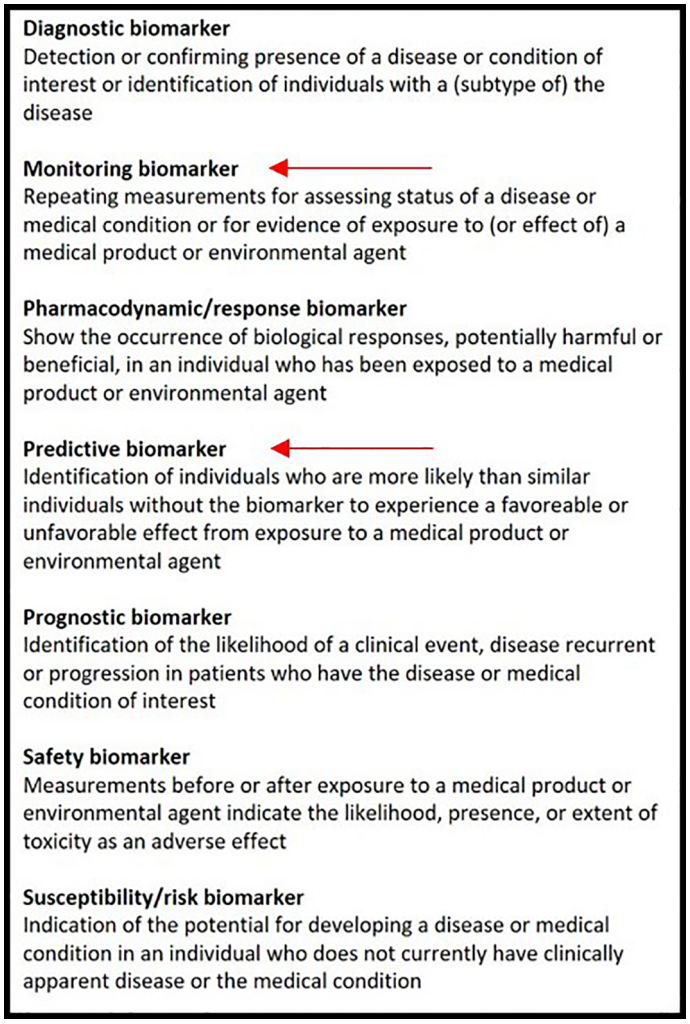

FIGURE 1
The biomarker classification according to the BEST biomarker categories. The red arrows indicate in which category EGFR-directed PET tracers could be included.

The current PET biomarkers can be categorized into 2 categories, i.e., those based on non-EGFR-directed tracers and those that are derived from EGFR TKI-based tracers.

2.2 Non-EGFR PET biomarkers in EGFR mutated NSCLC

The most widely-used tracer is ^{18}F-fluorodeoxyglucose (^{18}F-FDG), a radioactive analogue to glucose, that can quantify metabolic activity. In the past decade, many clinical studies attempted to establish the role of ^{18}F-FDG in evaluating the EGFR mutational status (33). A meta-analysis by Du et al. looked at studies that compared the lesional maximum of standardized uptake value (SUV_{max}) of ^{18}F-FDG uptake between wild-type and mutant EGFR and evaluated its value for predicting the EGFR status in NSCLC patients (33). In 15 studies (3574 patients), the pooled sensitivity and specificity was found to be low. The authors concluded that ^{18}F-FDG based SUV_{max} should be used with caution when predicting EGFR mutations in NSCLC (33). However, new studies are exploring the potential outcome of radiomics and artificial intelligence (AI) algorithms as biomarkers to assess the predictive capacity of ^{18}F-FDG PET. For example, Yin et al. demonstrated in a training data set of 198 NSCLC patients with a testing data set of 103 patients that their algorithm could predict EGFR mutations automatically with a ROC-AUC of 0.84 (95% CI, 0.75–0.90) (34). These developments may indicate an increasing role for radiomics and AI as new ^{18}F-FDG based biomarkers in the future, albeit, these algorithms need optimization and validation using larger cohorts.

In recent years, 3-deoxy-3-^{18}F-fluorothymidine (^{18}F-FLT) PET scans have generated interest in oncology. As opposed to ^{18}F-FDG, ^{18}F-FLT PET reflects cell proliferation (10, 35). This tracer is trapped intracellularly in the S-phase of the cell cycle (35). Elevated ^{18}F-FLT uptake of lesions could therefore be indicative of tumor cell proliferation and treatment-resistance. This supports the notion that ^{18}F-FLT could serve to generate treatment monitoring biomarkers. Indeed, studies using ^{18}F-FLT in EGFR mutation positive NSCLC have shown that a decrease of ^{18}F-FLT uptake in tumor lesions is associated with response to EGFR TKI treatment (10, 36, 37). As ^{18}F-FLT is nonspecific to EGFR mutations, the validation of ^{18}F-FLT-based monitoring biomarkers could be of interest for many cancer types as well.

Other non-EGFR PET tracers that have been investigated in EGFR mutation positive NSCLC patients, are ^{11}C-choline and *O*-(2-[^{18}F]fluoroethyl)-L-tyrosine(^{18}F-FET). ^{11}C-choline, a tracer mainly used in diagnostics of prostate cancer, is a component of phospholipids in the cell membrane (38). Phosphorylation of choline is upregulated in cancers through choline-kinase (38). Although ^{11}C-choline PET is used in the routine practice in other cancer types, results in NSCLC are discouraging (39–41). ^{18}F-FET has been used in diagnostics of brain tumors, including brain metastases of NSCLC, however, no studies were published on ^{18}F-FET in extracranial NSCLC tumors (42, 43).

2.3 EGFR PET biomarkers

2.3.1 Characteristics of EGFR PET tracers

For radiolabeling target-specific drugs such as EGFR TKI, the characteristics of the radionuclide that is used for labeling needs to be aligned with the pharmacokinetic properties of the parent compound. For example, using radionuclides with long-lived isotopes such as zirconium-89 ($t_{1/2}$ 78 hours) are best suited to label large molecules with slow pharmacokinetics like monoclonal antibodies, e.g., ^{89}Zr-cetuximab, however, inappropriate for labeling EGFR TKI. Since EGFR TKI are small molecules with relatively fast pharmacokinetics, i.e., fast target binding and rapid clearance from the circulation, using short-lived isotopes such as carbon-11 (t½ 20 min) or fluorine-18 (t½ 110 min) is more appropriate.

Also, instead of adding the radionuclide on the parent compound, substituting an existing carbon or fluorine atom of the TKI molecule will maintain the original pharmacokinetic (PK) behavior of the TKI resulting in a tracer that is equally specific as the original TKI. The choice whether carbon-11 or fluorine-18 is used for this inert substitution is based on the chemical structure of the parent compound (27, 31, 44).

Although tracers based on EGFR TKI that are in clinical use, when labeled inertly, provide the best PK behavior metrics to investigate tumor sensitivity to the respective TKI, the development of such tracers is inherently delayed, as clinical safety and efficacy data of the parent TKI need to be established. Moreover, the fast development of subsequent generations of TKI could disrupt the development of early generation TKI tracers and make them redundant. To illustrate this, a timeline indicating the approval of the 3 generations of EGFR TKI used in the clinical and their tracer counterparts is shown in Figure 2.

Clinical PET studies are not only being performed using EGFR PET tracers based on EGFR TKI, but also on tracers without treatment analogue. Many of these tracers without direct treatment analogue have been specifically developed for the purpose of imaging. These tracers, e.g., ^{18}F-MPG, ^{11}C-PD153035 and ^{18}F-IRS, show significant differences amongst themselves in kinetic characteristics, mainly in the binding affinity to the kinase domain (45–47).

2.3.2 Present EGFR TKI PET biomarkers

An overview of published clinical studies using EGFR PET tracers is given in Table 1. For ^{11}C-erlotinib and ^{18}F-afatinib, studies have shown that EGFR mutation positive patients can be identified and that tumor response to

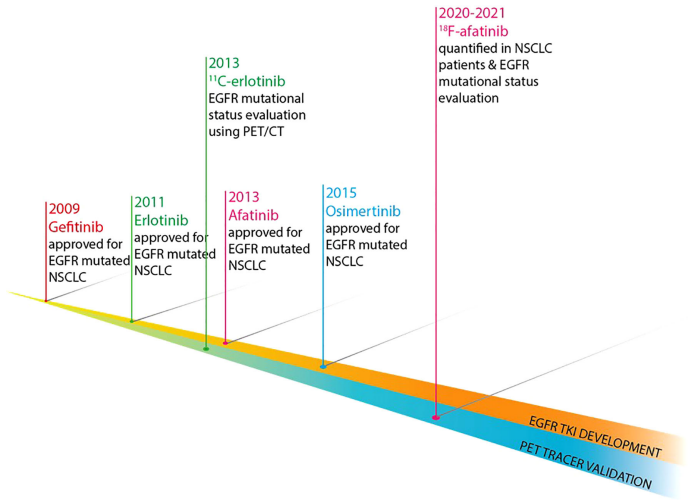

FIGURE 2
Development timeline of EGFR TKI and their respective EGFR-directed PET tracers. .

treatment using the corresponding EGFR TKI (27, 31) could be predicted using PET biomarkers. This was seen in patients with common and uncommon EGFR mutations. For ^{11}C-osimertinib, the clinical studies investigating its predictive value are still ongoing.

For EGFR PET tracers without treatment analogue, e.g., ^{18}F-MPG, ^{11}C-PD153035 and ^{18}F-IRS, studies have shown that tumor tracer uptake could be quantified and that this was predictive for the presence of an EGFR mutation and for TKI therapy response (45–47). Both ^{18}F-IRS and ^{11}C-PD153035

TABLE 1 Overview of clinical EGFR TKI PET studies.

Year	Tracer	N	Uptake parameter	Kinetic modeling?	Used as biomarker for EGFR status?	EGFR mutation in studies	Study
2008	^{11}C-PD153035	11	SUV	No	No	Exon 19 & exon 21 mutations	Yu et al. (48)
2009	^{11}C-PD153035	14	SUV_{max}	No	Yes	Exon 19 & exon 21 mutations	Yu et al. (49)
2010	^{11}C-PD153035	19	SUV_{max}	No	No	Unknown	Liu et al. (50)
2011	^{11}C-PD153035	21	SUV_{max}	No	No	Unknown	Meng et al. (45)
2011	^{11}C-Erlotinib	13	Radioactivity per mL tissue	No	No	Unknown	Memon et al. (51)
2013	^{11}C-Erlotinib	10	V_T	Yes	Yes	Exon 19 del	Bahce et al. (27)
2017	^{18}F-IRS	3	SUV_{max}	No	Yes	Exon 19 del	Song et al. (47)
2018	^{18}F-MPG	75	SUV_{max}	No	Yes	Unknown	Sun et al. (46)
2018	^{18}F-ODS2004436	20	SUV_{ratio}	No	Yes	Unknown	Cochet et al. (52)
2021	^{18}F-Afatinib	12	TBR_WB_{60-90}	Yes	Yes	Exon 19 deletion, exon 19 L747P insertion, exon 18 G719A point mutation, exon 18 G709T deletion	van de Stadt et al. (30)
2021	^{11}C-erlotinib	10	V_T & SUV_{mean}	Yes	Yes	Exon 19 deletion, L858R point mutation, G719S + S768I mutation, L861Q mutation	Petrulli et al. (53)

The EGFR mutational status as described in the study is shown.
SUV, standardized uptake value; V_T, volume of distribution; TBR_WB_{60-90}, tumor-to-whole-blood ratio in the time interval 60-90 minutes post-injection.

showed a close relation between tracer uptake (SUV_{max}) and EGFR expression, and for all three tracers a correlation between uptake (SUV_{max}) and treatment response was observed (45–47).

The overview in Table 1, comprising approximately 200 NSCLC patients, summarizes several study characteristics. When new tracers are introduced, the pharmacokinetic behavior of this tracer needs to be established by performing kinetic modeling. Kinetic modeling allows to better understand the obtained PET images and to quantify the tracer uptake using optimal dynamic parameters of uptake such as 'Distribution Volume' (V_T). For some tracers, this has been performed, as indicated in Table 1. In the absence of dynamic uptake parameters, usually simplified static uptake parameters such as 'Standardized Uptake Values' (SUVs) are used. For some tracers such as ^{11}C-erlotinib and ^{18}F-afatinib, the pharmacokinetic modeling has been published and, in these tracers, uptake parameters other than SUV have been suggested (29, 30, 53). In Table 2, tracer targets are listed for each tracer.

While this overview highlights the efforts done to investigate and discover the potential of the existing EGFR PET tracers and their biomarkers, it also highlights that data is scarce. From a clinical point of view, the question rises on what would be needed for EGFR PET biomarkers to be able to qualify in the routine clinical practice. To better understand the framework in which such a qualification occurs, we will below elaborate on the typical biomarker development process and how the current state of these tracers and their respective biomarkers relate to this process.

3 Challenges and opportunities in the development of EGFR PET biomarkers

3.1 Development process of PET biomarkers

To be able to qualify for use in the clinical practice, there are 3 main phases of development that a PET imaging biomarker must transition. See Figure 3, which is based on the consensus paper of the CRUK and the EORTC (59). In transitioning from one phase into another, biomarkers need to bridge several gaps. The first gap for a biomarker is to be able to enter the validation phase as a potential biomarker, fit to be tested for performance. In the validation phase, a biomarker needs to proof it is reliable and 'fit for purpose'. For the development of PET biomarkers, the 3 main validation tracks (analytical, clinical and cost-effectiveness validation) are typically developed in parallel and in an iterative manner. In the qualification phase, sufficient evidence will be needed to support the qualification of a biomarker for a specific context of use in drug development or routine clinical care. support qualification of a biomarker.

3.2 EGFR PET biomarker validation challenges

3.2.1 Analytical validation

The analytical validation track evaluates the measures related to biomarker precision, e.g., repeatability, reproducibility and technical bias, and the measures related to biomarker availability in the targeted patient group. The analytical validation, generally, does not consider the clinical utility of the biomarker, however, poor analytical features will hamper the clinical validation and qualification (59).

Ideally, new EGFR PET tracers for biomarking EGFR that are used in humans will undergo full kinetic modeling. This is an elaborate dynamic PET scanning procedure with arterial blood sampling and measurement of blood radioactivity and blood tracer metabolites. A dynamic PET scan is a continuous scan of 1 section of the body, where both the tumor and a large blood pool or vessel is included in the field of view (FoV), as depicted in Figure 4. Since conventional PET scanners have a limited (e.g., 18 cm) FoV, only a small part of the body where the tumor is located will be scanned continuously. The pharmacokinetic behavior over time of the tumor tracer concentration will be measured to produce a time-activity-concentration curve (TAC). Additionally, the radioactivity

TABLE 2 Key tracer targets for each tracer are shown.

Tracer	Key tracer targets	
^{11}C-PD153035	EGFR (wild-type and mutations), HER2	(54)
^{11}C-erlotinib	1st-generation TKI: common EGFR mutations (exon 19del, exon 21 L858R), partly wild-type EGFR, not T790M	(7)
^{18}F-IRS	Comparable to 1st-generation TKI: common EGFR mutations, no T790M	(48)
^{18}F-MPG	Common EGFR mutations, not wild type EGFR, not T790M	(47)
^{18}F-ODS2004436	Limited data is publicly available, targets wild type and exon 21 L858R, not T790M	(55, 56)
^{18}F-afatinib	2nd-generation TKI: common EGFR mutations (exon 19del, exon 21 L858R) + other ERBB family kinases, partly T790M	(57–59)
^{11}C-osimertinib	3rd-generation TKI: specifically developed for EGFR T790M mutation, common EGFR mutations, also uncommon non-exon20 insertions, not wild type EGFR	(20, 23)

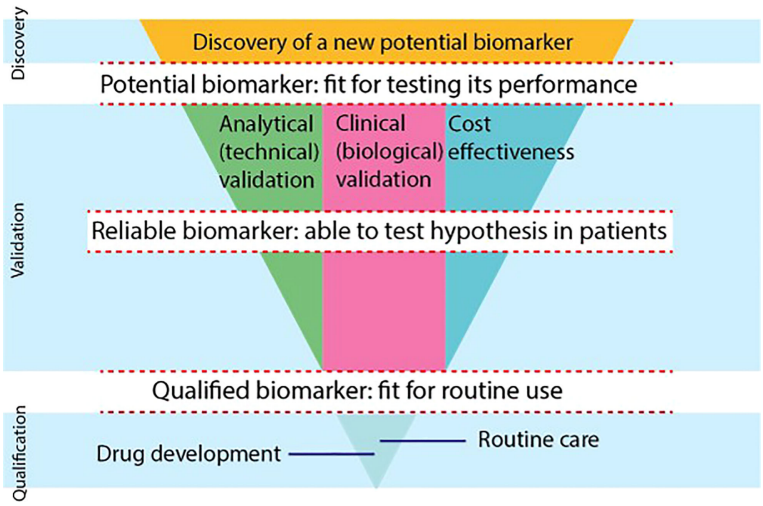

FIGURE 3
The biomarker development process is shown using a modified scheme, based on the consensus statement on biomarker development of the CRUK and EORTC (59). There are 3 phases of development (discovery, validation, qualification) that biomarkers go through. Biomarkers need to overcome gaps to become potential biomarkers, reliable biomarkers and qualified biomarkers. In the validation phase, 3 separate tracks will be evaluated in parallel and iteratively, i.e., the analytical, clinical and cost-effectiveness track. To be able to use a biomarker in drug development or in routine clinical care, biomarkers need to provide qualification evidence. (*) The FDA Evidentiary Framework provides recommendations that guide the evidence needed to support qualification, bridging the final gap to routine care and drug development in the qualification phase.

concentrations of the arterial blood pool over time will be measured to calculate the so-called blood 'input functions' using both blood samples from an arterial cannula, and PET image-derived blood pool data. Also, metabolites will be measured repeatedly *via* arterial blood samples to calculate the true parent tracer concentrations over time. Using the TACs, the blood input function and the metabolites data, the pharmacokinetic model that best describes the pharmacokinetic behavior of the tracer in the tumor will be established. This pharmacokinetic model yields various physiologic parameters, which can be used to select the optimal tracer uptake parameter to quantify the tracer uptake. These dynamic uptake parameters are considered the most precise biomarkers for tracer uptake. Only a few EGFR PET tracers such as ^{11}C-erlotinib and ^{18}F-afatinib have undergone full kinetic modeling.

To evaluate intra-patient repeatability is another step in the analytical validation of a biomarker to assure that biomarkers produce similar results when repeatedly measured in the same circumstances. This has been shown for tumor ^{11}C-erlotinib V_T, however, this crucial step is lacking in many other tracers.

Availability of short-lived EGFR PET tracers is limited due to the short half-life of their radionuclides. For examples, the half-life of carbon-11 is approximately 20 minutes, meaning that the scan must be performed in the same center where the tracer is produced and cannot be exported to other centers. The half-life of fluorine-18 is approximately 5 times longer (t ½ ~110 min), which allows shipping to external not-too-distant centers. Another factor limiting the availability is the scarcity of expertise to apply the complex algorithms used to interpret uptake. In the same vein, dedicated software with intuitive user-friendly interfaces are lacking.

3.2.2 Clinical validation

The clinical validation is a process in which the relationship of a biomarker to a clinical feature is evaluated. Biomarkers are typically linked to biological mechanisms of action at the tumor microenvironment. Ultimately, depending on the context of use, the clinical validation should lead to the identification of biomarkers that benefit clinical outcomes or improve the prevention, screening, staging, diagnosis, therapies, or care of patients (59).

Insights obtained in clinical validation studies will feedback into the analytical validation process in order to further optimize the technical aspect of the biomarker. This positive feedback loop highlights the interdependency between these two tracks. Another time-consuming factor in this (clinical) track is the fact that large, prospective clinical PET studies will only be initiated after analytical validation studies have established the precision and accuracy of the tracer as an EGFR biomarker.

The prompt introduction of new EGFR TKI therapy options and the rapid changes in the standard of care for these patients pose a risk on the EGFR PET tracer development, as most TKI-

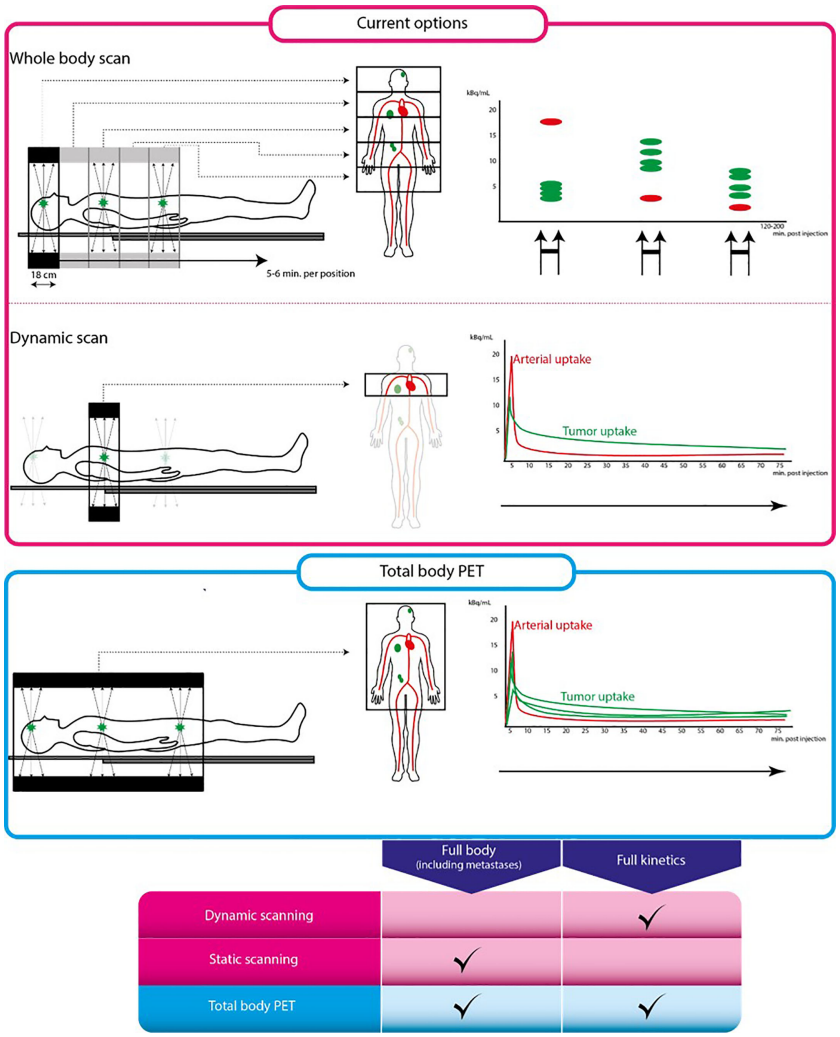

FIGURE 4
Conventional PET scan versus total body PET scan. From left to right: schematic representation of scan procedure, illustrations on the left are parts of the body that can be scanned using each scanning technique. Illustrations on the right are tracer uptake quantification differences for each technique. The pink box represent conventional PET scanning, the blue box represents total body PET. Table below shows characteristics of each scanning technique. Full kinetics indicates whether quantification using pharmacokinetic modeling is possible using this technique.

based tracers have a few years of delay vis-à-vis their therapeutic parents, which can lead to tracers become obsolete. This is highlighted by the timeline depicted in Figure 2: approval of afatinib dates back to 2013, whereas research regarding ^{18}F-afatinib was first published in 2020, a 7-year delay. In contrast, osimertinib was approved for clinical use in 2015, only 2 years after afatinib entered the market and 5 years before the first publication of ^{18}F-afatinib.

3.2.3 Cost-effectiveness

In the cost-effectiveness track, the costs associated with the use of biomarkers need to be assessed. To become a qualified biomarker for clinical use, these costs need to compare favorably to the existing alternative biomarkers such as bio specimen-derived biomarkers, e.g., liquid biopsies. Costs may become lower at a later stage after broad-scaled implementation (59).

The added advantage of the EGFR PET is to evaluate tumor EGFR TKI sensitivity when regular biopsies are not informative enough or for obtaining spatial insights in the tumor TKI sensitivity to guide decision-making. This technique is therefore used in addition to regular biopsy-techniques. Consequently, evaluating the cost-effectiveness for these situations is difficult. With further analytical and clinical optimization supported by upcoming PET technology and improved data processing algorithms, EGFR PET biomarkers hold promise to provide value for their costs. However, at the current stage, no EGFR PET tracer could be considered cost-effective, especially when compared to biopsy-techniques already widely-used in clinical practice.

3.3 Opportunities

The clinical implementation of EGFR PET biomarkers have been limited by the abovementioned challenges, however, recent developments in emerging new technologies are promising to help the biomarker validation process. Although technological advancements may seem to mainly benefit the technical validation and cost-effectiveness tracks, these optimizations feedback positively to the clinical track as well and therefore improve the full validation process. One of the developments that will advance the validation of EGFR PET biomarkers will be the large-scaled introduction of the so-called 'total body PET'.

3.3.1 Total body PET

The total body PET scanner refers to a new generation of commercially available PET-CT scanners that have a much larger axial FoV as compared to conventional state-of-the-art PET-CT systems with an axial FOV of less than 20cm. These new large-FoV PET-CT systems achieve ultra-high (40-to-200-fold higher) sensitivity and allow to visualize and quantify tracer uptake in all major internal organs in the body simultaneously (60–64). This provides numerous new imaging opportunities for patient care and research, since these total body PET-CT scanners will speed up the validation of EGFR PET biomarkers by optimizing their analytical validation and by supporting the clinical validation.

One of the advantages of the ultra-high sensitivity will be the possibility to use lower amounts of radioactivity per tracer injection, which will enable to lower the radiation burden to the patients (60, 64). This could make EGFR PET imaging biomarkers more suited for therapy monitoring through performing multiple PET-CT scans longitudinally.

For static tracer uptake parameters such as SUV, another advantage of the ultra-high sensitivity will be the shorter scan durations (currently 30-40 min per ^{18}F-FDG PET scan), which in turn will improve patient comfort. The optimal scan duration per EGFR tracer on the total body PET-CT scanner is not clear yet, but this could be as short as 20 seconds (a breath-hold) for some tracers. Short acquisition times could also significantly decrease possible *partial volume effects* caused by smearing the PET signal by the movement of small lesions, e.g., due to breathing-motions (60, 64). Also, this will reduce co-registration mismatch of the PET and CT data, e.g., because of patients moving on the scanner while scanning, which generates artefacts in the reconstructed PET data due to faulty CT-attenuation correction (60, 64). These improvements will increase the resolution and precision of the PET biomarkers, broadening their applicability.

For dynamic tracer uptake parameters, combining the large-axial FOV and the ultra-high specificity of the PET-CT system could greatly improve biomarker specificity, repeatability, and reproducibility. As compared to static PET studies, using dynamic PET studies allows to better characterize the pharmacokinetic (PK) behavior of short-lived tracers by generating dynamic tracer uptake parameters (i.e., biomarkers) that are more target specific and accurate than simplified static parameters (60–64). Typically, the limited axial FoV of the conventional PET-CT systems limits most dynamic scans to single organ studies. Also, for dynamic kinetic analysis a so-called 'arterial input function' is needed to describe the bioavailability of the radiotracer in blood. The total body PET-CT, covering all major organs and arterial blood pools (eliminating the need for an arterial cannula) could not only dynamically scan most tumor lesions and all major organs at once but could also provide a reliable image-based arterial input function, non-invasively and automatically, which could generate easily-accessible dynamic uptake parameters with higher specificity and precision (60, 64). Also, the large-FOV coverage will generate new insights on biodistribution in healthy organs, which may open avenues for discovering new PET biomarkers to predict toxicity or biomarkers to guide drug dosing.

Using the total body PET-CT would allow to address many of the analytical validation steps in a single PET study, while this would require many studies using the conventional PET system. Speeding up the analytical validation would significantly fasten the clinical validation as well. As less patients would be needed in the various validation steps of a biomarker, this would ultimately be more cost-effective, through shortening the delay between the introduction of a new EGFR TKI and its validation testing. As most of the tumor lesions, all the major organs and a significant part of the blood pool will be included in the dynamic scans, more comprehensive and automatable scanning and data processing algorithms will be developed. With such algorithms, uptake parameters will be produced more easily, and may require less effort from the PET physics personnel.

3.3.2 Further optimizations

With the advent of new PET technologies and improved data processing algorithms, radiolabeling new EGFR TKI could be of interest for pharmaceutical companies to learn about the biodistribution and PK behavior of their new EGFR TKI therapies at an early stage of development. For example, variations in the brain tissue penetration and uptake of TKI in the brain metastases could be of interest as there is quite some variability in the brain penetration of different TKIs (65). Also, blocking studies could be used to explore the optimal dosing to saturate all targets to support the optimal dosing strategy of a TKI (66, 67). The analytical validation associated with these pharmacological drug development projects could support the clinical validation effort as well.

4 Conclusion

The use of EGFR TKI PET tracers can generate predictive biomarkers to identify and monitor patients who will respond to

EGFR TKI therapies. Current EGFR TKI tracer biomarkers are still in a validation phase, where clinical and analytical improvements loop back iteratively. New developments such as the availability of large-FoV total body PET systems and more improved data processing algorithms can optimize the EGFR TKI PET biomarker validation process. Nevertheless, more evidence is needed for their qualification as predictive and monitoring biomarkers in drug development and routine clinical practice.

Author contributions

MY: supervision, edit and rewriting. HH: supervision, edit and rewriting. IB: supervision, edit and rewriting. All authors contributed to the article and approved the submitted version.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

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