Cell Signaling

Meyer et al., Cell Signal. 2024;2(1):14-22.

Commentary

Biomarkers for monoclonal antibody targeting EGFR in NSCLC: Challenges, current status, and future perspectives

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Received date: November 06, 2023
Accepted date: December 15, 2023

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Citation: Meyer ML, Hirsch FR. Biomarkers for monoclonal antibody targeting EGFR in NSCLC: Challenges, current status, and future perspectives. Cell Signal. 2024;2(1):14-22.

Abstract

Targeting EGFR has a long history in the treatment of non-small cell lung cancer (NSCLC). It was around the 2000s that it was reported that EGFR protein expression increased with bronchial dysplasia in high-risk smokers and patients with lung cancer. However, EGFR inhibitors were not effective in unselected patients with advanced NSCLC. After the identification of sensitizing *EGFR* mutations, tyrosine kinase inhibitors became the cornerstone of treatment for patients with *EGFR*-mutated NSCLC. However, other drugs were developed to target EGFR in the *EGFR*-wild type population, such as monoclonal antibodies. Cetuximab is an anti-EGFR monoclonal antibody, and has been a focus over the past two decades. Though not approved in NSCLC due to marginal and inconsistent effects in phase 3 trials, research aimed to discover biomarkers to identify a subgroup of the population that might benefit. This includes a composite score that evaluates histology, immunohistochemistry, and gene copy amplification. This article reviews the history of the development and discontinuation of monoclonal antibodies in NSCLC and discusses the role of biomarkers in the treatment of advanced *EGFR*-wild type NSCLC.

Keywords: Non-small cell lung cancer, Squamous carcinoma, EGFR, Monoclonal antibody, Cetuximab

Abbreviations: ADC: Antibody-Drug Conjugate; EGFR: Epidermal Growth Factor; FISH: Fluorescence In Situ Hybridization; IHC: Immunohistochemitry; mAb: Monoclonal Antibody; NGS: Next-Generation Sequencing; NS: Non Statistically Significant; NSCLC: Non-Small Cell Lung Cancer; NR: Not Reported; ORR: Overall Response Rate; OS: Overall Survival; PFS: Progression-free Survival; TKI: Tyrosine Kinase Inhibitor

Background

Non-small cell lung cancer (NSCLC) is the leading cause of cancer-related deaths [1]. However, significant improvements in the past decades have been achieved with improved outcomes [2-4]. Several mutations have been described as promoters of oncogenesis, and specific treatments are available, including those for Epidermal Growth Factor (*EGFR*) mutations [4]. Testing for mutations is recommended for every patient with advanced NSCLC, with the exception of patients with squamous NSCLC with a history of tobacco consumption [2,4]. The *EGFR* gene, also known as *ERBB1* or *HER1* (Human Epidermal growth factor Receptor 1) encodes the EGFR protein, a trans-membrane growth factor receptor [5]. Binding of EGFR triggers the activation of its intracellular tyrosine kinase, leading to signaling cascades of several oncogenic pathways (e.g. PI3K/AKT, MAPK, Ras/Raf/Mek/Erk, PLC-y/PKCJAK/STAT) [5,6]. EGFR protein overexpression has been found in premalignant cells, underlining its role in carcinogenesis [7,8].

Methods for EGFR Evaluation

EGFR status can be described using immunohistochemistry (IHC) to examine surface protein expression, fluorescence in situ hybridization (FISH) to assess gene copy number, or DNA sequencing to detect mutations as well as copy number variations (**Figure 1**).

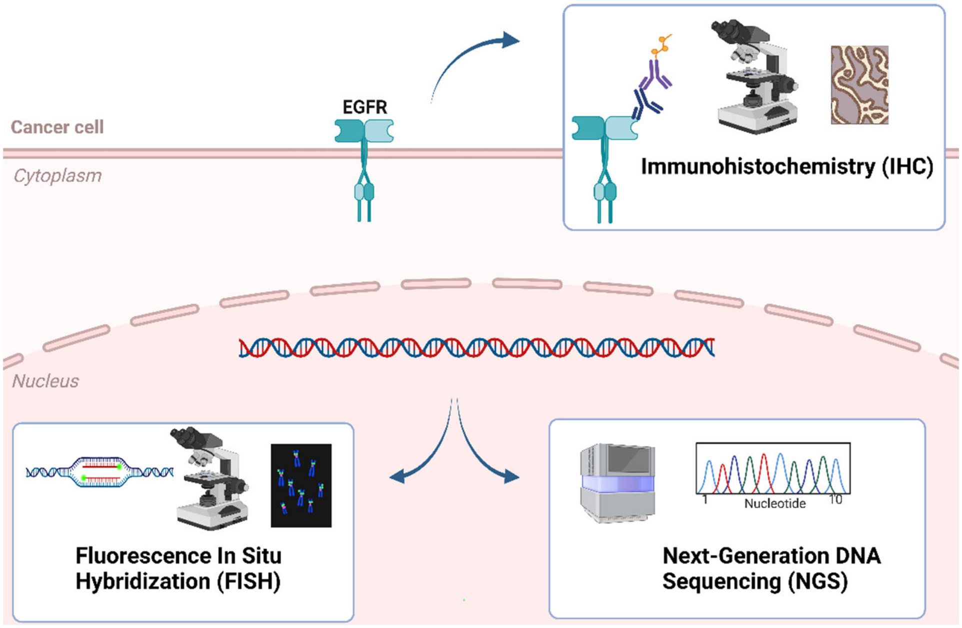

Figure 1. Techniques for EGFR evaluation.

EGFR protein expression studied with IHC is found in a majority of NSCLC, ranging from 50% to 90% of NSCLC, depending on the antibody and scoring technique used [9-11]. EGFR expression is reported to be higher in squamous NSCLC than in non-squamous histologies [11,12]. EGFR overexpression was not correlated with survival in a meta-analysis of 2185 patients with NSCLC [13]. However, due to variable staining methods and scoring systems, drawing definitive conclusions can be challenging [11].

Dysregulation of EGFR can also be detected using FISH, a technique that can determine whether the gene and the chromosome are amplified. The amplification can be either balanced (with the same number of gene copies as chromosomes) or unbalanced (with more gene copies than chromosomes); the latter condition is referred to as gene amplification or high gene copy number [14,15]. The Colorado Criteria were published to ensure homogeneous testing [15]. They define FISH positivity as EGFR FISH-positive in specimens that have (1) at least 40% of cells with at least 4 *EGFR* copies or (2) evidence of *EGFR* gene amplification, defined according to one of the following criteria: (a) an *EGFR/CEP7* ratio of at least 2, (b) the presence of gene clusters in at least 10% of tumor cells, and (c) at least 15 copies of *EGFR* in at least 10% of tumor cells [15]. In phase 2-3 trials of cetuximab and necitumumab, monoclonal antibodies targeting EGFR, gene amplification according to these criteria was found in 20-60% of advanced NSCLC (**Table 1**) [16-20].

DNA sequencing techniques, notably next-generation sequencing (NGS), is considered the gold standard for detecting and classifying *EGFR* mutations in patients with NSCLC [4]. The identification of specific *EGFR* mutations has proven to be the best predictor of the response to EGFR tyrosine kinase inhibitor (TKI) therapy [4]. NGS has progressively replaced IHC and FISH in the assessment of oncogenic drivers in advanced NSCLC, thanks to its broad coverage, accuracy, and efficiency [3,4]. Furthermore, gene amplification can also be assessed with NGS [21,22].

Targeting EGFR in Mutated and Wild-type EGFR NSCLC: TKI and Monoclonal Antibodies

EGFR TKIs were initially developed and tested in all-comers with NSCLC. However, in 2004, two studies showed that responses to gefitinib, an EGFR TKI, were correlated with mutations in the kinase domain of *EGFR*. A phase 3 trial of gefitinib versus chemotherapy in patients with advanced NSCLC also reported a doubling of progression-free survival (PFS) in patients with *EGFR* mutations [23]. A phase 3 trial later confirmed these results in 2010, demonstrating improved PFS with gefitinib over chemotherapy for patients with *EGFR*-mutated advanced NSCLC [24].

Since these results, the treatment of patients with *EGFR*-mutated NSCLC now primarily relies on the third-generation EGFR TKI osimertinib [2,4]. For patients with wild-type *EGFR* NSCLC, after it became clear that they did not benefit from EGFR TKIs, the question remained whether they could benefit from other drugs targeting EGFR, such as monoclonal antibodies, bispecific antibodies or antibody drug conjugates (ADCs). This is indeed the case in other cancers [25-27].

Cetuximab is a chimeric IgG1 monoclonal antibody that binds to EGFR and inhibits its tumor signaling activation [28]. The interaction between cetuximab and the receptor causes the internalization of the antibody-receptor complex, resulting in a reduction in overall EGFR expression, ultimately inhibiting growth and survival signal [28]. Cetuximab is approved for the treatment of colon cancer, head and neck carcinoma, and non-melanoma skin cancer [25-27].

Table 1. Phase 2-3 trials of cetuximab and necitumumab with chemotherapy in NSCLC [12,17-20,30-32,40,49,51-53,68,69].

Trial, Phase	Trial setting	Trial population	IHC +	IHC H-score ≥200	Oucome IHC high with EGFR mAb	FISH + (Colorado criteria)	Outcome FISH+ with EGFR mAb
FLEX, Phase 3	First line chemotherapy (cisplatin/ vinorelbine) +/- cetuximab	1125 patients with advanced NSCLC, 33% squamous	100% (85% of screened patients)	31%	Improved OS	37%	No significant difference
BMS 099, Phase 3	First- line chemotherapy (taxane/ carboplatin) +/- cetuximab.	676 patients with advanced NSCLC, 19% squamous	89%	NR	Trend to improved ORR (NS)	52%	No significant difference
BMS 100, Phase 2	First-line chemotherapy (gemcitabine/platinum) + cetuximab	131 patients with advanced NSCLC, 33% squamous	NR	NR	NR	NR	NR
LUCAS, Phase 2	First-line chemotherapy (cisplatin/ vinorelbine) +/- cetuximab	86 patients with advanced NSCLC expressing EGFR, 42% squamous	67%	NR	NR	NR	NR
SWOG S0342, Phase 2	First-line chemotherapy (gemcitabine/ cisplatin or carboplatin) + cetuximab (concurrent or sequential)	224 patients with advanced NSCLC, 17% squamous	NR	NR	NR	59%	Improved PFS and OS
SWOG S0536, Phase 2	First-line chemotherapy (carboplatin/paclitaxel) + cetuximab and bevacizumab +/- maintenance with cetuximab and bevacizumab	102 patients with advanced non-squamous NSCLC	73%	36%	Trend for better ORR, PFS, and OS (NS)	26%	No significant difference
SWOG 0819, Phase 3	First-line chemotherapy (carboplatin/paclitaxel) +/- bevavacizumab +/- cetuximab	1313 patients with advanced NSCLC, 24% squamous	NR	31%	Trend for improved OS (NS) Improved OS in squamous NSCLC and when combined to FISH+	41%	Trend for improved OS (NS) Improved OS in squamous NSCLC and when combined to IHC H-score ≥200
SQUIRE, Phase 3	First-line chemotherapy (cisplatin–gemcitabine) +/-necitumumab	1093 patients with advanced squamous NSCLC	95%	38%	Trend to improved OS (NS)	37%	Trend for improved OS (NS)
INSPIRE, Phase 3	First-line chemotherapy (pemetrexed/cisplatin) +/- necitumumab	633 patients with advanced non-squamous NSCLC	77%	41%	No significant differences	NR	NR

mAb: Monoclonal Antibody; NR: Not Reported; NS: Non Statistically Significant; OS: Overall Survival; PFS: Progression-Free Survival; ORR: Overall Response Rate

The association of cetuximab combined with chemotherapy in patients with advanced NSCLC was studied in several phase 2 and phase 3 trials [29-33]. Since these studies were conducted before *EGFR* mutations were identified as predictive factors for TKI treatment, patients were included regardless of their *EGFR* mutational status. Two meta-analyses of trials that added cetuximab to chemotherapy in the first-line treatment of patients with advanced NSCLC demonstrated a favorable benefit-to-risk ratio, with significantly improved overall survival [34,35]. However, due to limited benefits, cetuximab was not approved by regulatory authorities [36,37]. In the second-line setting, the combination of cetuximab and pemetrexed failed to improve outcomes compared to pemetrexed alone in advanced NSCLC [38].

Necitumumab is another monoclonal antibody that competitively inhibits EGFR [39]. The phase 3 INSPIRE trial evaluated the addition of necitumumab to chemotherapy in patients with advanced non-squamous NSCLC and did not find a survival improvement [40]. However, the SQUIRE trial, conducted in patients with advanced squamous NSCLC, demonstrated an improved overall survival (HR 0.84 [95% CI 0.74–0.96]; p=0.01), which led to necitumumab's approval in this setting [12].

Other anti-EGFR monoclonal antibodies, such as panitumumab and matuxumab, failed to demonstrate significant improvements in outcomes for patients with advanced NSCLC [41,42]. Nimotuzumab, on the other hand, was found to be non-inferior to docetaxel in the second line but was not approved. Nimotuzumab was also studied with chemoradiotherapy for patients with locally advanced NSCLC in a phase 2 trial, with acceptable safety and better outcomes in patients with squamous NSCLC [43]. However, its phase 3 trial in patients with advanced squamous NSCLC was discontinued early due to safety concerns [44].

In 2021, amivantamab, a bispecific monoclonal antibody targeting EGFR and MET, was approved by the FDA for the treatment of advanced NSCLC with *EGFR* exon 20 insertion mutations in the second line [45]. It is expected to move to the first-line setting following the results of the PAPILLON trial, demonstrating improved PFS when combined to chemotherapy, compared to chemotherapy alone [46]. The combination of amivantamab and lazertinib compared to osimertinib also recently demonstrated PFS improvement in patients with advanced NSCLC and classical *EGFR* mutations (exon 19 deletion and L858R mutation) [47].

Role of Biomarker

While trials that included patients without biomarker selection did not show consistent improvements with anti-EGFR monoclonal antibodies, research aimed at identifying patients who could benefit from these treatments, exploring various criteria. These criteria primarily included EGFR expression using IHC, *EGFR* gene amplification through FISH, *EGFR* or *KRAS* mutations, and histology [37,48].

IHC expression was used as selection criterion in a phase 2 trial with cetuximab, demonstrating a good safety profile [33], and the phase 3 FLEX trial was conducted to validate these results. The FLEX trial enrolled chemotherapy-naive patients with advanced EGFR-expressing NSCLC and randomized them to chemotherapy plus cetuximab or chemotherapy alone. The trial demonstrated a survival improvement, with a median overall survival (OS) of 11.3 months with the addition of cetuximab versus 10.1 months; HR for OS 0.871 (95% CI 0.762–0.996; p=0.044) [30]. Retrospective analyses in patients with high EGFR expression, defined as an H-score ≥200, showed improved OS in the chemotherapy plus cetuximab group compared to the chemotherapy alone group, with a median OS of 12.0 months versus 9.6 months; HR 0.73 (95% CI 0.58–0.93; p=0.011) [49]. However, even though there was a trend in favor of cetuximab combination in other trials of cetuximab and chemotherapy, such as the BMS099 and SWOG S0536 trials [20,50], H-score ≥200 was not a consistent biomarker among other trials (**Table 1**) [16].

EGFR copy number via FISH was also studied. A retrospective analysis of the phase 2 SWOG S0342 trial, which evaluated concurrent or sequential cetuximab plus chemotherapy in advanced NSCLC, reported that patients with a high *EGFR* copy number had a better outcome with cetuximab treatment [18].

This finding led to the design of the phase 3 trial SWOG S0819, aiming to validate FISH amplification as a biomarker. While the FISH-positive subgroup did indeed show a better survival with cetuximab (HR for OS 0.81 [95% CI 0.66–1.0], p= 0.048), the primary endpoint of improved PFS was not statistically significant (HR 0.92, [05% CI 0.75–1.12], p = 0.40) [16]. A subgroup analysis of patients with squamous histology and high *EGFR* copy number by FISH demonstrated an even more improved survival (HR for OS 0.58 [95% CI 0.36–0.86]; p=0.0071) [51]. Nevertheless, FISH amplification was not predictive in retrospective analyses in other phase 3 trials [17,19]. Thus, this finding raises the question whether the predictive biomarker in the *EGFR* wild-type patient population might be histology dependent. Indeed, patients with squamous NSCLC also tended to benefit from cetuximab in the FLEX trial [30]. As mentioned earlier, it was also among the squamous NSCLC that necitumumab was more effective [12].

Regarding genomic analyses, *EGFR* mutational status was not predictive for cetuximab, as observed in several studies [17,19,20]. This was also the case for *KRAS* mutations, unlike what is observed in colon cancer [17,19,25,52,53].

As we see, results conflicted between IHC and FISH. A new retrospective analysis of the SWOG S0819 was published in 2022, exploring the role of combining IHC and FISH scores in patients with squamous NSCLC. This analysis identified a subgroup of patients, i.e., those with high gene copy number and high EGFR protein expression, who had a significant survival improvement with cetuximab, with a median OS of 12.6 versus 4.6 months (HR for OS 0.32 [95% CI 0.18-0.59], p=0.0002) [52].

Have We Found the Perfect Predictive Algorithm?

These results are encouraging and may have identified the subgroup of patients who benefit from cetuximab. The limitation of combining FISH, IHC, and histology relies on the retrospective nature of its analysis. Validation in other trials and prospective assessments would be required to implement it in clinical practice.

Due to its limited results and the revolution brought by immunotherapy, the development of cetuximab in NSCLC was discouraged [37]. The patent for cetuximab expired in Europe in 2014 and in the USA in 2016, and cetuximab and panitumumab are now developed by the same company [54]. Therefore, it is unlikely to see new large trials of cetuximab in advanced NSCLC

[37]. Moreover, since panitumumab is approved for the treatment of squamous NSCLC, it's unlikely that cetuximab will have a future in NSCLC. Despite the consistent signal of effectiveness seen in all the trials involving cetuximab, it's disappointing to realize that the conclusions of biomarker research may have come too late [37].

Future Perspectives: Combination with Immunotherapy and Antibody-Drugs Conjugates

In the era of immunotherapy, a phase 2 trial combining avelumab and cetuximab with chemotherapy in patients with advanced squamous NSCLC demonstrated an acceptable safety profile, but overall response rate (ORR) was not improved [55]. A numerically higher ORR was observed in patients with *EGFR* FISH–positive tumors [55].

Necitumumab was also evaluated in combination with pembrolizumab in a phase 1b trial in advanced NSCLC, with acceptable safety and a modest benefit (ORR of 23%) [56]. There is an ongoing trial (NCT04285671) evaluating the combination of trastuzumab and necitumumab in patients with *EGFR*-mutated and TKI-resistant NSCLC. Currently, there is no ongoing trial involving necitumumab or cetuximab in the United States for patients with EGFR wild-type NSCLC, except for one trial with cetuximab in combination with PF-07284892, a SHP2 inhibitor (NCT04800822) [57].

Amivantamab, a bispecific antibody targeting EGFR and MET, was recently found to provide PFS benefit over osimertinib in the first-line setting for patients with sensitizing *EGFR*-mutated tumors [47]. It was also superior to chemotherapy in patients progressing after EGFR-TKI [58], and in patients with *EGFR* exon 20 mutations [46]. Whether this antibody might also benefit patients with wild-type *EGFR* NSCLC remains to be investigated. A study on xenograft models suggested activity of amivantamab in models with high EGFR expression (H-score ≥170) [59].

Antibody-drug conjugates (ADCs) are new treatments for NSCLC. They are engineered to connect a monoclonal antibody to a payload, typically a cytotoxic agent [60]. Several targets have been identified, with some of them being evaluated in all-comers patients, while others are "biomarker-dependent," requiring a previous analysis of the tumor, such as ADCs targeting HER2, c-MET, CEACAM5 and PK7 (**Table 2**) [61]. Trastuxumab deruxtecan, an ADC targeting HER2, has received FDA's accelerated approval for the treatment of patients with *HER2*-mutated NSCLC [61]. In all-comers patients with advanced NSCLC, a phase 3 trial of datopotamab deruxtecan (Dato-DXd), an ADC targeting TROP2, recently demonstrated that Dato-DXd improved PFS compared to docetaxel [62]. However, patients with squamous histology did not seem to benefit from Dato-DXd, highlighting once again the role of

Table 2. Main targets and ADCs studied in NSCLC [61,70,71].

Target	ADC	Payload	Population (biomarker) studied
HER2	Ado-trastuzumab emtansine	Emtansine (DM1)	HER2 mutation
	Trastuzumab-deruxtecan (T-DXd)	Deruxtacan (DXd)	HER2 mutation or overexpression
	A166	Duostatin-5	HER2 + (IHC) or HER amplification (ISH or NGS)
	XMT-1522	Auristatin F-hydroxypropylamide	HER2 + (IHC)
HER3	Patritumab-deruxtecan	Deruxtacan (DXd)	EGFR mutation and no biomarker
TROP2	Datopotamab-deruxtecan (Dato-DXd)	Deruxtacan (DXd)	No biomarker
	Sacituzumab-govitecan	SN38	No biomarker
	Sacituzumab tirumotecan (SKB264)	KL610023	No biomarker
CEACAM5	Tusamitamab ravtansine	DM4	CEACAM5 + of high level of circulating CEA
c-MET	Telisotuzumab vedotin	MMAE	c-MET1+ (IHC) of MET amplification (FISH)
B7-H3	Vobramitamab duocarmazine (MGC018)	DUBA	No biomarker
	Ifinatamab Deruxtecan (DS-7300)	Deruxtacan (DXd)	No biomarker
AXL	Enapotamab vedotin	MMAE	No biomarker
	Mecbotamab vedotin (BA3011)	MMAE	No biomarker
PK7	Cofetuzumab pelidotin	Auristatin-0101	PK7+ (IHC)
PVRL4	Enfortumab vedotin	MMAE	No biomarker
TF	Tisotumab-vedotin	MMAE	No biomarker
ROR2	Ozuriftamab vedotin (BA3021)	MMAE	No biomarker
GPNMB	Glembatumumab Vedotin	MMAE	Squamous NSCLC
NaPi2b	Upifitamab rilsodotin	AF-HPA	No biomarker
	Lifastuzumab vedotin	MMAE	No biomarker

histology [62]. Several trials are ongoing, and will probably redefine the treatment arsenal for NSCLC [60].

ADCs targeting EGFR are being evaluated. Serclutamab talirine and depatuxizumab mafodotin were studied in glioblastoma, but did not demonstrate benefit [63,64]. MRG003 is another ADC targeting EGFR with a monomethyl auristatin E (MMAE) payload and is currently being studied in a phase 3 trial in patients with head and neck cancer (NCT05751512). In small-cell lung cancer, a phase 2 trial is underway in patients with EGFR positive tumors (NCT04838548). A phase 1 trial of M1231, a bispecific ADC targeting EGFR and Mucin 1 was recently completed, and results should be published soon (NCT04695847).

The use of biomarkers to select patient population varies depending on the drug. The benefit of trastuzumab deruxtecan was clearly observed in patients with *HER2*-mutated NSCLC but was not correlated with HER2 IHC expression [65]. However, other ADCs targeting HER2 are studied in IHC-positive tumors [61]. Tusamitamab ravtansine, an ADC targeting CEACAM5, focuses on a population of patient with positive CEACAM5 expression, or elevated CEA levels [61]. Telisotuzumab vedotin, an anti-c-MET ADC is studied in patients with c-MET positive NSCLC or with MET amplification, while cofetuzumab pelidotin is studied in patients with PK7 positive tumors [61]. This reflects the importance of IHC and gene amplification testing in the current landscape of clinical trials.

Conclusion

The role and mechanisms of EGFR overexpression and gene amplification in wild-type *EGFR* tumors are still unclear and deserve further research. Although NGS is now recommended for the identification of targetable mutations in advanced NSCLC, it is currently not recommended routinely for patients with squamous NSCLC and a history of tobacco consumption [2,3]. Neither IHC nor FISH EGFR status is currently explored in clinical practice in patients with squamous NSCLC. Although the story of cetuximab in NSCLC might have ended, we believe that it is always worthwhile to explore new biomarkers to maximize benefits and minimize side effects. This is how we approach personalized medicine, aiming to provide the right treatment to the right patient. In the era of ADCs, we believe that assessing protein expression, gene amplification and mutations will help us identify the right candidates. We also see how it is crucial to make efforts to harmonize techniques, such as with the Colorado Criteria, to ensure homogeneity in clinical research.

Future research is needed concerning biomarkers in NSCLC to enhance treatment and outcomes. Liquid biopsies are becoming widely implemented and might address the challenge of tissue limitation for comprehensive profiling, as well as playing a role in early detection and monitoring treatment response [66]. Currently, several axes are being investigated, including molecular, structural, and immune biomarkers. Innovative approaches, such as analyses integrating multi-omic profiling, might play an important role [67].

Acknowledgment

The figure was created with Biorender.

Author Contributions Statement

MLM and FH wrote, edited and approved the manuscript.

Conflicts of Interest

MLM declares no conflict of interest. FRH: Scientific boards: AstraZeneca, Regeneron, Sanofi, Abbvie, G1 Therapeutics, Novartis, Merus Therapeutics, Merck, Novocure, NextCure, OncoHost. Patents: "EGFR protein expression and- copy number as predictive biomarkers for EGFR-directed therapies" (through University of Colorado)]. Sponsored research (through Icahn School of Medicine, Mount Sinai: Novartis. Through "Stand-Up-To Cancer": Jazz Pharmaceuticals.

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