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Opinion

Liquid Biopsy in *EGFR*-Mutated Advanced NSCLC from T790M to *MET* Amplification: Clinical Implications and Possibilities in the Resistance Setting

Luigi Della Gravara [1], Ciro Battiloro [2], Aniello Avellino [2], Francesca Caputo [3], Carmine D'Aniello [3] and Danilo Rocco [2,*]

[1] Department of Precision Medicine, Università degli Studi della Campania "Luigi Vanvitelli", 80138 Naples, Italy
[2] Department of Pulmonary Oncology, AORN dei Colli Monaldi, 80131 Naples, Italy
[3] Department of Medical Oncology, AORN dei Colli Monaldi, 80131 Naples, Italy
* Correspondence: danilorocc@yahoo.it

Abstract: According to the ESMO and ASCO clinical guidelines, the main role of liquid biopsy in *EGFR*+ advanced NSCLC patients is represented by T790M detection after erlotinib/gefitinib/afatinib progression. However, the general international expert consensus regards osimertinib as the preferred upfront treatment in this setting; therefore, this role has been scaled back in recent years. As of today, liquid biopsy has no ASCO or ESMO recommendation following first-line osimertinib; in the same vein, no targeted therapy has received ASCO or ESMO recommendation following post upfront Osimertinib progression. However, this standard could change in the near future. Therefore, adopting a clinical point of view, this paper aims to provide a comprehensive review on the previous, the current and the possible future role of liquid biopsy in the framework of the diagnostic–therapeutic algorithm of *EGFR*+ advanced NSCLC.

Keywords: NSCLC; *EGFR*; *MET*; osimertinib; liquid biopsy; NGS; cfDNA; ctDNA

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1. Current State of the Art for the Treatment of *EGFR*-Mutated Advanced NSCLC: The Role of Osimertinib

As of today, several different *EGFR* (epidermal growth factor receptor)-TKIs (tyrosine kinase inhibitors) are ASCO (American Society of Clinical Oncology)- and ESMO (European Society for Medical Oncology)-recommended for the first-line treatment of advanced NSCLC (non-small cell lung cancer) with activating *EGFR* mutations (i.e., exon 19 deletion and exon 21 L858R substitution): erlotinib (±bevacizumab or ramucirumab), afatinib, gefitinib (±carboplatin plus pemetrexed), osimertinib and dacomitinib, while icotinib is only ASCO-recommended. On the other hand, the only second-line ASCO- and ESMO-recommended *EGFR*-TKI in this subgroup of patients is represented by osimertinib, whose administration is limited to patients progressing on erlotinib/gefitinib/afatinib and presenting an exon 20 T790M mutation after re-biopsy or after cfDNA (cell-free DNA) testing via liquid biopsy [1,2].

However, due to the truly excellent efficacy data coming from the FLAURA study and to the vast amount of literature data reporting that approximately 30–60% of these patients do not present a T790M mutation after re-biopsy or cfDNA testing and that approximately 30% of these patients die without receiving a second-line treatment, the general international expert consensus regards osimertinib as the preferred upfront treatment in this setting. This notwithstanding, other *EGFR*-TKIs still have a role when osimertinib is not available or when it is not tolerated [3–14].

Osimertinib received its first-line recommendation thanks to the above-mentioned FLAURA study. In Phase III, a double-blind randomized clinical trial, 556 naïve *EGFR*+ advanced NSCLC patients were randomized (1:1) to receive osimertinib or gefitinib/erlotinib.

As a result, the experimental arm outclassed the control one according to every endpoint: mPFS (median progression-free survival): 18.9 months vs. 10.2 months (hazard ratio—HR—for disease progression or death: 0.46), ORR (objective response rate): 80% vs. 76%, DCR (disease control rate): 97% vs. 92% and mDOR (median duration of response): 17.2 months vs. 8.5 months. Furthermore, this benefit proved to be consistent in all the pre-specified subgroups, notably also in patients with CNS metastases: mPFS: 15.2 months vs. 9.6 months (HR for disease progression or death: 0.47). While mOS (median overall survival) data were not mature, a favorable trend was noted in favor of osimertinib (HR for death: 0.63). With reference to the safety and tolerability profile, the experimental arm was associated with less Grade 3–4 adverse events than the control one: 34% vs. 45% [14]. After a longer follow-up, the mOS results proved to be consistent with the initial findings: 38.6 months vs. 31.8 months (HR for death: 0.80); this benefit still applied to every pre-specified subgroup, notably also in patients with CNS metastases: HR for death: 0.83. Similarly, Grade $\geq$3 adverse events were still less frequent in osimertinib-treated patients: 42% vs. 47%. On a side note, it is worth mentioning that no large-scale head-to-head comparisons between osimertinib and afatinib/dacomitinib/icotinib has been conducted as of today [15].

On the other hand, osimertinib received its second-line recommendation due to the results coming from the AURA3 study. In the Phase III randomized trial, 419 *EGFR*+ T790M+ advanced NSCLC patients progressing after a first-line *EGFR*-TKI (erlotinib/gefitinib/afatinib) were randomized (2:1) to be administered osimertinib or pemetrexed + cis/carboplatin. At the time of data cut-off, the results clearly favored the former treatment: mPFS: 10.1 months vs. 4.4 months (HR for progression of disease or death: 0.30), ORR: 71% vs. 31%, DOR: 9.7 months vs. 4.1 months. Moreover, the survival benefit was robust, and thus, was reported in all the pre-specified subgroups of patients, remarkably also in CNS metastases patients: mPFS: 8.5 months vs. 4.2 months (HR for disease progression or death: 0.32). Grade $\geq$3 adverse events were less frequent in the experimental arm, when compared to the control one: 23% vs. 47%. At the time of data cut-off, OS data were still not mature [16]. After an extended follow-up, no statistically significant benefit in terms of OS was reported, most likely due to the high crossover rate of platinum-treated patients to osimertinib treatment. In fact, adjusting the OS data for crossover, the experimental treatment confirmed its superiority: 26.8 months vs. 15.9 months (HR for death: 0.54). No new safety signals were reported, and the experimental arm proved to be the most tolerable one again: Grade $\geq$3 adverse events: 37% vs. 48% [17].

2. Current State of the Art for the Treatment of *EGFR*-Mutated Advanced NSCLC: The Role of Liquid Biopsy

There are several advantages associated with the use of liquid biopsy: it is a safe, cost-effective, minimally invasive, easily-performed and easily-repeatable procedure, with a shorter turnaround time (i.e., the time between test request and the pathologist's report) when compared to real-world tissue-based techniques, whereas tissue biopsies are costly invasive procedures, with considerable risks of complications, limitations of serial assessment and with long turnaround times. By contrast, tissue biopsies allow histological evaluation, small-cell transformation detection and are highly standardized, sensitive and specific procedures; while liquid biopsy cannot assess tumor histology, it is still in a pre-standardization mastering phase and presents a limited sensitivity. In this vein, tissue-based and cfDNA-based NGS testing show a high concordance rate (75–90%), as well as a great specificity (90–95%) and an average sensitivity (40–50%); thus, while a positive finding on liquid biopsy can guide treatment choice, a negative finding warrants further testing [18–24].

The ESMO PMWG (Precision Medicine Working Group) recommends to profile a tissue or plasma sample from an advanced NSCLC patient using NGS (next generation sequencing) techniques in order to detect ESCAT (ESMO scale for clinical actionability of molecular targets) Level I alterations: *EGFR*, *ALK* (anaplastic lymphoma kinase), *ROS1*, *MET*, *RET* (rearranged during transfection), *NTRK* (neurotrophic tyrosine receptor kinase),

BRAF V600E. Similarly, the ASCO-endorsed IASLC (International Association for the Study of Lung Cancer) consensus paper states that upfront liquid biopsy (preferably via NGS techniques) may be considered in advanced NSCLC patients, especially if tissue is scarce, not available, or not obtainable in a timely fashion [25–27]. This notwithstanding, as the ESMO and ASCO clinical guidelines report, the main role of liquid biopsy is represented by T790M detection after erlotinib/gefitinib/afatinib progression; however, given the fact that a 90–100% specificity can be reached with current liquid biopsy techniques, while sensitivity results are still around 60–70%, a positive result after cfDNA testing is sufficient to detect T790M positivity; on the other hand, a negative result after cfDNA testing mandates a re-biopsy [1,2,28–31]. This role, however, has been definitely scaled back in light of the first-line osimertinib shift; as of today, liquid biopsy has no ASCO or ESMO recommendation following first-line osimertinib.

3. Challenges and Opportunities Ahead

In the same vein, it is imperative to mention that no targeted therapy has received ASCO or ESMO recommendation following post upfront osimertinib progression. In fact, while we presently understand the main resistance mechanisms behind post upfront osimertinib progression, no treatment, apart from standard chemotherapy ± immunotherapy, is ASCO- and ESMO-endorsed [1,2].

These resistance mechanisms can be categorized as on-target (i.e., *EGFR*-dependent) and off-target (i.e., *EGFR*-independent). Resistance mutations are the most frequent on-target resistance mechanism, and the C797S mutation represents the most reported mutation, accounting for approximately 7% of all the resistant cases. On the other hand, *MET* amplification is the most frequent off-target resistance mechanism, accounting for approximately 7–15% of all resistant cases; other off-target resistance mechanisms are represented by a histological switch from NSCLC to small-cell lung cancer (approximately 3–5% of cases) by epithelial-to-mesenchymal transition (approximately 3–5% of cases) and by oncogenic fusions, ALK, RET and BRAF being the most common (approximately 1–5% of cases) [32,33].

Thanks to favorable efficacy and safety results from early clinical studies [34–39], several different osimertinib-based combinations are currently being investigated in Phase II clinical trials in patients progressing after upfront osimertinib and presenting *MET* amplifications or C797S mutations following re-biopsy (Table 1).

In the Phase II NCT04606771 study, 56 (estimated enrollment) *EGFR*-mutated *MET*-amplified aNSCLC patients progressing after an osimertinib treatment (upfront or later lines) will be randomized 1:1 to be administered savolitinib (a *MET*-TKI) plus osimertinib or savolitinib plus placebo; in this study, *MET* amplification needs to be determined by FISH on tumor tissue. The primary endpoint is represented by ORR, and the study should be complete by February, 2024 [40].

Similarly, in the Phase II INSIGHT 2 study (NCT03940703), 120 (estimated enrollment) *EGFR*-mutated *MET*-amplified aNSCLC patients progressing after an upfront osimertinib treatment will be administered tepotinib (a *MET*-TKI) ± osimertinib; *MET* amplification can be determined by FISH on tumor tissue or by blood-based NGS. The primary endpoints are represented by dose-limiting toxicities (DLTs) and ORR, and the study should be complete by March, 2023 [41]. On the other hand, *EGFR*-mutated aNSCLC patients progressing after upfront osimertinib from Group A of the multi-arm Phase II ORCHARD study (NCT03944772) will receive an osimertinib-based combination according to the detected resistance mechanism following tissue re-biopsy: osimertinib plus savolitinib (*MET*-amplification), osimertinib plus gefitinib (C797S mutation), osimertinib plus necitumumab (an anti *EGFR* mAb; *EGFR*-amplification), osimertinib plus alectinib (an *ALK*-TKI *ALK*-rearrangement), osimertinib plus selpercatinib (a *RET*-TKI; *RET*-rearrangement). The primary endpoint is represented by ORR; the study should be complete by November, 2025 [42,43]. In the Phase II SAVANNAH (NCT03778229) study, 360 (estimated enrollment) *EGFR*-mutated *MET*-amplified/overexpressed aNSCLC patients progressing after upfront

osimertinib will be administered osimertinib plus savolitinib; *MET* amplification can only be determined by FISH and IHC on tumor tissue. The primary endpoint is represented by ORR, and the study should be complete by February, 2025 [44]. In an extremely recent press release, this combination was associated with very promising preliminary data in patients with high *MET* amplification/overexpression (IHC90+ and/or FISH10+): ORR: 49%, DCR: 74%, mDOR: 9.3 months, mPFS: 7.1 months [45].

Table 1. Phase II clinical trials investigating osimertinib-based combinations in *EGFR*-mutated advanced NSCLC patients progressing after upfront osimertinib.

Clinical Trial Identifier	Phase	Subset of Patients	Experimental Arm	Control Arm	Primary Objective(s)	Study Completion Date
NCT04606771	II	*EGFR*+ *MET*-amplified progressing after osimertinib	Savolitinib + osimertinib	Savolitinib + placebo	ORR	February 2024
NCT03940703 (INSIGHT 2)	II	*EGFR*+ *MET*-amplified progressing after upfront osimertinib	Tepotinib ± osimertinib	/	DLTs and ORR	March 2023
NCT03944772 (ORCHARD; group A)	II	*EGFR*+ progressing after upfront osimertinib presenting different resistance mechanisms	Osimertinib + savolitinib (*MET* amplification) Osimertinib + gefitinib (C797S mutation) Osimertinib + necitumumab (*EGFR*-amplification) Osimertinib + alectinib (*ALK*-rearrangement) Osimertinib + selpercatinib (*RET*-rearrangement)	/	ORR	November 2025
NCT03778229 (SAVANNAH)	II	*EGFR*+ *MET*-amplified/overexpressed progressing after upfront osimertinib	Savolitinib + osimertinib	/	ORR	February 2025

As the above-mentioned trials show, with the notable exception of the INSIGHT 2 trial, the vast majority of studies currently assessing new treatments for upfront osimertinib-resistant patients list only tissue re-biopsy among the inclusion criteria. However, liquid biopsy techniques (particularly NGS-based ones) show promise in detecting *MET* amplifications and C797S mutations [46]. Early single-patient experiences have shown that NGS liquid biopsy can reliably detect C797S mutations in osimertinib-progressing patients, and thus, guide subsequent-line treatment choices [47,48]. In the same vein, a recent small experience evaluating *MET* amplification via liquid biopsy techniques by Mondelo-Macía et al.

reported a very promising rate of concordance with tissue biopsy (91.67%), as well as a very notable sensitivity rate (>85%) [49].

In this vein, another topic of great interest is represented by the lack of standardization in terms of cut-offs to detect *MET* amplification, both for FISH and for NGS/PCR. For example, a gene copy number ≥ 5 and/or a *MET*/CEP7 ratio (mean MET per cell and chromosome 7 centromere ratio) ≥ 2 is the cut-off used in the aforementioned INSIGHT-2 trial [50]. However, other large experiences have adopted different FISH cut-offs: *MET*/CEP7 ratio ≥ 1.8, mean gene copy number per nucleus ≥ 6.0, $\geq$10% of tumor cells containing $\geq$15 *MET* copies, tight gene clusters in $\geq$10% of tumor cells [51–53]. Similarly, NGS cut-offs for *MET* amplification vary from a GCN (gene copy number) ≥ 4 or 5 to a GCN ≥ 10 [54–57]. These discrepancies make comparisons between studies and methods challenging, and thus, future standardization is needed, especially for NGS techniques.

4. Conclusions

Taking into account the impact of the T790M mutation, the liquid biopsy introduction into clinical practice revolutionized the pre-upfront osimertinib diagnostic–therapeutic algorithm for *EGFR*-mutated advanced NSCLC patients. However, new challenges have come along with the first-line shift of osimertinib, both in terms of diagnosis and of treatment. Several different new osimertinib-based combinations are being assessed in order to overcome resistance mechanisms in the framework of a mutation-tailored sequential algorithm; however, this approach renders re-biopsies mandatory. In this vein, liquid biopsy techniques could once again revolutionize our diagnostic–therapeutic landscape, allowing us to reduce the use of tissue-based re-biopsies and to better monitor disease evolution, thus choosing the optimal treatment [58,59].

In conclusion, while the currently available data are encouraging, we definitely look forward to the results of the above-mentioned trials (especially the ones from the INSIGHT 2 study) and to future larger ones, which are absolutely needed both to identify new effective targeted treatments and to validate and standardize liquid biopsy techniques.

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