Editorial Commentary

The APPLE trial in the evolving landscape of ctDNA monitoring

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Introduction

Activating epidermal growth factor receptor (*EGFR*) mutations are common in non-small cell lung cancer (NSCLC), reported in up to 50% of Asian and 12% of Caucasian patients (1). Tyrosine kinase inhibitors (TKIs) demonstrate longer progression-free survival (PFS) compared to platinum doublet chemo and have become the standard of care first-line treatment for patients with *EGFR* mutations (2). However, duration of response is limited as resistance mutations frequently develop approximately 9–13 months after treatment initiation. The T790M mutation occurs when threonine with methionine at amino acid position 790 in exon 20 of the *EGFR* gene and is found in up to 60% of patients (3). The T790M mutation has been shown to reduce first-generation TKI binding making tumors resistant to these targeted agents. Osimertinib is a third-generation TKI with activity against T790M resistant tumors.

The emerging role of ctDNA in lung cancer

In clinical practice, lack of tissue for molecular assessment can occur for a variety of reasons. Patients with mainly osseous disease have limited DNA for analysis after sample decalcification. Additionally, a biopsy may be contraindicated based on tumor location or patient comorbidities. In these cases, liquid biopsy with circulating tumor DNA (ctDNA) analysis can allow for molecular analysis and identification of targetable mutations. ctDNA also has the potential for dynamic monitoring of treatment efficacy (4) and early identification of resistance mutations (5). Tissue biopsy utility can also be limited by tumor heterogeneity and one biopsy site may not represent the genomic landscape of the entire tumor (6,7). Additional benefits of liquid biopsy include faster turnaround time for results, less invasive procedure, and ability to perform serial assessments (8).

Currently, treatment decisions are based on clinical (symptomatic) or radiographic disease progression. There is a paucity of data on using molecular progression (ctDNA) to influence treatment decisions and if this would impact clinical outcomes positively or negatively. Some authors have postulated that molecular progression may precede radiologic progression (5). Given the lack of data for comparison, it is unknown if radiographic progression is truly the optimal time to change to another line of therapy.

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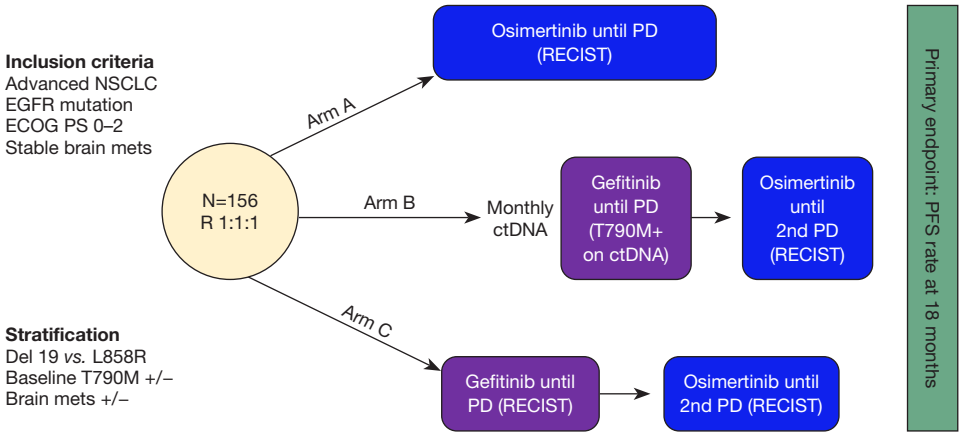

Figure 1 Study schema of the APPLE study. ctDNA, circulating tumor DNA; ECOG PS, Eastern Cooperative Oncology Group performance status; EGFR, epidermal growth factor receptor; brain mets, brain metastases; NSCLC, non-small cell lung cancer; PD, disease progression; PFS, progression-free survival; N, number; R, randomized; RECIST, response evaluation criteria in solid tumors.

Phase I AURA data

In the phase I AURA trial of 253 NSCLC patients who progressed on first generation EGFR TKI. Within patients with T790M mutations, objective response rate (ORR) was 61% with a median PFS of 9.6 months (9). Osimertinib efficacy was similar regardless of whether tissue or liquid biopsy was used for initial identification of *EGFR* mutation. Based on the findings of AURA extension (10) and AURA2 (11), osimertinib was initially Food and Drug Administration (FDA) approved for patients with *EGFR* T790M mutations in either blood or plasma in November 2015, prior to its first-line approval in April 2018.

APPLE study design

The APPLE trial is a randomized, open-label, multicenter phase II study of advanced *EGFR*-mutated and TKI-naïve NSCLC. The study was designed to evaluate the sequencing of TKIs gefitinib and osimertinib. Inclusion criteria for the study include Eastern Clinical Oncology Group (ECOG) performance status 0–2, treatment-naïve, and stable brain metastases without steroid use within the prior 7 days.

Disease progression was evaluated according to Response Evaluation Criteria In Solid Tumors 1.1 (RECIST). Plasma ctDNA was performed at a central laboratory, the Medical University of Gdansk Poland using the Cobas EGFR mutation test v2 (Roche Molecular Diagnostics, Rotkreuz, Switzerland). Patients had monthly plasma ctDNA T790M testing, and computed tomography (CT) scans every 8 weeks. The primary endpoint was 18-month PFS.

The study consisted of three intervention arms (*Figure 1*):

- Arm A: osimertinib 80 mg daily until disease progression by RECIST criteria.
- Arm B: gefitinib 250 mg daily until substitution of threonine with methionine at amino acid position 790 (T790M resistance mutation) positive status by ctDNA or disease progression by RECIST criteria, whichever came first, then switch to osimertinib 80 mg daily until disease progression by RECIST criteria.
- Arm C: gefitinib 250 mg daily until disease progression by RECIST criteria then switch to osimertinib 80 mg daily until second disease progression by RECIST criteria.

The APPLE trial was designed by the European Organization for Research and Treatment of Cancer (EORTC) Lung Cancer Group and funded by AstraZeneca.

APPLE trial results

Remon *et al.* reported initial findings of the APPLE trial including 52 and 51 patients randomized into arms B and C, respectively (12). Participants within arm B were a median of 69 years old, 71% were never smokers, and 31% had brain metastases at the time of study enrollment. *EGFR* mutation status consisted of exon 19 deletion in 64% and L858R mutation in 36%. Forty-seven patients were included in primary endpoint analysis. Participants in arm C were a median of 61 years old, 59% never smokers, and 28% had brain metastases at the time of enrollment. *EGFR*

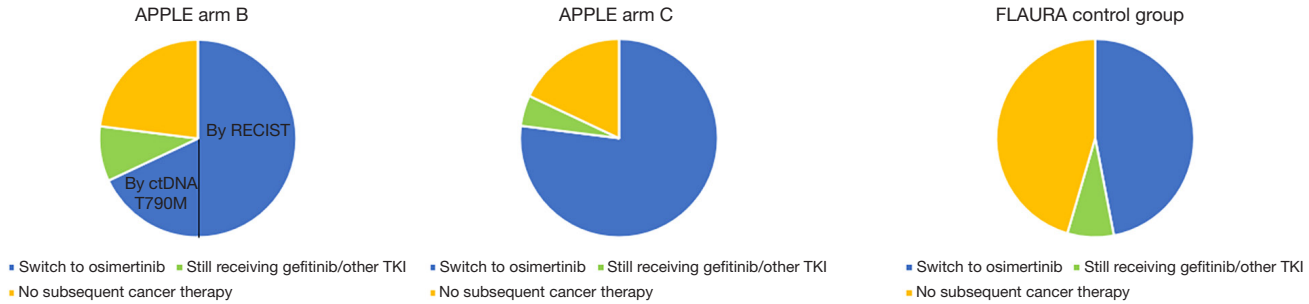

Figure 2 Comparison of subsequent therapies received in the APPLE study (arm B, arm C) and FLAURA. ctDNA, circulating tumor DNA; RECIST, response evaluation criteria in solid tumors; TKI, tyrosine kinase inhibitor.

mutation status was 65% exon 19 deletion and 35% L858R mutation. Within arm C, 44 patients were included in primary endpoint analysis of which 34 received osimertinib based on RECIST criteria.

Within arm B, 32 participants received osimertinib, 8 (25%) based on ctDNA and 24 based on RECIST criteria. The median time to molecular progression was 266 days.

There were 5 and 7 patients who did not meet eligibility criteria in arms B and C, respectively. As a result, the appropriate number of patients for initially planned power analysis was not met. The study team used PFSR-OSI-18 using the Kaplan-Meier technique with an 84% confidence interval (CI). Using this method, the study met its primary endpoint with PFSR-OSI-18 in arm B of 67.2% (84% CI: 56.4–75.9%) versus arm C of 53.5% (84% CI: 42.3–63.5%). The median PFS was 22.0 versus 20.0 months in arms B and C, respectively. The median overall survival (OS) was not reached in arm B but was 42.8 months in arm C. In patients with baseline brain metastases, median brain PFS in arms B and C respectively was 24.4 and 21.4 months.

The most common treatment-related adverse events (TRAE) include diarrhea, dry skin, and acneiform rash. During gefitinib treatment, rates of TRAE in arms B and C were 88.5% and 84.3%. Grade 3 or higher TRAE were more frequent in arm B (19.2%) compared to arm C (13.7%). While on osimertinib, TRAE was reported in 68% of patients in arm B and 52.8% of patients in arm C. Rates of grade 3 or higher TRAE were 8.6% versus 5.6% in arms B and C, respectively.

Discussion

The APPLE trial demonstrated that serial ctDNA monitoring of T790M mutation status was feasible. ctDNA monitoring of molecular progression resulted in an earlier switch to osimertinib in 25% of patients with a non-significant PFS and OS benefit. These findings challenge prior convention that more efficacious drugs should be used upfront to delay disease progression and instead suggest there may be a survival benefit from utilizing subsequent personalized therapy.

Although the study demonstrated improved PFS and OS results with ctDNA monitoring, the interpretation of these results is limited as the number of participants was not enough to calculate standard statistical results using a 95% CI. Given the modifications made to the statistical analysis, it is unclear if these results are clinically meaningful. The 18-month median OS of 87% and 77% in arms B and C, respectively is higher than what was previously reported in the FLAURA trial (13) despite having a higher percentage of participants with brain metastases (30% *vs.* 23%) (14). The benefit is also greater than reported real-world outcomes (15). These findings may also be explained by a higher percentage of patients in the ctDNA group with T790M mutations than the general population given the small sample size. Studies have shown the ORR for osimertinib is 60% in T790M mutated NSCLC versus 20% without the mutation (16,17). The authors suggest that this degree of benefit may be explained by at least 70% of patients switching to osimertinib in the APPLE trial compared to 47% of patients in the control arm of the FLAURA trial (*Figure 2*).

A recent systematic review and meta-analysis found that presence of ctDNA was associated with a statistically significant lower PFS [hazard ratio (HR) 2.34, 95% CI: 1.89–2.89] (18). When analyzing 7 studies looking at ctDNA collected at multiple timepoints, favorable survival was associated with ctDNA clearance, ctDNA decrease, or undetectable ctDNA (18). Another systematic review focusing on the impact of ctDNA in NSCLC patients

receiving targeted therapy showed that negative or early reduction in ctDNA correlated with improved PFS (HR 1.35; 95% CI: 0.83–1.87) (19). ctDNA has the potential to improve standard monitoring and treatment of cancer patients given the ease of obtaining a sample and faster turnaround time for results. Studies have proposed that ctDNA can be used to monitor treatment response (20) and assess development of resistance mutations in real-time (21). However, ctDNA has limited utility in low shedding tumors, low tumor burden, or isolated brain metastases. One study found a 65% increase in detection of actionable mutations when adding ctDNA compared to tissue analysis alone (22). Although the APPLE trial suggests that serial ctDNA is feasible and may improve clinical outcomes, given the small sample size additional trials are needed before serial ctDNA should be used in clinical decision-making.

Although there may be patients who were started with earlier generation EGFR TKIs prior to the approval of next generation TKIs and although we also acknowledge that access to third-generation EGFR TKIs such as osimertinib may not be possible in some countries or regions, as many, if not most, countries have adopted the use of third-generation EGFR TKIs for first-line treatment, unfortunately, the actual results of the APPLE study; serial monitoring of T790M in ctDNA to determine when to change therapy is of limited value. However, the concept of serial ctDNA may still have a future. If an oncogene has been detected, the variant allele frequency (VAF) may alter with treatment and may guide us in patient management. Alternatively, a development of a new genomic alteration (i.e., MET amplification) at the time of progression may also help steer subsequent therapy.

It must also be noted that the front-line treatment landscape of patients with *EGFR*-mutated NSCLC is rapidly evolving and includes intensification of therapy with chemotherapy (FLAURA2) or bispecific (MARIPOSA).

In the FLAURA2 trial patients received osimertinib plus platinum and pemetrexed (n=279) or osimertinib monotherapy (n=278) (13). At the time of enrollment 42 and 40 patients at central nervous system (CNS) metastases, respectively. Median PFS was improved 8.8 months in the combination therapy arm [25.5 months (95% CI: 24.7–not reached) versus 16.7 months (95% CI: 14.1–21.3)]. PFS benefit was consistent across all subgroups analyzed, however, effects were most pronounced in patients with CNS metastases. In this group, median PFS with combination therapy was 24.9 months (95% CI: 22.0–not reached) and with monotherapy was 13.8 months (95% CI: 11.0–16.7) with HR 0.47 (0.33–0.66). The combination regimen did result in increased grade 3 or higher toxicities (53% versus 11%). Most common grade 3 or greater TRAEs include anemia (20%), neutropenia (23%), and thrombocytopenia (14%).

In the MARIPOSA study, the combination of amivantamab plus lazertinib (n=429) was compared to osimertinib monotherapy (n=429) (23). There were 41% and 40% of patients with a history of brain metastases in each cohort, respectively. Median PFS with amivantamab plus lazertinib versus osimertinib was 23.7 months (95% CI: 19.1–27.7) and 16.6 months (95% CI: 14.8–18.5) respectively. Combination therapy resulted in better outcomes in patients with brain metastases (18.3 *vs.* 13.0 months) however this was less pronounced than the results in FLAURA2. Grade 3 and higher TRAEs were higher with amivantamab and lazertinib (75%) than with osimertinib monotherapy (43%).

While the primary results have shown promise, we await further details on the study including data on ctDNA, as it may be in these details that we find the molecular subsets of patients who would benefit the most from intensified therapy or who may be able to forgo the intensification.

Conclusions

The PFS and OS benefit with osimertinib in this study is higher than benefit reported in prior trials and in real-world outcomes which may be explained by the small sample size, a higher number of T790M mutations, or more patients switching to later generation targeted therapy. Given osimertinib has become standard of care in the front-line setting and many patient care centers may not have access to resources for ctDNA monitoring, these results are of limited value. As combination therapy is advancing to the front-line setting, ctDNA monitoring may have a role in identifying which patients require more intensive regimens and spare certain patients from unnecessary toxicity. The APPLE trial demonstrated that serial ctDNA monitoring is feasible and further research is needed on how ctDNA can impact clinical decision-making for a more patient-centered treatment approach.

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Footnote

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Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

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