

Molecular residual disease analysis of adjuvant osimertinib in resected *EGFR*-mutated stage IB–IIIA non-small-cell lung cancer

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Osimertinib—a third-generation epidermal growth factor receptor-tyrosine kinase inhibitor—is recommended as adjuvant therapy for resected stage IB–IIIA epidermal growth factor receptor-mutated non-small-cell lung cancer, based on significant disease-free survival (DFS) and overall survival improvement shown in the previously reported phase 3 ADAURA trial. A trend toward an increased DFS event rate after completion of 3 years adjuvant treatment in ADAURA suggests that some patients may benefit from longer adjuvant osimertinib treatment. We therefore explored whether tumor-informed, circulating tumor DNA-based, molecular residual disease (MRD) could predict recurrence in an exploratory post hoc analysis of 220 patients ($n = 112$ osimertinib; $n = 108$ placebo) from ADAURA. MRD preceded imaging DFS events in this study by a median of 4.7 (95% confidence interval, 2.2–5.6) months. DFS and MRD event-free rate at 36 months was 86% versus 36% for patients in the osimertinib versus placebo groups (hazard ratio, 0.23 (95% confidence interval, 0.15–0.36)). In the osimertinib group, DFS or MRD events were detected in 28 (25%) patients; most events occurred following osimertinib cessation (19 of 28, 68%) and within 12 months of stopping osimertinib (11 of 19, 58%). At 24 months after osimertinib, the DFS and MRD event-free rate was 66%. In this study, MRD preceded DFS events in most patients across both arms. DFS and MRD event-free status was maintained for most patients during adjuvant osimertinib treatment and posttreatment follow-up, with most MRD or DFS events occurring after osimertinib treatment discontinuation or completion. MRD detection could potentially identify patients who may benefit from longer adjuvant osimertinib, although this requires clinical confirmation. ClinicalTrials.gov identifier: [NCT02511106](https://clinicaltrials.gov/ct2/show/study/NCT02511106).

Osimertinib is a third-generation, central nervous system (CNS)-active epidermal growth factor receptor (EGFR)-tyrosine kinase inhibitor (TKI) (EGFR-TKI), with demonstrated efficacy in early and advanced-stage *EGFR*-mutated (EGFRm) (exon 19 deletion (Ex19del)/L858R) non-small-cell lung cancer (NSCLC)^{1–10}. Adjuvant osimertinib for a duration of 3 years is recommended for resected EGFRm stage IB–IIIA NSCLC, based on results from the phase 3 ADAURA trial^{11–13}. In the ADAURA primary analysis, adjuvant osimertinib demonstrated a statistically significant and clinically meaningful improvement in DFS versus placebo in patients following complete surgical resection of stage IB–IIIA EGFRm NSCLC, with or without previous chemotherapy⁹. DFS benefit was sustained in an updated analysis with a further 2-year follow-up², which translated to a significant improvement in overall survival (OS), with a 5-year OS rate of 88% in the osimertinib group compared with 78% in the placebo group, and an overall hazard ratio (HR) for death of 0.49 (95.03% confidence interval (CI) 0.34–0.70; $P < 0.001$)⁸. A trend toward an increased DFS event rate was observed after completion of 3 years of osimertinib treatment², suggesting that some patients may benefit from a longer treatment duration with the opportunity to further improve outcomes. However, determining which patients would benefit from a longer treatment duration remains unknown.

The standard surveillance paradigm following curative intent therapies includes serial radiographic imaging; however, this detects only macroscopic disease, is confounded by changes in normal tissue after treatment and the optimal scan frequency for surveillance is uncertain^{14–17}. Circulating tumor DNA (ctDNA) can be detected in liquid biopsies (for example, plasma), providing a minimally invasive diagnostic tool to assess tumor burden and genomic alterations¹⁸. MRD—the detection of ctDNA after treatment with curative intent—could be a biomarker for early disease recurrence, with the potential to inform treatment and prognosis¹⁹. Due to low tumor burden, and a concomitant low fraction of ctDNA in patients with early-stage disease, robust detection of ctDNA can be challenging. However, recent advances in next-generation sequencing (NGS) approaches have improved the limit of detection for ctDNA and increased the feasibility of successfully building bespoke tumor-informed MRD assays and applying them in this setting^{19–25}. A successful technological strategy, generally referred to as tumor-informed MRD testing, leverages the genomic profile from the patient's tumor to track an individualized set of tumor-specific alterations longitudinally in plasma ctDNA samples^{19,26–29}.

Several studies in patients with early-stage NSCLC who have undergone curative intent treatment support the use of tumor-informed MRD assays to predict radiographic clinical recurrence^{26–29}. Studies in patients with stage I–III NSCLC, such as a prospective study in 261 patients²⁹ and the LUCID study in 88 patients²⁸, showed that MRD detection preceded radiographic progression by a median of 3.4 months and 212.5 days (approximately 7 months), respectively. In a retrospective analysis to track previously identified tumor mutations in posttreatment (radiotherapy, chemotherapy and/or surgery) plasma from 40 patients with stage I–III NSCLC, ctDNA detection preceded radiographic progression in 72% patients by a median 5.2 months²⁷. Although studies are limited, these data suggest the potential utility of tumor-informed MRD assays to monitor MRD in early-stage NSCLC.

We performed an exploratory analysis to investigate whether plasma-based, tumor-informed MRD analysis could predict disease recurrence during and after adjuvant treatment, in a patient cohort ($n = 220$) from ADAURA. We then investigated whether genomic alterations detected in patient tumors before treatment correlate with molecular and clinical outcomes. Here we report a long-term longitudinal MRD analysis from a phase 3 randomized clinical trial in patients with resected EGFRm NSCLC.

Results

Patient characteristics

We performed a retrospective, post hoc exploratory analysis, independent of the statistical analysis plan of the ADAURA study⁹, to investigate whether plasma-based, tumor-informed MRD analysis could predict disease recurrence during and after adjuvant treatment in a patient subset ($n = 220$) from the phase 3 trial. Study design details have been published previously^{2,8,9}. Briefly, following complete surgical resection of primary NSCLC, patients received adjuvant chemotherapy (if determined necessary, per physician and patient choice) for up to four cycles. Patients were then randomized 1:1 to receive osimertinib (80 mg once daily (QD)) or placebo for a planned treatment duration of up to 36 months. Plasma samples were collected from day 1 of randomization, and longitudinally during the adjuvant and posttreatment follow-up periods. Baseline computed tomography (CT) scans were performed within 28 days before first dose. Subsequent assessments were performed during the adjuvant treatment and posttreatment follow-up periods (Fig. 1a).

Of 682 patients randomized in ADAURA, 239 (35%) had available matched tumor, germline DNA and plasma samples for development and testing of tumor-specific RaDaR panels²⁸ at the time of data cut-off (DCO) (Fig. 1b). Genomic DNA (gDNA) was unavailable for 397 patients due to patients declining germline testing, withdrawing consent or local restrictions preventing sample collection and tumor tissue was unavailable for 46 patients due to insufficient tissue slides or reparation. Subsequently, RaDaR panels were successfully designed and validated for 220 (92%) of the 239 patients across both the osimertinib ($n = 112$) and placebo ($n = 108$) groups and comprised the MRD analysis set. For the remaining 19 patients, whole-exome sequencing (WES) failed for seven patients because of poor DNA yield and/or quality or low sequencing depth, MRD panel design failed for 12 patients because of insufficient tumor-informed mutations detected during WES. An average (range) of 30 (9–47), 32 (8–48) and 32 (9–48) tumor-specific gene variants were included in the panels for patients with stage IB, stage II and stage IIIA disease, respectively (Extended Data Table 1).

Demographics, disease characteristics and outcomes were generally similar between patients in the MRD analysis set ($n = 220$) and overall ADAURA population ($N = 682$; Extended Data Table 2). However, in the MRD analysis set there were fewer patients with stage IB NSCLC (21%, 47 of 220) compared with the overall ADAURA population (32%, 216 of 682) because of insufficient tumor tissue available to meet the MRD panel requirements. There was also a lower representation of Asian patients in the MRD analysis set (45%, 98 of 220) compared with the overall ADAURA population (64%, 434 of 682) because of local restrictions on the export of human biological material. In the MRD analysis set, the DFS HR was 0.19 (95% CI, 0.12–0.29) and OS HR was 0.42 (95% CI, 0.24–0.74); in the overall ADAURA population, the DFS HR was 0.27 (95% CI, 0.21–0.34) and OS HR was 0.49 (95.03% CI, 0.34–0.70) for osimertinib versus placebo (DCOs were 11 April 2022 and 27 January 2023 for DFS and OS, respectively).

MRD status at randomization and MRD clearance

RaDaR panels were used to analyze the MRD status of longitudinal samples at randomization and throughout the adjuvant and posttreatment follow-up periods. MRD was considered 'detected' at any timepoint when ctDNA was detected for one or more mutations in a patient's tumor-specific RaDaR panel. Overall, 8% (18 of 220) of patients across both groups had MRD detected at randomization before study treatment (4% (5 of 112) receiving osimertinib compared with 12% (13 of 108) receiving placebo; Fig. 2a). Of the 18 patients with detected MRD at randomization, none had stage IB, 39% (7 of 18) had stage II, and 61% (11 of 18) had stage IIIA disease (Extended Data Table 3). Within the MRD analysis set, 60% (54 of 90) of patients with stage II and 77% (64 of 83) with stage IIIA disease received adjuvant chemotherapy (Fig. 2b). Among patients with stage II disease, MRD was detected at

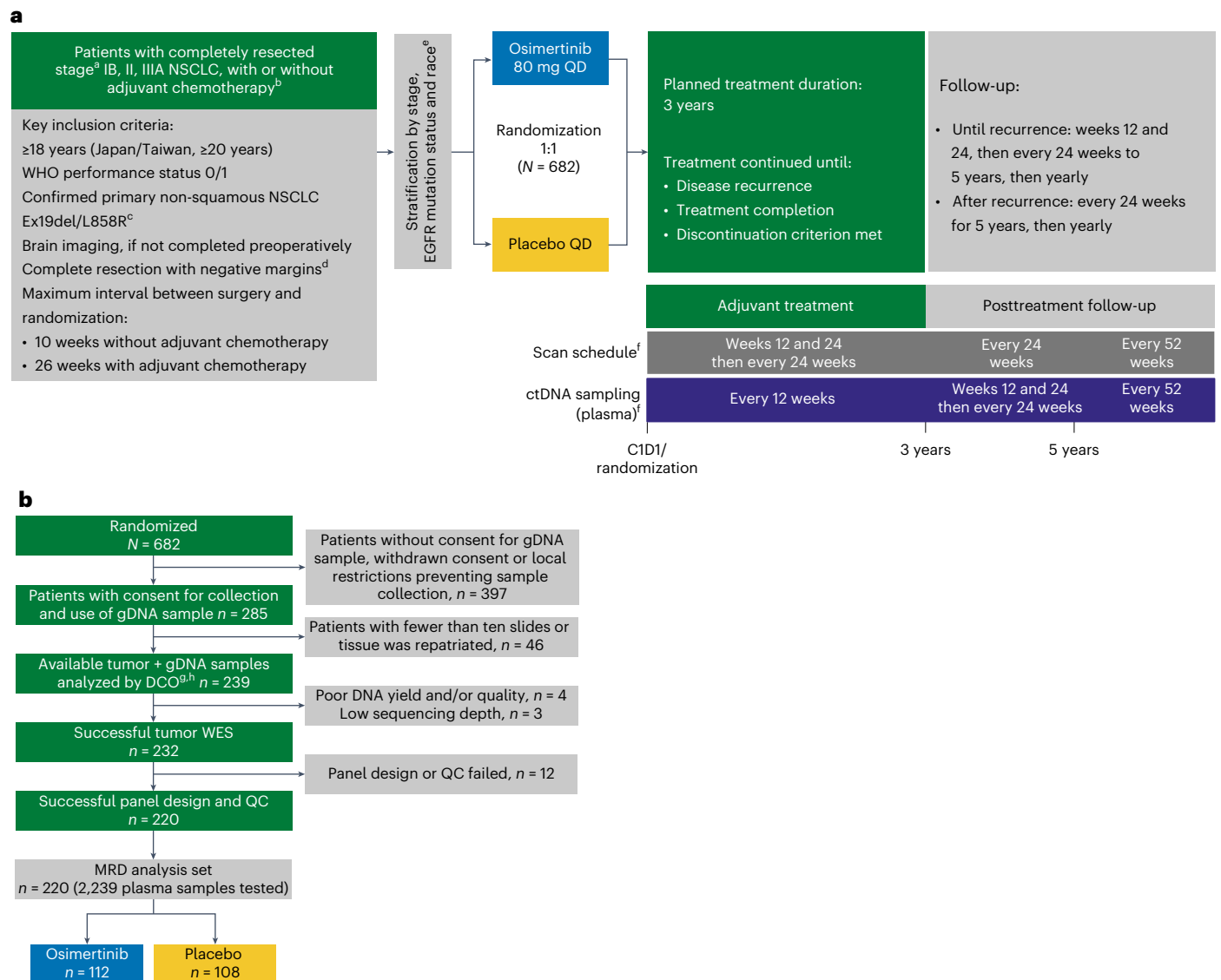


Fig. 1 | Study population and MRD analysis. a, ADAURA study design and sample collection schedules. **b**, Patient disposition for the MRD analysis set.

^aAt the time of recruitment, staging was determined by the AJCC Staging Manual 7th ed. ^bPreoperative, postoperative or planned radiotherapy was not allowed. ^cEGFR mutation status was centrally confirmed in tissue. ^dPatients received a CT scan after resection and within 28 days before treatment. ^ePatients were stratified by stage (AJCC 7th ed.⁴², IB versus II versus IIIA), EGFR mutational status

(Ex19del versus L858R) and race (Asian versus non-Asian). Patients with stage IB disease were not eligible in Japan. ^fCollected until disease recurrence or study end. ^gOf 682 patients randomized in ADAURA, 239 had matched tumor, plasma and germline DNA samples and were eligible for MRD analyses; ctDNA was not collected in China and some patients declined the optional germline sample. ^hDCO for MRD analysis was 25 March 2024. C1D1, cycle 1 day 1; QC, quality control; WHO, World Health Organization.

randomization in fewer patients who received previous chemotherapy, versus those who did not (1% (1 of 90) versus 7% (6 of 90), respectively). In patients with stage IIIA disease, MRD was detected at randomization at similar rates in patients who received previous chemotherapy versus those who did not (8% (7 of 83) versus 5% (4 of 83)), respectively.

Although the number of patients with MRD detected at randomization was small, it seemed to be associated with poor outcomes for both the osimertinib and placebo groups, with 17 of 18 patients experiencing a DFS event. The five patients with detected MRD at randomization in the osimertinib group generally had longer DFS compared with the 13 patients in the placebo group (Fig. 2c). Of the five patients with detected MRD in the osimertinib group, four had recurrence events. Three events were local and/or regional and one was a distant event; two occurred during adjuvant treatment and two after treatment. All 13 patients with MRD detected at randomization in the placebo group had recurrence events (local and/or regional $n = 7$, distant $n = 5$, death $n = 1$).

MRD was considered ‘cleared’ if, following the detection of MRD at randomization, subsequent samples within the next two timepoints (24 weeks from randomization) were determined to be MRD undetected. Four of the five patients in the osimertinib group with MRD detected at randomization cleared MRD; the remaining patient did not have samples available for analysis until week 60 of treatment (Fig. 2d). Of the four patients who cleared MRD detected at randomization, three had undetected MRD until the postadjuvant period after stopping osimertinib (Fig. 2c,e) and one had a re-emergence of detected MRD before the end of the planned treatment period (36 months) coinciding with a treatment interruption followed by dose reduction. Of the 13 patients with MRD detected at randomization in the placebo group, none had clearance during treatment or follow-up (Fig. 2d,e).

Longitudinal MRD analysis of plasma samples

MRD events were detected in 31% (68 of 220) of patients across both groups during the analysis period (randomization, on treatment

and posttreatment follow-up; Fig. 3). In the osimertinib group, 87% (97 of 112) of patients were MRD-free throughout the study compared with 51% (55 of 108) in the placebo group. Of patients with undetected MRD at randomization (osimertinib, 96% (107 of 112); placebo, 88% (95 of 108)), MRD-free status was maintained during the on-treatment and posttreatment follow-up periods in 91% (97 of 107) of patients in the osimertinib group compared with 58% (55 of 95) in the placebo group. Among the 15 patients in the osimertinib group with MRD events, most events occurred after stopping or completing treatment (67%, 10 of 15).

Prediction of DFS events with ctDNA-based tumor-informed MRD

We evaluated the performance of the RaDaR assay to predict disease recurrence by comparing the occurrence of postrandomization MRD events (molecular recurrence) with DFS events (clinical recurrence or death, whichever occurred first) during the on-treatment and post-treatment follow-up periods. MRD detection using RaDaR had a 65% (95% CI, 55–74) positive percent agreement (PPA; clinical sensitivity) with DFS events and a 95% (95% CI, 91–99) negative percent agreement (NPA; clinical specificity) with DFS-censored patients across groups (Extended Data Table 4). The positive predictive value (PPV) and negative predictive values (NPV) for MRD were 91% (95% CI, 84–98) and 78% (95% CI, 71–84), respectively. Among the patients who had a DFS event, there were no differences in prevalence of *EGFR* mutation subtype, race or smoking status according to the MRD status of detected versus not detected (Extended Data Table 5). However, numerically, there were more patients with stage IIIA disease, more patients treated with placebo and fewer patients with local and/or regional recurrence or CNS-only recurrence with detected MRD versus not detected (58% (36 of 62) versus 44% (15 of 34); 82% (51 of 62) versus 62% (21 of 34); 50% (29 of 58) versus 74% (23 of 31), respectively).

To determine whether longitudinal MRD monitoring could predict a DFS event, a lead-time analysis was performed for the 62 patients across both treatment groups with both a postrandomization DFS event and any detected MRD positive sample through the whole study period. Across both groups, MRD preceded a DFS event by a median of 4.7 (95% CI, 2.2–5.6) months (Fig. 4a).

In addition to tumor-specific mutations, *EGFR* mutations (Ex19del and L858R) were also included in each RaDaR panel to allow for longitudinal monitoring and correlations with both MRD and DFS events (Extended Data Fig. 1a). From an analysis set of 206 patients, *EGFR* mutation detection had 54% PPA and 42% PPA for DFS events and MRD detection, respectively, and a 92% NPA and 100% NPA for DFS-censored patients and MRD-not detected samples, respectively, across groups (Extended Data Table 6). Of the 40 patients with detected MRD, detected *EGFR* mutations and a DFS event, 19 patients had detected MRD ahead of detected *EGFR* mutations and 19 had detected MRD at the same time as detected *EGFR* mutations (Extended Data Fig. 1b). Only two patients had detected *EGFR* mutations ahead of detected MRD. Detected *EGFR* mutations preceded a DFS event by a median of 1.0 month (95% CI, 0.0–5.2) across groups but did not precede MRD detection in most patients (lead time of 0.0 months (95% CI, 2.9–0.0 months)).

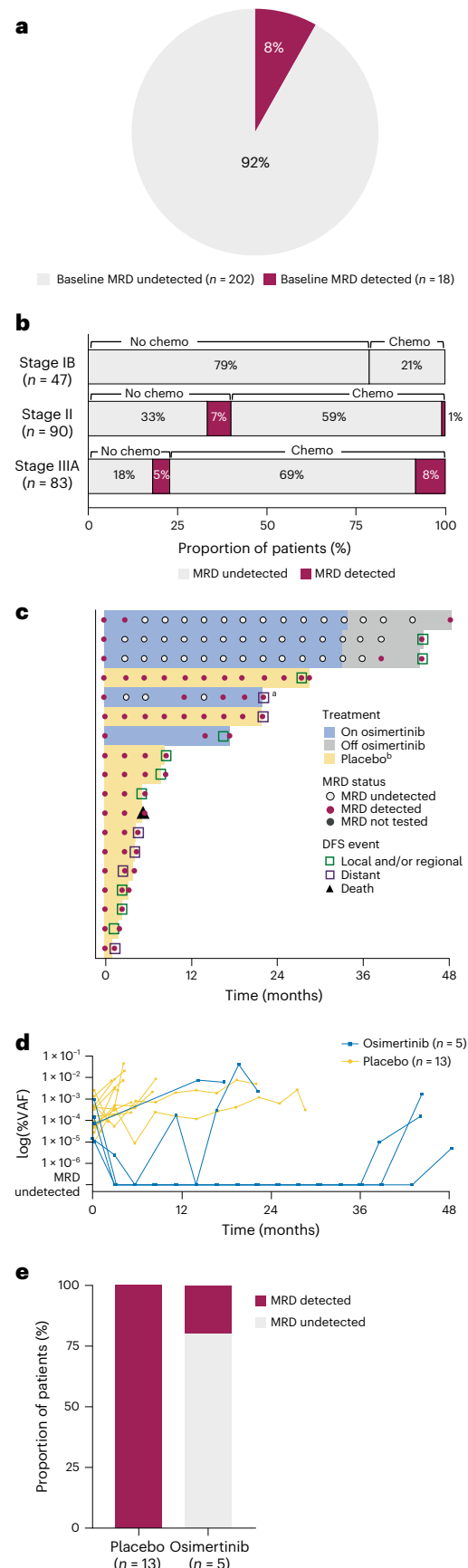
Fig. 2 | Clearance of baseline MRD. **a**, MRD events occurring at randomization in the MRD analysis set. **b**, MRD status at randomization by disease stage (stage IB, II and IIIA) and receipt of previous adjuvant chemotherapy in the MRD analysis set. **c**, Longitudinal analysis of MRD status (undetected or detected) and DFS outcomes (local and/or regional, distant and death) for patients with detected MRD at randomization across both the osimertinib and placebo groups.

d, Percentage variant allele frequency (VAF) detected in plasma samples over time among patients in the osimertinib ($n = 5$) and placebo ($n = 13$) groups with detected MRD at randomization. **e**, Proportion of patients in the osimertinib and placebo groups showing clearance of MRD detected at randomization.

^aCNS-only DFS event. ^bPatients received placebo for up to 36 months. Chemo, chemotherapy; VAF, variant allele frequency.

Effect of osimertinib on MRD and clinical disease recurrence

To further understand the benefit of osimertinib with respect to molecular or clinical disease recurrence, the combined endpoint of



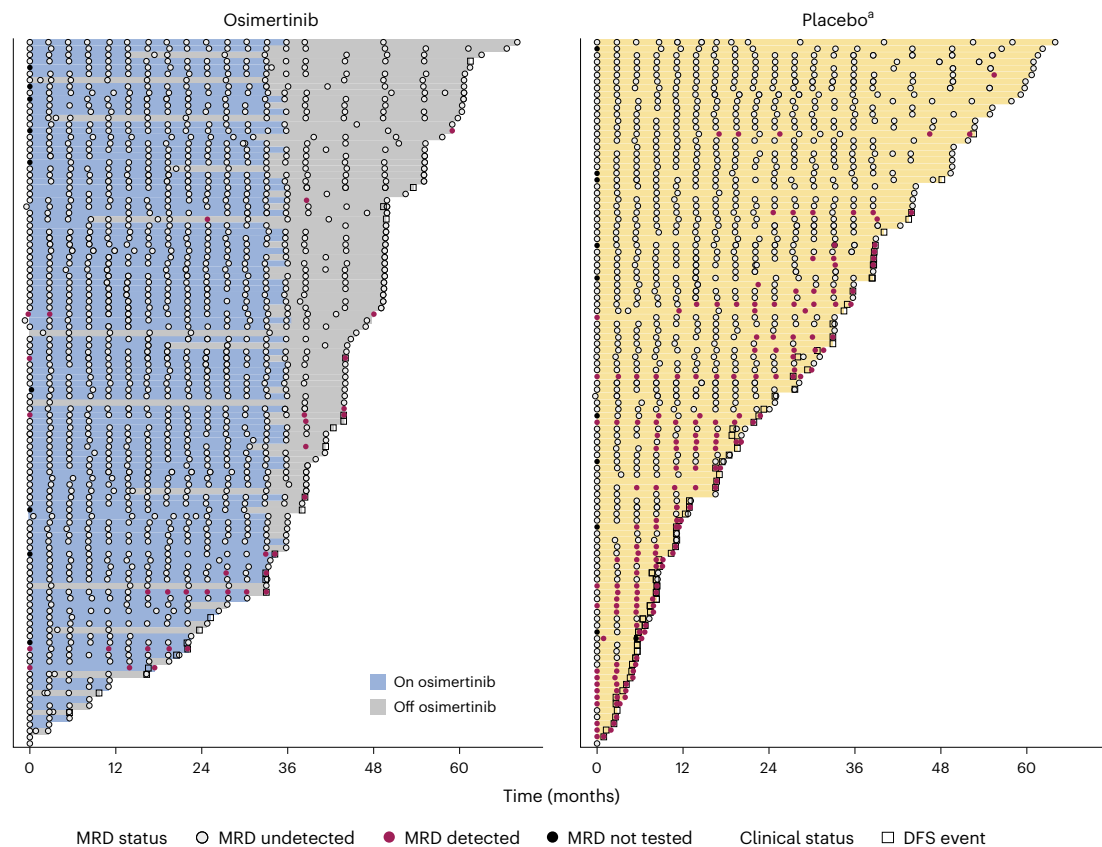


Fig. 3 | Longitudinal MRD analysis of plasma samples from patients in the MRD analysis set. Swimmer plots for the osimertinib and placebo groups showing the MRD status (undetected, detected or not tested) and clinical status (DFS

event) of patients in the MRD analysis set over time (randomization, treatment and posttreatment follow-up periods). ^aPatients received placebo for up to 36 months.

DFS (clinical recurrence or death) or MRD events was assessed from randomization to postrandomization MRD detection, disease recurrence or death (whichever occurred first). Median follow-up time from randomization was 44.2 months (95% CI, 42.4–49.1) and 19.1 months (95% CI, 11.1–28.3) in the osimertinib and placebo groups, respectively (Fig. 4b). The DFS and MRD event-free rate at 36 months on treatment was 86% (95% CI, 78–92) versus 36% (95% CI, 27–45) with an overall HR of 0.23 (95% CI, 0.15–0.36) for osimertinib versus placebo.

DFS or MRD events were detected at any time postrandomization in 25% (28 of 112) of patients in the osimertinib group compared with 69% (74 of 108) in the placebo group (Fig. 4c). In the osimertinib group, most (68%, 19 of 28) DFS or MRD events occurred after treatment discontinuation or completion and, of these, 58% (11 of 19) occurred within 12 months of treatment discontinuation or completion.

DFS or MRD events, whichever occurred first, were also assessed after patients discontinued or completed osimertinib treatment. In this analysis, there were 28 DFS or MRD events. Patients who experienced a DFS or MRD event ($n = 9$) or were censored ($n = 9$) before discontinuing osimertinib treatment were included in this analysis to avoid the introduction of survivor bias and had their time to DFS or MRD event in the treatment-free period set to day 1. Median follow-up time after osimertinib discontinuation was 11.7 months (95% CI, 8.3–13.9; Table 1). The DFS and MRD event-free rate at 12 and 24 months after osimertinib treatment was 80% (95% CI, 71–87) and 66% (95% CI, 53–77), respectively.

Genomic analysis at baseline

To determine whether baseline genomic alterations correlated with molecular and clinical outcomes, the baseline tumor WES data used

for MRD panel design ($n = 220$) were reanalyzed for known oncogenic driver alterations occurring in >1% of samples and compared with MRD and DFS events. Baseline tumor samples were obtained from surgery before use of chemotherapy. Excluding Ex19del and L858R *EGFR* mutations, *TP53* mutations had the highest prevalence, at 45% in the MRD analysis set (Fig. 5a). The frequencies of other co-occurring alterations associated with cell signaling, cell cycle progression and DNA damage repair were low (<7%). Across both treatment groups, *TP53* mutations occurred more frequently in the following: patients with detected MRD at randomization (MRD detected, 72% (13 of 18) versus not detected, 42% (85 of 202)); more advanced disease stage (stage IB, 28% (13 of 47) versus stage II, 46% (41 of 90) versus stage III, 53% (44 of 83)); and receipt of postsurgical adjuvant chemotherapy (yes, 55% (70 of 128) versus no, 30% (28 of 92), Fig. 5b). However, the benefit of adjuvant osimertinib versus placebo was similar irrespective of *TP53* status (*TP53* altered: HR, 0.28 (95% CI, 0.15–0.51); *TP53* wild type: HR, 0.17 (95% CI, 0.08–0.34)), seeming to represent a negative prognostic patient subgroup across both groups (Fig. 5c). Further, a multivariable analysis adjusting for *TP53*, disease stage and previous chemotherapy fitted separately for each treatment group, showed that patients with *TP53* alterations had a higher risk of DFS or MRD events versus patients with wild-type *TP53* (osimertinib: HR, 2.28 (95% CI, 0.96–5.64); placebo: HR, 1.33 (5% CI, 0.82–2.17); Extended Data Fig. 2).

Discussion

The detection of MRD by assaying tumor-derived mutations in ctDNA offers a sensitive tool to identify the risk of disease recurrence after curative intent treatment ahead of radiological evaluation in resected EGFRm NSCLC. If validated for clinical practice, systematic use of MRD monitoring has the potential to personalize postadjuvant patient

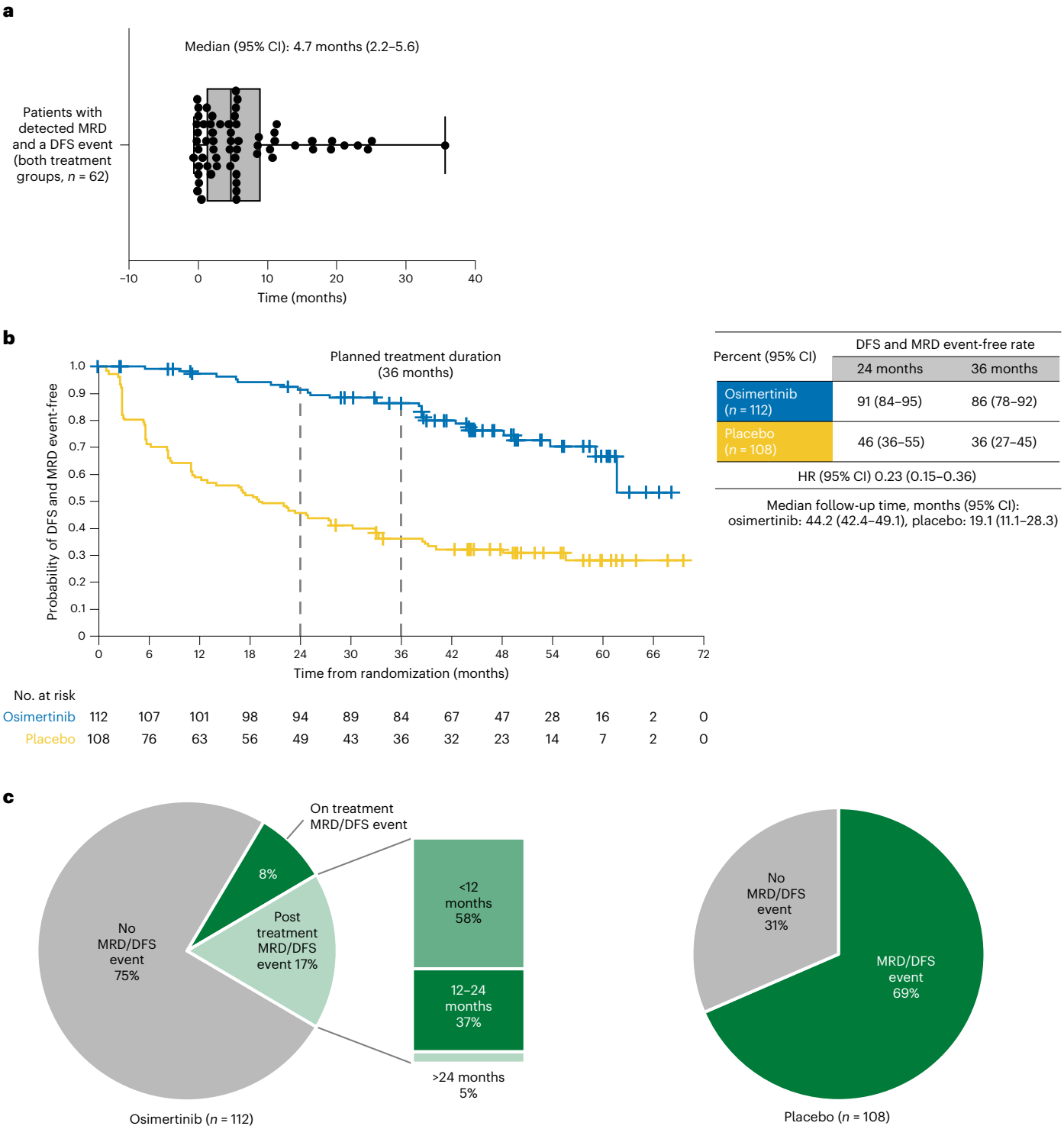


Fig. 4 | Effect of osimertinib on molecular and disease recurrence. **a**, Lead-time analysis of the median time from MRD detection to a DFS event performed for the 62 patients across both treatment groups with both a postrandomization DFS event and any plasma sample with detected MRD. Each point on the plot represents a single patient. The center vertical line represents the median time from MRD detection to a DFS event. The whiskers represent the minimum and maximum time from MRD detection to a DFS event and the box limits are representative of upper and lower quartiles. In this study, MRD preceded a DFS event by a median of 4.7 (95% CI, 2.2–5.6) months. **b**, Kaplan–Meier analysis in the MRD analysis set of the time from randomization to the combined endpoint of

DFS (clinical recurrence or death) or MRD events assessed postrandomization (whichever event occurred first) in the osimertinib and placebo groups. The DFS and MRD event-free rate is shown for landmark analyses at 24 and 36 months. HRs and 95% CIs were calculated using an unadjusted Cox proportional hazards model. **c**, DFS or MRD events occurring in the osimertinib versus placebo group. For the osimertinib group, DFS or MRD events occurring on treatment and after treatment discontinuation or completion are shown. Percentages were calculated by dividing the total number of MRD or DFS events by the total number of patients in each subgroup.

Table 1 | DFS or MRD events in the posttreatment follow-up period in the osimertinib group

Percent (95% CI)	DFS and MRD event-free rate in the posttreatment follow-up period	
	12 months after osimertinib discontinuation	24 months after osimertinib discontinuation
Osimertinib (n = 112)	80 (71–87)	66 (53–77)
Median follow-up after osimertinib discontinuation	11.7 months (8.3–13.9)	

DFS and MRD event-free rate, median follow-up and associated 95% CIs represent data from the 112 patients who discontinued treatment with osimertinib. At 12 and 24 months after osimertinib discontinuation, the DFS and MRD event-free rate was 80% and 66%, respectively. The 12- and 24-month landmarks were calculated using the Kaplan–Meier method from the time patients discontinued osimertinib treatment. Events in this analysis were DFS or MRD events (whichever came first) that occurred after osimertinib discontinuation (that is, time to MRD or DFS – total exposure + 1). Patients who experienced an MRD or DFS event (n = 9), or who were censored (n = 9) before discontinuing osimertinib treatment, had the time to MRD or DFS event in the treatment-free period set to day 1. The total number of DFS or MRD events in this analysis was 28.

care and improve outcomes by identifying high-risk patients who could benefit from restarting or intensifying treatment. In the future, with further sensitivity improvements, longitudinal MRD monitoring could also identify patients with low risk of recurrence after adjuvant therapy, potentially sparing them from further treatment and any associated drug toxicities. Therefore, understanding the predictive role and clinical utility of MRD detection is crucial to further improve outcomes for patients undergoing treatment for early and locally advanced NSCLC.

Here, we demonstrate the feasibility of performing an exploratory longitudinal ctDNA-based, tumor-informed MRD analysis in a cohort of patients with resected EGFRm NSCLC from ADAURA. As expected, the frequency of MRD detection at randomization was greater among subgroups with more advanced disease: stage IB 0%, stage II 39%, stage III 61%. Using RaDaR plasma ctDNA-based, tumor-informed MRD panels, we discovered that, for most patients, molecular recurrence based on detected MRD events, preceded clinical recurrence or death, with a median lead time of 4.7 months across both groups in this study. We note that this lead time is potentially related to the specific plasma collection and scan schedule employed in this study. Our results support previous studies showing MRD as a predictor of recurrence in patients with early-stage NSCLC following treatment with curative intent^{26–29}.

In ADAURA, a DFS and MRD event-free status was maintained for most (75%, 84 of 112) patients during adjuvant osimertinib treatment and posttreatment follow-up. Most DFS or MRD events occurred after osimertinib treatment discontinuation or completion (68%, 19 of 28) and within 12 months after osimertinib (58%, 11 of 19). Furthermore, the DFS and MRD event-free rate was high (66%) 24 months after completion or discontinuation of osimertinib treatment; this suggests that osimertinib can reduce or eradicate the micrometastatic component of the disease in some patients, leading to long-term benefit that persists after completion of adjuvant therapy. Longer follow-up is required to adequately assess this hypothesis. We can also hypothesize that, in the ADAURA setting, a subset of patients at risk of recurrence posttreatment completion may benefit from adjuvant treatment beyond 36 months or from treatment intensification via combination strategies. MRD monitoring may provide a powerful tool to personalize the use of postadjuvant treatment, although this requires prospective studies to fully elucidate the risk–benefit of such an approach.

In this analysis, only a small proportion (8%) of patients had detected MRD at randomization and this is consistent with a previous study in nonbiomarker-selected patients with early-stage NSCLC²⁹.

Although patients with detected MRD at randomization are likely to represent a high-risk subset with a poor prognosis, those who received adjuvant osimertinib had a greater rate of MRD clearance and a longer DFS compared with patients who received placebo. The large clinical benefit of adjuvant osimertinib demonstrated in ADAURA, combined with the relatively low rate of MRD detected at randomization samples and frequent occurrence (49%) of MRD in the placebo group, suggest that adjuvant osimertinib should not be limited to patients with detected MRD postoperatively. Additionally, an MRD event-free status was maintained for most patients during osimertinib treatment (87%, 97 of 112), with most MRD events occurring after stopping or completing treatment (67%, 10 of 15). Continued monitoring for detected MRD in the postadjuvant treatment follow-up period may identify a high-risk subset of patients who may benefit from further treatment. Although current MRD technologies are not sensitive enough to determine which patients with EGFRm NSCLC are disease-free postsurgery, continued undetected MRD status may represent a population for whom disease has been eradicated and no further treatment is necessary.

Previous studies in patients with resected NSCLC have focused on nonbiomarker-selected patient populations and shown a range of clinical sensitivity and specificity for longitudinal ctDNA detection to recurrence^{28,29}. The performance of MRD testing in resected EGFRm NSCLC, where tumor mutational burden has been reported to be low³⁰, is unknown. Assay sensitivity (65%) and specificity (95%) was similar in the ADAURA trial to the previous LUCID study²⁸ where RaDaR was also used. However, sensitivity and specificity of MRD assays can differ depending on the assay type, trial design and study population^{27,29,31}, and we anticipate that other MRD assays should yield similar findings.

The early clearance of *EGFR* mutations detected in plasma by droplet digital polymerase chain reaction (PCR) has been shown in the advanced setting to correlate with improved outcomes following treatment³². The use of droplet digital PCR to detect *EGFR* mutations could offer a less time-consuming and more cost-effective approach to monitor MRD than the use of tumor-informed panels in late-stage disease. In advanced EGFRm NSCLC, panel-based NGS has been shown to have comparable sensitivity to droplet digital PCR for detection of plasma *EGFR* Ex19del and L858R mutations³³. Thus, we customized the RaDaR panels to include *EGFR* mutations (Ex19del and L858R) to investigate whether detection of these mutations could identify early disease recurrence ahead of MRD detection. Whereas the detection of *EGFR* mutations could predict DFS events, *EGFR* mutation detection had a lower sensitivity and shorter median lead time than tumor-informed panel-based MRD. Among the 40 patients with both detected MRD and *EGFR* mutations, only two had detected *EGFR* mutations ahead of detected MRD. Although there will be a trade-off between assay performance and feasibility or cost implications in the real world, these findings reinforce the value of MRD detection using multiple tumor-informed gene variants in the context of resected EGFRm stage IB–IIIA NSCLC.

We used the combined MRD or DFS endpoint to assess whether patients with putative de novo *EGFR*-TKI resistance mutations have worse molecular and clinical outcomes. WES of baseline tumor samples (samples obtained from surgery before use of chemotherapy if given) from patients in the MRD analysis set showed that *TP53* mutations were the most common (45%) baseline genetic alteration concomitant with *EGFR* mutations. Adjuvant osimertinib provided clear benefit with or without co-occurring *TP53* alterations. After adjusting for the potentially confounding factors of disease stage and postsurgical chemotherapy in a multivariable analysis, baseline *TP53* alterations had a greater association with DFS or MRD events versus patients with wild-type *TP53* across both groups. Our data suggest that baseline *TP53* mutations may be prognostic within the resected EGFRm NSCLC setting. There is a lack of published data examining the prognostic and predictive value of *EGFR/TP53* comutations in patients with resected EGFRm NSCLC, and in the advanced setting the clinical importance

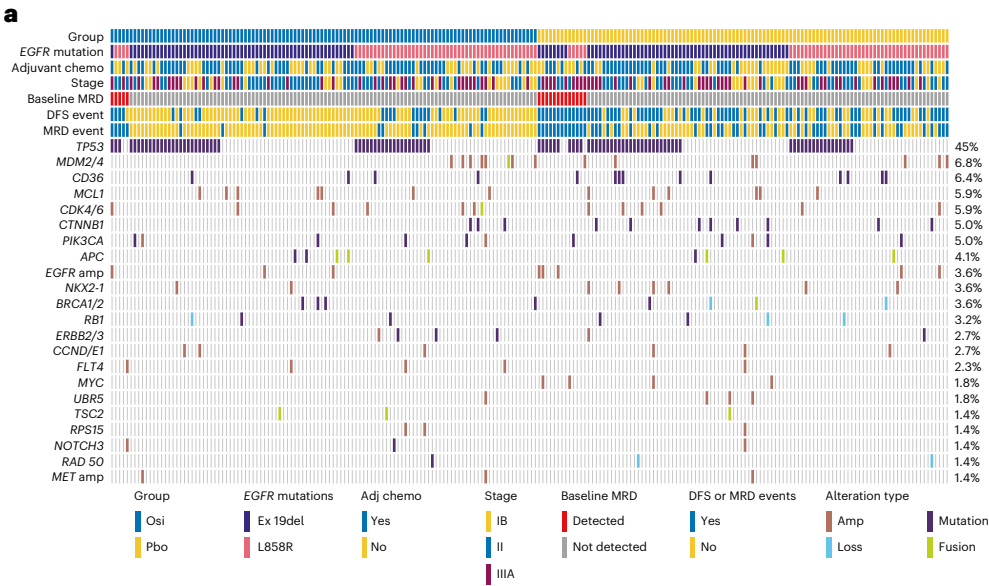
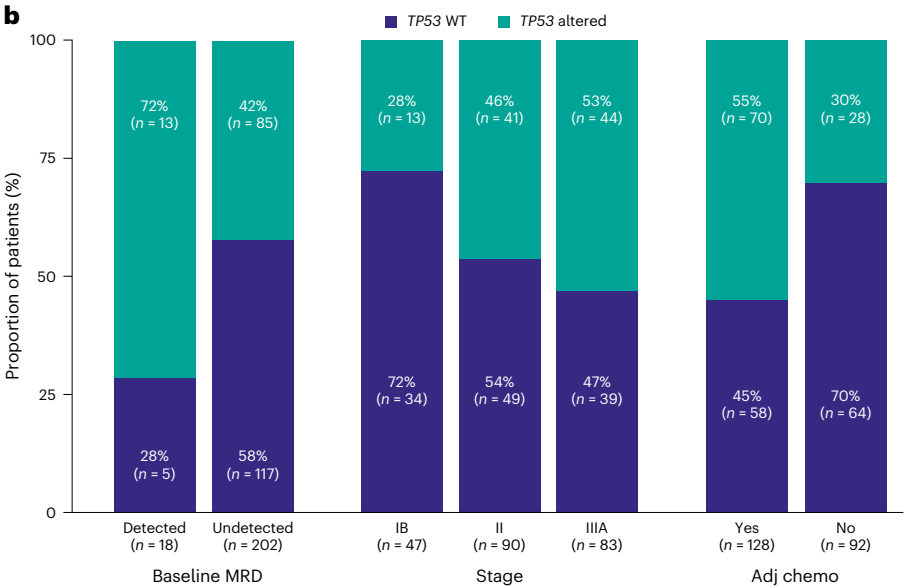
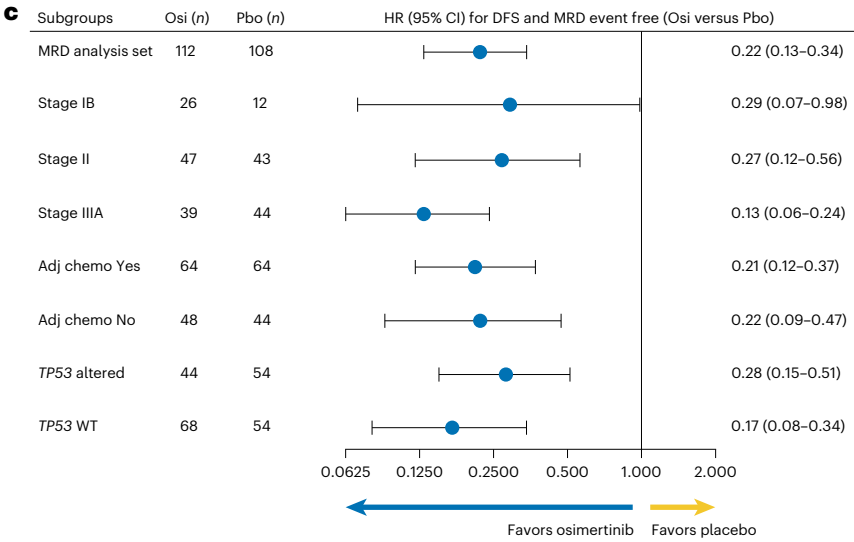


Fig. 5 | Genomic analysis of WES data from baseline tumor samples of patients in the MRD analysis set. **a**, OncoPrint for patients in the MRD analysis set showing oncogenic driver alterations (occurring in >1% of samples), MRD and DFS events, MRD status at randomization, adjuvant chemotherapy status and disease stage. **b**, Proportion of patients with *TP53* alterations by disease stage and receipt of previous adjuvant chemotherapy. **c**, Analysis of DFS and MRD event-free survival for osimertinib versus placebo for disease stage, receipt of previous

adjuvant chemotherapy and *TP53* mutation status. The number of patients varied in each subgroup as reported on the graph. The subgroup analyses were performed using a Cox proportional hazards model including treatment, subgroup and treatment by subgroup interaction. HR (blue dots) and 95% CI (horizontal lines) are shown for each subgroup. An HR of <1 favors osimertinib. Adj, adjuvant; amp, amplification; osi, osimertinib; pbo, placebo; WT, wild type.

of these computations is unclear³⁴. In advanced NSCLC, whereas some studies suggest that patients who harbor *EGFR/TP53* computations have a low response rate and poor prognosis to EGFR-TKI therapy^{35,36}, others have been unable to demonstrate this^{37–39}. Recently, patients with metastatic lung adenocarcinoma harboring *EGFR/TP53* computations were shown to be more likely to have mixed intrapatient tumor responses (responding and progressing lesions within one patient) to EGFR-TKIs compared with those with an *EGFR* mutation alone⁴⁰. Further analyses in larger cohorts of patients with early-stage EGFRm NSCLC are warranted to better understand the correlation between *TP53* mutations and adjuvant chemotherapy and disease stage, and the prognostic value of *TP53* mutations. The baseline rates for putative de novo EGFR-TKI resistance mutations, other than *TP53*, were generally low.

Key limitations of the MRD analysis were that only a subset of patients from ADAURA were assessed for MRD due to restrictions on sample availability, and the limited clinical sensitivity (65%) of the tissue-informed RaDaR MRD panels. However, there were few technical failures (9%), and the assay successfully detected MRD ahead of DFS for most patients where samples were available. Additionally, scan and plasma sampling schedules differed throughout the study, and scans were also performed less frequently than plasma sampling. The ability to predict disease recurrence using MRD should further improve as more advanced technologies with greater sensitivity are developed. Although MRD monitoring may offer a highly sensitive tool with a lead time between the detection of MRD events and clinical recurrence or death, MRD monitoring may not be amenable to all clinical situations. This is owing to the requirement for sufficient pretreatment primary tumor sample and the time required to build a validated tumor-informed MRD assay. However, given that the ADAURA regimen involves adjuvant osimertinib treatment for up to 36 months, this time provides a sufficient window to develop a robust patient-specific MRD assay for postadjuvant MRD monitoring. The postadjuvant treatment setting therefore represents a clinical scenario where prospective testing would be feasible to validate for future clinical practice.

This MRD analysis represents one of the largest patient samples so far analyzed in early-stage resected EGFRm NSCLC, with MRD measured longitudinally across a 3-year adjuvant and posttreatment follow-up period. Unanswered questions and important considerations for future analyses include: whether MRD analyses can be used to determine the optimal duration of adjuvant treatment; what the optimal frequency of MRD sampling is following adjuvant treatment completion; and how MRD detection can inform clinical intervention, including restarting adjuvant treatment or intensifying treatment via combination strategies for patients at high risk of recurrence. Similarly, de-escalation of treatments in lower-risk patients would be important, although our data have not identified any specific factors. Some of these questions should be addressed in the future analyses of the phase 2 TARGET (NCT05526755) trial, a single-arm study of 5 years of adjuvant osimertinib in patients with completely resected EGFRm stage II–IIIB NSCLC⁴¹.

In conclusion, this exploratory analysis of patients from the ADAURA trial suggests that plasma-based, tumor-informed MRD analysis may predict disease recurrence during adjuvant treatment and during posttreatment follow-up. We also show that a DFS and MRD event-free status was maintained for most patients during adjuvant

osimertinib treatment and over 24 months posttreatment. These observations suggest that MRD monitoring may inform clinical intervention, including restarting or intensifying treatment. Prospective studies to understand the optimal frequency of MRD monitoring following adjuvant treatment completion and the appropriate clinical interventions following MRD detection are warranted before this can be applied routinely to the real-world clinical setting.

Online content

Any methods, additional references, Nature Portfolio reporting summaries, source data, extended data, supplementary information, acknowledgements, peer review information; details of author contributions and competing interests; and statements of data and code availability are available at <https://doi.org/10.1038/s41591-025-03577-y>.

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Methods

Inclusion and ethics

The ADAURA protocol and amendments were approved by the relevant ethics committees. The trial was conducted in accordance with the provisions of the Declaration of Helsinki, the Good Clinical Practice guidelines of the International Council for Harmonisation, applicable regulatory requirements and the trial sponsor's (AstraZeneca) policy on bioethics and human samples; the protocol was approved by the relevant institutional review boards or ethics committees associated with each study center. All patients provided written informed consent.

ADAURA trial design

Full details of the phase 3, double-blind, placebo-controlled ADAURA trial have been reported previously^{2,8,9} and are available in the study protocol provided with ref. 9 at <https://www.nejm.org/>. Briefly, eligible patients were 18 years of age or older (20 years or older in Japan and Taiwan) and had postsurgical stage IB (T2a tumors >3 cm and ≤5 cm in size), II or IIIA NSCLC (classified according to the American Joint Committee on Cancer (AJCC) Cancer Staging Manual, 7th edn⁴²), with a centrally confirmed *EGFR* mutation (Ex19del/L858R) and a World Health Organization performance status of 0 or 1.

Following complete surgical resection of primary NSCLC, patients received adjuvant chemotherapy (if determined necessary, per physician and patient choice) for up to four cycles (at physician discretion), no more than 26 weeks postsurgery. Patients were stratified per disease stage (IB, II or IIIA), *EGFR* mutation type (Ex19del or L858R) and race (Asian or non-Asian), and then randomized 1:1 to receive osimertinib (80 mg QD) or placebo until disease recurrence, meeting a treatment discontinuation criterion or for up to 3 years of treatment. Patients not receiving adjuvant chemotherapy were randomized no more than 10 weeks after surgery.

Patient demographics for the overall ADAURA population included biological sex, which was determined based on the assigned definition rather than self-report. A breakdown of biological sex and age is provided for each treatment arm of the overall ADAURA population (Extended Data Table 2), the MRD analysis patient set by study group from ADAURA (Extended Data Table 2), patients who had detected MRD at randomization (Extended Data Table 3) and patients with DFS events by MRD status (Extended Data Table 5).

Endpoints and assessments

The primary endpoint was DFS (per investigator assessment) for patients with stage II or IIIA disease and was defined as the time from the date of randomization until the date of disease recurrence or death from any cause in the absence of disease recurrence. Secondary endpoints included DFS among patients with stage IB–IIIA disease. An exploratory endpoint was to evaluate the feasibility (defined as the success rate for assay creation) of using ctDNA profiling approaches for detection of MRD in early-stage NSCLC patients after completion of surgery (and chemotherapy in eligible patients).

Baseline CT or magnetic resonance imaging brain scans were performed within 28 days before dosing of study drug. Subsequent assessments were performed at week 12 and 24, then every 24 weeks during adjuvant treatment (up to 36 months), then every 24 weeks during the posttreatment follow-up period and then every 52 weeks until disease recurrence or death, or study end.

Plasma samples were collected from day 1 of randomization, before dosing of study drug (postsurgery and adjuvant chemotherapy, if received). Longitudinal plasma samples were collected every 12 weeks during adjuvant treatment (up to 36 months), then at week 12 and 24 and every 24 weeks during the posttreatment follow-up period (up to 60 months from randomization) and then every 52 weeks until disease recurrence or death, or study end. Formalin-fixed, paraffin-embedded (FFPE) tumor tissue specimens were available from surgical or

diagnostic primary tumor biopsies. Whole blood samples for germline analysis were collected at any time on study.

MRD detection

RaDaR MRD analysis (NeoGenomics) was performed as outlined in ref. 28. Briefly, DNA was extracted from matched FFPE tissue sections and buffy coat was derived from the whole blood samples.

WES of sheared DNA was performed on tissue DNA. Patient tumor-specific variants were ranked and prioritized for inclusion into custom primer panels containing common population-specific single nucleotide polymorphisms, for internal sample quality control during NGS testing. Apart from tumor-specific variants, sensitizing *EGFR* mutations (Ex19del and L858R) were included in the RaDaR panels to monitor their dynamics over time. Custom panels were validated by multiplex PCR and library sequencing using the matching tumor and germline DNA from each patient to exclude germline mutations and clonal hematopoiesis of indeterminate potential variants. Cell-free DNA was extracted from longitudinal plasma samples. Amplified libraries were generated using the validated custom panels and high-depth NGS was performed. Sequencing data FASTQ files were demultiplexed using *bc12fastq*; reads were then aligned to the GRCh38/hg38 assembly of the human genome and processed to identify primer pairs, and to count mutant and reference bases. A statistical model was used to assess the significance of the observed counts for each variant and the information was integrated over the entire set of variants from an individual sample to obtain evidence of detected MRD (ctDNA positive) or undetected MRD (ctDNA negative) at the sample level. The model also generated an estimated variant allele frequency for each sample.

An 'MRD detected' classification was made in alignment with the standard NeoGenomics RaDaR assay algorithm when plasma ctDNA tested positive according to the integrated statistical assessment of mutations in the RaDaR panels. MRD was considered 'detected' at any timepoint, when its integrated statistical assessment was above a preset threshold as defined during analytical development⁴³. MRD was considered 'cleared' if, following the detection of MRD at randomization, subsequent samples within the next two timepoints (24 weeks from randomization) were determined to be MRD undetected. Lead times were defined as the date of the DFS event minus the date of the first postrandomization timepoint with MRD detected. Postrandomization timepoints were used for the lead-time analysis to allow for MRD clearance with osimertinib treatment.

Genomic analysis at baseline

The genomic data used for pipeline analyses were from WES of FFPE tumor DNA samples described in the above 'MRD detection' section. FASTQ files were processed using pipeline software bcbio-nextgen (<https://doi.org/10.5281/zenodo.3564938>). Reads were aligned to the hg38 reference using bwa mem v.0.7.17. All software were run using best-practice parameters established within the bcbio workflow. Variant calling was performed using VarDict v.1.8.2 (ref. 44), down to a variant allele frequency of 1% (before filtering and curation) and variant effects were annotated by snpEff v.5.0. Filtering of noncancer variants (common polymorphisms) was performed per VarDict best practice. Copy number analysis for both exon- and whole-genome level was performed using Seq2C (<https://github.com/AstraZeneca-NGS/Seq2C>) for each gene in the panel⁴⁵. The change in the normalized log₂ values was used to determine potential copy number changes.

Statistical analysis

No statistical methods were used to predetermine sample sizes but our sample sizes are larger than those in refs. 27,28, or similar to those in ref. 29 as reported in relevant previous studies.

Baseline demographics and disease characteristics of the MRD analysis set were calculated using the number of patients in each treatment group. DFS for the MRD analysis set was analyzed using a log-rank

test stratified by disease stage (AJCC 7th ed., II and IIIA, or IB, II and IIIA), *EGFR* mutational status (Ex19del/L858R) and race (Asian or non-Asian); the same analysis method that was applied to the primary analysis. DFS events that did not occur within two scheduled visits (plus visit window) of the last evaluable assessment (or randomization) were censored and therefore excluded in the number of events. Patients who had evidence of disease at study entry were censored at day 1. DCO for DFS was 27 January 2023. Due to the low number of events, OS for the MRD analysis set was analyzed using an unstratified log-rank test. DCO for OS was 11 April 2022.

This analysis of MRD is a post hoc exploratory analysis separate from the prespecified primary and secondary endpoints of the ADAURA study. These analyses were not included in the statistical analysis plan (SAP) and therefore *P* values have not been generated⁹.

The Kaplan–Meier approach was used to calculate DFS and MRD event-free rates at 24 and 36 months on treatment for the osimertinib and placebo groups. HR for the combined endpoint of DFS or MRD events in the osimertinib versus placebo group was calculated using an unadjusted Cox proportional hazards model that contained only treatment as a covariate, with the Efron method used to control for ties. An HR of <1 favored osimertinib. Profile-likelihood CIs were also determined. The combined endpoint of DFS (clinical recurrence or death) or MRD events was assessed postrandomization from randomization to postrandomization MRD detection, disease recurrence or death (whichever occurred first). Any patient not known to have had a postrandomization DFS or MRD event was censored at the last follow-up visit when confirmed (date of first postrandomization MRD or DFS event or censoring – date of randomization + 1).

The Kaplan–Meier approach was also used to calculate DFS and MRD event-free rates in the posttreatment follow-up period for osimertinib. The 12- and 24-month post-osimertinib discontinuation landmarks were calculated from the time patients discontinued or completed osimertinib treatment. Events in this analysis were DFS or MRD events (whichever occurred first) that occurred after osimertinib discontinuation (time to MRD/DFS – total exposure + 1). Patients who experienced an MRD or DFS event (*n* = 9) or were censored (*n* = 9) before discontinuing osimertinib treatment, had the time to MRD or DFS event in the treatment-free period set to day 1. SAS v.9.4M7 was used for statistical analysis.

Reporting summary

Further information on research design is available in the Nature Portfolio Reporting Summary linked to this article.

Data availability

To protect the privacy and confidentiality of patients in this study, clinical data are not made publicly available in a repository or in the supplementary material. Data underlying the findings described in this manuscript may be obtained in accordance with AstraZeneca's data sharing policy described at <https://astrazenecagrouptrials.pharmacm.com/ST/Submission/Disclosure>. Data for studies directly listed on Vivli can be requested through Vivli at www.vivli.org. Data for studies not listed on Vivli could be requested through Vivli at <https://vivli.org/members/enquiries-about-studies-not-listed-on-the-vivli-platform/>. AstraZeneca Vivli member page is also available outlining further details: <https://vivli.org/ourmember/astrazeneca/>. hg38 reference: NCBI RefSeq assembly (v.GCF_000001405.40).

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Author contributions

R.S.H., C.G., Y.-L.W., J.R., R.H. and M. Tsuboi contributed to the conception and design of the work. R.S.H., C.G., K.L., M.M., M.-J. A., D.M.K., M.P., P.B., A.M., C.M., J.R., R.H., M. Tiseo and M. Tsuboi contributed to the acquisition, analysis or interpretation of data for the work. All authors drafted the work or revised it critically for important intellectual content, provided final approval of the version to be published and agree to be accountable for all aspects of the work ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

Competing interests

R.S.H. declares board of directors for Immunocore; consulting fees from AbbVie, AstraZeneca, Bolt Biotherapeutics, Bristol Myers Squibb, Candel Therapeutics, Inc., Checkpoint Therapeutics, Cybrexa Therapeutics, Eli Lilly and Company, EMD Serono, Genentech, Gilead, I-MAB Biopharma, Immune-Onc Therapeutics, Inc., Immunocore, Janssen, Merck and Company, Mirati Therapeutics, NextCure, Normunity, Novartis, Ocean Biomedical, Inc., Pfizer, Regeneron Pharmaceuticals, Revelar Biotherapeutics, Inc., Roche, Sanofi, and Seattle Genetics; and funding from AstraZeneca, Eli Lilly and Company, Genentech/Roche and Merck and Company. T.J. declares consulting fees from Amgen, AstraZeneca, AstraZeneca/MedImmune, Boehringer Ingelheim, Bristol Myers Squibb, Gilead Sciences, Ignyta, Merck KGaA, Merck Sharp and Dohme, Novartis, Pfizer, PharmaMar, Roche/Genentech, Seagen and Specialised Therapeutics; honoraria from AstraZeneca/MedImmune, Bristol Myers Squibb, Merck Sharp and Dohme, and Roche/Genentech; and travel and accommodation expenses from AstraZeneca, Boehringer Ingelheim, Bristol Myers Squibb, Merck Sharp and Dohme, and Roche. C.G. declares advisory board for AstraZeneca and Merck Sharp and Dohme; consulting fees and honoraria from AstraZeneca, Boehringer Ingelheim, Daiichi-Sankyo, Pfizer, and Sanofi; and speaker's bureau and travel, accommodation, expenses from AstraZeneca, Boehringer Ingelheim, and Roche. J.W.G. declares consulting fees from AbbVie, AstraZeneca, Bristol Myers Squibb, Genentech, Gritstone bio, Janssen, Jazz Pharmaceuticals, Lilly, Pfizer, Puma Biotechnology, Regeneron and Turning Point Therapeutics; and funding from AbbVie, Advaxis, AstraZeneca/MedImmune, Bristol Myers Squibb, Genentech/Roche, Lilly, Pfizer and Spectrum Pharmaceuticals (to institution). T.K. declares funding from AbbVie, Amgen, ArriVent, AstraZeneca, Bayer, BeiGene, Blueprint, Bristol Myers Squibb, Chugai Pharmaceutical Co., Ltd., Daiichi-Sankyo, Eli Lilly, Gilead, GSK, Haihe, Janssen, Merck Biopharma, Merck Sharp and Dohme, Novartis, Pfizer, Regeneron and Takeda; and honoraria from AstraZeneca, Chugai Pharmaceutical Co., Ltd., Daiichi-Sankyo, Merck Biopharma, and Merck Sharp and Dohme.

K.L. declares consulting fees from Amgen, AstraZeneca, Biocad, Pfizer and Roche; and speaker's bureau from Amgen, AstraZeneca, Biocad, Pfizer, Roche and Swixx BioPharma. L.B. declares advisory board for AstraZeneca, Bristol Myers Squibb, Janssen, Roche, Merck Sharp and Dohme and Novartis; funding from AstraZeneca; and speaker's bureau from AstraZeneca, Bristol Myers Squibb, Lilly, Merck, Merck Sharp and Dohme, Novartis and Roche. M. Tiseo declares consulting fees from Amgen, AstraZeneca, Boehringer Ingelheim, Bristol Myers Squibb, Daiichi-Sankyo, Eli Lilly, Janssen, Merck, Merck Sharp and Dohme, Novartis, Pfizer, Roche, Sanofi and Takeda; funding from AstraZeneca, Boehringer Ingelheim and Roche; and travel and accommodation expenses from Amgen and Takeda. M.M. declares consulting fees from Amgen, AstraZeneca, BeiGene, Boehringer Ingelheim, Bristol Myers Squibb, Helsinn Therapeutics, Janssen, Lilly, Merck Sharp and Dohme, Novartis, Pfizer, Pierre Fabre, Roche, Sanofi and Takeda; funding from AstraZeneca, Bristol Myers Squibb and Roche (to institution); and travel and accommodation expenses from AstraZeneca, Merck Sharp and Dohme, Pfizer and Roche. M.D. declares advisory board for AstraZeneca, Boehringer Ingelheim, Bristol Myers Squibb, Merck Sharp and Dohme, Pfizer and Roche and lecture fees from AstraZeneca, Boehringer Ingelheim, Bristol Myers Squibb, Merck Sharp and Dohme, Pfizer and Roche. M.-J.A. declares advisory role for Alpha Pharmaceuticals, Amgen, AstraZeneca, Lilly, Merck, Merck Sharp and Dohme, Novartis, Ono Pharmaceutical, Co. Ltd., Roche, Takeda and Yuhan Corporation; and invited speaker for Amgen, AstraZeneca, Lilly, Merck, Merck Sharp and Dohme, Novartis, Ono Pharmaceutical, Co., Ltd., Roche, Takeda and Yuhan Corporation. D.M.K. declares advisory board for Amgen, AstraZeneca, Boehringer Ingelheim, Bristol Myers Squibb, Johnson and Johnson/Janssen, Merck Serono, Merck Sharp and Dohme, Pfizer, Roche/Genentech and Takeda; and consulting fees from Amgen, AstraZeneca, Boehringer Ingelheim, Bristol Myers Squibb, Johnson and Johnson/Janssen, Merck Serono, Merck Sharp and Dohme, Pfizer, Roche/Genentech and Takeda. M.P. declares consulting fees from Amgen, AstraZeneca, Boehringer Ingelheim, Bristol Myers Squibb, Chugai Pharmaceutical Co., Ltd., Daiichi-Sankyo, Eisai, GSK, Gritstone bio, IPSEN, Janssen, Lilly, Merck Sharp and Dohme, Novartis, Novocure, Pfizer, Roche/Genentech, Sanofi and Takeda; funding from AstraZeneca, Boehringer Ingelheim, Roche and Takeda (to institution); other relationship with AstraZeneca; and travel and accommodation expenses from AstraZeneca, Bristol Myers Squibb, Chugai Pharmaceutical, Merck Sharp and Dohme, Pfizer, Roche and Takeda. V.S. declares consulting fees from Astellas, AstraZeneca, Eisai, Merck Sharp and Dohme, Novartis, Pfizer, Roche and Takeda; funding from Astellas, AstraZeneca, Daiichi-Sankyo, Merck Sharp and Dohme, Novartis, Roche and Takeda (to institution); invited speaker from Astellas, AstraZeneca, Eisai, Merck Sharp and Dohme, Novartis, Pfizer, Roche and Takeda. M.Ö. declares advisory board for Astellas,

Merck Sharp and Dohme and Regeneron/Sanofi; advisory role for AstraZeneca and Merck Sharp and Dohme; honoraria from Astellas, Janssen and Novartis; invited speaker for Regeneron (nonfinancial); local principal investigator for AstraZeneca, BeiGene, Bristol Myers Squibb, Daiichi-Sankyo, Merck Sharp and Dohme, Pfizer, Regeneron and Roche; membership with ASCO and ESMO; and speaker's bureau, steering committee member, and travel and accommodation, expenses from AstraZeneca. P.B. declares employment and stock/shares with AstraZeneca. A.M., Y.R. and C.M. declare employment and stock/shares with AstraZeneca. J.R. declares employment and stock/shares with AstraZeneca; inventor on patents held by UT MD Anderson Cancer Center licensed to Spectrum Pharmaceuticals for treatment of EGFR/HER2 exon 20 mutant cancers and inventor on patent held by UT MD Anderson Cancer Center regarding EGFR mutation subtypes and methods of treatment (nonfinancial). R.H. declares employment and stock/shares with AstraZeneca and inventor on patent US11066709B2 (nonfinancial). M. Tsuboi declares advisory role with AstraZeneca KK, Chugai Pharmaceutical Co., Ltd., Daiichi-Sankyo, Merck Sharp and Dohme and Novartis; funding from AstraZeneca KK, Bristol Myers Squibb KK, Eli Lilly Japan, Ono Pharmaceutical Co., Ltd., Merck Sharp and Dohme, MiRXES and Novartis (to institution); and lecture fees from Amgen KK, AstraZeneca KK, Bristol Myers Squibb KK, Chugai Pharmaceutical Co., Ltd., Daiichi-Sankyo, Eli Lilly Japan, Johnson and Johnson Japan, Medtronic Japan, Merck Sharp and Dohme, Novartis, Ono Pharmaceutical Co., Ltd. and Taiho Pharma. Y.-L.W. declares consulting fees from AstraZeneca, Boehringer Ingelheim, Roche and Takeda; funding from Boehringer Ingelheim, Bristol Myers Squibb, Roche and Pfizer (to institution); and honoraria from AstraZeneca, Boehringer Ingelheim, Bristol Myers Squibb, BeiGene Beijing, Hengrui Pharmaceutical, Merck Sharp and Dohme, Pfizer and Roche.

Additional information

Extended data is available for this paper at

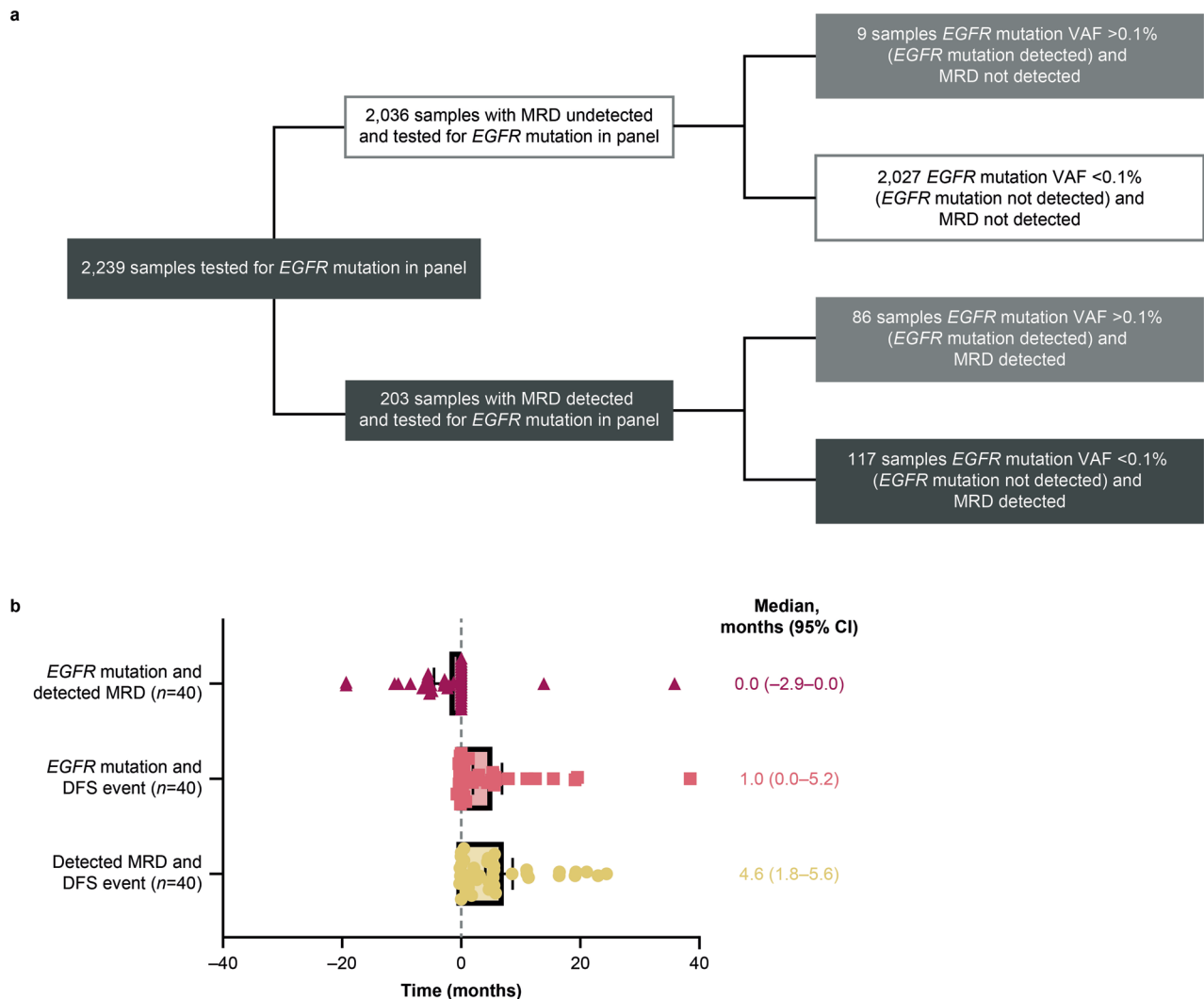
<https://doi.org/10.1038/s41591-025-03577-y>.

Supplementary information The online version contains supplementary material available at <https://doi.org/10.1038/s41591-025-03577-y>.

Correspondence and requests for materials should be addressed to Roy S. Herbst or Yi-Long Wu.

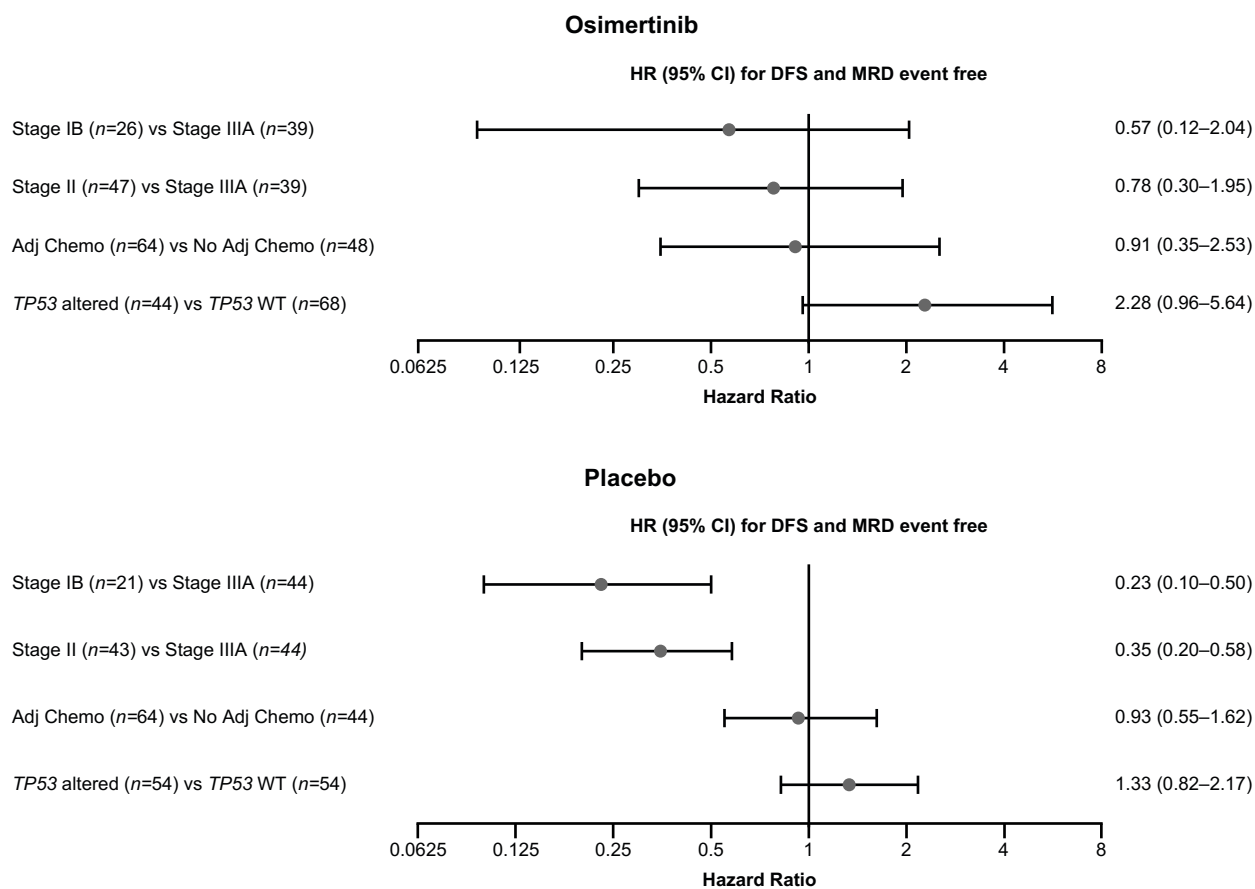
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Extended Data Fig. 1 | Comparison of *EGFR* mutation (Ex19del and L858R) and MRD assay performance. a, Flow chart showing samples included as part of the *EGFR* mutation analysis. **b**, Lead-time analysis demonstrating median time from *EGFR* mutation detection to MRD detection, *EGFR* mutation detection to a DFS event, and MRD detection to a DFS event performed for the 40 patients across both groups with a post-randomization DFS event, any sample with detected MRD, and any sample with detected *EGFR* mutation. *EGFR* mutation

preceded detected MRD by a median of 0.0 (95% CI -2.9-0.0) months, *EGFR* mutation preceded a DFS event by a median of 1.0 (95% CI 0.0-5.2) month and MRD preceded a DFS event by a median of 4.6 (95% CI 1.8-5.6) months. Each point on the plot (triangle/square/circles) represents a single patient. The whiskers represent the minimum and maximum lead time between events and the box limits are representative of the 95% CI.



Extended Data Fig. 2 | Multivariable analysis adjusting for disease stage, adjuvant chemotherapy status and *TP53* mutation type on the DFS or MRD combined endpoint fitted separately for each group. The number of patients

varied in each subgroup as reported on the graph. HRs and 95% CIs were calculated using the Cox proportional hazards model. The HRs (represented by black dots) and 95% CIs (horizontal lines) are shown for each subgroup.

Extended Data Table 1 | Average number of genetic variants included in tumor-specific RaDaR panels by disease stage (AJCC 7th edition)

	Average	Minimum	Maximum	Number of patients
All stages	31.4	8	48	220
Stage IB	29.9	9	47	47
Stage II	31.6	8	48	90
Stage IIIA	32.0	9	48	83

Extended Data Table 2 | Baseline demographics, disease characteristics, and outcomes for the MRD analysis set (n=220) and full ADAURA patient population (N=682)

Characteristics, n (%) ^a	MRD analysis set		Overall ADAURA population ⁹	
	Osimertinib (n=112)	Placebo (n=108)	Osimertinib (n=339)	Placebo (n=343)
AJCC stage ^b : IB / II / IIIA	26 (23) / 47 (42) / 39 (35)	21 (19) / 43 (40) / 44 (41)	107 (32) / 115 (34) / 117 (35) ^c	109 (32) / 116 (34) / 118 (34)
EGFR mutation at randomization ^d : Ex19del / L858R	62 (55) / 50 (45)	62 (57) / 46 (43)	185 (55) / 153 (45)	188 (55) / 155 (45)
Adjuvant chemotherapy: yes / no	64 (57) / 48 (43)	64 (59) / 44 (41)	202 (60) / 137 (40)	207 (60) / 136 (40)
Age: median, years	66	68	64	62
Race: Asian / non-Asian	50 (45) / 62 (55)	48 (44) / 60 (56)	216 (64) / 123 (36)	218 (64) / 125 (36)
Sex: male / female	36 (32) / 76 (68)	24 (22) / 84 (78)	109 (32) / 230 (68)	95 (28) / 248 (72)
Smoking history: yes / no	43 (38) / 69 (62)	32 (30) / 76 (70)	108 (32) / 231 (68)	86 (25) / 257 (75)
Outcomes				
Osimertinib vs placebo				
DFS: HR (95% CI)	0.19 (0.12–0.29) ^e		0.27 (0.21–0.34) ²	
OS: HR (95% CI)	0.42 (0.24–0.74) ^f		0.49 (0.34–0.70) ⁸	

^aUnless otherwise specified; ^bAt the time of recruitment, staging was determined by the AJCC Staging Manual 7th edition; ^cPercentages may not total to 100 due to rounding; ^dCentral test; ^eDFS was analyzed using a log-rank test stratified by disease stage (AJCC 7th edition, IB versus II versus IIIA), EGFR mutational status (Ex19del versus L858R), and race (Asian versus non-Asian); the same analysis method applied to the primary analysis; ^fDue to the low number of events OS for the MRD analysis set was analyzed by an unstratified log-rank test. DFS and OS for the overall ADAURA population was analyzed by a stratified log-rank-test. AJCC, American Joint Committee on Cancer; CI, confidence interval; DFS, disease-free survival; EGFR, epidermal growth factor receptor; Ex19del, exon 19 deletion; HR, hazard ratio; MRD, molecular residual disease; OS, overall survival.

Extended Data Table 3 | Baseline demographics and disease characteristics of patients with MRD detected at randomization (*n*=18) compared with all patients in the MRD analysis set (*n*=220)

Characteristics, n (%)	Patients with MRD detected at randomization (<i>n</i> =18)	MRD analysis set (<i>n</i> =220)
AJCC stage ^a : IB / II / IIIA	0 (0) / 7 (39) / 11 (61)	47 (21) / 90 (41) / 83 (38)
<i>EGFR</i> mutation ^b : Ex19del / L858R	9 (50) / 9 (50)	124 (56) / 96 (44)
Adjuvant chemotherapy: yes	8 (44)	128 (58)
Age: median, years	68	66
Race: Asian / non-Asian	6 (33) / 12 (67)	98 (45) / 122 (55)
Sex: male / female	7 (39) / 11 (61)	60 (27) / 160 (73)
Smoking history: yes / no	7 (39) / 11 (61)	75 (34) / 145 (66)

^aAt the time of recruitment, staging was determined by the AJCC Staging Manual 7th edition. ^b*EGFR* mutation status was centrally confirmed in tissue. AJCC, American Joint Committee on Cancer; *EGFR*, epidermal growth factor receptor; Ex19del, exon 19 deletion; MRD, molecular residual disease.

Extended Data Table 4 | Sensitivity and specificity for the RaDaR MRD assay

		MRD status, ^b <i>n</i>	
		Detected (<i>n</i> =68)	Undetected (<i>n</i> =152)
DFS status, ^a <i>n</i>	Event (<i>n</i> =96)	62	34
	Censor (<i>n</i> =124)	6	118
Concordance of MRD with DFS, ^c % (95% CI)			
PPA (sensitivity)		65 (55–74)	
NPA (specificity)		95 (91–99)	
PPV		91 (84–98)	
NPV		78 (71–84)	
OPA		82 (77–87)	

^aScans were performed across both groups at week 12 and 24, every 24 weeks until 60 months, and then every 52 weeks thereafter until disease recurrence or study end. ^bPlasma samples were collected across both groups every 12 weeks during first 36 months, every 24 weeks until 60 months, and then every 52 weeks thereafter until disease recurrence or study end. ^cMRD was the test variable and DFS was the response for concordance calculations. CI, confidence interval; DFS, disease-free survival; MRD, molecular residual disease; NPA, negative percentage agreement; NPV, negative predictive value; OPA, overall percentage agreement; PPA, positive percentage agreement; PPV, positive predictive value.

Extended Data Table 5 | Baseline demographics and disease characteristics of patients with DFS events (n=96) by MRD status

Characteristics, n (%)	Patients with DFS events and MRD detected (n=62)	Patients with DFS events and MRD undetected (n=34)
AJCC stage ^a : IB / II / IIIA	6 (10) / 20 (32) / 36 (58)	6 (18) / 13 (38) / 15 (44)
<i>EGFR</i> mutation ^b : Ex19del / L858R	31 (50) / 31 (50)	19 (56) / 15 (44)
Race: Asian / non-Asian	29 (47) / 33 (53)	19 (56) / 15 (44)
Smoking history: yes / no	21 (34) / 41 (66)	14 (41) / 20 (59)
Treatment group: Osimertinib / placebo	11 (18) / 51 (82)	13 (38) / 21 (62)
DFS location ^c : Distant ± CNS, Local / regional or CNS only	29 (50) / 29 (50)	8 (26) / 23 (74)

^aAt the time of recruitment, staging was determined by the AJCC Staging Manual 7th edition. ^b*EGFR* mutation status was centrally confirmed in tissue. ^cPatients with a DFS event but location of recurrence is unknown were excluded from the analysis of DFS location. AJCC, American Joint Committee on Cancer; CNS, central nervous system; DFS, disease-free survival; *EGFR*, epidermal growth factor receptor; Ex19del, exon 19 deletion; MRD, molecular residual disease.

Extended Data Table 6 | Sensitivity and specificity for *EGFR* mutation detection in the MRD analysis set

		<i>EGFR</i> mutation			Concordance of <i>EGFR</i> mutation with DFS, % (95% CI)	
		Detected	Undetected	Total		
DFS	Event	47	40	87	PPA (sensitivity)	54.0 (43.6–64.5)
	Censor	9	110	119	NPA (specificity)	92.4 (87.7–97.2)
		56	150	206	PPV	83.9 (74.3–93.5)
					NPV	73.3 (66.3–80.4)
		<i>EGFR</i> mutation			Concordance of <i>EGFR</i> mutation with MRD, % (95% CI)	
		Detected	Undetected	Total		
MRD	Detected	86	117	203	PPA (sensitivity)	42.4 (35.6–49.2)
	Undetected	9	2,027	2,036	NPA (specificity)	99.6 (99.3–99.8)
		95	2,144	2,239	PPV	90.5 (84.6–96.4)
					NPV	94.5 (93.6–95.5)

CI, confidence interval; DFS, disease-free survival; MRD, molecular residual disease; NPA, negative percentage agreement; NPV, negative predictive value; OPA, overall percentage agreement; PPA, positive percentage agreement; PPV, positive predictive value.

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☒	☐ For null hypothesis testing, the test statistic (e.g. <i>F</i> , <i>t</i> , <i>r</i>) with confidence intervals, effect sizes, degrees of freedom and <i>P</i> value noted <i>Give P values as exact values whenever suitable.</i>
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Software and code

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Data collection	No software was used. Code Availability Statement Not applicable. No unreported custom computer code or algorithm was used to generate the results.
Data analysis	R version 4.4 was used for data visualization bcbio-nextgen (https://doi.org/10.5281/zenodo.3564938) was used to process FASTQ files bwa mem v0.7.17. was used to align sequencing reads to the hg38 reference SAS version 9.4M7 was used for statistical analysis VarDict v1.8.2 was used to call variants snpEff v5.0 was used to annotate variant effects Seq2C (https://github.com/AstraZeneca-NGS/Seq2C) was used to analyze copy number for both exon- and whole-gene level

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Data Availability Statement

In order to protect the privacy and confidentiality of patients in this study, clinical data are not made publicly available in a repository or the supplementary material of the article. Data underlying the findings described in this manuscript may be obtained in accordance with AstraZeneca's data sharing policy described at <https://astrazenecagrouptrials.pharmacm.com/ST/Submission/Disclosure>.

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hg38 reference: NCBI RefSeq assembly (version GCF_000001405.40).

Research involving human participants, their data, or biological material

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Reporting on sex and gender

Patient demographics for the overall ADAURA population included biological sex, which was determined based on the assigned/methodology definition rather than self-report. A breakdown of biological sex and age is provided for each treatment arm of the overall ADAURA population (Extended Data Table 2), the molecular residual disease (MRD) analysis patient set by study group from ADAURA (Extended Data Table 2), patients who had detected MRD at randomization (Extended Data Table 3) and patients with disease-free survival (DFS) events by MRD status (Extended Data Table 5). Findings were similar across both sexes. Reporting individual-level data was not included in consent; therefore, de-identified data are reported. Thirty percent of patients in the overall ADAURA patient population were male and 70% were female. EGFR-mutant NSCLC occurs more frequently in female patients, therefore this bias was expected and representative of the disease subtype.

Reporting on race, ethnicity, or other socially relevant groupings

Patient demographics for the overall ADAURA population reports the percent of patients who self-identified as Asian as a variable (45% of patients included). Race was a predefined stratification factor for the ADAURA study.

Population characteristics

Enrolled patients were 18 years of age or older (20 years or older in Japan and Taiwan) and had post-surgical stage IB (T2a tumors >3 cm and ≤5 cm in size), II, or IIIA non-small cell lung cancer (NSCLC) (classified according to the AJCC Cancer Staging Manual, 7th edition), with a centrally confirmed EGFR mutation (Ex19del/L858R) and a World Health Organization performance status of 0 or 1. Complete resection of the primary NSCLC was mandatory. Administration of standard postoperative adjuvant chemotherapy before randomization was allowed but not mandatory; decisions about whether patients would receive adjuvant chemotherapy were made by the physician and the patient and were made before trial enrollment. Treatment with preoperative, postoperative, or planned radiation therapy was not allowed. (Wu et al. N Engl J Med 2020;383:1711-1723)

Recruitment

Eligible patients were recruited by investigators at study sites in China, Japan, Russian Federation, Taiwan, South Korea, Thailand, Italy, Spain, Vietnam, United States, Australia, Brazil, Germany, Poland, Israel, Turkey, France, Hong Kong, Canada, Romania, Ukraine, Hungary, Sweden and Belgium. While patients were recruited from China in the clinical study, patients from China were excluded from the exploratory analysis due to local restrictions. Due to tissue requirements for the assay, there was a bias in the exploratory analysis for patients with more advanced stage disease (stage II-IIIa disease vs stage Ib), as those patients had more tissue available.

Ethics oversight

The trial was conducted in accordance with the provisions of the Declaration of Helsinki, the Good Clinical Practice guidelines of the International Council for Harmonisation, applicable regulatory requirements, and the trial sponsor's policy on bioethics and human samples, and the protocol was approved by the relevant institutional review boards or ethics committees associated with each study center. All patients provided written informed consent. Full details of the trial have been reported previously in Wu et al. N Engl J Med 2020;383:1711-1723 and are available in the study protocol provided at NEJM.org. A redacted version of the study protocol is available at: <https://www.astrazenecaclinicaltrials.com/study/D5164C00001/> and https://cdn.clinicaltrials.gov/large-docs/06/NCT02511106/Prot_000.pdf

Note that full information on the approval of the study protocol must also be provided in the manuscript.

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Life sciences study design

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Sample size	No sample size calculation was performed for this exploratory analysis of molecular residual disease (MRD). Patient sample size was based on available available matched tumor, germline DNA, and longitudinal plasma samples and successful validation of tumor-informed RaDaR panels, such that 220 patients from ADAURA were successfully analyzed for MRD. Specifically, of 682 patients randomized in ADAURA, 239 (35%) had available matched tumor, germline DNA, and plasma samples for development and testing of tumor-specific RaDaR panels at the time of data cut-off. RaDaR panels were successfully designed and validated for 220 (92%) of the 239 patients across both the osimertinib (n=112) and placebo (n=108) groups and comprised the MRD analysis set. However, the original ADAURA study sample sizes were designed to have 80% power to detect a hazard ratio of 0.70 with a two-sided alpha level of 5% with 247 disease recurrence events or deaths in 490 patients with stage II-IIIa disease (Wu et al. N Engl J Med 2020;383:1711-1723). Samples for exploratory MRD analysis were based on patient consent and local country restrictions on sample usage/data export.
Data exclusions	Only patients with sufficient matched tumor, germline DNA, and plasma samples to meet the requirements of the RaDaR molecular residual disease (MRD) platform were included (n=239 patients). Patients from China were excluded as plasma samples were unable to be exported for analysis. Of the 239 patients, RaDaR panels were successfully designed for 220 patients. For the remaining 19 patients, whole-exome sequencing (WES) failed for seven patients because of poor DNA yield and/or quality or low sequencing depth, MRD panel design failed for 12 patients because of insufficient tumor-informed mutations detected during WES.
Replication	No specific measures were taken to verify the reproducibility of the findings. Due to the limited sample availability for each patient it was not feasible to perform repeat analyses of the samples.
Randomization	Patients were stratified per disease stage (IB, II, or IIIA), EGFR mutation type (Ex19del or L858R), and race (Asian or non-Asian), and then randomized 1:1 to receive osimertinib (80 mg QD) or placebo until disease recurrence, meeting a treatment discontinuation criterion, or for up to 3 years of treatment.
Blinding	ADAURA was double-blinded but no blinding was used in these exploratory analysis of molecular residual disease

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☒	☐ Palaeontology and archaeology
☒	☐ Animals and other organisms
☐	☒ Clinical data
☒	☐ Dual use research of concern
☒	☐ Plants

Methods

n/a	Involved in the study
☒	☐ ChIP-seq
☒	☐ Flow cytometry
☒	☐ MRI-based neuroimaging

Clinical data

Policy information about [clinical studies](#)

All manuscripts should comply with the ICMJE [guidelines for publication of clinical research](#) and a completed [CONSORT checklist](#) must be included with all submissions.

Clinical trial registration	NCT02511106
Study protocol	Full details of the trial have been reported previously in Wu et al. N Engl J Med 2020;383:1711-1723 and are available in the study protocol provided at NEJM.org. A redacted version of the study protocol is available at https://cdn.clinicaltrials.gov/large-docs/06/NCT02511106/Prot_000.pdf
Data collection	Patients were enrolled in the study globally at 185 study centers in 24 countries across Europe, Asia-Pacific, North America and South

Data collection	America from November 2015 to February 2019 (Wu et al. N Engl J Med 2020;383:1711-1723). In these exploratory analyses of molecular residual disease (MRD), formalin-fixed, paraffin-embedded tumor tissue specimens (used to design the RaDaR panels) were available from surgical or diagnostic primary tumor biopsies collected prior to randomization and plasma samples (for MRD detection) were collected longitudinally up until overall survival data cut-off (January 27, 2023) MRD data for the MRD analysis set were analyzed with cut-off dates of April 11, 2022 for disease-free survival, and January 27, 2023 for overall survival.
Outcomes	<p>As this was an exploratory analysis of molecular residual disease (MRD), no pre-defined primary or secondary outcome measures were assessed. Longitudinal plasma samples were collected every 12 weeks during adjuvant treatment (up to 36 months), then at week 12 and 24 and every 24 weeks during the post-treatment follow-up period (up to 60 months from randomization), and then every 52 weeks until disease recurrence or death, or study end. Disease-free survival (DFS; per investigator assessment) for patients with stage II or IIIA disease was defined as the time from the date of randomization until the date of disease recurrence or death from any cause in the absence of disease recurrence. DFS was assessed by baseline computed tomography or magnetic resonance imaging brain scans, which were performed within 28 days prior to dosing of study drug. Subsequent assessments were performed at week 12 and 24, then every 24 weeks during adjuvant treatment (up to 36 months), then every 24 weeks during the post-treatment follow-up period, and then every 52 weeks until disease recurrence or death, or study end.</p> <p>To assess MRD, tumor-specific RaDaR panels, designed based on patient tumor formalin-fixed paraffin-embedded samples, were used to detect circulating tumor DNA (ctDNA) in patient plasma samples at baseline and throughout the adjuvant and post-treatment follow-up periods. An 'MRD detected' classification was made in alignment with the standard NeoGenomics RaDaR assay algorithm when plasma ctDNA tested positive according to the integrated statistical assessment of mutations in the RaDaR panels. MRD was considered 'detected' at any time point when its integrated statistical assessment was above a pre-set threshold as defined during analytical development. MRD was considered 'cleared' if, following the detection of MRD at baseline, subsequent samples within the next two time points (24 weeks from baseline) were determined to be MRD undetected.</p> <p>To understand the benefit of osimertinib with respect to molecular or clinical disease recurrence, the combined endpoint of DFS (clinical recurrence or death) or MRD events was assessed from randomization to post-baseline MRD detection, disease recurrence, or death (whichever occurred first). The Kaplan–Meier approach was used to calculate DFS and MRD event-free rates at 24 and 36 months on treatment for the osimertinib and placebo groups. Hazard ratio for the combined endpoint of DFS or MRD events in the osimertinib versus placebo group was calculated using an unadjusted Cox proportional hazards model that only contained treatment as a covariate, with the Efron method used to control for ties.</p>

Plants

Seed stocks	<i>Report on the source of all seed stocks or other plant material used. If applicable, state the seed stock centre and catalogue number. If plant specimens were collected from the field, describe the collection location, date and sampling procedures.</i>
Novel plant genotypes	<i>Describe the methods by which all novel plant genotypes were produced. This includes those generated by transgenic approaches, gene editing, chemical/radiation-based mutagenesis and hybridization. For transgenic lines, describe the transformation method, the number of independent lines analyzed and the generation upon which experiments were performed. For gene-edited lines, describe the editor used, the endogenous sequence targeted for editing, the targeting guide RNA sequence (if applicable) and how the editor was applied.</i>
Authentication	<i>Describe any authentication procedures for each seed stock used or novel genotype generated. Describe any experiments used to assess the effect of a mutation and, where applicable, how potential secondary effects (e.g. second site T-DNA insertions, mosaicism, off-target gene editing) were examined.</i>