Adjuvant chemotherapy for stage IA–IIA non-squamous, non-small-cell lung cancer identified as molecular high-risk by a 14-gene expression profile (AIM-HIGH): an international, randomised, phase 3 trial

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Summary

Background Survival for non-small-cell lung cancer (NSCLC) remains unacceptably low, even in stage IA–IIA. Current guidelines recommend adjuvant treatment for patients considered to be at high risk in stages IB and IIA, but suggest criteria that have not been validated to predict benefit. A previously validated, CLIA-certified 14-gene expression profile has identified patients with high-risk non-squamous NSCLC tumours in stages IA–IIA who benefitted from adjuvant chemotherapy in a non-randomised prospective study. In this prespecified interim analysis, we aimed to assess the efficacy and safety of platinum-based adjuvant chemotherapy in patients with stage IA–IIA molecular high-risk non-squamous NSCLC in a randomised trial.

Methods AIM-HIGH, a randomised, phase 3 trial, was done at 45 centres in France, Germany, and the USA. Patients aged 18 years or older with stage IA–IIA non-squamous NSCLC, an adequate tumour sample, and an Eastern Cooperative Oncology Group performance status of 0–1 underwent risk stratification with the 14-gene assay. Patients with a molecular high risk, defined as those receiving a high-risk or an intermediate-risk score, were randomly assigned (1:1) to four cycles of platinum-based adjuvant chemotherapy (using local institutional standard of care regimens) or observation. Randomisation was stratified according to age, sex, and tumour size of 4 cm or more. The primary outcomes for the study and for this prespecified interim analysis were 48-month and 24-month disease-free survival, respectively, in the modified intention-to-treat (mITT) population, which was defined as randomly assigned patients who continued to meet eligibility criteria either at chemotherapy initiation or at random assignment to observation; an early interim analysis was prespecified to detect a large difference between groups. This trial is registered at ClinicalTrials.gov, NCT01817192, and is closed to enrolment.

Findings Between Sept 11, 2020, and Feb 7, 2025, 449 patients were enrolled and underwent risk stratification. 236 patients with molecular high risk were randomly assigned to chemotherapy (n=124) or observation (n=112). At the time of the prespecified interim analysis, 87 patients were evaluable in the mITT population (47 [54%] males and 40 [46%] females; median age 63 years [IQR 52–74]) in the chemotherapy group and 107 (58 [54%] males and 49 [46%] females; 66 years [56–76]) in the observation group. 48 (55%) patients in the chemotherapy group and 58 (54%) patients in the observation group had stage IA disease; 34 (39%) and 44 (41%), respectively, had stage IB disease, and five (6%) and five (5%), respectively, had stage IIA disease. Six (3%) of 200 patients in the mITT population had died at the time of the interim analysis. 24-month disease-free survival was 96% (95% CI 92–100) with adjuvant chemotherapy versus 79% (70–90) with observation (hazard ratio 0·22 [0·06–0·76]; p=0·0087).

Interpretation The 14-gene assay identified patients with molecular high risk who benefitted from adjuvant chemotherapy. Use of the assay to determine eligibility for adjuvant therapy in stage IA–IIA non-squamous NSCLC has the potential to substantially improve otherwise persistently poor outcomes.

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Introduction

Despite recent progress in the treatment of locally advanced and metastatic non-small-cell lung cancer (NSCLC) with targeted therapies and immuno-oncology,[1] early-stage outcomes remain dismal compared with many solid cancers, including breast and colon cancers.[2] A recent randomised trial comparing operative approaches to very early-stage NSCLC (peripheral tumours, stage IA1 and IA2) observed 5-year disease-free survival of only 64%, regardless of the surgical approach.[3]

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See Online for appendix

Research in context

Evidence before this study

We searched PubMed for research articles published in English with the search terms "non-small cell lung cancer", "molecular profile", "adjuvant therapy", "high-risk", and "early stage" from database inception to April 23, 2025. This search identified multiple studies of the adjuvant treatment of non-small-cell lung cancer (NSCLC) that documented the potential benefit of chemotherapy, immunotherapy, and targeted therapy for post-operative, residual NSCLC. The majority of those studies, however, generally did not demonstrate a benefit to current stage I–IIA—and particularly to stage IA—patients with NSCLC from adjuvant intervention in the absence of an effective risk discriminator that could segregate patients most likely cured by surgery alone. A single-centre, non-randomised study has provided preliminary evidence that a 14-gene expression profile can provide this type of predictive risk segregation in patients with stage I–IIA non-squamous NSCLC.

Added value of this study

The results reported here provide international, multicentre, prospective, randomised evidence of the predictive value of the 14-gene expression profile in identifying patients at stage I–IIA with non-squamous NSCLC who derive substantial improvement in disease-free survival from platinum doublet adjuvant chemotherapy. The magnitude of the effect observed in a prespecified interim analysis renders the likelihood that these molecular high-risk, very early-stage tumours are resistant to adjuvant intervention to be very low, and provides a basis for larger-scale implementation of this strategy to reduce persistently high rates of early recurrent disease.

Implications of all the available evidence

Based on the current incidence of stage IA–IIA non-squamous NSCLC and its recently reported outcomes, the evidence now indicates that molecular risk segregation of stage I–IIA non-squamous NSCLC with the 14-gene expression profile can help to prevent disease recurrence and death in tens of thousands of patients annually worldwide. As recent and ongoing studies evaluate new approaches for the adjuvant treatment of NSCLC, it will be important to integrate this predictive risk segregation into the design and analysis of these studies.

Seminal studies from the mid-2000s first documented improved survival with post-operative, adjuvant chemotherapy in NSCLC.[4–7] Benefit was confined to stages II and III (equivalent to current stages IIB–III); meta-analyses indicated equivocal or worse outcomes in unstratified N0 patients (current stages IA–IIA).[4–9] Although recent studies of adjuvant tyrosine kinase inhibitors have shown improvement in stage IB–III patients with activating *EGFR* mutations (about 10–15% of European and North American populations and 40% of Asian populations), the most significant benefits were again seen in stages II–III, and no benefit has been demonstrated in stage IA.[10,11] Benefit from the addition of immunotherapy to either neoadjuvant or adjuvant chemotherapy has similarly been demonstrated essentially in stage II–III disease, and has not affected either the treatment paradigm or survival of stage I NSCLC.[12–17]

These results, together with persistently high distant recurrence and 5-year mortality rates in unstratified patients with N0 NSCLC, indicate that a third to a half of these patients harbour minimal, often microscopic disease that can result in fatal recurrence and yet is likely amenable to chemotherapeutic intervention. The previous absence of effective risk stratification has resulted in negative or equivocal results when attempting to treat the overall N0 population.[4–9] Given the increasing proportion of patients diagnosed with small, N0 lung cancers due to gradual increases in screening and incidental diagnoses,[18,19] up to 100 000 or more patients with early stage disease worldwide are likely being undertreated annually and succumb to recurrent NSCLC as a result.

Seminal studies of adjuvant chemotherapy that treated entire populations of patients with stage IA–IIA NSCLC might have failed due to their inability to discriminate large proportions of those patients without undetectable metastasis who were cured by surgery alone. In 2012, we reported large-scale, independent, international, blinded validations (involving about 1500 patients) of a 14-gene expression profile that accurately stratified patients with early-stage non-squamous NSCLC into high-risk, intermediate-risk, and low-risk groups, and that outperformed all conventional pathological and clinical risk factors.[20–22] A lingering question remained as to whether cancers receiving high-risk and intermediate-risk scores might prove recalcitrant to adjuvant intervention despite their apparent otherwise biological similarity to bulkier stage II and III disease that is known to respond. Preliminary prospective data from a single-centre, non-randomised study indicated a substantial improvement in disease-free survival and freedom from recurrence when patients designated by this assay as molecular high risk (defined as receiving either a high-risk or intermediate-risk score) were treated with platinum-based adjuvant chemotherapy.[20–23]

AIM-HIGH (Adjuvant Intervention in Molecular HIGH-risk patients) aimed to definitively assess the efficacy and safety of platinum-based adjuvant chemotherapy in patients with stage IA–IIA molecular high-risk non-squamous NSCLC. We report a prespecified interim analysis of disease-free survival.

Methods

Study design and participants

The international, prospective, randomised, phase 3 AIM-HIGH trial was conducted at 45 centres in France, Germany, and the USA (appendix p 11). Inclusion criteria

were age 18 years or older, an Eastern Cooperative Oncology Group (ECOG) performance status of 0–1, candidacy for adjuvant chemotherapy according to local institutional standards, life expectancy and likely compliance with follow-up of 5 years, willingness to comply with protocol procedures including adjuvant chemotherapy if randomly assigned to that group of the study, histologically documented completely resected (R0) stage I or IIA non-squamous NSCLC (per the eighth edition of the TNM staging system), and an adequate tumour tissue sample for the 14-gene assay for risk stratification. Patients with a pathological diagnosis of pure squamous, pure small-cell, or pure neuroendocrine histology (or any combination of only these three histologies); pregnancy or lactation; unwillingness to use an effective means of contraception; history of systemic chemotherapy or anticancer agent within 5 years before enrolment; history of radiotherapy to the chest in the immediate pre-operative or post-operative period; history of other malignancy within 5 years with the exceptions of adequately treated carcinoma-in-situ of the cervix, non-melanoma skin cancer, localised prostate cancer treated locally, and ductal carcinoma-in-situ of the breast treated surgically; history of treatment with any investigational drug or participation in another clinical trial within 28 days of enrolment; known hypersensitivity to treatment agents; or active infection or any other disease that contraindicated the use of systemic cytotoxic chemotherapy were excluded. Sex was self-reported by study participants as male or female.

In France, all study activities were organised and managed by the Intergroupe Francophone de Cancérologie Thoracique. The trial protocol (appendix) was approved by competent authorities in France and Germany, and by local ethical committees when required. In the USA, Advarra served as a central institutional review board, with the addition of local centre institutional review board approval where required by local institutional policy. All patients provided written informed consent. This trial is registered with ClinicalTrials.gov, NCT01817192, and is closed to enrolment.

Randomisation and masking

A computer-generated randomisation table was provided by an independent statistician. Enrolled patients who were classified as having a molecular high risk, defined as those receiving a high-risk or intermediate-risk score on the 14-gene assay, were randomly assigned (1:1 initially, but later 2:1 to account for chemotherapy refusals and withdrawals) by their local centre using this table to either adjuvant chemotherapy or observation. Randomisation was stratified according to age (<65 years *vs* ≥65 years, sex and tumour size (<4 cm *vs* ≥4 cm). An independent, masked statistician verified the results of the primary interim disease-free survival analysis.

Procedures

A formalin-fixed, paraffin-embedded tumour sample for each patient was shipped to a central, CLIA-certified laboratory for performance of the 14-gene expression profile (RiskReveal, Razor Genomics, Nashville, TN, USA). This assay measures gene expression in such samples using quantitative PCR with a turnaround time of less than 14 days. An algorithm combines expression levels to produce a risk score of high, intermediate, or low. The development of the assay and its robust clinical and technical validation have been previously described;[22,24] the assay has not undergone material modification since its original development and definitive validation. A brief description of the assay and the genes measured is in the appendix (pp 1–2). Patients randomly assigned to adjuvant chemotherapy were treated with platinum doublets according to the local standard of care. Four cycles were planned, and dose reductions or modifications were undertaken as needed according to local standards. Patients in the observation group did not undergo any post-operative treatment; both the chemotherapy and observation groups were followed up at 6-month intervals with a chest CT scan and physical examination. Per protocol, patients with low risk underwent routine surveillance by their treating physicians and will be assessed for recurrence and vital status only once at the end of the study.

Outcomes

The primary outcomes for the study and for this prespecified interim analysis were 48-month and 24-month disease-free survival, respectively, in the modified intention-to-treat (mITT) population. Disease-free survival was assessed locally and defined as the time from randomisation to recurrence of the primary disease, exclusive of new primary lung cancer, or death from any cause. mITT was defined as randomly assigned patients who continued to meet eligibility criteria either at chemotherapy initiation or at random assignment to observation. Although not prespecified for the interim analysis, key secondary analyses of the study were disease-free survival in the intention-to-treat (ITT) population, which was defined as all randomly assigned patients, and in the per-protocol population, which was defined as the subset of the mITT population who either had at least 2 months of follow-up if randomly assigned to observation or completed at least two cycles of treatment if randomly assigned to chemotherapy. Although not prespecified for the interim analysis, other secondary outcomes included overall survival and time to recurrence (TTR), defined as the length of time from randomisation to documented disease recurrence (with death as a competing risk). For TTR, recurrences that were first diagnosed at the time of death were treated as recurrences at the date of death. For the interim analysis, a post-hoc exploratory analysis was also conducted using the TTR endpoint. Adverse events were reported by each

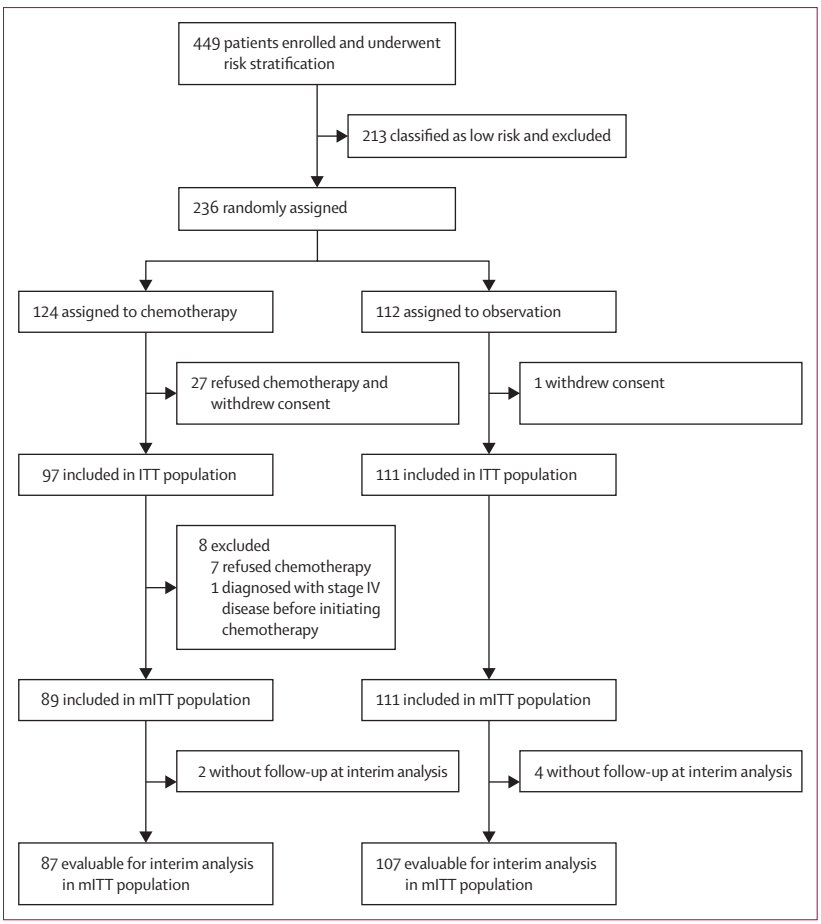

Figure 1: **Trial profile**
ITT=intention to treat. mITT=modified ITT.

centre and were adjudicated both locally by investigators according to the National Cancer Institute Common Terminology Criteria for Adverse Events (version 5.0) grading system, and centrally by the study medical monitor. Laboratory toxicities were defined on the basis of local laboratory normal ranges and National Cancer Institute Common Terminology Criteria for Adverse Events (version 5.0).

Statistical analysis

For the planned final primary analysis, we expected 47·7% disease-free survival at 4 years in the observation group and an improvement to 62% with chemotherapy. On the basis of this estimate, derived from the results of seminal studies of adjuvant chemotherapy in NSCLC,[4–7] we expected an improvement of median disease-free survival from 3·75 years to 5·8 years, with a hazard ratio (HR) of 0·65, in favour of chemotherapy. After accounting for a 10% dropout due to withdrawal, ineligibility, and loss to follow-up, the study had a target accrual of 550 evaluable patients, with approximately 86% power to detect an improvement in 4-year disease-free survival with a two-sided overall study α value of 0·05.

The adjuvant treatment effect from previous studies[4–7] that formed the basis of these conservative estimates was observed only in patients with current stage IIB–III NSCLC, whose burden of disease is substantially larger than that of patients with stage IA–IIA NSCLC studied in this trial. Given the larger magnitude of treatment effect observed in our preliminary, non-randomised study in this latter population of patients with molecular high-risk stage IA–IIA NSCLC,[20,23] an interim analysis was also prespecified and designed to detect a HR closer to 0·2 observed in that preliminary study. The primary goals of prespecifying this interim analysis were both to curtail the accrual of an unnecessarily large cohort of patients and, if positive, to make randomised evidence of the susceptibility of molecular high-risk stage IA–IIA non-squamous NSCLC to adjuvant chemotherapy available to clinicians well before the study would have finished enrolment and analysis at its primary accrual target. The interim analysis was prespecified to take place when at least 15 disease-free survival events had been observed.

Two potential interim analyses were prespecified in the protocol based on number of events: the first with an α value of 0·02, and the second with an α value of 0·015 to be undertaken only in the event that the first interim analysis did not show significantly improved disease-free survival with an alpha of 0·02. To control the overall type I error, penalisation for multiple comparisons yielded a two-sided significance level of 0·03 for the final analysis to be conducted at the end of the study given the single interim analysis. Study enrolment was also prespecified to be halted in the event of a positive interim analysis; the final enrolment of about 200 patients at the time of the interim analysis yielded a study power of more than 90% assuming an HR as high as 0·55.

The prespecified interim analysis included all follow-up as of Dec 12, 2024. Kaplan–Meier analysis using a right-censored dataset and the log-rank test were used to compare the two groups when assessing the primary and secondary endpoints. The reverse Kaplan–Meier method was used to estimate median follow-up time for each group. Univariate Cox regression analyses were used to explore the associations between the effect of chemotherapy and clinical subgroups, including age, sex, smoking history, ECOG status, stage, minimally invasive surgery, type of surgical resection, and *EGFR* status on disease-free survival. Significance was evaluated with the log-rank test in the univariate analyses and the Wald test in the multivariate analyses, using a prespecified two-sided α value of 0·05. In a post-hoc exploratory analysis, a Fisher's exact test was used to compare recurrence events in patients who received chemotherapy within 3·5 months of tissue diagnosis versus those who received chemotherapy at or after 3·5 months. The safety analysis population included all patients who received at least one dose of chemotherapy. Analyses were conducted using the programming language R (version 4.3.2 for Linux). An external data and safety monitoring board

(DSMB) met twice annually to review the safety and progress of the study and to make recommendations regarding study conduct. Upon review of the interim analysis results, the DSMB recommended a halt to further enrolment as prespecified in the protocol.

Role of the funding source

The funder of the study had no role in data collection, nor did it have access to enter or modify data in the study database, data from which were available to multiple independent academic investigators. The funder similarly did not have any role in local site endpoint adjudication. Data monitoring was undertaken both by independent contractors of the sponsor and by independent academic monitors. The funder did participate in the study design, data analysis, data interpretation, and in the writing of the report.

Results

From Sept 11, 2020, to Feb 7, 2025, 449 patients were enrolled in the study (figure 1). 14-gene molecular risk stratification classified 213 (47%) patients as having low risk; the remaining 236 (53%) patients with either intermediate-risk or high-risk scores were defined as molecular high risk. Patients with a molecular high risk were randomly assigned to either chemotherapy (n=124) or observation (n=112). 28 patients withdrew consent after randomisation (27 in the chemotherapy group and one in the observation group). Among the remaining 208 randomly assigned patients, seven refused to begin their assigned chemotherapy and one was found to have stage IV disease before initiating chemotherapy; these eight patients were excluded from the mITT population (appendix p 3).

Table 1 lists baseline characteristics of the mITT population. The two groups were similar in all clinicopathological characteristics assessed. 58 (65%) of 89 patients in the chemotherapy group and 77 (69%) of 111 patients in the observation underwent minimally invasive resection, with 15 (17%) and 19 (17%), respectively, undergoing sublobar resection (segmentectomy). 49 (55%) patients in the chemotherapy group and 61 (55%) patients in the observation group had stage IA disease; 35 (39%) and 45 (41%), respectively, had stage IB disease, and five (6%) and five (5%), respectively, had stage IIA disease.

Patients in the chemotherapy group received local institutional standard of care for adjuvant treatment of NSCLC (table 1). 46 (52%) patients received cisplatin, 40 (45%) received carboplatin, and three (3%) received a combination of the two. 66 (74%) patients completed all four planned cycles, and 77 (87%) completed at least three cycles. There were no chemotherapy protocol deviations, and dose reductions were made per protocol in three (27%) of 11 patients who received just two cycles, in two (18%) of 11 patients who received three cycles, and in 15 (23%) of 65 patients who received all four cycles. One patient in the chemotherapy group was treated with

	Adjuvant chemotherapy group (n=89)	Observation group (n=111)
Age, years	63 (52–74)	66 (56–76)
Sex		
Female	42 (47%)	52 (47%)
Male	47 (53%)	59 (53%)
Smoking		
History		
Yes	80 (90%)	96 (86%)
No	8 (9%)	15 (14%)
Unknown	1 (1%)	0
Status		
Never	8 (9%)	15 (14%)
Former	53 (60%)	69 (62%)
Current	27 (30%)	27 (24%)
Unknown	1 (1%)	0
Pack-years, months	35 (5–65)	37 (2–67)
ECOG performance status		
0	60 (67%)	72 (65%)
1	28 (31%)	38 (34%)
Unknown	1 (1%)	1 (1%)
Histology		
Adenocarcinoma	86 (97%)	103 (93%)
Large cell	0	3 (3%)
Mixed	3 (3%)	5 (5%)
Stage		
IA1	3 (3%)	10 (9%)
IA2	24 (27%)	28 (25%)
IA3	22 (25%)	23 (21%)
IB	35 (39%)	45 (41%)
IIA	5 (6%)	5 (5%)
Resection		
Approach		
Minimally invasive	58 (65%)	77 (69%)
Open	31 (35%)	34 (31%)
Technique		
Segmentectomy (sublobar)	15 (17%)	19 (17%)
Lobectomy (lobar)	72 (81%)	92 (83%)
Sleeve lobectomy	1 (1%)	0
Bilobectomy	1 (1%)	0
Adjuvant chemotherapy		
Carboplatin plus pemetrexed	31 (35%)	NA
Cisplatin plus pemetrexed	26 (29%)	NA
Cisplatin plus vinorelbine	18 (20%)	NA
Carboplatin plus paclitaxel	4 (4%)	NA
Carboplatin plus vinorelbine	4 (4%)	NA
Other	6 (7%)	NA

Data are median (IQR) and n (%). ECOG=Eastern Cooperative Oncology Group. mITT=modified intention to treat. NA=not applicable.

Table 1: **Baseline clinical and pathological characteristics of the mITT population**

	Any grade	Grade 1	Grade 2	Grade 3	Grade 4
Nausea	51 (57%)	33 (37%)	16 (18%)	2 (2%)	0
Fatigue	32 (36%)	22 (24%)	8 (9%)	2 (2%)	0
Anaemia	23 (26%)	13 (14%)	7 (8%)	3 (3%)	0
Asthenia	23 (26%)	14 (16%)	8 (9%)	1 (1%)	0
Constipation	23 (26%)	19 (21%)	3 (3%)	1 (1%)	0
Diarrhoea	18 (20%)	13 (14%)	5 (6%)	0	0
Neutropenia	15 (17%)	4 (4%)	4 (4%)	6 (7%)	1 (1%)
Dyspnoea	12 (14%)	8 (9%)	4 (4%)	0	0
Headache	10 (11%)	8 (9%)	2 (2%)	0	0
Decreased appetite	10 (11%)	5 (6%)	3 (3%)	2 (2%)	0
Vomiting	9 (10%)	4 (4%)	4 (4%)	1 (1%)	0
Cough	9 (10%)	7 (8%)	2 (2%)	0	0

Data are n (%). Adverse events reported in at least 10% of the patients in the chemotherapy group are shown, according to the National Cancer Institute Common Terminology Criteria for Adverse Events (version 5.0) grading system and preferred term.

Table 2: **Adverse events in the chemotherapy group (n=89)**

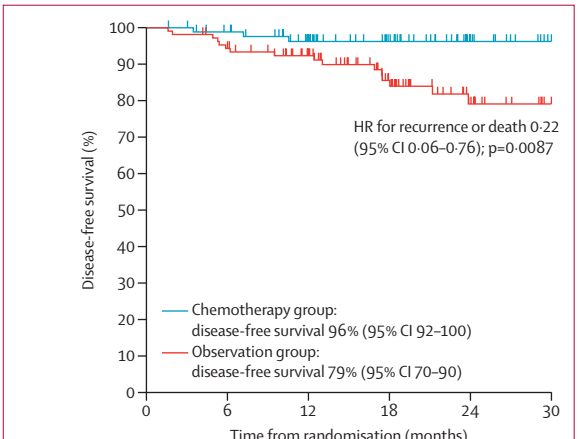

Figure 2: **Kaplan–Meier survival analysis of disease-free survival in the evaluable mITT population**
HR=hazard ratio. mITT=modified intention to treat.

osimertinib (80 mg per day) after four cycles of carboplatin plus pemetrexed; none of the patients received immunotherapy during the study. Adverse events encountered during the administration of chemotherapy during this study included nausea, fatigue, anaemia, gastrointestinal symptoms, and neutropenia (table 2; appendix pp 4–6). One (1%) death was related to chemotherapy.

At the time of the prespecified interim analysis, in the mITT population, follow-up data had been collected for 87 patients (47 [54%] males and 40 [46%] females; median age 63 years [IQR 52–74]) in the chemotherapy group and 107 patients (58 [54%] males and 49 [46%] females; 66 years [56–76]) in the observation group; these 194 patients (appendix pp 7–8) were assessed for the primary endpoint of disease-free survival. At 24 months, 19 disease-free survival events were observed among the mITT population when the interim analysis was conducted: 16 in the observation group and three in the chemotherapy group. Median follow-up was 19·5 months (IQR 12·3–27·0) in the chemotherapy group and 19·0 months (12·9–24·9) in the observation group. 24-month disease-free survival was 96% (95% CI 92–100) in the chemotherapy group and 79% (70–90) in the observation group (figure 2). Median disease-free survival was not reached in either group. The overall disease-free survival HR was 0·22 (95% CI 0·06–0·76; p=0·0087), indicating a 78% lower risk of disease recurrence or death after adjuvant platinum-doublet treatment in patients with very early-stage disease and molecular high risk. Multivariate Cox proportional hazards modelling controlling for age, sex, and tumour size of 4 cm or more in the evaluable mITT population demonstrated a similar disease-free survival HR of 0·22 (95% CI 0·06–0·77; p=0·018; table 3). None of the other clinical covariates demonstrated a significant association with disease-free survival (table 3).

Six (3%) of 200 patients in the mITT population had died at the time of the interim analysis: two (2%) of 89 patients in the chemotherapy group, including one (1%) patient who died of complications of chemotherapy and one who died of recurrent disease, and four (4%) of 111 patients in the observation group, including one patient who died of myocardial infarction without recurrence and three who died of recurrent disease.

Although this interim analysis was not powered for multiple subgroup analyses or for analysis of overall survival, an exploratory subgroup analysis revealed a pattern favouring disease-free survival in the chemotherapy group (figure 3). ITT analysis, although not prespecified as part of the interim analysis, was undertaken and revealed a consistent improvement in disease-free survival associated with randomisation to adjuvant chemotherapy (20 overall events, HR 0·29 [95% CI 0·10–0·86]; p=0·018; appendix p 12). Although the number of events in patients in stages IB and IIA did not support Kaplan–Meier or log-rank analyses in this interim analysis, subgroup analysis of patients in stage IA did reveal a significant improvement in disease-free survival (HR 0·15 [95% CI 0·02–1·16]; p=0·035; appendix 13). TTR analysis of the mITT population similarly indicated an HR of 0·16 (95% CI 0·04–0·69; p=0·0049), favouring adjuvant intervention in patients with molecular high risk.

Next-generation sequencing was obtained off-protocol by local investigators for 48 (54%) of 89 patients in the chemotherapy group and 59 (53%) of 111 patients in the observation group in the mITT population. In a post-hoc analysis, activating *EGFR* mutations were found in six (13%) patients in the chemotherapy group and in five (8%) patients in the observation group for whom *EGFR* sequencing was undertaken. In the observation group,

12 (22%) of the *EGFR*-negative and one (20%) of the *EGFR*-positive patients either had recurrence or died, while two (5%) of the *EGFR*-negative patients and none of the *EGFR*-positive patients in the chemotherapy group had a disease-free survival endpoint. In an exploratory analysis, *EGFR* status in this cohort did not appear to be related to disease-free survival, and the response of patients with a molecular high risk to chemotherapy (HR 0·20 [95% CI 0·04–0·88, p=0·033]) remained stable when controlling for *EGFR* mutation status (appendix p 9).

An additional post-hoc exploratory analysis further revealed that all recurrences in the chemotherapy group occurred when initiation of chemotherapy was longer than 3·5 months after histological diagnosis of the primary tumour (p=0·042 *vs* initiation of therapy within 3·5 months; appendix p 10).

Discussion

To our knowledge, the results reported here are the first from a randomised trial to confirm that molecular high-risk designation based on a 14-gene prognostic assay is predictive of a significant benefit from adjuvant platinum-based chemotherapy in stage IA–IIA non-squamous NSCLC.

The most effective strategy to achieve long-term survival in NSCLC continues to involve complete resection of disease diagnosed at an early stage. Unlike many other solid tumours, however, conventional staging in NSCLC has failed to effectively identify early-stage populations without micrometastatic or subclinical disease who can reliably be cured with complete resection. In contrast to breast and colon cancers, for example, for which stage I survival is more than 90%,[2] 5-year survival of patients with NSCLC remains about 75% in stage IA and even lower in stage IB, despite multiple revisions of the TNM staging system.[25,26] Recurrence of residual disease that was undetectable at the time of surgery is associated with most of this mortality.[3] Although adjuvant chemotherapy improves survival in locally advanced and regional NSCLC,[4–9] this opportunity for improved survival is not currently afforded to patients with stage IA disease, and only to patients with stage IB–IIA based on subjective assessment of high-risk status without evidence-based risk factors that predict benefit from adjuvant therapy.[27] As a result, many thousands of patients die each year from initially early-stage NSCLC without having had any potentially life-saving adjuvant intervention.

The 14-gene expression profile used in this study has had extensive validation over the past decade and reliably identifies patients at high risk even in the earliest stages of non-squamous NSCLC.[20–23] Given the novelty of molecular risk stratification in this disease, however, widespread recommendation for the use of this assay to identify patients in stages IA–IIA for potentially toxic adjuvant intervention has awaited evidence from a randomised trial that the undetectable micrometastases in these patients with a molecular high risk respond to current systemic therapies. A preliminary, non-randomised, single centre study provided the first indication that the risk of recurrence in these patients can be reduced by as much as 80%.[23] The current study has provided multicentre, international, prospective, randomised evidence of this significant survival benefit from adjuvant systemic therapy in patients with a molecular high risk identified

	Univariate analysis		Multivariate analysis	
	HR (95% CI)	p value	HR (95% CI)	p value
Chemotherapy	0·22 (0·06–0·76)	0·0087	0·22 (0·06–0·77)	0·018
Age ≥65 years	2·27 (0·86–5·98)	0·088	1·85 (0·69–4·96)	0·22
Male sex	1·15 (0·46–2·87)	0·77	1·25 (0·49–3·19)	0·64
Tumour size ≥4 cm	2·10 (0·49–9·12)	0·31	3·36 (0·74–15·26)	0·12

Clinical covariates used to stratify patients during randomisation were included in the model. Significance was assessed using the log-rank test for the univariate model and the Wald test for the multivariate model. HR=hazard ratio. mITT=modified intention to treat.

Table 3: **Univariate and multivariate Cox proportional hazards models for 24-month disease-free survival in the mITT population**

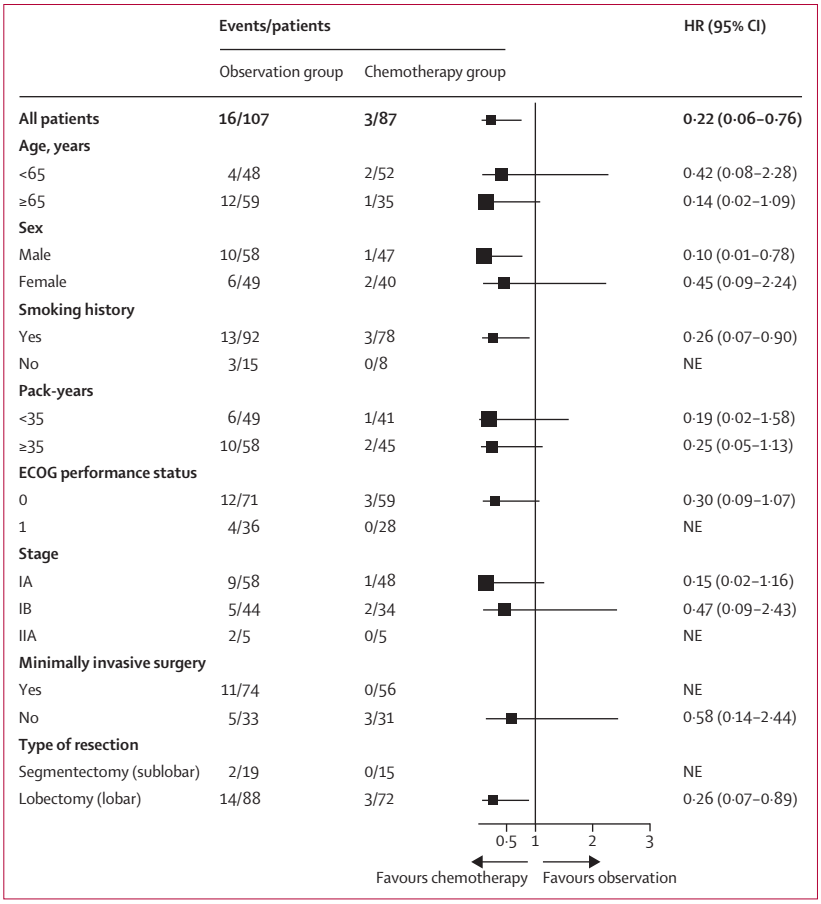

Figure 3: **Forest plot of the subgroup analysis in the mITT population**
Box size is proportional to the SE of the HR estimate, with a smaller box denoting a more precise estimate. For subgroups with no data displayed (NE), events (recurrence or death) were only observed in the observation group; no events were observed in the chemotherapy group. HR=hazard ratio. mITT=modified intention to treat. NE=not estimable.

by the 14-gene assay. The spectrum and incidences of adverse events were similar to those reported for NSCLC chemotherapy in previous studies,[4–7,9] and the 1% incidence of chemotherapy-related death was similarly consistent with 1–6-month mortality previously reported for the adjuvant treatment of NSCLC.[28]

Despite a gradual decline in the incidence of lung cancer in certain countries, this disease remains by far the greatest global cancer killer.[2] There is also an ongoing shift in diagnosis from advanced NSCLC, in which long-term survival continues to be difficult to achieve, to early-stage disease that is amenable to resection.[18,19] An interim analysis of this trial was designed to provide the earliest reliable signal of efficacy of adjuvant chemotherapy for patients with molecular high-risk disease.

One unexpected limitation of this study was the refusal of a substantial proportion of patients to comply with random assignment to chemotherapy, despite instituting a regimen of multiple confirmations of informed consent before randomisation. This pattern of refusal was observed across numerous centres in all three countries and probably reflected a common fear of chemotherapy and its potential toxicity with perception of relatively low benefit from the adjuvant treatment of early-stage NSCLC. This perception might become outdated in light of the results reported here. Refusal was not related to the patients' own experience of treatment, as they all withdrew consent before initiating chemotherapy. No patient was removed from the primary analysis because of a refusal to continue treatment after initiating chemotherapy. Analysing the mITT population addressed the impact of chemotherapy refusal. As a post-hoc sensitivity analysis, however, we also conducted a secondary ITT analysis, which was consistent with mITT results. If bias introduced by mITT analysis did influence the magnitude of the benefit observed, it is unlikely to account for the majority of that large benefit. Given the widespread nature of the refusal observed across numerous high-volume centres, it is also unlikely that this type of study could be conducted without encountering this limitation.

The interim analysis of this study has additional limitations. The positive result from this interim analysis depended on a large reduction in disease recurrence; the magnitude of this benefit will be further assessed and refined in about 3–5 years with the final analysis of more mature study data. This interim analysis is also not powered to support subgroup analyses or other secondary analyses that will await longer-term follow-up. The post-hoc analysis of patients with stage IA disease should be recognised as exploratory only and interpreted with caution. Despite these limitations, prespecification of the interim analysis was elected both to avoid drastic over-accrual beyond the 200 evaluable randomly assigned patients necessary for more than 90% power even with an expected HR of 0·55; and in consideration of the trial's central objective, which was randomised assessment of whether 14-gene molecular high-risk tumours are resistant to adjuvant intervention. Given the very low likelihood that the very large effect observed could have resulted entirely from the limitations of the study discussed here, these interim results render the possibility of complete disease resistance unlikely.

The analysis of circulating tumour DNA in the development of bespoke assays for the detection of minimal residual disease (MRD) for various cancers has garnered much attention in the past 5 years.[29] MRD technology has been associated with earlier diagnosis of recurrent NSCLC in several experimental studies of longitudinal testing after resection,[27,29] generally preceding radiographic diagnosis by several months. The sensitivity even of recent generations of MRD technology, however, has not exceeded about 50% in identifying patients with residual disease within the treatment decision-making time period after surgery that could support the implementation of adjuvant therapy.[29,30] Furthermore, this early post-operative sensitivity has not yet been established in the context of very early-stage disease, when risk stratification is most needed. In this study, recurrence after adjuvant therapy was observed only when initiation of chemotherapy exceeded 3·5 months after initial NSCLC diagnosis. Although this post-hoc observation does not carry the statistical weight of the study's primary conclusion, it might reflect the impact of immediate post-operative risk stratification and rapid implementation of systemic therapy on the subsequent efficacy of adjuvant intervention. Whereas MRD might prove to be valuable for early detection of downstream adjuvant treatment failures either after surgery for more advanced stage tumours or in conjunction with risk stratification and post-operative intervention for very early-stage disease, it is unlikely that assessment of MRD alone immediately after surgery for early-stage disease will result in the same improvement in survival reflected in the results of the current study.

As adjuvant interventions in NSCLC improve for identifiable subpopulations of patients positive for *EGFR*, *ALK*, and other mutation and PD-L1-positive patients, particularly with the advent of novel adjuvant targeted and immunotherapies, the need for effective risk discrimination in very early-stage disease becomes even more pronounced—both for the effective treatment of all patients with high-risk stage I disease and to avoid over-treatment of the large proportion of these patients who are at low-risk for disease recurrence. There is no scientific or clinical rationale to suggest that patients with molecular high risk in very early-stage disease will be any less responsive to more recent innovations than observed in this study with conventional chemotherapy. Consistent with previous observations in a cohort of 150 prospectively studied but non-randomised patients, the benefit of adjuvant intervention in patients with molecular high risk was independent of *EGFR* mutations status.[23] In addition, although the current study was designed to assess outcomes in its cohort of 213 patients with low risk

only at the end of study follow-up, previous, prospective analysis of another cohort of 149 patients with low risk indicated 5-year freedom from recurrence of 95%;[23] and recent re-analysis of that separate cohort indicated that this degree of 5-year survival remained stable with median follow-up of longer than 64 months (Kratz JR, unpublished). Despite ongoing trials of targeted therapy in unstratified patients with stage I disease,[31] it will therefore remain a question whether all patients with stage I disease and targetable mutations or immunotherapy markers derive benefit from adjuvant targeted therapy.[23] It is more likely that only a subgroup of patients with stage I disease at high risk of recurrence will benefit significantly from targeted therapies and immunotherapies. It still remains a priority, therefore, to risk stratify patients with early-stage disease now and in the future, both in investigational and clinical contexts.

Despite unacceptably high recurrence rates after resection of very early-stage NSCLC, the absence of effective risk stratification in earlier studies of adjuvant intervention contributed to their failure to demonstrate a benefit in current stages IA–IIA; that failure continues to prevent the effective application of adjuvant therapy for large numbers of patients who succumb to recurrent disease as a result. The results reported here demonstrate that a 14-gene assay effectively predicts benefit from adjuvant chemotherapy in patients with high-risk of recurrence. The use of this predictive assay allows for improved selective application of adjuvant therapy and might lead to improved freedom from disease and longer-term survival for this growing population of patients with early-stage NSCLC.

Contributors

DRS, VW, ICA, LG, FG, OB, FBB, GR-B, CD, FG, AB, WZ, JLT, SVH, DRG, MR, and PH were investigators involved in patient accrual and acquisition of data. MAG was the medical monitor. VW, JC, HvdL, GAW, JRK, DMJ, and MJM participated in study design and study monitoring. VW, JRK, and MJM verified the underlying data; VW, JC, JRK, and MJM were involved in data analysis and data interpretation. All authors had full access to all the data in the study and had final responsibility for the decision to submit for publication.

Declaration of interests

VW has received honoraria or travel support from Amgen, AstraZeneca, Bristol Myers Squibb (BMS), Pfizer, Janssen, Merck Sharp &Dohme (MSD), Roche, and Sanofi, and has served on data monitoring or advisory boards for Amgen, AstraZeneca, Regeneron, Ipsen, Johnson & Johnson, and Daiichi. OB has consulted for MSD and Takeda, and has received honoraria or travel support from AstraZeneca, MSD, and Pfizer. MAG has consulted for AstraZeneca, Atreca, Bicycle, BMS, Cardinal Health, Catalyst, Genentech/Roche, Genzyme/Sanofi, Gilead, Guardant, Invitae, Johnson & Johnson, Merus, Natera, Nuvalent, OncoHost, Regeneron, and Summit, and has served on data monitoring boards for Samsung Bioepis. JRK and DMJ have consulted for Razor Genomics. MJM serves as an officer and director of Razor Genomics. All other authors declare no competing interests.

Data sharing

The study protocol, statistical analysis plan, and informed consent form are available upon request made to trial.info@encoreclinical.org. Proposals for accessing the study data collected and analysed for this interim report, including individual participant data and a data dictionary defining each field in the set, will be considered by the study Steering Committee, beginning from the date of publication and continuing for 12 months. Access based on proposal approval will require a signed data access agreement. Proposals can be addressed to trial.info@encoreclinical.org.

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