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ABSTRACT

Introduction No predictive clinical or genetic markers have been identified or validated for antiangiogenic agents in lung cancer. We aimed to identify a predictive clinical marker of benefit for nintedanib, an angiokinase inhibitor, using data from two large second-line non-small cell lung cancer Phase III trials (LUME-Lung 1 ([LL1] and LUME-Lung 2).

Methods Predictive marker identification was conducted in a multi-step process using data from both trials; a hypothesis was generated, confirmed and validated. Statistical analyses included a stepwise selection approach, a recursive partitioning method and the evaluation of HRs, including treatment-by-covariate interactions. The marker was finally validated using a prospectively defined hierarchical testing procedure and treatment-by-covariate interaction for overall survival (OS) based on LL1.

Results Time since start of first-line therapy (TSFLT) was identified as the only predictive clinical marker. A cut-off of 9 months was chosen for further analysis, based on HRs and recursive partitioning. The prospectively defined final validation using OS data from LL1 established the strong relationship between TSFLT and treatment with nintedanib. Patients with adenocarcinoma with TSFLT <9 months showed a greater survival benefit (median OS 10.9 vs 7.9 months, HR 0.75 [95% CI 0.60–0.92]; p=0.0073) compared with patients in the TSFLT >9 months group (median OS 17.0 vs 15.1 months, HR 0.89 [95% CI 0.66–1.19]).

Conclusions Patients with shorter TSFLT derive a greater progression-free survival and OS benefit from nintedanib. This clinical marker could be used for patient selection and further investigation is warranted regarding pathways promoting aggressive tumour growth and antiangiogenic tyrosine kinase inhibitor benefit.

INTRODUCTION

The classification of advanced non-small cell lung cancer (NSCLC) includes the determination of histological and molecular subtypes that influence therapeutic decisions.[1 2] Histology is recognised as an important factor in treatment selection, as

Key questions

What is already known about this subject?
► The classification of advanced non-small cell lung cancer (NSCLC) includes the determination of histological and molecular subtypes, which influence therapeutic decisions.
► No predictive clinical or molecular markers have been identified or validated for antiangiogenic agents in patients with advanced NSCLC.

What does this study add?
► This is the first study to identify a predictive clinical marker for antiangiogenic therapy in patients with advanced NSCLC.
► Predictive clinical markers of benefit for nintedanib, an angiokinase inhibitor, were evaluated using data from two large second-line NSCLC phase III trials (LUME-Lung 1 and LUME-Lung 2).
► A high threshold for success was set for developing the marker hypothesis, by requiring that any clinical marker should meet the criteria of being both prognostic of outcome in the control arms and predictive of treatment benefit in the investigational arms of the LUME-Lung trials. An additional threshold was that the observation made in one trial had to be validated in an independent trial in patients with the same histology.
► Time since start of first-line therapy (TSFLT) was shown to be the only prognostic and predictive clinical marker for treatment benefit with nintedanib.

How might this impact on clinical practice?
► TSFLT is the first independently validated clinical marker for antiangiogenic treatment in NSCLC, and is predictive and prognostic of treatment success with nintedanib.
► A shorter TSFLT is indicative that more aggressive tumours with a worse prognosis have a greater relative benefit from treatment with nintedanib.
► Further validation and characterisation of this clinical marker in future studies should help to define its potential utility in daily practice, as well as direct the search for a potential genetic marker to a narrower population.

different histologies have distinct patterns of genomic alterations[3] and differential responses to treatments.[4] Combination chemotherapies, including the vascular endothelial growth factor (VEGF) antibody bevacizumab, have shown different overall survival (OS) benefits based on histology.[5 6] The lack of validated clinical or molecular markers that can predict clinical benefit from VEGF receptor (VEGFR) inhibitors in patients with advanced NSCLC remains a significant unmet need. Identifying clinical markers that are predictive of antiangiogenic treatment effect would be particularly beneficial as it could help to guide future research into novel molecular markers, as well as potential pathways driving therapeutic resistance.

Nintedanib is an oral, triple angiokinase inhibitor of VEGFR1–3, platelet-derived growth factor receptor (PDGFR)-α/β, fibroblast growth factor receptor (FGFR)1–3, RET and Flt3.[7] Nintedanib combined with docetaxel is approved in the European Union and other countries for the treatment of patients with locally advanced, metastatic or locally recurrent NSCLC of adenocarcinoma tumour histology after first-line chemotherapy, and has been approved as monotherapy for the treatment of patients with idiopathic pulmonary fibrosis. The LUME-Lung 1 study (NCT00805194; 1199.13) assessed nintedanib–docetaxel versus placebo–docetaxel in patients with advanced or recurrent NSCLC who had relapsed or failed one prior line of chemotherapy, and was the first phase III study to show a survival benefit of an add-on treatment versus an active comparator in a broad adenocarcinoma population.[8] A separate study in patients with NSCLC showed similar findings; ramucirumab, a monoclonal antibody targeting the VEGFR2 receptor, significantly improved OS in patients with primarily non-squamous cell carcinoma when added to docetaxel.[9] Based on these promising data in molecularly unselected patients, there is a need to identify which patients may benefit most from antiangiogenic treatment.

Indeed, patient selection is particularly relevant with the increasing availability of different treatment options, such as immunotherapies, for patients progressing after first-line therapy. Although pembrolizumab is approved for patients with metastatic NSCLC after first-line therapy in a specific population of patients whose tumours express programmed death-ligand 1 (PD-L1), nivolumab is an available treatment option without a requirement for PD-L1 expression testing. However, PD-L1 expression levels do impact on outcome with nivolumab; the benefits of nivolumab over docetaxel were comparable regardless of PD-L1 expression status in squamous cell histology,[10] whereas the magnitude of benefit appeared greater among patients whose tumours expressed PD-L1 than among those whose tumours did not express PD-L1 in patients with non-squamous histology NSCLC.[11] When combined with the observation that patients with non-squamous histology and low tumour PD-L1 expression (<50%) may be at higher risk of death within the first 3 months of treatment with nivolumab,[12] PD-L1

expression levels should be a consideration in the treatment selection for both immunotherapy agents. Given that the majority of patients with NSCLC do not have high levels of PD-L1 expression,[11] factors that can be used to guide treatment choice in these patients are particularly welcome.

Against this background and concurrent with LUME-Lung 1, another phase III trial, LUME-Lung 2 (NCT00806819; 1199.14), assessed nintedanib–pemetrexed versus placebo–pemetrexed in patients with advanced or recurrent non-squamous NSCLC who had relapsed or failed one prior line of chemotherapy.[13] Based on a preplanned futility analysis of investigator-assessed progression-free survival (PFS), the independent Data Monitoring Committee (DMC) recommended that this trial be stopped for futility because achievement of the primary endpoint was deemed to be unlikely.[13] Follow-up was continued for previously accrued patients, and the DMC recommended exploring whether specific patients might benefit from nintedanib. Despite failing the futility analysis, subsequent analysis of centrally reviewed PFS, the primary study endpoint, demonstrated a statistically significant improvement in the nintedanib–pemetrexed arm (median, 4.4 vs 3.6 months, respectively; HR 0.83 (95% CI 0.70–0.99); p=0.0435),[13] indicating benefit from treatment with nintedanib–pemetrexed.

The objective of this analysis was to identify a predictive clinical marker of nintedanib benefit that could be used by clinicians to select patients most likely to benefit from the addition of nintedanib to docetaxel after first-line chemotherapy. Detailed analysis of the LUME-Lung 2 data was conducted to identify a predictive and prognostic marker for nintedanib benefit, which was validated using data from the LUME-Lung 1 study. Identifying clinical markers that are predictive of antiangiogenic treatment effect would be particularly beneficial as it could help to guide future research into novel molecular markers, as well as potential pathways driving therapeutic resistance.

MATERIALS AND METHODS

Patients
LUME-Lung 1 and 2 were independent phase III trials in patients with stage IIIB/IV or recurrent NSCLC after failure of first-line chemotherapy, and Eastern Cooperative Oncology Group (ECOG) performance status (PS) 0–1[8 13] (figure 1). Only patients with relapse or failure of one previous first-line chemotherapy regimen were allowed to enter the study (in the case of recurrent disease one additional prior regimen was allowed for adjuvant, neo–adjuvant or neo–adjuvant plus adjuvant therapy). Both trials were approved by local independent ethics committees or institutional review boards at each site, and by all relevant regulatory bodies.

LUME-Lung 1 assessed the efficacy and safety of nintedanib–docetaxel (n=655) versus placebo–docetaxel (n=659) in all histological subtypes of NSCLC,
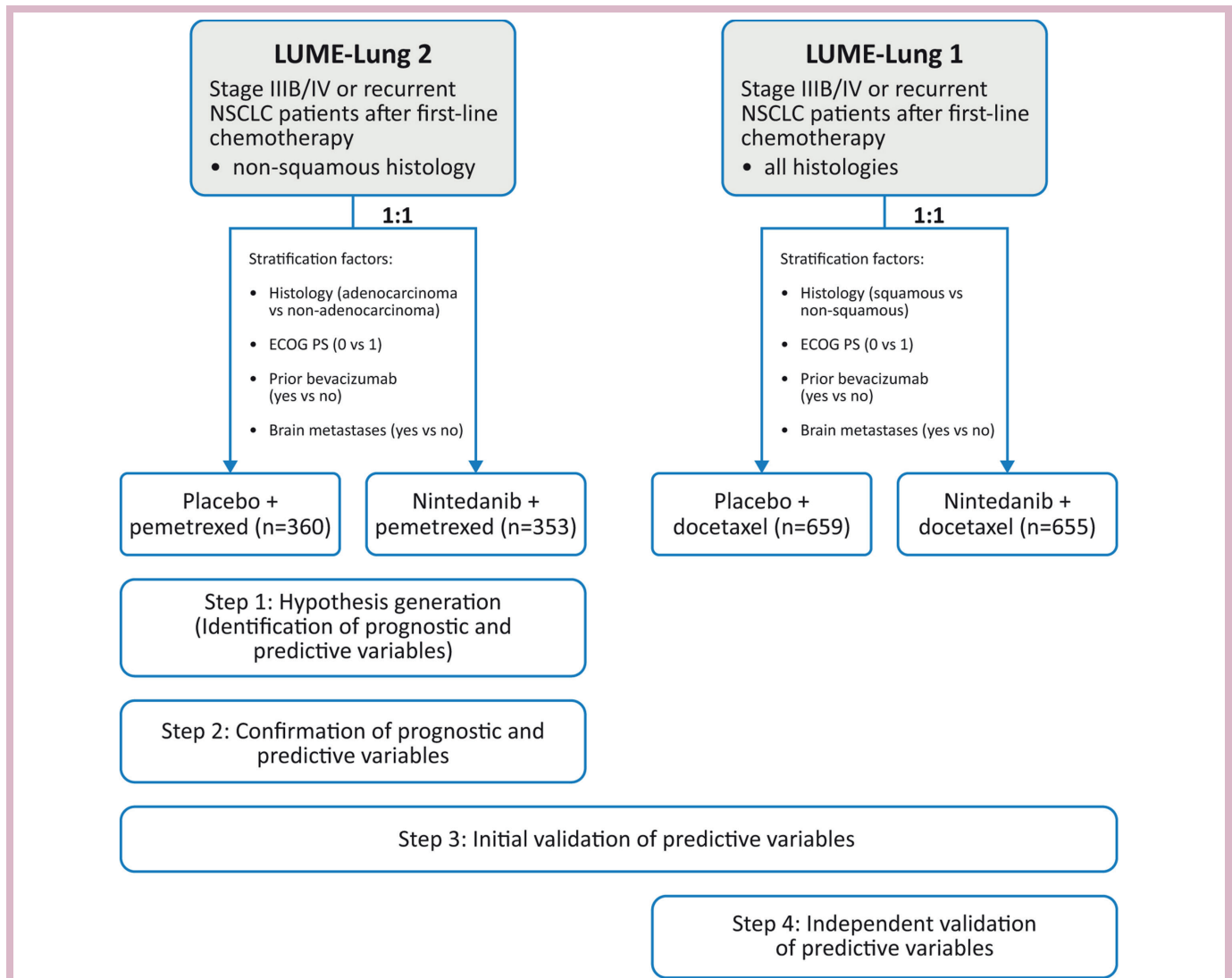
Figure 1 Overview of studies and clinical marker identification process. ECOG PS, Eastern Cooperative Oncology Group performance status; NSCLC, non-small cell lung cancer.

whereas LUME-Lung 2 assessed nintedanib–pemetrexed ($500\,mg/m^2$; n=353) versus placebo–pemetrexed (n=360) in patients with non-squamous NSCLC.

In both trials, demographics and baseline characteristics were balanced between treatment groups. Median age was ~60 years and just under one-third of patients were aged ≥65 years old. In LUME-Lung 1, 73% of patients were men, whereas this proportion was slightly lower in LUME-Lung 2 (57%). Median time since diagnosis in both trials was approximately 9 months and the large majority of patients (>97%) had received platinum-based first-line therapy. In LUME-Lung 1, 4% of patients had received first-line bevacizumab; this proportion was somewhat higher in LUME-Lung 2 (8%). Further details about the patient populations in these trials have been reported previously.[8 13]

In both trials, histology was a stratification factor (determined locally): squamous versus non-squamous in LUME-Lung 1 and adenocarcinoma versus non-adenocarcinoma in LUME-Lung 2. Among the patients with non-squamous NSCLC, patients with adenocarcinoma represented the majority of the study population (87% in LUME-Lung 1% and 94% in LUME-Lung 2). The primary endpoint in both trials was PFS by central independent review according to modified Response Evaluation Criteria in Solid Tumours version 1.0. OS was the key secondary endpoint. In both trials, patient demographics and oncological history were balanced between the treatment arms for the intention-to-treat populations and for the respective major histologies.

Clinical marker identification

Following the recommendation of the DMC, a systematic analysis of the LUME-Lung 2 trial was predefined and implemented to identify the patient population that gained most benefit from nintedanib treatment. For the clinical marker to be considered valid,[14 15] and to reduce the likelihood of falsely identifying a subgroup of patients benefiting from nintedanib, it was predefined that the marker was required to be both prognostic and predictive for PFS and

OS, and had to be confirmed with the independent set of LUME-Lung 1 data for PFS and OS.

By first identifying prognostic markers and testing only those for predictivity, the number of potential predictive variables was reduced and the chance of false-positive findings was decreased. A predefined range of patient baseline variables was explored to determine whether they were prognostic (table 1). These variables had been prespecified in the protocol or had been identified as potentially prognostic factors in the literature when the study was stopped because of futility.

Predictive marker identification was conducted in a four-step process, using data from both trials (figure 1). First, a hypothesis was generated by identifying prognostic and predictive variables using data from the LUME-Lung 2 trial. Second, any prognostic and predictive variables identified from hypothesis generation were confirmed using additional data from LUME-Lung 2. Third, initial validation of the variable as predictive of nintedanib benefit was conducted using OS data from LUME-Lung 2 (internal validation) and data from the LUME-Lung 1 trial (external validation). As a final validation step, the prespecified key secondary endpoint of OS in the LUME-Lung 1 study was extended beyond the original specifications of the analysis plan to validate time since first-line therapy as a predictor of treatment benefit.

Because histology is recognised as an important factor in treatment selection for patients with advanced NSCLC, confirmation and validation of the marker was conducted in the non-squamous (LUME-Lung 2 only) and the adenocarcinoma patient populations; these represented the largest populations in both studies. This ensured the comparability of the results and reduced any population bias to a minimum.

STATISTICAL METHODS
Analysis of prognostic variables
Two different statistical methods were used to identify prognostic variables: (1) a stepwise selection approach using Cox proportional hazards modelling and (2) a recursive partitioning tree approach (see online supplementary appendix for further details).[16]

Cox proportional hazards modelling included all prespecified baseline variables in a stepwise selection model. The two major stratification factors (based on patient numbers) used in the LUME-Lung trials (ECOG PS [0 vs 1] and tumour histology [adenocarcinoma vs non-adenocarcinoma]) were included in the model as stratification factors. The critical value for inclusion or exclusion of a potentially prognostic variable was the significance level of 0.05.

Prognostic analyses were conducted using PFS data from the control arm of the LUME-Lung 2 study. Investigator-assessed PFS was used during hypothesis generation and centrally assessed PFS data were used during confirmation.

Table 1 Investigated prognostic variables

Baseline characteristic	Randomisation stratification factor	Prespecified in both protocols
Tumour histology (LUME-Lung 1: squamous vs non-squamous; LUME-Lung 2: adenocarcinoma vs non-adenocarcinoma)	✓	✓
Baseline ECOG performance status (0 vs 1)	✓	✓
Prior bevacizumab therapy (yes vs no)	✓	✓
Brain metastases at baseline (yes vs no)	✓	✓
Liver metastases (yes vs no)	✗	✓
Sex (men vs women)	✗	✓
Age (<65 vs ≥65 years)	✗	✓
Best response to first-line chemotherapy (CR/PR/SD vs PD/unknown/NA/missing)	✗	✓
Concomitant bisphosphonates at baseline (yes vs no)	✗	✓
Disease stage at diagnosis (<IIB/IV vs IIIB vs IV)	✗	✓
Region (LUME-Lung 1: Asia vs rest of world; LUME-Lung 2: Asia vs non-Asia)	✗	✓
Time between start of first-line therapy and randomisation into the trial ('TSFLT') (months)	✗	✓
Adrenal metastases (yes vs no)*	✗	✓
Number of metastatic organs at baseline*	✗	✓
Lactate dehydrogenase level at baseline* (>1 x ULN vs ≤1 x ULN)†	✗	✓

*Additionally identified from the literature.
†Lactate dehydrogenase (LDH) levels at baseline were transformed into a binary variable (>1 × ULN vs ≤1 × ULN) because the range of LDH values was very limited (5th percentile, 0.5; 95th percentile, 2.0). This categorisation of LDH levels has been used previously.[47,48]
✓=yes; ✗=no; CR, complete response; ECOG, Eastern Cooperative Oncology Group; NA, not available; PD, progressive disease; PR, partial response; SD, stable disease; TSFLT, time since start of first-line therapy; ULN, upper limit of normal.

Analysis of predictive variables

To investigate whether prognostic variables were also predictive for the treatment effect, the interaction between these variables and treatment was examined by a stepwise selection approach using Cox proportional hazards modelling with data from LUME-Lung 2.

For the calculation of HRs and 95% CIs, the Cox proportional hazards model was stratified by the LUME-Lung 2 stratification factors: ECOG PS (0 vs 1), tumour histology (adenocarcinoma vs non-adenocarcinoma), brain metastases at baseline (yes vs no) and prior treatment with bevacizumab (yes vs no). Investigator-assessed PFS was used during hypothesis generation and centrally assessed PFS data were used during confirmation. Formal interaction tests were used for categorical covariates; treatment-by-covariate interaction was evaluated at a significance level of 0.2.

Hazard ratio-by-treatment interaction plots

Assessment of treatment interaction for continuous variables was evaluated by calculating HRs for various covariate levels, which were depicted visually by HR-by-treatment interaction plots.[17][18] No formal interaction tests were performed; the covariate values below which the HR point estimate was <1 and for which the width of the 95% CI was small were considered to define a subgroup of patients with greater treatment benefit.

HR-by-treatment interaction plots were used throughout the analyses and in the validation of the interaction between treatment effect and continuous variables, using the OS data from LUME-Lung 2 (internal validation) and OS data from LUME-Lung 1 (external validation).

FINAL VALIDATION

The final analysis of the key secondary endpoint OS of the LUME-Lung 1 trial was planned to be extended to validate this potential clinical marker by prospectively introducing hierarchical testing as follows[8] : (1) patients as defined by the predictive marker within the identified tumour histology groups (non-squamous or adenocarcinoma), (2) patients with the identified tumour histology groups (non-squamous or adenocarcinoma) and (3) the entire study population. In order to define clearly the beneficial group of patients for the first step of this testing procedure, any predictive continuous marker would require dichotomisation by an appropriate cut-point. With this hierarchy, each of the three hypotheses could be tested at the prespecified significance level only if the previous null hypothesis in the testing sequence had been rejected (and after a statistically significant result for the primary endpoint PFS in the primary and follow-up PFS analyses for LUME-Lung 1 had been achieved). The overall alpha level for OS followed a Lan–DeMets spending function with an O'Brien–Fleming shape parameter[19] to preserve an overall two-sided alpha level of 0.05. The significance level for each step of this final OS analysis was 0.04984, and depended on the number of deaths that had accrued at the time of the interim OS analysis (n=423 OS events) and the number of deaths that had accrued by the time of the final OS analysis (n=1121 OS events).

In order to validate the predictivity of the clinical marker, the treatment interaction should be confirmed — for binary variables, by repeating the interaction test; and for continuous variables, by evaluating the HR–interaction plot. In addition, the estimated OS treatment benefit from the first hierarchical testing step should be greater compared with the OS treatment benefit of the second testing step and of the last testing step.

RESULTS

Hypothesis generation based on LUME-Lung 2

Stepwise selection analyses of the placebo arm of the LUME-Lung 2 study identified TSFLT (defined as time between start of first-line therapy and randomisation into the trial; HR 0.96 [95% CI 0.94–0.98; p=0.0001]), male sex (HR 1.33 [95% CI 0.94–1.87; p=0.1045]), lower baseline lactate dehydrogenase (LDH) levels (HR 1.34 [95% CI 1.07–1.66; p=0.009]) and having never smoked (HR 1.59 [95% CI 1.10–2.30; p=0.0135]) as potentially prognostic for longer PFS.

Recursive partitioning also identified TSFLT as potentially prognostic, with an optimal binary split for TSFLT of 7.8 months (see online supplementary figure 1A).

The HR-by-treatment interaction plot of TSFLT based on investigator-assessed PFS showed that patient benefit from nintedanib treatment was higher with shorter TSFLT. The upper limit of the 95% CI approached a HR of 1.0 at TSFLT values between 8 and 9 months. A cut-off of 9 months (ie, TSFLT <9 months vs TSFLT ≥9 months) was chosen for further exploratory analysis, as the 95% CIs were narrowest around this point and similar to the cut-off identified by recursive partitioning.

Further analysis revealed that TSFLT was the only predictive marker for the treatment effect of nintedanib in combination with pemetrexed. The HR for patients with TSFLT <9 months was 0.76 (95% CI 0.58–0.98). Owing to the high frequency of patients with adenocarcinoma in the LUME-Lung 2 study, this finding was mainly driven by these patients (HR 0.96 [95% CI 0.78–1.17], nintedanib–pemetrexed vs placebo–pemetrexed).

Confirmation of the predictive marker

Stepwise selection analysis of centrally assessed PFS in the LUME-Lung 2 placebo arm identified TSFLT (HR 0.95 [95% CI 0.93–0.98; p<0.0001]), baseline LDH levels (HR 1.52 [95% CI 1.23–1.89; p=0.0001]), region (Asian vs non-Asian) (HR 1.53 ([95% CI 1.12–2.10; p=0.0073]) and presence/absence of liver metastases (HR 1.42 [95% CI 0.97–2.08; p=0.0676]) as potentially prognostic variables. Recursive partitioning analysis of centrally assessed PFS in the LUME-Lung 2 placebo arm confirmed TSFLT as a prognostic variable, with an optimal binary split point of 8.2 months (see online supplementary figure 1B).
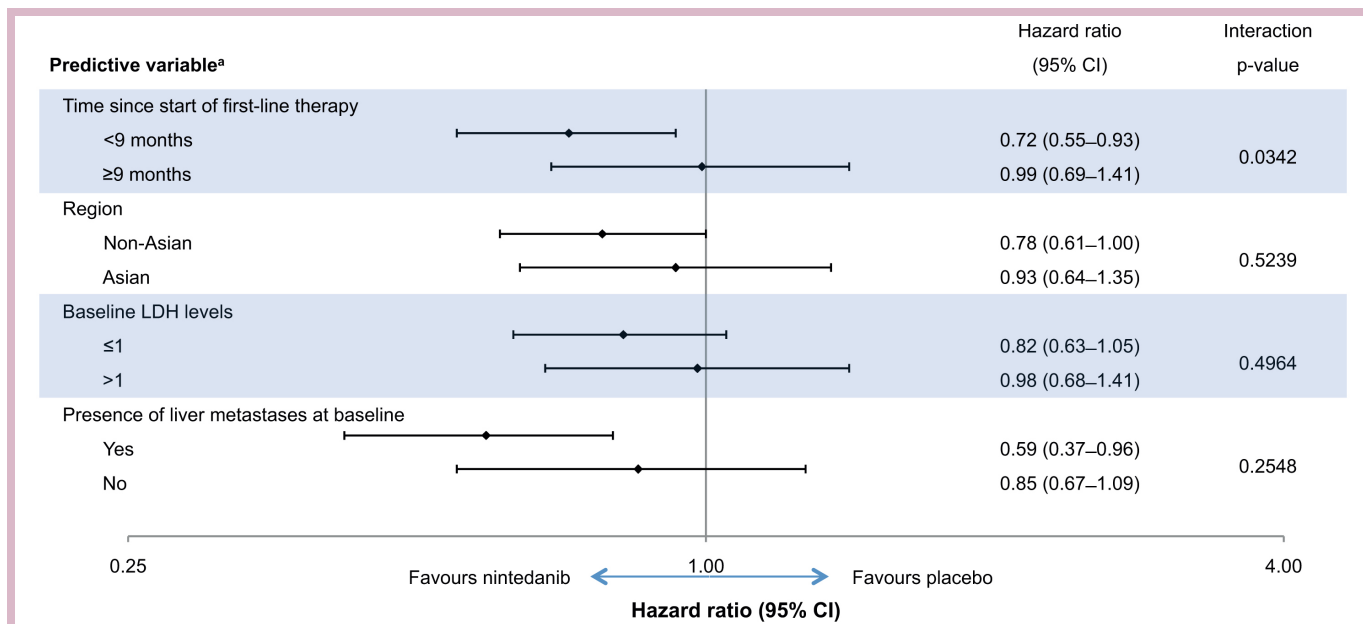
Figure 2 Clinical marker confirmation using centrally assessed progression-free survival data in patients with advanced non-small cell lung cancer with non-squamous histology treated in LUME-Lung 2. Treatment-by-covariate interaction tests for HRs using data from patients treated with nintedanib–pemetrexed. LDH, lactate dehydrogenase. [a]HRs and CIs obtained from a model stratified by Eastern Cooperative Oncology Group performance status (0 vs ≥1), brain metastases at baseline (yes vs no), prior treatment with bevacizumab (yes vs no) and tumour histology (adenocarcinoma vs non-adenocarcinoma).

Further analysis showed that TSFLT was also the only predictive variable of nintedanib benefit; the p values of the treatment-by-covariate interaction tests for other variables were all above 0.2 (figure 2).

The HR–treatment interaction plot confirmed the relationship between HR and TSFLT, with the upper limit of the 95% CI approaching a HR of 1.0 at TSFLT values between 8 and 9 months. This finding was confirmed in analysis of patients with adenocarcinoma tumour histology (figure 3A), and Kaplan–Meier curves demonstrated a significant treatment benefit (figure 4A).

Initial validation of the predictive marker

The interaction between treatment effect and TSFLT was validated using the OS data from LUME-Lung 2: the shorter the TSFLT, the better the treatment effect, as indicated by a decreasing HR with shorter TSFLT in patients with either non-squamous or adenocarcinoma histology tumours. In both patients with non-squamous histology with TSFLT <9 months and patients with adenocarcinoma histology with TSFLT <9 months, the HR for OS was 0.84 (95% CI 0.62–1.14).

The HR-by-treatment interaction plots based on centrally assessed PFS and the interim OS for the patients with non-squamous cell carcinoma in LUME-Lung 1 validated the relationship between TSFLT and HR observed in the LUME-Lung 2 data. The same relationship between TSFLT and HR was observed in patients with adenocarcinoma for centrally assessed PFS (figure 3B) and the interim OS.

For patients with adenocarcinoma histology and TSFLT <9 months, the HR for centrally assessed PFS was 0.63 (95% CI 0.48–0.83) (figure 4B) and the HR for interim OS was 0.79 (95% CI 0.55–1.14). PFS benefit was also observed in patients with TSFLT <6 months (see online supplementary figure 2A). Of note, in the patients with squamous cell carcinoma, no relationship between this marker and either PFS or OS treatment effects was observed.

Final validation

The first testing step for the final OS data from LUME-Lung 1 established that patients with adenocarcinoma with TSFLT <9 months showed a greater survival benefit. In these patients, nintedanib–docetaxel significantly prolonged median OS by 3 months compared with placebo–docetaxel, and reduced the risk of death by 25% (figure 4C) (median final OS 10.9 months [95% CI 8.5–12.6] vs 7.9 months [95% CI 6.7–9.1], HR 0.75 [95% CI 0.60–0.92]; p=0.0073). In the group with TSFLT ≥9 months, the HR for OS was 0.89 (95% CI 0.66–1.19; p=0.4239, median OS 17.0 vs 15.1 months).

The assessment of OS HRs for various levels of TSFLT in the LUME-Lung 1 study shows that patients with adenocarcinoma with the shortest TSFLT derived significant treatment benefit for OS when treated with nintedanib–docetaxel (figure 3C). More importantly, however, this confirmed that the overall population of patients with adenocarcinoma derived a treatment benefit, as the estimated HR stayed below 1, irrespective of the TSFLT. Of note, the upper limit of the 95% CI did cross 1 at around the time of 12 months since start of first-line therapy.

In line with the predictive effect of TSFLT, patients with TSFLT <6 months (median final OS 9.5 months
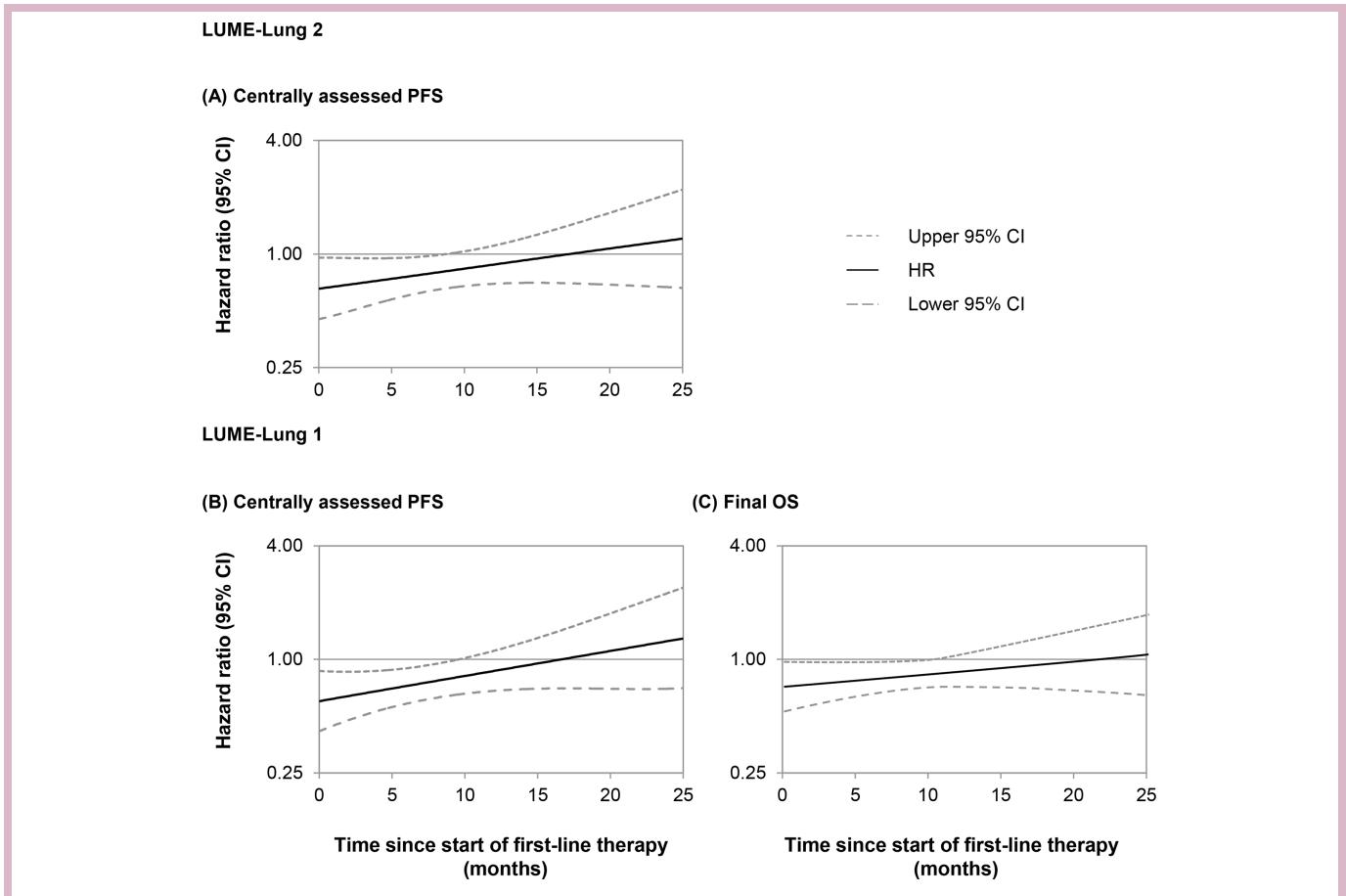
Figure 3 HR by treatment interaction plots for time since start of first-line therapy in patients with advanced adenocarcinoma histology. (A) Centrally assessed PFS (primary analysis) in patients treated with nintedanib–pemetrexed in LUME-Lung 2, (B) centrally assessed PFS (primary analysis) and (C) final OS in patients treated with nintedanib–docetaxel in LUME-Lung 1. OS, overall survival; PFS, progression-free survival.

[95% CI 6.4–12.0] vs 7.5 months [95% CI 5.9–8.6], HR 0.73 [95% CI 0.55–0.98]; p=0.0327) (see online supplementary figure 2B) and patients who had only progressive disease as best response during first-line treatment (median OS 9.8 months [95% CI 6.1–15.5] vs 6.3 months [95% CI 5.0–8.1], HR 0.62 [95% CI 0.41–0.94]) showed a greater survival benefit (see online supplementary figure 2C).

Additional evaluation of the data showed that TSFLT was strongly correlated with time to disease progression on first-line therapy in both studies (Pearson correlation coefficient for the nintedanib arm: 0.93 for LUME-Lung 2 [follow-up data] and 0.97 for LUME-Lung 1 [final OS data]).

Discussion

Using data from two large, independent trials, we have identified and independently confirmed TSFLT as a prognostic and predictive clinical marker of nintedanib benefit in patients with pretreated advanced NSCLC of adenocarcinoma histology receiving concomitant chemotherapy with docetaxel or pemetrexed. A rigorous methodology was used to ensure the validity of TSFLT as a clinical marker. This clinical marker specifically defined a population of patients with adenocarcinoma

histology and TSFLT <9 months; this population experienced a 3-month improvement in median OS and more than double the median PFS compared with placebo–docetaxel in LUME-Lung 1. Confirmation of the cut-off point for TSFLT of 9 months was also validated using an additional methodology (see online supplementary appendix). Taken together with the OS benefits reported in the LUME-Lung 1 study for the overall adenocarcinoma population, these findings suggest that early progressors with adenocarcinoma histology may benefit relatively more from nintedanib-based combination treatment. To our knowledge, this is the first report of a predictive clinical marker of benefit for an antiangiogenic tyrosine kinase inhibitor in patients with NSCLC, which has been validated in an independent trial. TSFLT represents an easy-to-assess parameter and, given that shorter time is associated with more aggressive tumour characteristics, TSFLT represents a clinically relevant factor that can be used to guide treatment choice.

Results from the REVEL study also confirm TSFLT as a potential clinical marker for the efficacy of antiangiogenic therapy in patients with advanced NSCLC; TSFLT was one of the most predictive factors for ramucirumab in the second-line NSCLC setting.[9] Interestingly, the
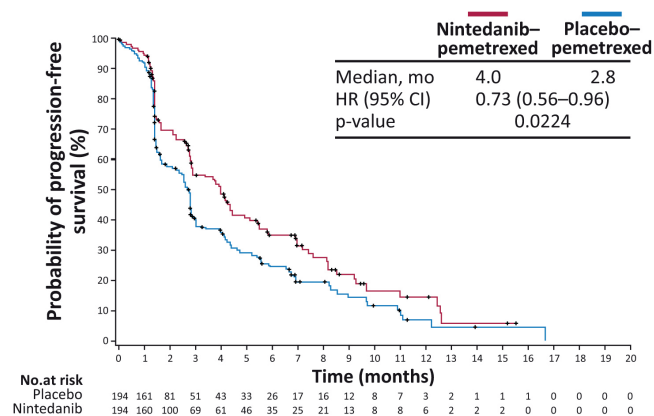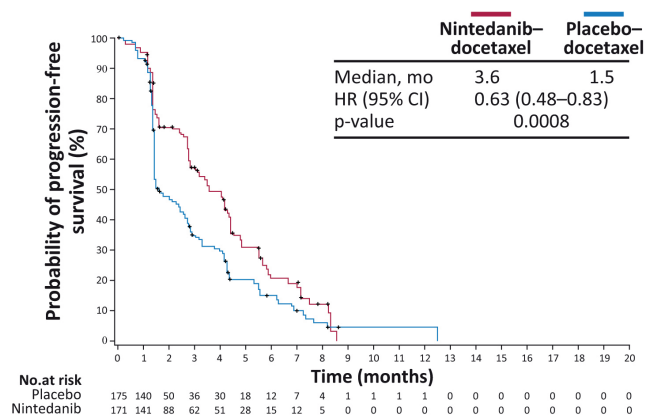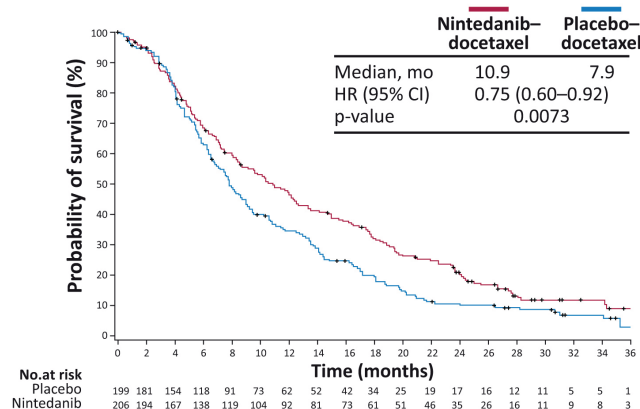
Figure 4 Kaplan–Meier analysis of PFS and OS in patients with advanced non-small cell lung cancer with adenocarcinoma histology with time since start of first-line therapy <9 months. (A) Centrally assessed PFS (primary analysis) in patients treated in LUME-Lung 2, (B) centrally assessed PFS (primary analysis) and (C) final OS in patients treated in LUME-Lung 1. OS, overall survival; PFS, progression-free survival.

opposite is true for the anti-PD-L1 inhibitor nivolumab; OS benefit with nivolumab treatment was greatest in patients with non-squamous NSCLC with >6 months since completion of their most recent platinum-based doublet chemotherapy regimen.[11] Additional *post–hoc* analyses have shown that nivolumab-treated patients with poorer prognostic features and/or aggressive disease, when combined with low tumour PD-L1 expression (<50%), may be at higher risk of death within the first 3 months of treatment.[12] As such, TSFLT may be a useful factor to guide second-line treatment selection.

Time to progression or time since completion of therapy has been used in other trials to characterise early-progressing or refractory NSCLC patient populations using similar time frames to the 9-month cut-off identified here.[20–23] From a clinical perspective, a cut-off of 9 months since start of first-line therapy to define early progressors is also logical, given the average duration of first-line platinum-containing chemotherapy (~4.5–5 months) and the timing of routine CT scanning after the end of the first-line regimen (6–8-week intervals). Furthermore, time to

progression is an established clinical marker in ovarian cancer.[24]

We hypothesise that there is a scientific rationale to explain the benefit of nintedanib-based treatment that is observed in adenocarcinoma early progressors, which is related to the biology of rapidly progressing tumours as well as the unique biology of adenocarcinoma. Rapidly progressing tumours contain a large proportion of proliferating cells and require high levels of oxygen and nutrients. As such, the growth of these tumours may particularly depend on the development of new blood vessels, which may render them more sensitive to the effects of antiangiogenic treatment than slowly progressing tumours. This hypothesis is supported by an analysis of gene expression. Clinical outcome data derived from a large NSCLC database were analysed using a gene set enrichment analysis that was applied to robust multi-array average normalised expression data generated by the Director's Challenge Consortium.[25] That analysis showed that genes relevant to cell proliferation are more frequently expressed in lung adenocarcinomas

from patients with early disease progression, indicating a large fraction of proliferating cells.[26] The potential ability of nintedanib to provide sustained blockade of the major angiokinases involved in tumour vascularisation (VEGFR-1–3, PDGFR-α/β and FGFR-1–3[7]) may enhance the effectiveness of combination treatment and help to overcome the compensatory angiogenic signalling that has been observed when anti-VEGF/VEGFR agents are used alone.[27] We cannot exclude other possible reasons for the benefit of nintedanib-based treatment in adenocarcinoma early progressors, and further study would be needed to do so. However, we have observed no other obvious clinical features of these patients that could contribute to the effect seen.

As shown here, histological subtype has been linked to the therapeutic outcome of patients with NSCLC in other studies, including the E4599 study with bevacizumab,[6] the REVEL study with ramucirumab[9] and meta-analyses;[28 29] all have shown an OS benefit with antiangiogenic compounds in combination with a taxane in patients with non-squamous/adenocarcinoma NSCLC. Potential differences in treatment effect in different histologies may relate to the molecular profile of adenocarcinoma, which is largely different from squamous cell cancer histology.[30–34]

There have been extensive research efforts to identify predictive markers of benefit from antiangiogenic therapy in NSCLC. Hypertension has been suggested as a predictive marker for efficacy, but has not been validated and recent meta-analyses showed no association.[35] In our analysis, LDH levels were identified as a prognostic marker, as observed previously in several cancers,[36] including NSCLC.[37] However, LDH levels were not predictive for the effect of nintedanib here, as has been reported with some antiangiogenic agents in metastatic colorectal cancer.[38–40]

Other studies have also shown a link between markers associated with more aggressive tumours/worse prognosis and greater relative benefit from antiangiogenic treatment.[41–43] High baseline VEGF levels are associated with worse prognosis, and they are predictive of benefit from treatment with bevacizumab in patients with NSCLC[42] or gastric cancer,[41] although they were not predictive in other studies.[44] Similarly, high baseline levels of interleukin-6 predicted poor prognosis in patients with renal cell carcinoma, but greater relative benefit from the antiangiogenic pazopanib.[43] The antiangiogenic agent vandetanib combined with docetaxel has been shown to have a higher activity in pretreated NSCLC with a high epidermal growth factor receptor (*EGFR*) gene copy number or activating *EGFR* mutations than docetaxel alone; however, this effect is most likely linked to its dual EGFR inhibitory effect.[45] Low baseline circulating VEGF levels were also identified in an exploratory analysis as a predictive marker for vandetanib efficacy.[46]

The LUME-Lung trials were largely comparable in patient characteristics and study design[8 13] and the reported efficacy data in the control arms were comparable with those reported previously for second-line therapies. Thus, both studies were considered to be adequate for justifying the identification and validation of the clinical marker. Limitations of this analysis include the fact that assessment of predictive clinical markers was not a primary or key secondary endpoint in these studies, and the early termination of the LUME-Lung 2 study.

In conclusion, TSFLT was shown to be the only prognostic and predictive clinical marker for the treatment effect of nintedanib combined with either docetaxel or pemetrexed in patients with advanced NSCLC of adenocarcinoma histology progressing following chemotherapy. This clinical marker defined that patients with shorter TSFLT who have a poorer prognosis derive a greater PFS and OS benefit from nintedanib. This marker could be used for patient selection, and further investigation is warranted regarding pathways promoting aggressive tumour growth and angiogenic tyrosine kinase inhibitor benefit.

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Time since start of first-line therapy as a predictive clinical marker for nintedanib in patients with previously treated non-small cell lung cancer

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