TG4010 immunotherapy and first-line chemotherapy for advanced non-small-cell lung cancer (TIME): results from the phase 2b part of a randomised, double-blind, placebo-controlled, phase 2b/3 trial

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Summary

Background MUC1 is a tumour-associated antigen expressed by many solid tumours, including non-small-cell lung cancer. TG4010 is a modified vaccinia Ankara expressing MUC1 and interleukin 2. In a previous study, TG4010 combined with chemotherapy showed activity in non-small-cell lung cancer and the baseline value of CD16, CD56, CD69 triple-positive activated lymphocytes (TrPAL) was shown to be potentially predictive of TG4010 efficacy. In this phase 2b part of the phase 2b/3 TIME trial, we further assess TG4010 in combination with first-line chemotherapy and use of the TrPAL biomarker in this setting.

Methods In this phase 2b part of a randomised, double-blind, placebo-controlled, phase 2b/3 trial, we recruited previously untreated patients aged 18 years or older with stage IV non-small-cell lung cancer without a known activating *EGFR* mutation and with MUC1 expression in at least 50% of tumoural cells. Patients were randomly allocated (1:1) by an external service provider to subcutaneous injections of 10^8 plaque-forming units of TG4010 or placebo from the beginning of chemotherapy every week for 6 weeks and then every 3 weeks up to progression, discontinuation for any reason, or toxic effects, stratified according to baseline value of TrPAL (≤ or > the upper limit of normal [ULN]) and, in addition, a dynamic minimisation procedure was used, taking into account chemotherapy regimen, histology, addition or not of bevacizumab, performance status, and centre. Patients, site staff, monitors, the study funder, data managers, and the statistician were masked to treatment identity. The primary endpoint was progression-free survival, assessed every 6 weeks, to validate the predictive value of the TrPAL biomarker. If patients with TrPAL values of less than or equal to the ULN had a Bayesian probability of more than 95% that the true hazard ratio (HR) for progression-free survival was less than 1, and if those with TrPAL values of greater than the ULN had a probability of more than 80% that the true HR for progression-free survival was more than 1, the TrPAL biomarker would be validated. We did primary analyses in the intention-to-treat population and safety analyses in those who had received at least one dose of study drug and had at least one valid post-baseline safety assessment. Monitors, site staff, and patients are still masked to treatment assignment. This trial is registered with ClinicalTrials.gov, number NCT01383148.

Findings Between April 10, 2012, and Sept 12, 2014, we randomly allocated 222 patients (TG4010 and chemotherapy 111 [50%]; placebo and chemotherapy 111 [50%]). In the whole population, median progression-free survival was 5·9 months (95% CI 5·4–6·7) in the TG4010 group and 5·1 months (4·2–5·9) in the placebo group (HR 0·74 [95% CI 0·55–0·98]; one-sided p=0·019). In patients with TrPAL values of less than or equal to the ULN, the HR for progression-free survival was 0·75 (0·54–1·03); the posterior probability of the HR being less than 1 was 98·4%, and thus the primary endpoint was met. In patients with TrPAL values of greater than the ULN, the HR for progression-free survival was 0·77 (0·42–1·40); the posterior probability of the HR being greater than 1 was 31·3%, and the primary endpoint was not met. We noted grade 1–2 injection-site reactions in 36 (33%) of 110 patients in the TG4010 group versus four (4%) of 107 patients in the placebo group. We noted no grade 3 or 4 nor serious adverse events deemed to be related to TG4010 only. Four (4%) patients presented grade 3 or 4 adverse events related to TG4010 and other study treatments (chemotherapy or bevacizumab) versus 11 (10%) in the placebo group. No serious adverse event was related to the combination of TG4010 with other study treatments. The most frequent severe adverse events were neutropenia (grade 3 29 [26%], grade 4 13 [12%] in the TG4010 group *vs* grade 3 22 [21%], grade 4 11 [10%] in the placebo group), anaemia (grade 3 12 [11%] *vs* grade 3 16 [15%]), and fatigue (grade 3 12 [11%], grade 5 one [1%] *vs* grade 3 13 [12%]; no grade 4 events).

Interpretation TG4010 plus chemotherapy seems to improve progression-free survival relative to placebo plus chemotherapy. These data support the clinical value of the TrPAL biomarker in this clinical setting; because the primary endpoint was met, the trial is to continue into the phase 3 part.

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Research in context

Evidence before this study

We did a search of PubMed using the terms "cancer vaccine", "antineoplastic combined chemotherapy protocols", and "lung cancer" from Jan 1, 1995, to July 31, 2015, with no language restrictions. Combination of vaccines with antineoplastic drugs is potentially synergistic through different mechanisms: inhibition of regulatory T cells, release of tumour antigens, and modification of the biological environment of the tumours. However, to the best of our knowledge, addition of a targeted vaccine to chemotherapy in advanced non-small-cell lung cancer has not been tested clinically apart from two previous phase 2 studies of TG4010 for this indication. One of these previous randomised phase 2 studies tested TG4010 in addition to cisplatin and gemcitabine. The study met its primary endpoint based on 6-month progression-free survival and suggested the predictive value of activated natural killer cells for TG4010 activity in this setting. On the basis of another PubMed search with the terms "killer cells natural" and "predictive value of the tests", also from Jan 1, 1995, to July 31, 2015, with no language restrictions, the predictive value of the concentration of activated natural killer cells for the efficacy of a cancer immunotherapy has not been mentioned elsewhere.

Added value of this study

The phase 2b part of the TIME study presented here aimed to both substantiate the activity of TG4010 in combination with present first-line chemotherapy regimens and also further assess the predictive value of baseline activated natural killer cells, now called triple-positive activated lymphocytes (TrPAL). This study has reproduced the findings from a previous randomised study testing addition of TG4010 to first-line chemotherapy in advanced non-small-cell lung cancer: TG4010 if added to chemotherapy improves efficacy metrics, consisting of progression-free survival, overall survival, and the proportion of patients achieving an overall response, and in the 75% of patients with the lowest TrPAL values, TG4010 provides a benefit, whereas in the 25% with the highest values, it does not. During the period of time corresponding to this study, targeted vaccines have not been convincingly successful in treatment of advanced non-small-cell lung cancer, be it either MAGE-A3 or tecemotide, two peptide-based vaccines given as monotherapy. TG4010 belongs to the class of genetic vaccines and is given in combination with chemotherapy.

Implications of all the available evidence

Addition of a genetic targeted cancer vaccine like TG4010 to chemotherapy appears feasible, well tolerated, and active. Similar studies should take into account the baseline value of TrPAL in the analysis of data. The results collected with TG4010 in advanced non-small-cell lung cancer also justify exploration of the combination of TG4010 with immune checkpoint blockers in this setting.

Introduction

Non-small-cell lung cancer is the most frequent cause of cancer-related deaths.[1] Most tumours have a non-squamous histology, and the proportion of this type of tumours is still growing. Present first-line treatment of advanced stage disease is based on a platinum-based doublet chemotherapy regimen. In patients with *EGFR* mutations or ALK rearrangements, targeted treatments are the preferred option. The goal of treatment of advanced disease is to improve the duration of survival while quality of life is maintained.[2,3] Immunotherapy has proven its ability to change the course of neoplastic diseases in a substantial manner, with some patients showing durable responses.[4] Some cancer immunotherapy products, such as therapeutic vaccines, induce development of a cellular immune response against the tumour, whereas others, such as immune checkpoint blockers, suppress negative regulatory pathways that prevent the antitumoural immune response from being fully active.

TG4010 (Transgene, Illkirch, France) consists of a suspension of a recombinant modified vaccinia Ankara that codes for the MUC1 tumour-associated antigen and interleukin 2.[5] The MUC1 protein is overexpressed in lung cancer and many other epithelial tumours to which it offers a selective advantage. In tumours as compared with healthy tissues, the MUC1 protein seems aberrantly glycosylated and this glycosylation is the source of new antigens that make it an immune target. The full-length MUC1 protein expressed by TG4010 in the cytoplasm of modified vaccinia Ankara-infected cells shares epitopes associated with tumoural MUC1 against which it induces a cellular immune response. This immunisation is potentiated by danger signals related to the viral nature of the product and by local co-expression of interleukin 2 at the injection site.

The combination of TG4010 with first-line chemotherapy for advanced stage non-small-cell lung cancer has been tested in two previous randomised studies.[6,7] Findings from these studies have shown that the combination was feasible and safe; the studies met their respective efficacy endpoints, which were progression-free survival at 6 months[6] and the proportion of patients achieving a response.[7] A biomarker programme associated with the study by Quoix and colleagues[6] identified a low baseline value of CD16, CD56, CD69 triple-positive activated lymphocytes (TrPAL), mainly a phenotype of activated natural killer cells, as being predictive of TG4010 activity in combination with chemotherapy. In the previous clinical study by Quoix and colleagues,[6] the 75% of patients with the lowest values of TrPAL benefited from addition of TG4010 to chemotherapy, whereas the 25%

with the highest values did not. Natural killer cells positively regulate development of an adaptive immune reaction through close interactions with dendritic and effector T cells, up to a specific level of activation. If their level of activation becomes too high, they shift toward an immune-suppressive behaviour and limit development of an adaptive immune response.[8] They are reactive to the presence of tumoural cells and viral infections; these two elements are at play in patients with non-small-cell lung cancer repeatedly receiving the live virus TG4010.

The TIME trial is a randomised, controlled, phase 2b/3 trial in previously untreated patients with advanced stage non-small-cell lung cancer, aiming to substantiate the activity of TG4010 in combination with first-line chemotherapy and the clinical usefulness of the TrPAL biomarker. We present here the results of the phase 2b part of the study.

Methods

Study design and participants

In the phase 2b part of this randomised, double-blind, placebo-controlled, phase 2b/3 trial, we recruited patients from 45 centres located in France, Belgium, the UK, Italy, Spain, Hungary, Poland, Israel, and the USA (appendix p 9). Patients were eligible for study inclusion if they had histologically confirmed, stage IV (according to the Union Internationale Contre le Cancer) non-small-cell lung cancer without a known activating *EGFR* mutation. MUC1 expression, analysed by immunohistochemistry of a tumour specimen, had to be present in at least 50% of the tumoural cells (Clone H23; Ventana Medical Systems, Tucson, Arizona, USA). Patients had to be aged at least 18 years, be previously untreated for the advanced stage of the disease, have a good general status (performance status 0 or 1 according to the Eastern Cooperative Oncology Group), and have adequate haematological and biochemical characteristics, including albuminaemia at 30 g/L or higher. We excluded patients with central nervous system metastases unless they were surgically removed or irradiated with no residual disease. We did not permit previous history of any malignancy (except for basal-cell carcinoma of the skin or cervical intraepithelial neoplasia) within 5 years. Additionally, patients had to have at least one measurable site of disease with a CT scan or MRI as defined by Response Evaluation Criteria In Solid Tumors (RECIST [version 1.1]).

The study was approved in each country by the appropriate regulatory bodies and independent ethics committees or institutional review boards. Patients provided written informed consent before entering the screening process. The study was done under the oversight of an independent data monitoring committee in accordance with the principles of the Declaration of Helsinki and the Good Clinical Practice guidelines of the International Conference on Harmonisation.

Randomisation and masking

Patients were randomly allocated (1:1) by an external service provider (Cenduit) to receive TG4010 or placebo through a web-based system. We stratified randomisation according to baseline value of TrPAL (≤ or > the upper limit of normal [ULN]) and, in addition, used a dynamic minimisation procedure taking into account chemotherapy regimen (cisplatin-based or carboplatin-based), histology, addition or not of bevacizumab (squamous, non-squamous without bevacizumab, or non-squamous with bevacizumab), Eastern Cooperative Oncology Group performance status (0 or 1), and centre. The dynamic minimisation used a stochastic treatment allocation algorithm based on the variance method; to minimise imbalance, treatment was assigned with a probability of 0·8, or 0·5 in case of a tie. The minimisation algorithm used a random number sequence to allocate the treatment, including a random factor for allocation, with a probability of 80%.

All study treatments were delivered by the hospital staff of the participating centres. In this double-blind study, at the investigator's site, only kit and vial numbers were visible, with no mention of whether it was TG4010 or placebo. None of the site staff (investigators, pharmacist, or study staff), monitors, or patients were informed of the study drug given. We collected data through web-based case report forms. The study funder, data managers, and statistician (BB) were masked to treatment identity during the study and until the date of the analysis. Monitors, site staff, and patients are still masked.

See Online for appendix

Procedures

All patients were to receive a platin-based doublet of chemotherapy. Chemotherapy regimen was chosen by the investigator: paclitaxel and carboplatin (whatever histology), pemetrexed and cisplatin (for non-squamous tumours), or gemcitabine and cisplatin (for squamous tumours), and given at standard doses for up to six cycles. We allowed bevacizumab and maintenance treatment with pemetrexed or erlotinib according to labelling. We gave TG4010 at a dose of 10^8 plaque-forming units or the matching placebo by subcutaneous injections from the beginning of chemotherapy once a week for 6 weeks and then every 3 weeks up to progression, premature discontinuation due to any reason (eg, an adverse event), or toxic effects. The placebo was the formulation buffer of TG4010.

We did physical examination, including vital signs, at each injection visit. We recorded bodyweight and performance status every 3 weeks. We analysed haematology variables every week for 6 weeks and every 3 weeks thereafter until end of treatment. We did biochemistry tests (alanine aminotransferase, aspartate aminotransferase, lactate dehydrogenase, alkaline phosphatase, total bilirubin, serum protein and albumin, electrolyte [Na^+, K^+, Cl^-, and Ca^{2+}], creatinine, and

C-reactive protein concentration) every 3 weeks until end of treatment. We assessed tumour response using CT scanning on the basis of RECIST 1.1 every 6 weeks until documented progression or for a period of 9 months after the start of study treatment, whichever occurred first. Beyond 9 months of treatment, we did assessments every 12 weeks until documented disease progression. We based clinical decisions and tumour assessments for analysis on local review.

The TrPAL test is being developed as a companion diagnostic for TG4010. It analyses, with flow cytometry, the percentage of TrPAL in the total number of lymphocytes gated on CD45 positivity and cell shape. The real-time version of the test that we used is run on whole blood instead of peripheral blood mononuclear cells that were used in the previous study by Quoix and colleagues.[6] The quartile-based threshold that allowed discovery of the TrPAL biomarker could not be used anymore because of a change in the method of measurement from frozen to fresh blood between the original study and this study and so a new threshold therefore had to be defined to classify patients at study entry for the purpose of stratification. We based this threshold as a first approximation on the ULN (95th percentile) by sex (8·9% in men and 6·3% in female) of a distribution of TrPAL values obtained in a healthy volunteer population (369 people; 224 [61%] men and 145 [39%] women; data not shown). However, in the previous study,[6] the threshold predictive of TG4010 activity corresponded to the first three quartiles of the patient distribution (75%). To reproduce this important finding, we therefore planned a prespecified analysis of the results of this study as well, with a TrPAL cutoff based on the third quartile (Q3) of the TrPAL distribution in the patients screened for the study (cutoffs different to those used in the previous study[6]). The threshold based on Q3 could only be established at the end of patients' screening periods and therefore was not available at study launch for classification of patients. We identified Q3 as the optimum cutoff for classification of patients according to their TrPAL values, which reproduces the observation made in the previous study.[6] ULN was used to classify patients for stratification at study entry before the Q3 could be calculated; however, after the study was completed, the Q3 threshold could be used and therefore results are presented using this threshold.

Outcomes

The primary outcome was progression-free survival, defined as the time from the date of randomisation to the date of first documented tumour progression or death by any cause, whichever occurs first. If patients with TrPAL values at baseline of less than or equal to the ULN had a Bayesian probability of more than 95% that the true hazard ratio (HR) for progression-free survival was less than 1, and if patients with TrPAL values of greater than the ULN had a probability of more than 80% that the true HR for progression-free survival was more than 1, the TrPAL biomarker would be validated. Secondary outcomes were the proportion of patients achieving an overall response, duration of response, overall survival, safety, and time to overall response.

We defined overall response as the proportion of patients whose best overall response is either a complete or partial response (response confirmed in a subsequent assessment at least 4 weeks later) according to RECIST version 1.1. Duration of response applied only to patients whose best overall response was a complete or partial response. The start date was the date of first documented response (complete or partial) and the end date was the date of first documented disease progression or death due to underlying cancer. We defined overall survival as the time from the date of randomisation to the date of death by any cause. After the end of study treatments, we followed up patients for survival every 3 months. We assessed safety with the National Cancer Institute Common Terminology Criteria for Adverse Events (version 4.03). We reported occurrence of adverse events and serious adverse events. On achievement of the primary endpoint in this phase 2b part, the TIME trial is to continue into its phase 3 part, with overall survival as the primary outcome.

Statistical analysis

We based analysis of the primary outcome of the study for validation of the TrPAL biomarker on a Bayesian normal conjugate model for the log of the HR for progression-free survival and did the analysis separately in patients with TrPAL values at baseline of less than or equal to the ULN and greater than the ULN. This model integrates the results of both the previous randomised study[6] and this study. For patients with a TrPAL value of less than or equal to the ULN, at least 154 patients should have been enrolled; the recruitment was to be put on hold until we did the efficacy analysis. At that time, assuming that the true value of the HR was 0·6 (as noted in the previous study), after 89 events of progression, the conditional power that the HR is less than 1 with compelling evidence (95% probability or more) is higher than 90%. In patients with a TrPAL value of greater than the ULN, recruitment was to be put on hold after accrual of 52 patients and at least 38 events were needed to show, with an 80% conditional power, that the HR was more than 1; in the case that this condition was not satisfied, the probability that the HR was less than 1 had to be calculated. We calculated Bayesian probabilities in each subgroup of patients according to their TrPAL value on the basis of ULN values and, in a prespecified sensitivity analysis, repeated the calculation using the Q3 cutoff. In an exploratory analysis, we also calculated progression-free survival not stratified by TrPAL value.

Because of the delayed effect of immunotherapies, we assessed fixed-time percentages, such as progression-free survival at 9 months. In a post-hoc exploratory

analysis, we assessed expression of PD-L1 with immunohistochemistry of pretreatment tumour specimens if available, using clone E1L3N (Cell Signaling, Danvers, Massachusetts, USA) for this purpose. All results apart from overall survival data presented in this report are based on a cutoff date of Dec 15, 2014, at a moment when the prespecified number of progression-free survival events needed to do the analyses had occurred in both stratification groups. We updated overall survival data with a cutoff date of July 6, 2015.

We censored progression-free survival if we did not note any progression or death at the cutoff date for analysis or at the date when a further antineoplastic treatment (other than those planned in the protocol) was started. The censoring date was the date of the last evaluable tumour assessment before the cutoff date or start of further antineoplastic treatment. If we noted a progression-free survival event after two or more missing or non-evaluable tumour assessments, then we censored the date of progression at the latest-occurring evaluable tumour assessment before missing assessments; for progression noted after a single missing or non-evaluable tumour assessment, we used the actual date of disease progression. Establishment of progression was based on local assessments of baseline and post-baseline scans and was to be established by assessment of target and non-target disease at baseline according to RECIST (version 1.1), using the same methods throughout the study. We investigated the appearance of new lesions if clinically indicated. If a patient was not known to have died, we censored overall survival at the date of last contact.

We present progression-free survival, overall survival, and duration of response using Kaplan-Meier curves. We used unstratified log-rank tests to compare treatment groups and estimated unstratified HRs with corresponding 95% CIs using a Cox regression model. We deemed a one-sided p value of less than 0·025 significant. Preplanned subgroup analyses included patients with TrPAL values of less than or equal to Q3 and those with both TrPAL values of less than or equal to Q3 and a non-squamous tumour.

The intention-to-treat population consisted of all randomly allocated patients. Following the intention-to-treat principle, we analysed patients according to the treatment and stratum that they were assigned to at randomisation. The intention-to-treat population was the primary population in the assessment of efficacy. The safety population consisted of all patients who received at least one dose of any component of the study treatment (study drug, chemotherapy, or bevacizumab) and had at least one valid post-baseline safety assessment. We analysed patients according to the treatment that they actually received. We analysed patients never treated with TG4010 or placebo separately. Thus, safety results are provided in patients having received at least one injection.

We did analyses with SAS version 9.3. This trial is registered with ClinicalTrials.gov, number NCT01383148.

Role of the funding source

The study was designed by the funder of the study. Monitoring, management, and analysis of the data were done by service providers under the supervision of the funder. Data collection was done by a contract research organisation. The funder was associated with writing of the report. The corresponding author had full access to all of the data in the study and had final responsibility for the decision to submit for publication. Several employees of the funder had access to the unmasked data after analysis (CH, TP, AT, GLa, and BB).

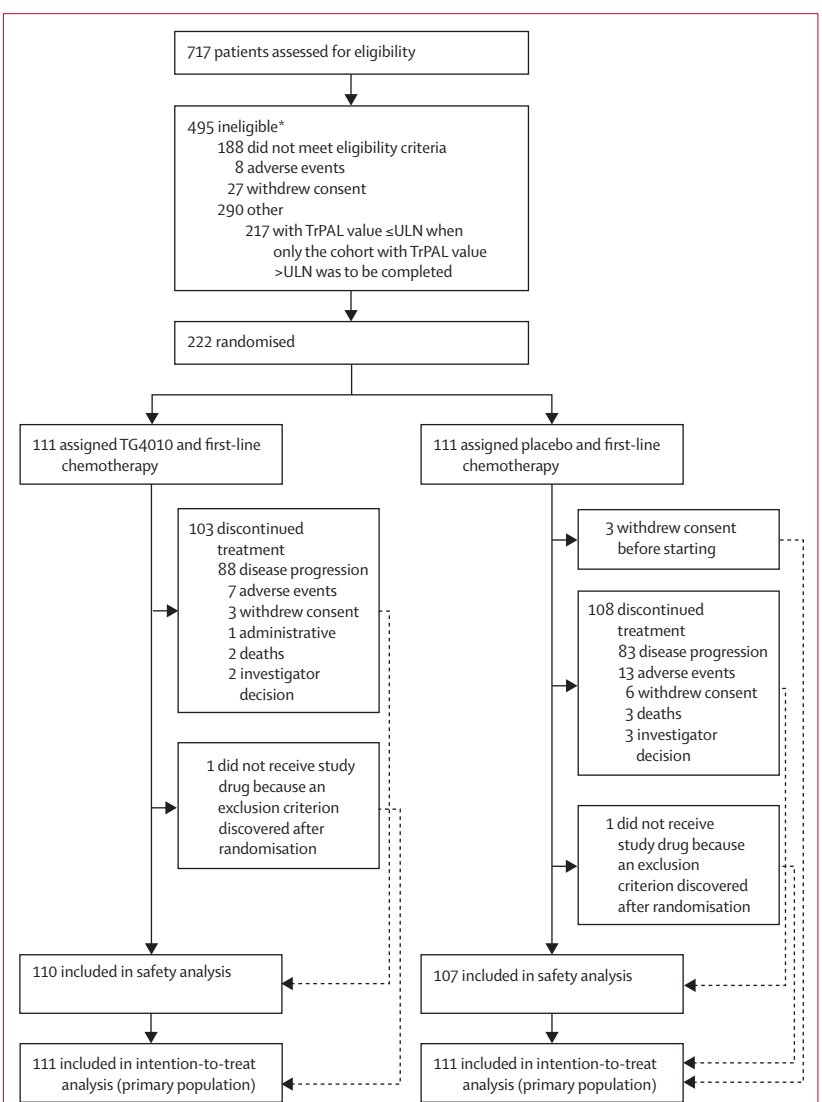


***Figure 1*: Trial profile**
TrPAL=CD16, CD56, CD69 triple-positive lymphocytes. ULN=upper limit of normal. *Patients could have been excluded for several reasons.

Results

Between April 10, 2012, and Sept 14, 2014, we enrolled 222 patients (111 [50%] per treatment group; figure 1). As of Dec 15, 2014 (median follow-up 18·2 months [IQR 16·8–23·5] since randomisation), most patients had discontinued the study; 11 (5%) patients had not yet progressed and were still undergoing treatment (eight [7%] in the TG4010 group and three [3%] in the placebo group). 217 (98%) patients received at least one dose of TG4010 or placebo (TG4010 110 [99%]; placebo 107 [96%]).

	TG4010 (n=111)	Placebo (n=111)
Sex		
Male	72 (65%)	70 (63%)
Female	39 (35%)	41 (37%)
Age (years)	63 (57–68)	59 (54–66)
Histological type		
Squamous	13 (12%)	13 (12%)
Adenocarcinoma	95 (86%)	90 (81%)
Other*	3 (3%)	8 (7%)
Non-squamous	98 (88%)	98 (88%)
ECOG performance status		
0	33 (30%)	35 (32%)
1	77 (69%)	76 (68%)
Missing	1 (1%)	0
Smoking status		
Never smoker	7 (6%)	12 (11%)
Ex or present smoker	104 (94%)	99 (89%)
TrPAL value		
≤ULN	85 (77%)	85 (77%)
>ULN	26 (23%)	26 (23%)
≤Q3	71 (64%)	76 (68%)
>Q3	40 (36%)	35 (32%)

Data are n (%) or median (IQR). ECOG=Eastern Cooperative Oncology Group. ULN=upper limit of normal. Q3=quartile 3. *Large-cell carcinoma, undifferentiated carcinoma, and other rare non-squamous subtypes.

Table 1: **Baseline characteristics**

Baseline characteristics were well balanced between the two treatment groups except for age, with a median age lower in the placebo group (table 1). Most patients had a non-squamous tumour (table 1), showing the epidemiology of non-small-cell lung cancer, but also the expression of MUC1, which is more frequent and stronger in adenocarcinoma than in squamous carcinoma (appendix p 7). The number of patients who received each type of chemotherapy and the number who received a maintenance treatment are described in the appendix (p 1). Overall, 106 (48%) of 222 patients had an unknown status for EGFR-activating mutation at baseline. Further treatment after discontinuation of study treatment is presented in the appendix (p 2).

Because of the slower than expected recruitment of patients with a TrPAL value of greater than the ULN, the final analysis was delayed until the necessary number of events was obtained in both groups (Dec 15, 2014). At this time, we recorded 151 events in the less than or equal to ULN group instead of the 89 planned in the protocol. The power of the analysis with 151 progression-free survival events if the late separation of the curves is integrated is equal to 92% and, therefore, not substantially different from the 90% expected. The Bayesian probability that the HR for progression-free survival was less than 1 in patients with a TrPAL value of less than or equal to the ULN was 98·4% and therefore the primary outcome was met in this group (table 2). In patients with a TrPAL value of greater than the ULN, the probability that the HR was more than 1 was 31·3%, therefore the primary outcome was not met in this group. Because we used the ULN-based cutoff as a first approximation, we also calculated the Bayesian probabilities with use of the Q3 cutoff (7·0% in men and 5·35% in women), in line with the observation made in the previous randomised trial (table 2).[6]

With a classic frequentist analysis (exploratory analysis), progression-free survival was significantly improved in the TG4010 group compared with the placebo group when not stratified by TrPAL value (table 3). In patients with TrPAL values of

	TrPAL value ≤ULN	TrPAL value >ULN	TrPAL value ≤Q3	TrPAL value >Q3
% of TrPAL				
Male	1·0–8·9% (4·30/113)	8·9–19·9% (9·90/29)	1·0–7·0% (3·90/98)	7·1–19·9% (9·30/44)
Female	1·1–6·2% (3·20/57)	6·5–10·4% (7·40/23)	1·1–5·0% (3·00/49)	5·6–10·4% (7·20/31)
Progression-free survival events				
TG4010	76/85 (89%)	21/26 (81%)	62/71 (84%)	35/40 (88%)
Placebo	75/85 (88%)	22/26 (85%)	67/76 (88%)	30/35 (86%)
HR (95% CI)	0·75 (0·54–1·03)	0·77 (0·42–1·40)	0·68 (0·48–0·96)	0·91 (0·56–1·47)
Posterior probability				
HR <1	98·4%	68·7%	99·5%	55·3%
HR >1	1·6%	31·3%	0·5%	44·7%

Data are min–max% (median/n), n/N (%), or %. TrPAL=CD16, CD56, CD69 triple-positive lymphocytes. ULN=upper limit of normal. Q3=quartile 3. HR=hazard ratio.

Table 2: **Bayesian analyses**

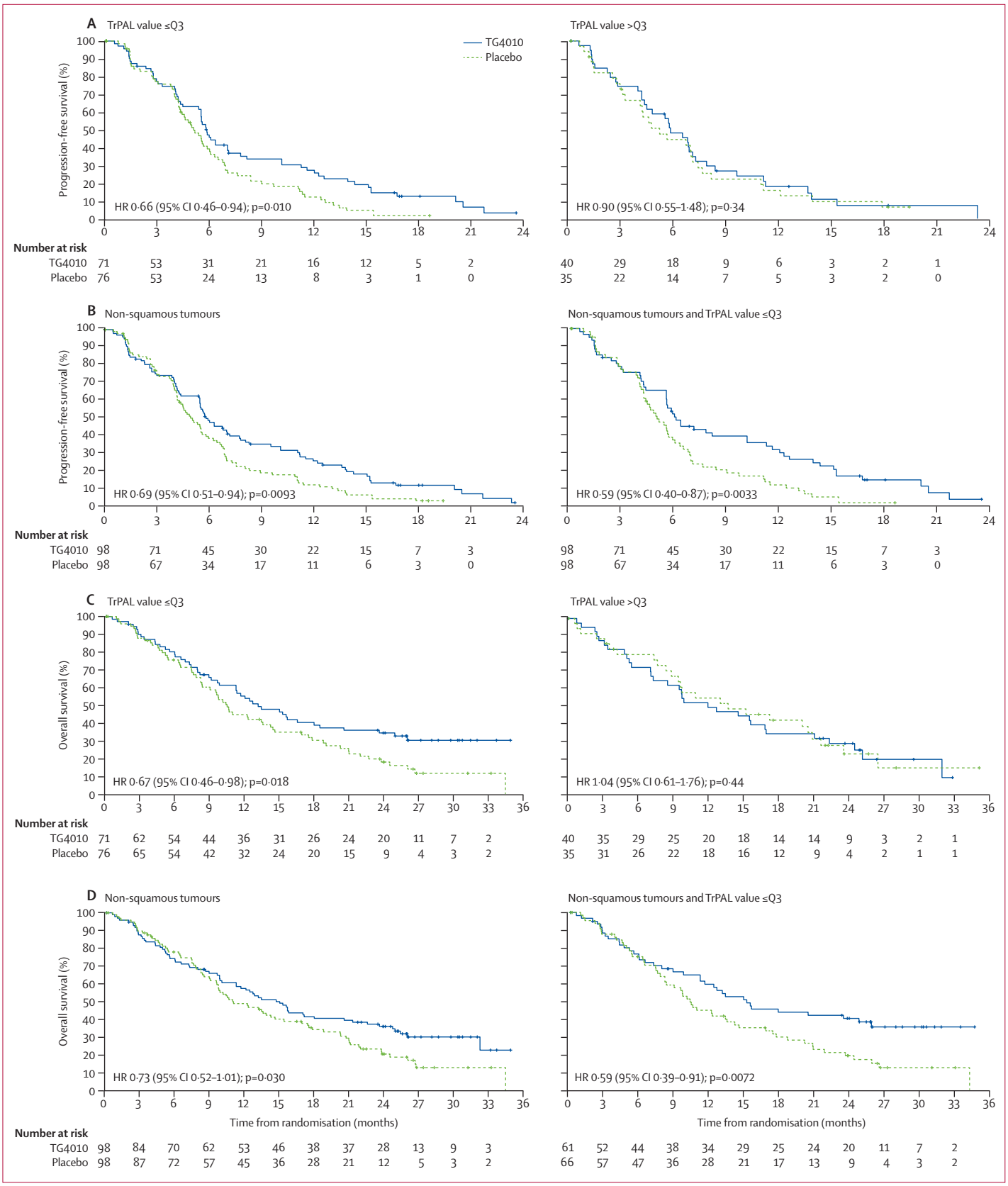
	Whole population (n=222)		TrPAL value ≤Q3 (n=147)		TrPAL value >Q3 (n=75)		Non-squamous tumours (n=196)		Non-squamous tumours and TrPAL value ≤Q3 (n=127)	
	TG4010 (n=111)	Placebo (n=111)	TG4010 (n=71)	Placebo (n=76)	TG4010 (n=40)	Placebo (n=35)	TG4010 (n=98)	Placebo (n=98)	TG4010 (n=61)	Placebo (n=66)
Progression-free survival										
Progression-free survival events	97 (87%)	97 (87%)	62 (87%)	67 (88%)	35 (88%)	30 (86%)	84 (86%)	86 (88%)	52 (85%)	59 (89%)
Progression-free survival (months)	5·9 (5·4–6·7)	5·1 (4·2–5·9)	5·8 (5·3–7·0)	5·0 (4·2–5·9)	5·7 (4·1–7·0)	5·1 (3·1–7·0)	5·8 (5·5–7·2)	5·0 (4·2–5·8)	6·0 (5·5–10·1)	4·9 (4·2–5·8)
9 month progression-free survival	31% (22–40)	20% (13–29)	33% (23–45)	20% (11–30)	27% (14–41)	22% (10–38)	35% (26–45)	19% (12–28)	39% (27–52)	19% (10–29)
Hazard ratio	0·74 (0·55–0·98)	..	0·66 (0·46–0·94)	..	0·90 (0·55–1·48)	..	0·69 (0·51–0·94)		0·59 (0·40–0·87)	..
p value (one-sided)	0·019	..	0·010	..	0·343	..	0·0093		0·0033	..
Overall survival										
Deaths	78 (70%)	87 (78%)	47 (66%)	62 (82%)	31 (78%)	25 (71%)	66 (67%)	77 (79%)	37 (61%)	54 (82%)
Overall survival (months)	12·7 (9·8–16·4)	10·6 (9·5–14·3)	13·0 (9·7–18·4)	10·4 (8·2–14·1)	12·4 (7·3–17·0)	13·7 (8·8–21·0)	14·6 (11·1–20·4)	10·8 (9·5–14·5)	15·1 [11·1–25·9]	10·3 (8·1–14·1)
18 month overall survival	37% (28–46)	34% (25–43)	39% (27–50)	30% (20–41)	35% (21–50)	43% (26–59)	40% (31–50)	34% (25–44)	44% (31–56)	30% (19–41)
Hazard ratio	0·78 (0·57–1·06)	..	0·67 (0·46–0·98)	..	1·04 (0·61–1·76)	..	0·73 (0·52–1·01)		0·59 (0·39–0·91)	..
p value (one-sided)	0·055	..	0·018	..	0·44		0·030		0·0072	..
Proportion achieving overall response										
Proportion of patients achieving overall response	44 (40%)	32 (29%)	28 (39%)	24 (32%)	16 (40%)	8 (23%)	39 (40%)	27 (28%)	24 (39%)	20 (30%)
p value (one-sided)	0·030	..	0·079	..	0·060	..	0·015	..	0·064	..
Complete responses	1 (1%)	0	0	0	1 (3%)	0	1 (1%)	0	0	0
Partial responses	43 (39%)	32 (29%)	28 (39%)	24 (32%)	15 (38%)	8 (23%)	38 (39%)	27 (28%)	24 (39%)	20 (30%)
Stable disease	49 (44%)	54 (49%)	32 (45%)	35 (46%)	17 (43%)	19 (54%)	41 (42%)	51 (52%)	26 (43%)	32 (48%)
Progressions	13 (12%)	15 (14%)	8 (11%)	11 (15%)	5 (13%)	4 (11%)	13 (13%)	12 (12%)	8 (13%)	9 (14%)
Unknown	5 (5%)	10 (9%)	3 (4%)	6 (8%)	2 (5%)	4 (11%)	5 (5%)	8 (8%)	3 (5%)	5 (8%)

Data are n (%), median (95% CI), % (95% CI), or hazard ratio (95% CI). TrPAL=CD16, CD56, CD69 triple-positive lymphocytes. Q3=quartile 3.

Table 3: **Efficacy results**

less than or equal to the Q3, progression-free survival was significantly improved by addition of TG4010 to chemotherapy, whereas we noted no benefit in patients with TrPAL values of greater than the Q3. Progression-free survival was significantly improved in patients with non-squamous tumours, but we noted the highest benefit in the subgroup of patients with both a TrPAL value of less than or equal to the Q3 and a non-squamous tumour (HR 0·59 [95% CI 0·40–0·87]; p=0·0033; table 3, figure 2). In this group, overall survival was significantly improved as well and by the same magnitude (0·59 [0·39–0·91]; p=0·0072).

Patients with both a TrPAL value of less than or equal to the Q3 and a non-squamous tumour are of special interest because the favourable TrPAL immune profile is associated with a histological type of tumour that expresses MUC1 (the target of TG4010) strongly and almost constantly (appendix p 7). The proportion of patients achieving a confirmed response was higher for patients given TG4010 (44 [40%] of 111) than for those given placebo (32 [29%] of 111; table 3). Time to response did not seem different between patients who had received TG4010 or placebo (figure 3). We noted delayed responses beyond 20 weeks in five (11%) of 44 patients given TG4010 and none in the responding patients given placebo. The duration of response was longer in patients who received TG4010 (median 30·1 weeks [95% CI 21·9–43·1]) than in those who received placebo (18·7 weeks [14·9–36·4]; figure 3). 15 (34%) of 44 patients are still in response at 1 year in the TG4010 group versus six (19%) of 32 in the placebo group. More patients in the TG4010 group (38 [39%] of 98) than in the placebo group (29 [30%] of 98) have been able to receive maintenance chemotherapy with


A
TrPAL value ≤Q3
TG4010
Placebo
Progression-free survival (%)
HR 0·66 (95% CI 0·46–0·94); p=0·010
TrPAL value >Q3
HR 0·90 (95% CI 0·55–1·48); p=0·34
Number at risk
TG4010 71 53 31 21 16 12 5 2
Placebo 76 53 24 13 8 3 1 0
40 29 18 9 6 3 2 1
35 22 14 7 5 3 2 0
B
Non-squamous tumours
Progression-free survival (%)
HR 0·69 (95% CI 0·51–0·94); p=0·0093
Non-squamous tumours and TrPAL value ≤Q3
HR 0·59 (95% CI 0·40–0·87); p=0·0033
Number at risk
TG4010 98 71 45 30 22 15 7 3
Placebo 98 67 34 17 11 6 3 0
98 71 45 30 22 15 7 3
98 67 34 17 11 6 3 0
C
TrPAL value ≤Q3
Overall survival (%)
HR 0·67 (95% CI 0·46–0·98); p=0·018
TrPAL value >Q3
HR 1·04 (95% CI 0·61–1·76); p=0·44
Number at risk
TG4010 71 62 54 44 36 31 26 24 20 11 7 2
Placebo 76 65 54 42 32 24 20 15 9 4 3 2
40 35 29 25 20 18 14 14 9 3 2 1
35 31 26 22 18 16 12 9 4 2 1 1
D
Non-squamous tumours
Overall survival (%)
HR 0·73 (95% CI 0·52–1·01); p=0·030
Non-squamous tumours and TrPAL value ≤Q3
HR 0·59 (95% CI 0·39–0·91); p=0·0072
Time from randomisation (months)
Time from randomisation (months)
Number at risk
TG4010 98 84 70 62 53 46 38 37 28 13 9 3
Placebo 98 87 72 57 45 36 28 21 12 5 3 2
61 52 44 38 34 29 25 24 20 11 7 2
66 57 47 36 28 21 17 13 9 4 3 2

pemetrexed (patients with a non-squamous tumour), and this finding is consistent with the fact that more patients in the TG4010 group (61 [62%] of 98) than in the placebo group (48 [49%] of 98) were not in progression after the third radiological reassessment done after six cycles of chemotherapy (or week 18).

In view of the importance of PD-L1 as a potential biomarker for another class of immunotherapies, we established its expression in tumour samples from study patients (160 [72%] patients assessable for PD-L1 tumour cell expression and 137 [62%] assessable for PD-L1 tumour-infiltrating immune cell expression; exploratory analysis; appendix p 8). We noted a non-significant improvement in progression-free survival in the TG4010 group compared with the placebo group whether or not the tumour cells express PD-L1. However, there was a significant difference in progression-free survival between the TG4010 and placebo groups in patients with a low level of PD-L1 expression in the immune infiltrate (HR 0·61 [95% CI 0·39–0·96]; p=0·015), but not in those with tumours that had an intermediate or high level of PD-L1 expression in the immune infiltrate (appendix p 8).

More patients in the placebo group (30 [28%] of 108) had at least one dose change for chemotherapy than in the TG4010 group (20 [18%] of 111; appendix p 2). However, the median number of cycles and duration of chemotherapy treatment were equivalent in both treatment groups. Only one (1%) patient discontinued study treatment because of a grade 2 adverse event (fatigue) related to TG4010 (table 4). We noted no excess of adverse events and serious or severe (grade 3 or higher) adverse events in the patients who received TG4010 compared with placebo. Grade 1–2 injection-site reactions were the most frequent adverse events associated with TG4010. We noted no grade 3 or 4, serious, or fatal adverse events related to TG4010 only. Four (4%) of 110 patients given TG4010 presented with grade 3 or 4 treatment-related adverse events related to TG4010 and other study treatments (chemotherapy or bevacizumab) as compared with 11 (10%) of 107 in the placebo group. In the TG4010 group, all were of a frequency of less than or equal to 2%. For adverse events of grade 3 or 4 deemed related to study treatment, including chemotherapy, and present in more than 1% of patients, we noted neutropenia in two (2%) patients in the TG4010 and chemotherapy group versus five (5%) in the placebo and chemotherapy group, and fatigue in one (1%) in the TG4010 and chemotherapy group versus two (2%) in the placebo and chemotherapy group. None of these adverse events were fatal. No serious adverse event was related to the combination of TG4010 with other study treatments. The most frequent severe adverse events were neutropenia, anaemia, and fatigue, and we deemed all related to chemotherapy or underlying disease. All adverse events per grade occurring in at least 10% of patients for grades 1–2 and all patients for grades 3–5 are given in the appendix (pp 3–6).

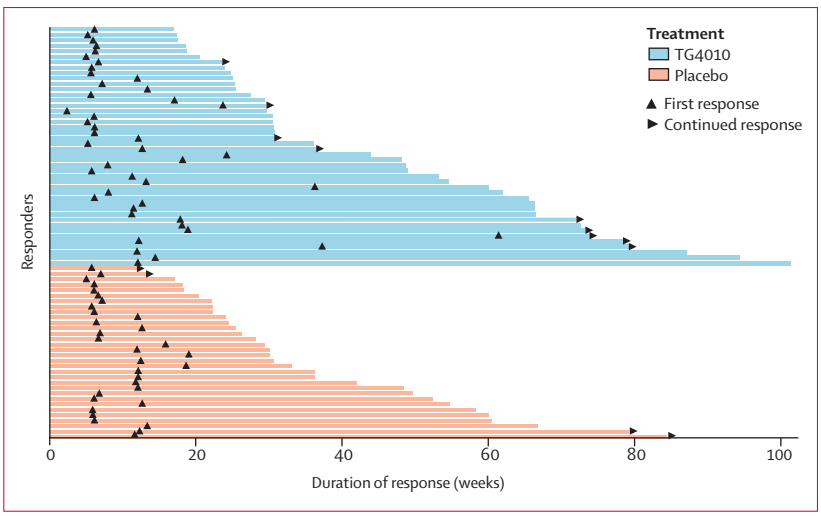


***Figure 3*: Response profile**
Duration of response by treatment group in patients with confirmed response.

Discussion

In this phase 2b part of the TIME trial, we have shown that addition of TG4010 to first-line chemotherapy in advanced stage non-small-cell lung cancer significantly improves progression-free survival, particularly in patients with a TrPAL value of less than or equal to the ULN on the basis of a Bayesian analysis. In patients with a TrPAL value of greater than the ULN, the Bayesian analysis did not validate the TrPAL biomarker. Similarly, when the lower cutoff values for TrPAL at baseline based on Q3 of the screened patients were used, we noted significant improvements of both progression-free survival and overall survival in patients with TrPAL values of less than or equal to the Q3, especially in those with a non-squamous tumour, whereas in those with TrPAL values of greater than the Q3, we noted no benefit in either progression-free or overall survival. We have also shown that progression-free survival is significantly improved in the whole study population with a classic frequentist analysis. Additionally, our results show that addition of TG4010 to chemotherapy improves the proportion of patients achieving a response and is associated with delayed and durable responses consistent with the mechanism of action of TG4010. On the basis of these results, the trial is to continue into the phase 3 part.

Our results reproduce the observation made in the previous randomised phase 2b trial[6] and further support the predictive value of the TrPAL biomarker in patients receiving TG4010 along with first-line chemotherapy, at least in non-small-cell lung cancer. The finding that patients with low values of TrPAL and, hence, activated

***Figure 2*: Progression-free and overall survival**
Progression-free survival according to (A) TrPAL value and (B) non-squamous tumours. Overall survival according to (C) TrPAL value and (D) non-squamous tumours. Crosses denote censored patients. TrPAL=CD16, CD56, CD69 triple-positive lymphocytes.

	TG4010 and chemotherapy (n=110)				Placebo and chemotherapy (n=107)			
	Grade 1–2	Grade 3	Grade 4	Grade 5	Grade 1–2	Grade 3	Grade 4	Grade 5
Any serious adverse event	12 (11%)	29 (26%)	6 (6%)	18 (16%)	27 (25%)	35 (33%)	7 (7%)	14 (13%)
Any adverse event	108 (98%)	70 (64%)	21 (19%)	18 (16%)	104 (97%)	71 (66%)	18 (17%)	14 (13%)
Preferred term*								
Fatigue	56 (51%)	12 (11%)	0	1 (1%)	50 (47%)	13 (12%)	0	0
Nausea	51 (46%)	4 (4%)	0	0	43 (40%)	4 (4%)	0	0
Anaemia	43 (39%)	12 (11%)	0	0	27 (25%)	16 (15%)	0	0
Injection-site reaction	36 (33%)	0	0	0	4 (4%)	0	0	0
Vomiting	30 (27%)	4 (4%)	0	0	33 (31%)	10 (9%)	0	0
Neutropenia	30 (27%)	29 (26%)	13 (12%)	0	19 (18%)	22 (21%)	11 (10%)	0
Diarrhoea	27 (25%)	2 (2%)	0	0	20 (19%)	4 (4%)	0	0
Dyspnoea	25 (23%)	5 (5%)	0	0	9 (8%)	7 (7%)	0	0
Decreased appetite	23 (21%)	1 (1%)	0	0	24 (22%)	4 (4%)	0	0
Weight decreased	20 (18%)	2 (2%)	0	0	18 (17%)	1 (1%)	0	0
Thrombocytopenia	19 (17%)	8 (7%)	6 (6%)	0	16 (15%)	4 (4%)	2 (2%)	0
Headache	14 (13%)	0	0	0	10 (9%)	2 (2%)	0	0
Abdominal pain	13 (12%)	2 (2%)	0	0	11 (10%)	0	0	0
Back pain	13 (12%)	1 (1%)	0	0	11 (10%)	3 (3%)	0	0
Alopecia	11 (10%)	2 (2%)	0	0	8 (8%)	0	0	0
Hypertension	9 (8%)	0	0	0	10 (9%)	5 (5%)	0	0
Stomatitis	8 (7%)	2 (2%)	0	0	13 (12%)	1 (1%)	0	0
Musculoskeletal pain	8 (7%)	2 (2%)	0	0	4 (4%)	0	0	0
Hypokalaemia	7 (6%)	1 (1%)	0	0	10 (9%)	5 (5%)	0	0
Tumour pain	7 (6%)	3 (3%)	0	0	5 (5%)	2 (2%)	0	0
Leucopenia	5 (5%)	4 (4%)	1 (1%)	0	6 (6%)	6 (6%)	1 (1%)	0
Respiratory tract infection	5 (5%)	3 (3%)	0	1 (1%)	4 (4%)	0	0	1 (1%)
Metastatic pain	4 (4%)	0	0	0	2 (2%)	3 (3%)	0	0
Pulmonary embolism	4 (4%)	4 (4%)	0	0	1 (1%)	3 (3%)	0	0
Haemoptysis	3 (3%)	2 (2%)	0	0	8 (8%)	2 (2%)	0	0
Hyponatraemia	3 (3%)	4 (4%)	0	0	3 (3%)	4 (4%)	1 (1%)	0
Dehydration	3 (3%)	4 (4%)	0	0	3 (3%)	1 (1%)	0	0
Lymphopenia	3 (3%)	1 (1%)	0	0	2 (2%)	5 (5%)	0	0
Pneumonia	2 (2%)	2 (2%)	0	1 (1%)	6 (6%)	4 (4%)	0	0
Lung infection	2 (2%)	2 (2%)	0	0	2 (2%)	0	0	0
Erysipelas	2 (2%)	2 (2%)	0	0	0	0	0	0
γ-glutamyl transferase increased	2 (2%)	2 (2%)	0	0	0	0	0	0
Renal failure	1 (1%)	1 (1%)	1 (1%)	0	8 (8%)	2 (2%)	1 (1%)	0
Febrile neutropenia	0	2 (2%)	1 (1%)	0	0	7 (7%)	2 (2%)	0
Peripheral ischaemia	0	2 (2%)	0	0	0	1 (1%)	0	0
Superior vena cava syndrome	0	0	0	0	0	3 (3%)	0	0
Hypophosphataemia	0	0	0	0	0	2 (2%)	0	0

Adverse events classified by the Common Terminology Criteria for Adverse Events (version 4.03) in all patients who received at least one dose of TG4010 or placebo. Patients with adverse events in more than one category are counted separately in each category. *Reported as adverse events of grades 1–2 occurring in at least 10% or grade 3 or 4 occurring in more than 1% of patients in either treatment group.

Table 4: **Adverse events**

natural killer cells benefit from TG4010 immunotherapy, whereas those with high values do not, is consistent with the dual role of natural killer cells in regulation of the development of an adaptive immune response. This concept, however, cannot be generalised to other viral-based immunotherapy products before having tested it. In previous phase 2 studies,[7,9,10] the association between the clinical activity of TG4010 and the cellular immune response against MUC1 has been shown.

The cutoff value of the TrPAL test is a crucial element to properly identify patients who are likely to benefit from addition of TG4010 to chemotherapy and those who are not. The present version of the TrPAL test in which ULN in healthy subjects is used as a cutoff segmented

the study population at 85% of patients instead of the expected 75% and might have led to a suboptimum classification. The power of the analysis with 151 progression-free survival events if the late separation of the curves is integrated is equal to 92% and, therefore, not substantially different from the 90% expected.

This study allows the definition of optimised cutoffs for each sex for continuation of the trial into its phase 3 part and full validation of the test. The fact that overall survival in patients with a TrPAL value of greater than Q3 who received TG4010 was shorter than in those that received placebo has already been noted in the previous randomised clinical trial,[6] and this previous study was part of the biomarker discovery. Unfortunately, the numbers of patients with TrPAL values of greater than Q3 are small and the 95% CIs very large, precluding any definitive conclusion at present in this group. A TrPAL value of greater than Q3 has also never been a prognostic factor, meaning that neither in the previous study[6] nor in this study have patients in the placebo group shown a shorter survival if they had a TrPAL value of greater than Q3 than if they had a TrPAL value of less than or equal to Q3. The TrPAL biomarker so far has behaved exclusively as a biomarker predictive of the benefit or not of addition of TG4010 to chemotherapy in non-small-cell lung cancer.

For both progression-free and overall survival, the benefit of addition of TG4010 to chemotherapy is characterised by a delayed effect, with an initial overlap of survival curves before they separate and stay separate over time.[11] This typical pattern was initially noted with other immunotherapy products like ipilimumab[12] or sipuleucel-T.[13] Indeed, unlike chemotherapy, which acts directly on the tumour, cancer immunotherapies exert their effects on the immune system and show new kinetics that involve building of a cellular immune response followed by changes in tumour burden or patient survival.

Consistent with previous studies, an increased proportion of patients achieving an overall response was noted when TG4010 was added to chemotherapy. In line with the delayed effect noted for some immunotherapies and as shown in figure 3, delayed responses continue to occur in the TG4010 group after 20 weeks, which is not the case with chemotherapy and placebo. Additionally, more patients experience durable responses and long-term survival with TG4010 plus chemotherapy than do those in the placebo group, and the duration of response is substantially longer than with chemotherapy plus placebo. Durable responses noted with immunotherapy products are supposed to express the presence of an immune reaction keeping tumour growth under control.

The safety profile of TG4010 if combined with chemotherapy was favourable, as already evidenced by the two previous phase 2 studies[6,7] with no immune-mediated adverse events. Minor-to-moderate injection-site reactions were reported by about a third of the patients; neither fatigue nor fever were reported in excess. In the previous randomised study,[6] more serious adverse events and a worse outcome were noted in patients with higher TrPAL values having received TG4010, whereas this observation could not be substantiated in this study. One explanation could be introduction of an additional inclusion criterion in this study: we defined a lower limit for albuminaemia of 30 g/L to exclude from the study patients with poor nutritional status and cachexia.

A new class of immunotherapy products blocking the PD-L1 checkpoint pathway has substantially changed the course of the disease in some patients with different malignancies, including non-small-cell lung cancer.[14,15] Retrospective analysis of whether the activity of TG4010 in this study was affected by the expression level of PD-L1 in the available tumour specimens therefore seemed of interest. The immunohistochemical definitions and thresholds of positivity for PD-L1 have not yet been unanimously defined. At first glance, TG4010 seems to manifest its activity whether the tumour samples express PD-L1 or not. Whether administration of TG4010 increases the immune infiltrate in the tumours or increases the expression of PD-L1 would be interesting to establish in future clinical studies.

The phase 3 part of the study is needed to fully validate the clinical usefulness of the TrPAL biomarker for the prescription of TG4010 in patients with advanced non-small-cell lung cancer. The fact that the study has achieved its primary outcome justifies continuing the assessment of the combination of TG4010 with chemotherapy, especially in patients with non-squamous tumours, along with the development of the TrPAL companion test. In parallel, the complementary mechanisms of action of TG4010 and immune checkpoint blockers support testing their combined administration.

Contributors

EQ, GLa, BB, and J-ML designed the study. AT, CH, and GLa did the study procedures. JA did pathology and immunohistochemistry. EQ, HL, GLo, FF, CC, ZP, RG, CO, AS, AK, JTB, VW, EF, DD, and AM collected data. TP and BB managed data. EQ, GLa, BB, and J-ML interpreted data. BB did statistical analysis. EQ, GLa, BB, and J-ML wrote the report. EQ, HL, GLo, FF, CC, ZP, RG, CO, AS, AK, JTB, VW, EF, DD, and AM reviewed the report.

Declaration of interests

GLa, BB, AT, CH, TP, and J-ML are employees of Transgene, the funder of the study. EQ and ZP report advisory board participation for Transgene. JTB reports grants from AstraZeneca, Eli Lilly, Novartis, Abbvie, Amgen, Genentech, and Incyte, outside the submitted work. EF reports personal fees from Eli Lilly, Pfizer, Roche, Boehringer Ingelheim, AstraZeneca, Bristol-Myers Squibb, Merck Sharp & Dohme, and Novartis, outside the submitted work. All other authors declare no competing interests.

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