Pembrolizumab plus trastuzumab in trastuzumab-resistant, advanced, HER2-positive breast cancer (PANACEA): a single-arm, multicentre, phase 1b–2 trial

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Summary

Background HER2-positive breast cancers usually contain large amounts of T-cell infiltrate. We hypothesised that trastuzumab resistance in HER2-positive breast cancer could be mediated by immune mechanisms. We assessed the safety and anti-tumour activity of pembrolizumab, a programmed cell death protein 1 (PD-1) inhibitor, added to trastuzumab in trastuzumab-resistant, advanced HER2-positive breast cancer.

Methods We did this single-arm, multicentre, phase 1b–2 trial in 11 centres based in five countries. Eligible participants were women aged 18 years or older, who had advanced, histologically confirmed, HER2-positive breast cancer; documented progression during previous trastuzumab-based therapy; an Eastern Cooperative Oncology Group performance status of 0 or 1; and a formalin-fixed, paraffin-embedded metastatic tumour biopsy for central assessment of programmed cell death 1 ligand 1 (PD-L1) status. In phase 1b, we enrolled patients with PD-L1-positive tumours in a 3+3 dose-escalation of intravenous pembrolizumab (2 mg/kg and 10 mg/kg, every 3 weeks) plus 6 mg/kg of intravenous trastuzumab. The primary endpoint of the phase 1b study was the incidence of dose-limiting toxicity and recommended phase 2 dose; however, a protocol amendment on Aug 28, 2015, stipulated a flat dose of pembrolizumab of 200 mg every 3 weeks in all Merck-sponsored trials. In phase 2, patients with PD-L1-positive and PD-L1-negative tumours were enrolled in parallel cohorts and received the flat dose of pembrolizumab plus standard trastuzumab. The primary endpoint of the phase 2 study was the proportion of PD-L1-positive patients achieving an objective response. This trial is registered in ClinicalTrials.gov, number NCT02129556, and with EudraCT, number 2013-004770-10, and is closed.

Findings Between Feb 2, 2015, and April 5, 2017, six patients were enrolled in phase 1b (n=3 received 2 mg/kg pembrolizumab, n=3 received 10 mg/kg pembrolizumab) and 52 patients in phase 2 (n=40 had PD-L1-positive tumours, n=12 had PD-L1-negative tumours). The data cutoff for this analysis was Aug 7, 2017. During phase 1b, there were no dose-limiting toxicities in the dose cohorts tested. Median follow-up for the phase 2 cohort was 13·6 months (IQR 11·6–18·4) for patients with PD-L1-positive tumours, and 12·2 months (7·9–12·2) for patients with PD-L1-negative tumours. Six (15%, 90% CI 7–29) of 40 PD-L1-positive patients achieved an objective response. There were no objective responders among the PD-L1-negative patients. The most common treatment-related adverse event of any grade was fatigue (12 [21%] of 58 patients). Grade 3–5 adverse events occurred in 29 (50%) of patients, treatment-related grade 3–5 adverse events occurred in 17 (29%), and serious adverse events occurred in 29 (50%) patients. The most commonly occurring serious adverse events were dyspnoea (n=3 [5%]), pneumonitis (n=3 [5%]), pericardial effusion (n=2 [3%]), and upper respiratory infection (n=2 [3%]). There was one treatment-related death due to Lambert-Eaton syndrome in a PD-L1-negative patient during phase 2.

Interpretation Pembrolizumab plus trastuzumab was safe and showed activity and durable clinical benefit in patients with PD-L1-positive, trastuzumab-resistant, advanced, HER2-positive breast cancer. Further studies in this breast cancer subtype should focus on a PD-L1-positive population and be done in less heavily pretreated patients.

Funding Merck, International Breast Cancer Study Group.

Introduction

Breast cancers that overexpress the human epidermal growth factor receptor 2 (*HER2* or *ERBB2*) oncogene are treated with drugs that target the HER2 pathway. Trastuzumab, a humanised monoclonal antibody against HER2, has transformed the natural history of this disease, with recent therapeutic advances such as pertuzumab and trastuzumab emtansine (T-DM1) and neratinib further improving overall survival for patients with this breast cancer subtype.[1–3] Although overall survival for

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See Comment page 316

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See Online for appendix

Research in Context

Evidence before this study

We searched PubMed for clinical trials or studies published in English between Jan 1, 2010, and June 1, 2018, assessing checkpoint blockade and HER2-directed therapy in patients with advanced HER2-positive breast cancer, using the search terms "HER2 positive", "breast", "checkpoint blockade", and "trastuzumab". To the best of our knowledge, this is the first report of the anti-tumour activity and safety of an anti-programmed cell death protein 1 (PD-1) antibody in combination with trastuzumab in the treatment of patients with advanced HER2-positive breast cancer resistant to previous trastuzumab containing therapies. A previous study examined a programmed cell death 1 ligand 1 (PD-L1) inhibitor without trastuzumab and reported no objective responses.

Added value of this study

In this single-arm, multicentre study, pembrolizumab combined with trastuzumab was well tolerated by patients with documented progression and resistance to trastuzumab and trastuzumab-combination therapies, and had durable anti-tumour activity, especially in those patients with PD-L1-positive tumours and tumour-infiltrating lymphocytes assessed by biopsy of metastatic lesions.

Implications of all the available evidence

Our results suggest that immune-mediated mechanisms of trastuzumab resistance are important in patients with HER2-positive breast cancer. Targeting the PD-1 pathway with pembrolizumab in combination with anti-HER2 drugs could prove to be an effective treatment option, particularly in patients who test positive for immune infiltrates. Our data support further development of this treatment approach for patients with HER2-positive breast cancer in large randomised clinical trials.

most patients with early-stage disease is impressive,[4] a few patients still relapse and others still present with incurable stage 4 disease. Hence, further research to improve overall survival in patients with advanced disease is still warranted in this disease subtype.

Substantial quantities of lymphocytic infiltrate have been observed in primary HER2-positive tumours, with increasing amounts associated with achieving a pathological complete response and having improved disease-free survival and overall survival.[5] Trastuzumab has been shown to have immune mechanisms of action involving innate and adaptive immunity.[6,7] Preclinical studies have suggested that the combination of trastuzumab with drugs targeting immune checkpoints could overcome trastuzumab resistance.[8] High expression of programmed cell death protein 1 (PD-1) and programmed cell death 1 ligand 1 (PD-L1), and other checkpoint molecules have been observed in tumour-infiltrating lymphocytes in this setting.[8,9] Hence, we hypothesised that drugs targeting the PD-1 pathway could be active in patients with HER2-positive breast cancer and trastuzumab-resistant disease.

Pembrolizumab is an anti-PD-1 antibody with robust anti-tumour activity as a monotherapy in multiple settings and an acceptable safety profile, with US Food and Drug Administration (FDA) approval in several solid tumour types. The use of drugs targeting the PD-1 pathway in breast cancer is thus far limited because of the absence of supporting studies[10–12] and the fact that the anti-tumour activity of these checkpoint inhibitors seems somewhat modest in comparison with that seen in more immunogenic tumour types.

We did a combined phase 1b dose-escalation and phase 2 study to determine the safety and anti-tumour activity of pembrolizumab plus trastuzumab in patients with trastuzumab-resistant, HER2-overexpressing, advanced breast cancer. A signal of activity would support the concept that upregulation of checkpoint molecules and subsequent immune evasion might be mechanisms of trastuzumab resistance. A previous phase 1 study[11] reported no objective responses in 26 patients with HER2-positive breast cancer unselected for PD-L1 status treated with the PD-L1 inhibitor avelumab as monotherapy. To the best of our knowledge, this is the first report of a clinical trial investigating PD-1 inhibition with trastuzumab in this subtype of breast cancer.

Methods

Study design and participants

PANACEA (IBCSG 45-13/BIG 4-13/KEYNOTE-014) was a single-arm, multicentre, phase 1b–2 trial done in 11 centres based in Australia, Austria, France, Belgium, and Italy (appendix p 1).

Eligible patients were female, aged 18 years or older, and had advanced (unresectable locoregional, or metastatic), histologically confirmed, HER2-positive breast cancer; documented progression during previous trastuzumab-based therapy; an Eastern Cooperative Oncology Group performance status of 0 or 1; a left ventricular ejection fraction of 50% or more; measurable disease as per Response Evaluation Criteria in Solid Tumours (RECIST, version 1.1); adequate haematological, biochemical, and liver function; and a life expectancy of more than 3 months. Of note, eligible patients had to submit a formalin-fixed, paraffin-embedded tumour biopsy for central confirmation of HER2 positivity or *ERBB2*-amplification, and central assessment of PD-L1 status. Key exclusion criteria were a history of non-infectious pneumonitis; an autoimmune disorder requiring immunosuppressive drugs in the past 2 years; a history of hepatitis, autologous, or allogeneic transplant; HIV; or active tuberculosis. Patients were required to

have adequate washout from major surgery (≥4 weeks), radiotherapy (≥2 weeks), chemotherapy (≥3 weeks), and other anti-HER2 therapy, apart from trastuzumab (≥3 weeks). The complete eligibility criteria are listed in the study protocol, available in the appendix (p 6).

The study was done in accordance with Good Clinical Practice guidelines and the Declaration of Helsinki. The study protocol was approved by independent ethics committees at all 11 academic centres and the IBCSG ethics committee, and is available in the appendix (p 6). All patients provided written, informed consent before undertaking study procedures. The study was overseen by an independent International Breast Cancer Study Group (IBCSG) data and safety monitoring committee.

Procedures

In the phase 1b, dose escalation portion of the trial, two doses of pembrolizumab were used: 2 mg/kg and 10 mg/kg, with a fallback dose of 1 mg/kg, given every 3 weeks. Trastuzumab was administered at the standard dose (6 mg/kg every 3 weeks). The drug combination was administered intravenously on the first day of a 3-week (21-day) cycle. When the phase 2 part of the study started, because of an internal decision by Merck, a flat 200 mg dose of pembrolizumab was introduced across all pembrolizumab trials, irrespective of sponsor, and the study protocol was amended on Aug 28, 2015. In phase 2, patients were therefore treated with a flat dose of intravenous pembrolizumab 200 mg every 3 weeks, with 6 mg/kg of trastuzumab every 3 weeks.

In all cohorts, pembrolizumab was continued until disease progression, unacceptable toxicity, withdrawal of consent, or completion of 2 years of treatment. For isolated CNS progression, study treatment could be continued after appropriate local therapy as per investigator's decision. No dose reductions were allowed for either pembrolizumab or trastuzumab. Pembrolizumab was withheld for drug-related toxicities and severe or life-threatening adverse events until events resolved to grade 1 severity. Dose interruptions were also permitted in the case of medical or surgical events or logistical reasons not related to study therapy (eg, elective surgery, unrelated medical events, or patient vacation). Patients with interrupted doses were to resume trial treatment within 3 weeks of the next scheduled dose.

A tumour biopsy from a metastatic lesion was required for central determination of HER2, oestrogen receptor status, proportion of tumour-infiltrating lymphocytes, and PD-L1 status, as well as for future biomarker analyses. This could be freshly obtained or taken in the year prior to study enrolment. HER2, oestrogen receptor status, and quantity of tumour-infiltrating lymphocytes measured as the proportion of tumour stromal infiltration on haematoxylin and eosin-stained slides using a pre-defined method,[13,14] were centrally reviewed at the IBCSG central pathology office in Milan, Italy. PD-L1 immunohistochemical status was assessed centrally with formalin-fixed paraffin embedded tissue at the Merck Laboratories. PD-L1 status for the first 79 patients was assessed with the QualTek immunohistochemistry assay (QualtTek Molecular Laboratories, Santa Barbara, CA, USA) in which PD-L1 staining in at least 1% of tumour cells or any staining in stroma were defined as positive. The assay was changed to the Dako IHC 22C3 pharmDx Q² Solutions assay (Q2 Solutions, West Lothian, UK) on May 6, 2016, because this assay was introduced across all trials involving pembrolizumab. The measure of expression was the combined positive score, defined as the ratio of PD-L1–positive cells (tumour cells, lymphocytes, and macrophages) out of the total number of tumour cells × 100. PD-L1 positivity was defined as a combined positive score of 1 or more (previously reported as, and equivalent to, combined positive score ≥1%). To assess concordance between the two assays, samples with sufficient remaining tissue that were assessed with the QualTek assay were re-assessed with the Q² Solutions assay. Tumour assessments were done at screening; restaging was done at 12 weeks (after cycle 4), 18 weeks, 24 weeks, and every 12 weeks thereafter. CT of the chest, abdomen, and pelvis was used to measure tumour response according to RECIST 1.1. Objective response and disease progression were confirmed with subsequent imaging at least 4 weeks later; all responses were centrally reviewed. Clinically stable patients with progressive disease, as per RECIST 1.1, who were judged by the investigator to be clinically benefiting from pembrolizumab and trastuzumab, could remain on treatment until deemed to be clinically progressing.

Haematological and biochemical assessments were done at screening and within 3 days before day 1 of each cycle, and at the end of treatment. Safety assessments were done at each study visit, and serum and plasma were collected at every restaging assessment. Safety data were summarised for all patients who received at least one dose of pembrolizumab and trastuzumab. We summarised the incidence of events that were new or worsening from the time of first dose of treatment, according to system organ class or preferred term, severity (as per Common Terminology for Adverse Events, version 4.0), type of adverse event, and relation to study treatment. Each adverse event was reported according to the worst grade recorded for each patient.

Adverse events were monitored throughout follow-up and for 30 days after treatment end (90 days for serious adverse events). All immune-mediated events were reported, regardless of cause attributed by the investigator. Cardiac toxicity was monitored using left ventricular ejection fraction assessments done every 12 weeks or as clinically indicated. Survival status for all patients was updated just before the database lock for final analysis. The study database was locked and data were retrieved on Aug 7, 2017.

Outcomes

The primary endpoint of the phase 1b study was the incidence of dose-limiting toxicities to establish the recommended dose of pembrolizumab plus trastuzumab. The primary endpoint of the phase 2 study was the proportion of PD-L1-positive patients who achieved an objective response.

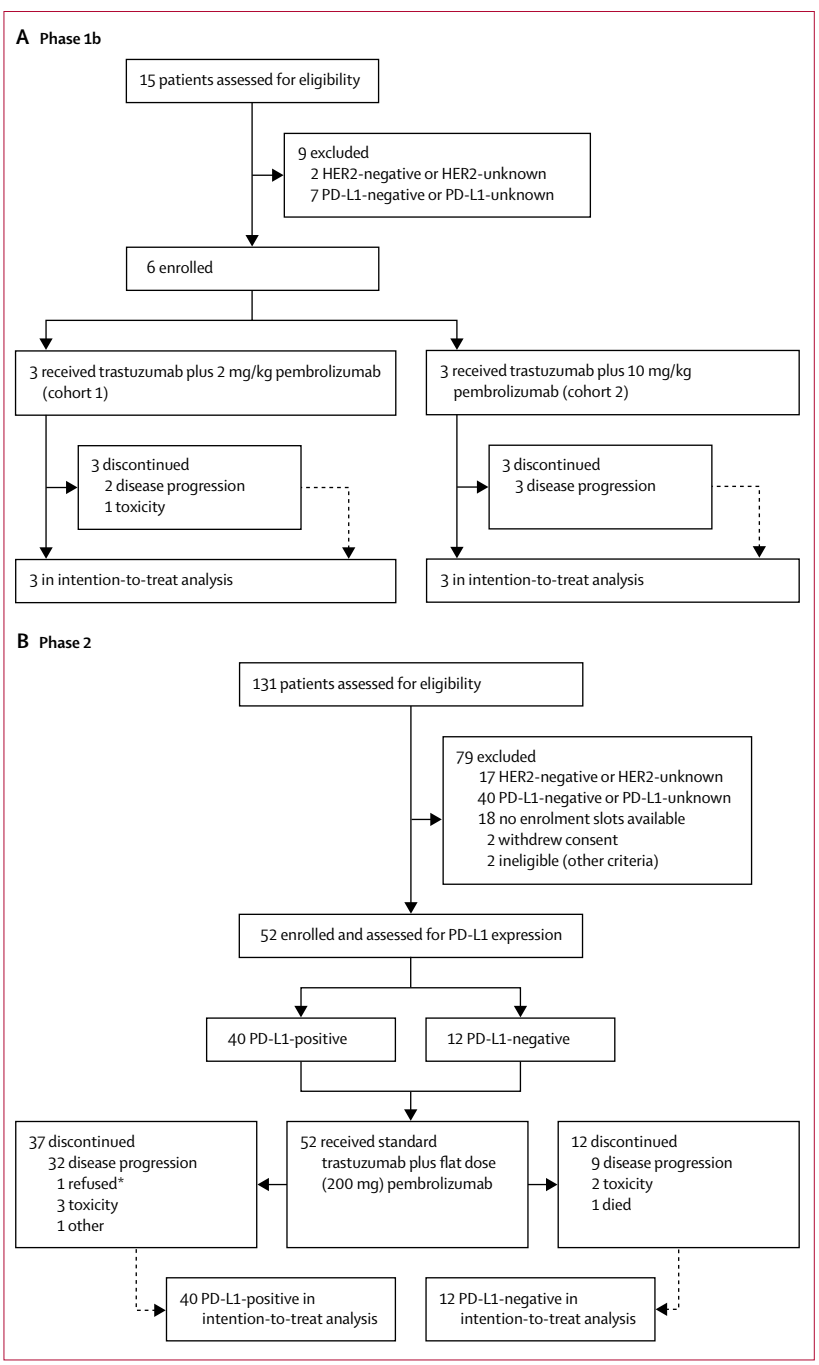

Figure 1: **Trial profile**
PD-L1=programmed cell death 1 ligand 1. *This patient refused further study treatment and assessments.

The secondary endpoints were the proportion of PD-L1-negative patients who achieved an objective response, safety and tolerability, disease control (defined as best overall response of confirmed complete or partial response, or stable disease for 24 weeks or more), duration of response (the time interval between first observation of objective response and progression or death from any cause), time to progression (the time interval between start of study treatment and progression), progression-free survival (time from start of treatment to disease progression or death), and overall survival (time from start of treatment to death).

The main correlative objective was to determine the primary and secondary endpoints according to quantity of tumour-infiltrating lymphocytes, quantified with haematoxylin and eosin-stained slides from a recently obtained biopsy, with the hypothesis that patients with more tumour-infiltrating lymphocytes would derive more benefit from the drug combination. Correlative objectives described were prespecified in the protocol (appendix p 6).

Statistical analysis

The phase 1b stage of the trial was based on a 3+3 dose-escalation design to determine the recommended phase 2 dose of combination therapy, defined as the highest dose level of pembrolizumab with standard dose trastuzumab at which <33% (0 of three patients, or 0 or 1 of six patients) experienced a dose-limiting toxicity in cycle 1. As per protocol, once dose escalation for pembrolizumab reached 10 mg/kg, no further escalation occurred. Dose-limiting toxicity was defined as an adverse event or abnormal laboratory value that was deemed to be treatment-related and unrelated to disease or disease progression, occurred within the first cycle of treatment, and was either a grade 3 or worse non-haematological adverse event lasting at least 1 week, any grade 4 haematological toxicity, or any adverse event resulting in a delay of more than 14 days to start cycle 2.

In the phase 2 stage, a Simon optimal two-stage design was used to estimate the sample size for patients with PD-L1-positive disease: the null hypothesis of a true objective response of 7% was tested against a one-sided alternative objective response of 22%.

As per protocol amendment on Jan 11, 2016, the trial design was changed to enrol a second parallel cohort of patients with PD-L1-negative disease. Eligibility criteria otherwise remained the same. For patients with PD-L1-positive tumours, the optimal two-stage design was based on a total of 40 evaluable patients, and the null hypothesis was rejected if a total of six or more objective responses were observed. This design yields a type I error rate of 0·05 (target type I error of 0·05) and power of 85% (target type II error of 0·15) when the true objective response is 22%. The proportion of patients with PD-L1-positive disease who had an objective response was presented with a two-sided, 90% CI that

was calculated with the method of Atkinson and Brown,[15] which allowed for the two-stage design. For patients with PD-L1-negative tumours, a single-stage design with enrolment of 15 patients was used to compare a null response of 1% with a desirable response of 20%. The decision rule was based on no responses: the drug combination would not be considered worthy of further investigation if no patients had objective response. If the true proportion of patients achieving a response was 20%, the probability that zero responses would be observed in 15 patients was 0·035 (target type II error of 0·05 and type I error for the design is 0·14). The proportion of patients with PD-L1-negative disease with an objective response was presented with a two-sided, 90% exact binomial confidence interval.

We assessed safety in all patients who received at least one dose of pembrolizumab plus trastuzumab, and activity endpoints for the phase 2 intention-to-treat population.

Given that the flat 200 mg pembrolizumab dose was similar to the phase 1b doses, we decided to also combine all PD-L1-positive patients for the activity analyses. This analysis was not prespecified. The aggregated proportion of patients with objective response and PD-L1-positive tumours (in both phase 1b and phase 2) is summarised with a two-sided, 90% exact binomial CI.

We summarised the distributions of time-to-event outcomes using the Kaplan-Meier method. Point estimates of 6-month and 12-month outcomes, and medians, are accompanied with 90% CIs, which we estimated using log-log transformation; comparisons were based on log-rank tests. Assessment of discordance between the QualTek and Q² Solutions PD-L1 assay results was based on McNemar's test. We summarised percentage change in tumour measurements using a waterfall plot. We based comparisons of baseline tumour-infiltrating lymphocytes according to PD-L1 status, site of biopsy, or response classification on Wilcoxon rank-sum or Kruskal-Wallis tests.

We did a post-hoc exploratory analysis of tumour-infiltrating lymphocyte quantity by site of biopsy.

We did statistical analyses using SAS (version 9.4). There were no corrections for multiple comparisons.

This trial is registered with ClinicalTrials.gov, number NCT02129556, and with the European Medicines Agency's European Clinical Trials database, number 2013-004770-10.

Role of the funding source

IBCSG and Merck funded the study. Merck contributed to the study design, data interpretation, and manuscript draft review, and approved the final version of the manuscript, but was not involved in data interpretation or writing of the report. IBCSG was responsible for the conduct of the trial, data management, data collection, and data analysis. The principal investigators (SL and FA) were involved in all aspects of study design, data analyses and interpretation, wrote the first draft of the manuscript and approved the final version of the manuscript in conjunction with IBCSG and the academic steering committee. AG-H and MMR had full access to the raw data. The corresponding author had full access to all data in the study and had the final responsibility to submit for publication.

	Phase 1b PD-L1-positive group (n=6)	Phase 2 PD-L1-positive group (n=40)	Phase 2 PD-L1-negative group (n=12)
Median age (range), years	49 (38–57)	49 (28–72)	57 (43–61)
Eastern Cooperative Oncology Group performance status			
0	2 (33%)	25 (63%)	5 (42%)
1	4 (66%)	15 (38%)	7 (58%)
Menopausal status			
Postmenopausal	4 (66%)	24 (60%)	11 (92%)
Premenopausal	2 (33%)	13 (33%)	1 (8%)
Oestrogen receptor status			
Negative (<1% expression)	4 (66%)	23 (58%)	6 (50%)
Positive (≥1% expression)	2 (33%)	17 (43%)	6 (50%)
Previous trastuzumab-containing therapy	6 (100%)	40 (100%)	12 (100%)
Additional anti-HER2 therapy			
No	1 (17%)	6 (15%)	0
Yes (at least one)	5 (83%)	34 (85%)	12 (100%)
Trastuzumab emtansine	4 (67%)	29 (73%)	9 (75%)
Pertuzumab	3 (50%)	10 (25%)	4 (33%)
Other (eg, lapatinib, afatinib, neratinib)	1 (17%)	17 (43%)	8 (67%)
Previous chemotherapy (anthracycline or taxane)	6 (100%)	40 (100%)	12 (100%)
Biopsy site of metastatic lesion			
Lymph node	3 (50%)	6 (15%)	2 (17%)
Breast	1 (17%)	5 (13%)	1 (8%)
Skin	1 (17%)	8 (20%)	3 (25%)
Liver	1 (17%)	9 (23%)	1 (8%)
Lung	0	11 (28%)	3 (25%)
Other (sternum, supra-clavicular fossa, vulva)	0	1 (3%)	2 (17%)
Median time from diagnosis of metastatic breast cancer to enrolment (IQR), months	15·7 (13·7–28·0)	40·0 (22·0–51·9)	71·5 (28·3–109·7)

Data are n (%), unless otherwise specified. PD-L1=programmed cell death 1 ligand 1.

Table 1: **Baseline characteristics**

Results

Between Feb 2, 2015, and April 5, 2017, 146 patients were screened and 58 were enrolled across the two phases of the trial (figure 1). In the phase 1b stage, all patients were PD-L1-positive. In the phase 2 stage, 40 patients had PD-L1-positive tumours and 12 patients had PD-L1 negative tumours. Recruitment to the PD-L1-negative cohort was stopped before 15 patients were enrolled because of slow accrual of eligible patients. Three patients in the phase 2, PD-L1-positive cohort were deemed ineligible after medical eligibility review, but were treated and followed-up per protocol and included in the analysis. The first patient's pregnancy test was not done

	Phase 1b, 2 mg/kg (n=3)			Phase 1b, 10 mg/kg (n=3)			Phase 2, PD-L1-positive (n=40)			Phase 2, PD-L1-negative (n=12)		
	Grade 1–2	Grade 3	Grade 4	Grade 1–2	Grade 3	Grade 4	Grade 1–2	Grade 3	Grade 4	Grade 1–2	Grade 3	Grade 4
Anaemia	2 (67%)	0	0	0	2 (67%)	0	5 (13%)	1 (3%)	0	1 (8%)	1 (8%)	0
Pericardial effusion	0	0	0	0	0	0	0	1 (3%)	0	0	0	0
Taketsubo cardiomyopathy	0	0	1 (33%)	0	0	0	0	0	0	0	0	0
Vertigo	0	0	0	0	1 (33%)	0	0	0	0	0	0	0
Retinal vascular disorder	0	0	0	0	0	0	0	1 (3%)	0	0	0	0
Visual field defect	0	0	0	0	0	0	0	1 (3%)	0	0	0	0
Diarrhoea	2 (67%)	0	0	0	0	0	7 (18%)	0	0	2 (17%)	0	0
Gastroenteritis	0	1 (33%)	0	0	0	0	0	0	0	0	0	0
Nausea	2 (67%)	0	0	0	0	0	9 (23%)	0	0	2 (17%)	0	0
Vomiting	1 (33%)	1 (33%)	0	0	0	0	2 (5%)	0	0	1 (8%)	0	0
Death (disease progression)	0	0	0	0	0	0	0	0	0	0	0	0
Fatigue	2 (67%)	0	0	1 (33%)	0	0	11 (28%)	0	0	5 (42%)	0	0
Pain	0	0	0	0	0	0	7 (18%)	0	0	1 (8%)	0	0
Bile duct dilatation	0	0	0	0	0	0	0	0	1 (3%)	0	0	0
Autoimmune disorder	0	0	0	0	0	0	0	0	0	0	1 (8%)	0
Catheter-related infection	0	0	0	0	0	0	0	1 (3%)	0	0	0	0
Sepsis	0	0	0	0	0	0	0	0	1 (3%)	0	0	0
Upper respiratory infection	1 (33%)	0	0	1 (33%)	0	0	1 (3%)	1 (3%)	0	0	0	0
Alanine aminotransferase increased	1 (33%)	0	0	0	0	0	3 (8%)	1 (3%)	0	0	0	0
Alkaline phosphatase increased	0	0	0	0	0	0	1 (3%)	1 (3%)	0	0	1 (8%)	0
Aspartate aminotransferase increased	0	0	0	0	0	0	2 (5%)	1 (3%)	0	0	0	0
Blood bilirubin increased	0	0	0	0	0	0	0	1 (3%)	0	0	0	0
Drug-induced liver injury	0	0	0	0	0	0	0	0	1 (3%)	0	0	0
Gamma glutamyl transferase increased	0	0	0	0	0	0	2 (5%)	1 (3%)	1 (3%)	0	0	1 (8%)
Lipase increased	0	0	0	0	0	0	2 (5%)	1 (3%)	0	0	0	0
Lymphocyte count decreased	0	1 (33%)	0	0	0	0	1 (3%)	0	0	0	0	0
Hyperuricaemia	0	0	0	0	0	0	0	0	0	0	1 (8%)	0
Arthralgia	1 (33%)	0	0	1 (33%)	0	0	5 (13%)	0	0	1 (8%)	0	0
Myalgia	1 (33%)		0	1 (33%)		0	3 (8%)			1 (8%)		
Aphasia	0	0	0	0	0	0	0	1 (3%)	0	0	0	0
Cognitive disturbance	0	0	0	0	0	0	0	1 (3%)	0	0	0	0
CNS metastasis	0	1 (33%)	0	0	0	0	0	0	0	0	0	0
Headache	1 (33%)	0	0	1 (33%)	0	0	7 (18%)	0	0	1 (8%)	0	0
Paraesthesia	0	0	0	0	0	0	0	0	0	0	1 (8%)	0
Vasovagal reaction	0	0	0	0	0	0	0	1 (3%)	0	0	0	0
Anxiety	0	0	0	1 (33%)	0	0	0	1 (3%)	0	1 (8%)	0	0
Acute renal impairment	0	0	0	0	0	0	0	1 (3%)	0	0	0	0
Cough	1 (33%)	0	0	0	0	0	9 (23%)	0	0	0	0	0
Dyspnoea	0	1 (33%)	0	1 (33%)	1 (33%)	0	8 (20%)	0	0	0	2 (17%)	1 (8%)
Pleural effusion	0	0	0	0	0	0	0	2 (5%)	0	0	0	0

(Table 2 continues on next page)

	Phase 1b (2 mg/kg) (n=3)			Phase 1b (10 mg/kg) (n=3)			Phase 2, PD-L1-positive (n=40)			Phase 2, PD-L1-negative (n=12)		
	Grade 1–2	Grade 3	Grade 4	Grade 1–2	Grade 3	Grade 4	Grade 1–2	Grade 3	Grade 4	Grade 1–2	Grade 3	Grade 4
(Continued from previous page)												
Pneumonitis	0	0	0	0	0	0	1 (3%)	1 (3%)	0	1 (8%)	0	1 (8%)
Cutaneous rash	0	0	0	0	0	0	0	1 (3%)	0	0	0	0
Hypertension	0	0	0	0	0	0	2 (5%)	3 (8%)	0	0	1 (8%)	0
Intracranial hypertension	0	0	0	0	0	0	0	1 (3%)	0	0	0	0
Thromboembolic event	0	0	0	0	0	0	0	0	0	0	1 (8%)	0

Data are n (%). Includes grade 1–2 adverse events occurring in at least 10% of patients, and all grade 3–5 events. There were no grade 5 adverse events in phase 1b (2 mg/kg) or phase 2, PD-L1-positive groups. There were two grade 5 events: one in the phase 1b (10 mg/kg) group (death due to disease progression) and one in the phase 2, PD-L1-positive group (Lambert-Eaton syndrome). PD-L1=programmed cell death 1 ligand 1.

Table 2: **Adverse events**

within 72 h of treatment start; however, subsequent pregnancy tests were negative. For the second patient, two laboratory screening tests (international normalised ratio and prothrombin time) were not done. The third patient did not have data recorded for her primary tumour. Baseline characteristics of the study population are summarised in table 1.

In phase 1b, there were no dose-limiting toxicities, cardiovascular toxic effects, or deaths (table 2). Although the aim of phase 1b was to determine a recommended dose for phase 2, a flat dose was chosen as per a protocol amendment on Aug 28, 2015, because of an internal decision by Merck. At the time of data cutoff, median follow-up was 25·7 months (IQR 25·6–25·8) and five (83%) of the six patients had discontinued treatment. One objective response (complete response) was confirmed (one [17%] of six patients). This patient, who received 2 mg/kg, continued without progression, but ceased pembrolizumab therapy after 35 cycles because of a grade 3 immune-related adverse event (gastritis). One patient, who received 10 mg/kg, continued taking study therapy for nearly 18 months after a complete response in her target lesions, and after isolated CNS progression, was treated with surgery and whole-brain radiotherapy after cycle 4.

Median follow-up for PD-L1-positive patients in phase 2 was 13·6 months (IQR 11·6–18·4). Six (15%; 90% CI 7–29) of 40 PD-L1-positive patients had a centrally confirmed objective response (table 3; figure 2). Two patients were not evaluable for response. One patient discontinued treatment before tumour evaluation and in the second, the tumour could not be measured upon scan. One further patient had an unconfirmed partial response. Disease control was achieved in ten (25%) of 40 patients (90% CI 14–39). Median progression-free survival was 2·7 months (90% CI 2·6–4·0) with estimated 6-month progression-free survival of 25% (15–37) and 12-month progression-free survival of 12% (5–22; figure 3A). 15 deaths were reported, seven due to disease progression; one due to glioblastoma multiforme as a second, non-breast malignancy; and seven had no cause of death ascertained. Median overall survival was not reached (90% CI 13·1–not reached); 6-month overall survival was estimated to be 87% (75–94) and 12-month survival was estimated to be 65% (50–76; figure 3B).

	PD-L1-negative, phase 2 (n=12)	PD-L1-positive, phase Ib (n=6)	PD-L1-positive, phase 2 (n=40)	All PD-L1-positive patients, phase 1b–2 (n=46)
Objective response	0 (0%; 0–18)	1 (17%; 1–58)	6 (15%; 7–29)	7 (15%; 7–27)
Disease control*	0 (0%; 0–18)	1 (17%; 1–58)	10 (25%; 14–39)	11 (24%; 14–36)
Best overall response				
Complete response	0	1 (17%)	1 (3%)	2 (4%)
Partial response	0	0	5 (13%)	5 (11%)
Stable disease	2 (17%)	0	7 (18%)	7 (15%)
Progressive disease	9 (75%)	5 (83%)	25 (63%)	30 (65%)
Not evaluable	1 (8%)	0	2 (5%)	2 (4%)

Data are n (%; 90% CI), or n (%). Objective responses were confirmed by repeat imaging 4–6 weeks later. PD-L1=programmed cell death 1 ligand 1. *Includes patients who achieved an objective response or had stable disease as their best response for 24 weeks or more.

Table 3: **Best response, by PD-L1 status**

For the phase 2 PD-L1-negative cohort, the median follow-up was 12·2 months (IQR 7·9–12·2). There were no objective responses, and no patients achieved disease control (appendix p 2). Median progression-free survival was 2·5 months (90% CI 1·4–2·7), with an estimated 6-month progression-free survival of 13% (90% CI 2–35) and 12-month progression-free survival of 0% (figure 3A). Nine deaths were recorded; five were due to disease progression, one was due to Lambert-Eaton syndrome, and three had no cause of death ascertained. Median overall survival was 7·0 months (90% CI 4·9–9·8); the estimated 6-month overall survival was 64% (36–82) and the estimated 12-month overall survival was 12% (1–36; figure 3B).

For the post-hoc combined phase 1b and phase 2 PD-L1-positive cohort (n=46), there were seven objective responses (15%) and four (8%) additional patients with stable disease. Median time to response was 2·7 months (90% CI 2·6–4·0), corresponding with the first restaging

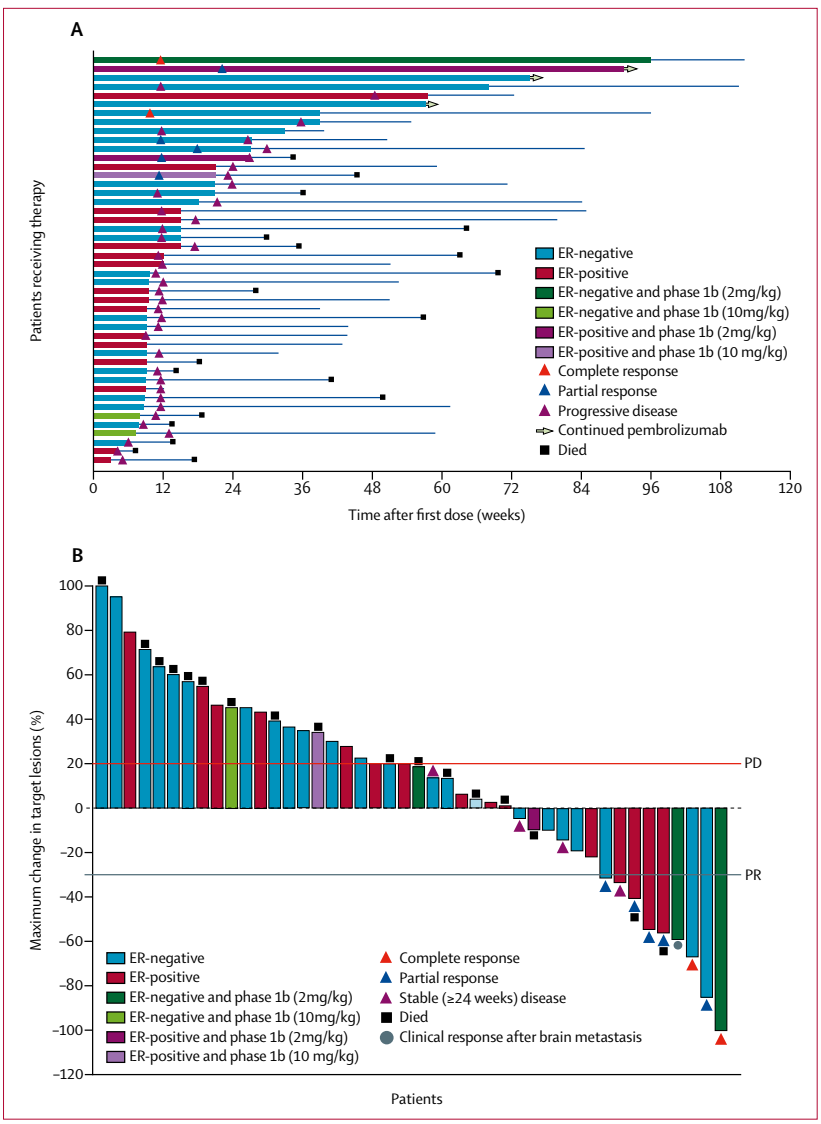

Figure 2: **Tumour response (A) and reduction in target lesions (B) in patients with PD-L1-positive tumours**
(A) Swimmer's plot showing patterns of response in patients with PD-L1-positive tumours. Solid bars indicate treatment duration according to oestrogen receptor status. Lines show duration of follow-up after treatment cessation. (B) Waterfall plot of all patients with PD-L1-positive tumours showing confirmed maximum reduction in target lesions, according to investigator assessment. The line at –30% indicates partial response threshold according to Response Evaluation Criteria in Solid Tumours version 1.1. Bars show maximum reduction from baseline sum of longest dimension by best confirmed response. ER=estrogen receptor. PD-1=programmed cell death protein 1. PD-L1=programmed cell death 1 ligand 1

scan. Median duration of response from time of first observation was 3·5 months (2·7–not reached) and median duration of disease control was 11·1 months (6·2–not reached) among 11 patients.

15 (34%) of the 44 patients with PD-L1-positive tumours and with at least one post-baseline scan had reductions in their target lesions size from baseline (figure 2B). 44 of 46 patients were evaluable, since two patients did not have a post-baseline reassessment of their target lesions. Of the patients with greater than 30% reduction: one patient (circle) had a brain metastasis detected at first restaging but had a response in all target lesions and remained in the study; one patient had greater than 30% reduction in target lesions but was not confirmed and hence this patient is indicated as stable disease; one patient indicated as complete response with less than 100% reduction in tumour was a nodal response, with lymph nodes smaller than pathological in size (<10 mm short axis). In total, there were 39 progression events and 19 deaths among the 46 patients with PD-L1-positive tumours. Ten deaths were due to disease progression, one due to glioblastoma multiforme as a second, non-breast malignancy, and cause was not ascertained in the remaining eight.

In a post-hoc analysis of the combined PD-L1-positive cohort, median progression-free survival was 2·7 months (90% CI 2·6–4·0). 6-month progression-free survival was 24% (14–35) and 12 month progression-free survival was 13% (6–22).

56 (97%) of 58 patients had one or more new or worsening adverse events during the trial (table 2). 41 (71%) had a treatment-related adverse event, of whom 17 (29%) had a treatment-related adverse event of grade 3 or worse. The most common treatment-related adverse events of any grade were fatigue (12 [21%] of 58), diarrhoea (eight [14%]), and arthralgia (eight [14%]). Grade 3–5 adverse events occurred in 29 (50%) of patients. The most commonly occurring serious adverse events were dyspnoea (n=3 [5%]), pneumonitis (n=3 [5%]), pericardial effusion (n=2 [3%]), and upper respiratory infection (n=2 [3%]). There were 13 treatment-related, severe adverse events in 11 (19%) patients. One patient had two episodes of Takotsubo cardiomyopathy and one patient had two occurrences of cutaneous toxicity. The remaining nine patients reported pneumonitis (two [3%]), a drug-induced liver injury (one [2%]), dyspnoea (one [2%]), autoimmune disorder (one [2%]), upper respiratory infection (one [2%]), cognitive disturbance (one [2%]), vertigo (one [2%]), and diarrhoea (one [2%]). 11 (19%) of 58 patients had any-grade immune-mediated adverse events; those occurring in two or more patients were thyroid dysfunction (four cases: two hypothyroid and two hyperthyroid [7%]), pneumonitis (n=2 [3%]), and autoimmune hepatitis (n=2 [3%]). Five (8%) patients had treatment-related adverse events that led to treatment discontinuation. These were gastritis (n=1 [2%]), autoimmune hepatitis (n=2 [3%]), and pneumonitis (n=1 [2%]), with one patient's death deemed to be treatment-related (Lambert-Eaton syndrome; appendix p 4).

For our correlative analyses, we examined the association between baseline proportion of tumour-infiltrating lymphocytes in the metastatic lesion, PD-L1 status, site of biopsy, and anti-tumour activity (figure 4). It was possible to measure the proportion of pre-treatment tumour-infiltrating lymphocytes in 41 (89%) of 46 patients in the PD-L1-positive cohort and in nine (75%) of 12 patients in the PD-L1-negative cohort.[16,17] Of note,

38 (83%) patients in the PD-L1-positive cohort had a fresh biopsy taken for the study, and all of the patients had PD-L1 testing on a metastatic lesion biopsy that was less than 1 year old. Unlike primary HER2-positive breast cancers, we observed low levels of tumour-infiltrating lymphocytes (median 1·5%, IQR 0–5). There were significantly higher levels of tumour-infiltrating lymphocyte in the PD-L1-positive cohort than in the PD-L1-negative cohort (figure 4A). In a post-hoc analysis, there were significant differences in the amount of tumour-infiltrating lymphocyte infiltrate by site of biopsy, with higher quantities in lung and breast lesions than in liver and skin biopsies (figure 4B). Finally, there was significantly greater lymphocytic infiltration in objective responders and in patients with disease control, (figure 4C, 4D).

Using the Q² Solutions assay, we re-assessed 61 samples that were initially assessed with the QualTek assay. There was no evidence of significant discordance according to the immunohistochemistry assay used (McNemar's p=0·47): seven (24%) of 29 samples that were positive according to the QualTek assay tested negative with the Q² Solutions assay. Notably, in the discordant group there were no objective responders.

Discussion

Pembrolizumab plus trastuzumab was safe and showed preliminary anti-tumour activity in patients with locally advanced, unresectable, or metastatic HER2-positive breast cancer and previous resistance to trastuzumab-based therapies, particularly in patients who were PD-L1 positive. Seven (15%) of 46 PD-L1-positive patients achieved an objective response, and a further four (8%) patients achieved stable disease for more than 6 months as their best response, with 11 (24%) of PD-L1-positive patients achieving disease control. The mean duration of disease control was 11·1 months (90% CI 6·2–not reached), suggesting that, in selected patients, disease control without chemotherapy is feasible. By contrast, there were no objective responders in the PD-L1-negative group. In addition to PD-L1 status, tumour-infiltrating lymphocyte levels in the metastatic lesion might also identify patients who can benefit from this combination therapy. Notably, overall survival seemed to improve in the PD-L1-positive compared with the PD-L1 negative cohort, despite extensive previous treatment and documented progression with previous trastuzumab-containing therapies. Therefore, the preliminary subgroup analyses of this study suggest that immune mechanisms might be important in trastuzumab resistance and selection for patients who are PD-L1-positive, with high tumour-infiltrating lymphocyte levels in future studies of metastatic HER2-positive disease testing HER-2 blockade combined with anti-PD-1 or anti-PD-L1 drugs seems warranted.

Pembrolizumab plus trastuzumab was well tolerated in this population, with the expected frequency of

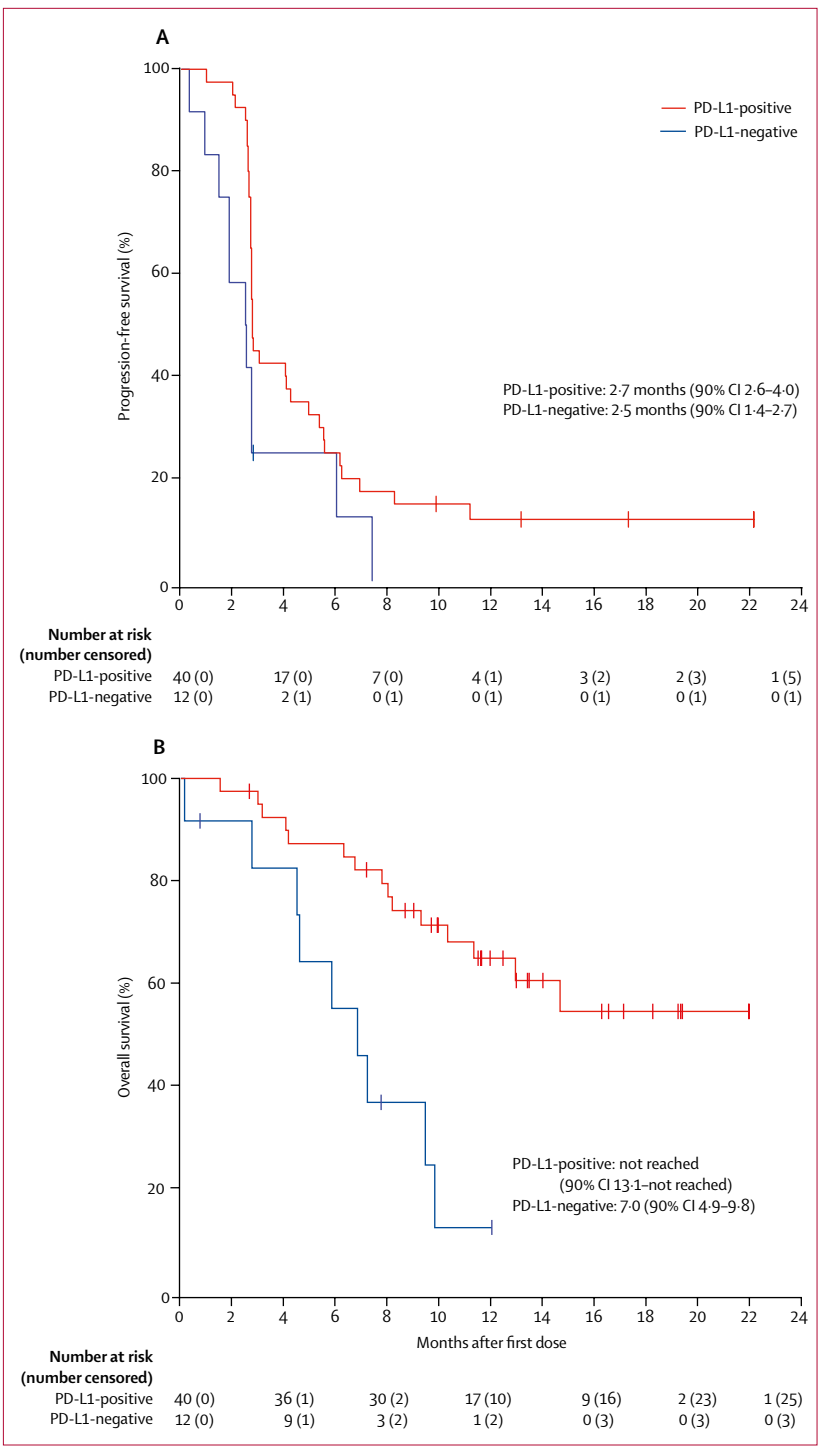

Figure 3: **Kaplan-Meier estimates of progression-free survival (A) and overall survival (B) in patients enrolled to phase 2**
PD-L1=programmed cell death 1 ligand 1.

treatment-related grade 3 or higher and immune-related adverse events.[18,19] Given that most of the 58 patients in this study had many previous lines of chemotherapy and anti-HER2 therapy, the acceptable tolerability profile

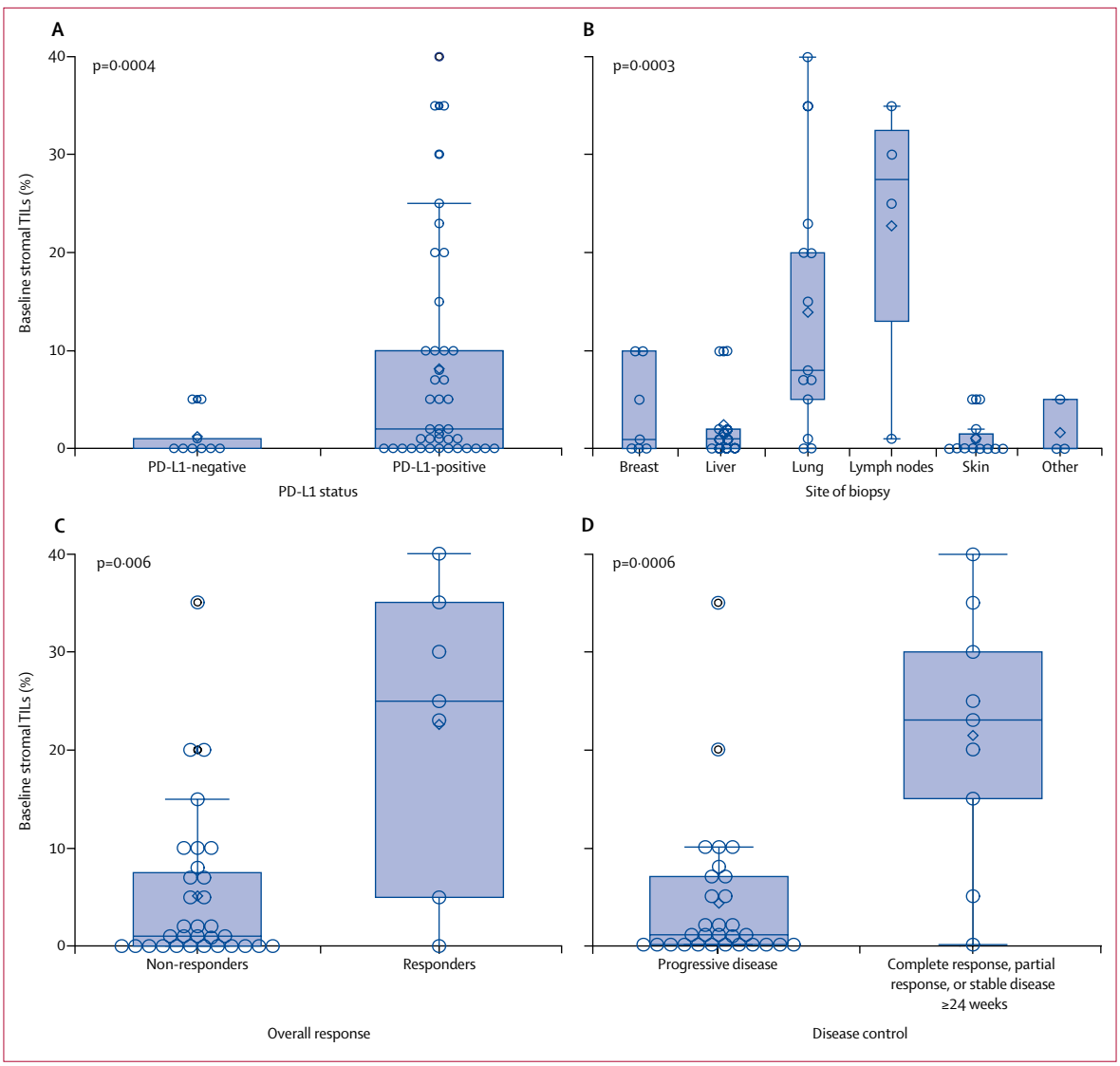

Figure 4: **Exploratory analysis of tumour-infiltrating lymphocytes**
Association between baseline tumour-infiltrating lymphocyte level and PD-L1 status (A), biopsy site (B), and anti-tumour activity (C, D). Diamonds indicate group mean. Large blue circles represent individual datapoints; small black circles indicate values that are more than 1·5 times the IQR. PD-L1=programmed cell death 1 ligand 1. TILs=tumour-infiltrating lymphocytes.

and long duration of some of the responses support further development of pembrolizumab (and other anti-PD-1 or anti-PD-L1 therapies) in this breast cancer subtype.

The data in many solid tumour types are mixed for the clinical use of PD-L1 expression as a treatment stratification marker in the setting of anti-PD-1 or PD-L1 monotherapy. PD-L1 protein expression seems to be highly correlated with the presence of an immune infiltrate in breast cancer.[9] Hence, it is probably not surprising in this study that response was associated both with levels of pre-existing immune infiltrate, as well as PD-L1 status. Our study is unique in this sense as it provides a contemporary assessment of PD-L1 status from a metastatic lesion, and these data support diminishing effective anti-tumour immunity in the advanced disease setting. Previous responses to anti-PD-1 or PD-L1 monotherapy in triple-negative breast cancer and HER2-positive breast cancer in the advanced setting are also modest, particularly in patients who have had previous treatment, and without selection according to PD-L1 status.[10,12] Tumour-infiltrating lymphocyte levels might also be lower in the more advanced and pre-treated setting, but can also enrich for responders in the metastatic, triple-negative breast cancer setting.[20] This finding suggests that baseline tumour-infiltrating lymphocyte levels, as simply quantified on a diagnostic slide from a fresh biopsy, could have clinical use in

further enriching the breast cancer population most likely to respond to treatment.[20] Our data suggest potentially important differences in the metastatic microenvironment, suggesting that baseline tumour-infiltrating lymphocyte levels from a metastatic lesion can identify patients with a higher chance of responding to pembrolizumab and trastuzumab. Our data suggest that a subset of patients who are positive for PD-L1 and tumour-infiltrating lymphocytes will be the group most likely to benefit in future studies of checkpoint inhibition and trastuzumab or trastuzumab-based therapies.

The crucial question now relates to how the number of responders to immunotherapy in the advanced setting can be improved. Large tumour burden and line of treatment currently seem to be associated with ineffective host anti-tumour immunity. The inclusion of a potent anti-tumour therapy targeting the HER2 pathway with further chemotherapy will probably be needed in this context. Addition of other immune targeting drugs (eg, innate immunity and T-cell agonists), additional HER2-targeting drugs, and combinations with chemotherapy also warrant consideration.[21]

There are limitations to this study. The absence of a control group restricts comparisons, particularly of quality of life against standard chemotherapy options, as well as on overall survival outcomes. The small size of this study means that a larger randomised study will be required to definitively define the role of such drugs in this setting. The patients enrolled were heavily pre-treated, which, in retrospect, is probably not the best setting in which to investigate immunotherapy drugs. Baseline characteristics differed depending on PD-L1 status, which probably affected survival outcomes, but could also reflect the more aggressive biology of PD-L1-negative disease. Halfway through recruitment, there was a a change in PD-L1 assay, but this did not appear to influence the reported activity results. Since we enrolled patients in the era of effective anti-HER2 therapy (eg, previous T-DM1, pertuzumab), this makes time-to-event comparisons with historical controls difficult. Correlative investigations are ongoing to further characterise responders, such as by tumour mutation burden, T-cell repertoire, or RNA sequencing. Regardless of these limitations, our findings set the stage for further studies and show that the combination of trastuzumab plus pembrolizumab is active and has acceptable tolerability as late-line treatment in patients with advanced, HER2-positive breast cancer, particularly for those who have PD-L1-positive tumours with tumour-infiltrating lymphocytes present in a recent tumour biopsy.

Contributors

SL, AG-H, TB, GC, HB, MR-P, RM, MJS, MCo, GV, MMR, and FA are responsible for substantially contributing to the conception and design, or analysis and interpretation of data. SL, AG-H, AG, TB, RH, GC, MCa, LB, HB, GJ, RB, MR-P, RK, MJS, ADL, MCo, GV, MMR, and FA are responsible for drafting the Article or revising it critically for important intellectual content. SL, AG-H, TB, RH, GC, MCa, LB, HB, GJ, RB, RM, MJS, ADL, MCo, GV, MMR, and FA agree to be accountable for all aspects of the work. All authors are responsible for final approval of the version to be published.

Declaration of interests

SL receives research funding to her institution, outside the submitted work, from Novartis, Bristol-Myers Squibb, Roche-Genentech, Puma Biotechnology and Pfizer; and has acted as consultant (uncompensated), outside the submitted work, to Seattle Genetics, Pfizer, Novartis, Bristol-Myers Squibb, Merck Sharp & Dohme, and Roche-Genentech. TB receives grants, personal fees, and non-financial support, outside the submitted work, from Roche, Novartis, AstraZeneca, and Pfizer. RH receives advisory board member and speaker honorarium, outside the submitted work, from Merck Sharp & Dohme, AstraZeneca, Novartis, Bristol-Myers Squibb, and Roche. MCa received grants and personal fees, during the conduct of the study, from Novartis; and personal fees, outside the submitted work, from Pfizer, AstraZeneca, and Lilly. LB receives grants, outside the submitted work, from Celgene; personal fees, outside the submitted work, from AstraZeneca, Celgene, Eisai, Ipsen, Pfizer, Pierre Fabre, Novartis, Roche, and Puma Biotechnology; and non-financial support, outside the submitted work, from Ipsen. HB receives personal fees, outside the submitted work, from Abbvie, Astellas, Bayer, and Pfizer; and travel grants, outside the submitted work, from Pfizer and Roche. GJ receives grants, outside the submitted work, from Novartis, Roche, Amgen, Bristol-Myers Squibb, and Merck Sharp & Dohme; personal fees, outside the submitted work, from Novartis, Roche, Lilly, Celgene, Amgen, Bristol-Myers Squibb, Pfizer, Puma, Daiichi-Sankyo, and AstraZeneca; and non-financial support, outside the submitted work, from Novartis, Roche, Lilly, Bristol-Myers Squibb, and AstraZeneca. RB receives grants, outside the submitted work, from Roche and Novartis; personal fees from Roche, Novartis, Merck Sharp & Dohme, Pfizer, Pierre Fabre, and Eli-Lilly; and other, outside the submitted work, from Roche and Pfizer. MJS receives research support from Bristol-Myers Squibb, Tizona Therapeutics and Aduro Biotech. ADL receives grants, outside the submitted work, from AstraZeneca, Pierre Fabre, and Pfizer; and personal fees, outside the submitted work, from AstraZeneca, Bayer, Celgene, Daichii-Sankyo, Eisai, Genomic Health, Ipsen, Lilly, Novartis, Pierre Fabre, Pfizer, and Roche. MCo receives advisory board fees, outside the submitted work, from AstraZeneca, Pierre Fabre, Pfizer, OBI Pharma, Puma Biotechnology, and Celldex; and honoraria, outside the submitted work, from Novartis. GV receives advisory board fees, outside the submitted work, from Merck Sharpe & Dohme, Roche/Genetech, AstraZeneca, and Bristol-Myers Squibb. MMR received grants (to the International Breast Cancer Study Group) and personal fees, during the conduct of the study, from Merck Sharpe & Dohme; and personal fees and non-financial support, outside the submitted work, from Bristol-Myers Squibb. FA receives grants, outside the submitted work, from AstraZeneca, Pfizer, Lilly, Roche, and Novartis. All other authors declare no competing interests.

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