Second-line therapy with nivolumab plus ipilimumab for older patients with oesophageal squamous cell cancer (RAMONA): a multicentre, open-label phase 2 trial

Matthias P Ebert, Nadja M Meinidl-Beinker, Tobias Gutting, Martin Maenz, Johannes Betge, Nadine Schulte, Tianzuo Zhan, Philip Weidner, Elke Burgermeister, Ralf Hofheinz, Arndt Vogel, Stefan Angermeier, Claus Bolling, Maike de Wit, Ralf Jakobs, Meinolf Karthaus, Gertraud Stocker, Peter Thuss-Patience, Tobias Leidig, Timo Gaiser, Jakob N Kather, Nicolai Haertel

Summary

Background The overall survival of patients with advanced and refractory oesophageal squamous cell carcinoma, mostly aged 65 years and older, is poor. Treatment with PD-1 antibodies showed improved progression-free survival and overall survival. We assessed the safety and efficacy of combined nivolumab and ipilimumab therapy in this population.

Methods This multicentre, open-label, phase 2 trial done in 32 sites in Germany included patients aged 65 years and older with oesophageal squamous cell carcinoma and disease progression or recurrence following first-line therapy. Patients were treated with nivolumab (240 mg fixed dose once every 2 weeks, intravenously) and continued with nivolumab and ipilimumab (nivolumab 240 mg fixed dose once every 2 weeks and ipilimumab 1 mg/kg once every 6 weeks, intravenously). The primary endpoint was overall survival, which was compared with a historical cohort receiving standard chemotherapy in the intention-to-treat population. This study is registered with ClinicalTrials.gov, NCT03416244.

Findings Between March 2, 2018, and Aug 20, 2020, we screened 75 patients with advanced oesophageal squamous cell carcinoma. We enrolled 66 patients (50 [76%] men and 16 [24%] women; median age 70·5 years [IQR 67·0–76·0]), 44 (67%) of whom received combined nivolumab and ipilimumab therapy and 22 (33%) received nivolumab alone. Median overall survival time at the prespecified data cutoff was 7·2 months (95% CI 5·7–12·4) and significantly higher than in a historical cohort receiving standard chemotherapy (p=0·0063). The most common treatment-related adverse events were fatigue (12 [29%] of 42), nausea (11 [26%]), and diarrhoea (ten [24%]). Grade 3–5 treatment-related adverse events occurred in 13 (20%) of 66 patients. Treatment-related death occurred in one patient with bronchiolitis obliterans while on nivolumab and ipilimumab treatment.

Interpretation Patients aged at least 65 years, with advanced oesophageal squamous cell carcinoma might benefit from combined nivolumab and ipilimumab therapy in second-line treatment.

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Introduction Oesophageal squamous cell carcinoma is the sixth leading cause of cancer-related deaths worldwide.¹ Most patients with oesophageal squamous cell carcinoma are aged 65 years and older and present with locally advanced stages and severe comorbidities.² In advanced stages, most patients undergo platinum-based chemotherapy; whereas second-line treatment, which was not standardised until 2017, relied mostly on taxanes.³ Immunotherapy, using antibodies directed against immune checkpoints (PD-1, PD-L1, and CTLA-4), has become a promising new treatment option with profound responses in squamous cell cancers (anal and head and neck cancers).⁴ Similarly, promising results were reported in phase 2 and phase 3 trials of patients with oesophageal squamous cell carcinoma.⁵⁶ Three phase 3 trials showed favourable results for PD-1 inhibitors as second-line therapy in patients with oesophageal squamous cell carcinoma. KEYNOTE-181 compared pembrolizumab with the investigator’s chemotherapy choice in patients with both oesophageal squamous cell carcinoma and adenocarcinoma of the oesophagus. In this trial, cancers expressing PD-L1 with a combined positive score of 10 or greater showed an improved overall survival from 6-7 months to 9-3 months (hazard ratio [HR] 0·69 [95% CI 0·52–0·93]). Interestingly, overall survival improved significantly in the overall oesophageal squamous cell carcinoma population, independent of combined positive score (8·2 months vs 7·1 months: HR 0·78 [0·63–0·96]). The activity of PD-1 inhibitors in oesophageal squamous cell carcinoma beyond first-line treatment was also shown in the ATTRACTION-3 trial with nivolumab. In this randomised, multicentre, phase 3 trial, patients with chemorefractory oesophageal
We searched PubMed from inception up to Nov 1, 2021, using the terms “oesophageal squamous cell cancer”, “advanced OR unresectable OR metastatic oesophageal squamous cell cancer”, “second line OR refractory”, and “PD-1 inhibitor OR PD-L1 inhibitor” for articles reporting the results from clinical trials in patients with unresectable oesophageal squamous cell cancer and progression after first-line therapy, who were subsequently treated with PD-1 or PD-L1 inhibitors. Using the same keywords, we also searched the congress websites of the American Society of Clinical Oncology and the European Society of Medical Oncology for abstracts and reports from November, 2019, to November, 2021. We found four prospective clinical trials that enrolled patients with oesophageal squamous cell carcinoma following progressive disease after first-line treatment. A phase 2 study from Asia including heavily pre-treated patients with oesophageal squamous cell carcinoma who received PD-1 inhibitor (nivolumab), reported an objective response in 11 (17%) of 64 patients and stable disease in 16 (25%) patients; median overall survival was 10·8 months (IQR 4·9–14·3). Three phase 3 trials showed favourable results for PD-1 inhibitors as second-line therapy for oesophageal squamous cell carcinoma. KEYNOTE-181 compared pembrolizumab with the investigator’s chemotherapy choice in patients with both oesophageal squamous cell carcinoma and adenocarcinoma of the oesophagus. In this trial patients with cancers expressing PD-L1 tumour proportion score of at least 5% showed an improved overall survival with pembrolizumab. The activity of PD-1 inhibitors in oesophageal squamous cell carcinoma beyond first-line treatment was also confirmed in the ATTRACTION-3 trial with nivolumab. In this randomised, multicentre, phase 3 trial, patients with chemorefractory oesophageal squamous cell carcinoma received either nivolumab or chemotherapy (either docetaxel or paclitaxel). Overall survival in the nivolumab group was significantly higher than in the control group. The RATIONALE 302 trial also reported improved overall survival for patients treated with humanized IgG4 anti-PD-1 tislelizumab versus the investigator’s chemotherapy choice.

To our knowledge, the RAMONA trial is the first prospective, multicentre, phase 2 trial using a dual checkpoint inhibitor combination therapy in patients aged 65 years and older with oesophageal squamous cell carcinoma from Europe. All other trials enrolled primarily Asian patients and younger patients and so far a dual anti PD-1 and CTLA-4 treatment strategy has not been investigated in this cancer. Thus, there has been an urgent medical need to prospectively assess dual PD-1 and CTLA-4 inhibition in a European patient cohort. Moreover, safety and toxicity of checkpoint inhibitors within an older patient population is unknown. Our trial, for the first time, describes the efficacy and shows safety of dual PD-1 and CTLA-4 inhibition in this vulnerable patient population. Overall survival time at the prespecified data cutoff was significantly higher than in a historical control group receiving standard chemotherapy. Furthermore, PD-L1 tumour proportion score of at least 5% was associated with long-term progression-free survival and sustained response.

Our trial shows that nivolumab and ipilimumab is a safe and effective novel treatment option for older patients with oesophageal squamous cell cancer beyond first-line therapy. This dual checkpoint inhibition therapy could be a new second-line option for older patients with oesophageal squamous cell carcinoma, especially within the group of patients with a PD-L1 tumour proportion score of at least 5%.

**Methods**

**Study design and participants**

The RAMONA study, a multicentre, open-label phase 2 trial, has been described in detail previously. Patients were recruited from 32 selected study sites in Germany. Briefly, eligibility criteria included adults aged 65 years and older at recruitment, with histologically confirmed oesophageal squamous cell carcinoma, independent of PD-1 or PD-L1 expression status, and progression beyond first-line therapy (including chemoradiation or systemic chemotherapy). Refractory disease was defined as progressive or recurrent disease as confirmed by CT or MRI scan. Baseline laboratory tests included assessment of white blood cell, neutrophil, and platelet counts; haemoglobin; alanine aminotransferase; aspartate aminotransferase; and bilirubin and serum creatinine and creatinine clearance. Patients’ functionality and geriatric

Research in context

**Evidence before this study**

squamous cell carcinoma received either nivolumab or chemotherapy (either docetaxel or paclitaxel). Overall survival in the nivolumab group was significantly higher than in the control group (10-9 months vs 8-4 months; HR 0-77 [0·62–0·96]). Lastly, the RATIONALE 302 trial reported improved overall survival for patients treated with humanised IgG4 anti-PD-1 tislelizumab versus the investigator’s chemotherapy choice (8-6 months vs 6-3 months; HR 0-70 [95% 0·57–0·85]). Together, these randomised controlled trials confirmed PD-1 inhibition activity as a second-line therapy for oesophageal squamous cell carcinoma. However, generalisation of these findings is limited because most patients were Asian, and the role of PD-1 inhibition in older patients is largely unknown. Furthermore, the role and efficacy of combination therapies with anti-PD-1 and anti-CTLA-4 antibodies in oesophageal squamous cell carcinoma is unknown. Hence, we designed a prospective trial in Germany to assess the efficacy and toxicity of nivolumab and ipilimumab in patients with oesophageal squamous cell carcinoma aged 65 years and older, beyond first-line therapy.

**Added value of this study**

To our knowledge, the RAMONA trial is the first prospective, multicentre, phase 2 trial using a dual checkpoint inhibitor combination therapy in patients aged 65 years and older with oesophageal squamous cell carcinoma from Europe. All other trials enrolled primarily Asian patients and younger patients and so far a dual anti PD-1 and CTLA-4 treatment strategy has not been investigated in this cancer. Thus, there has been an urgent medical need to prospectively assess dual PD-1 and CTLA-4 inhibition in a European patient cohort. Moreover, safety and toxicity of checkpoint inhibitors within an older patient population is unknown. Our trial, for the first time, describes the efficacy and shows safety of dual PD-1 and CTLA-4 inhibition in this vulnerable patient population. Overall survival time at the prespecified data cutoff was significantly higher than in a historical control group receiving standard chemotherapy. Furthermore, PD-L1 tumour proportion score of at least 5% was associated with long-term progression-free survival and sustained response.

**Implications of all the available evidence**

Our trial shows that nivolumab and ipilimumab is a safe and effective novel treatment option for older patients with oesophageal squamous cell cancer beyond first-line therapy. This dual checkpoint inhibition therapy could be a new second-line option for older patients with oesophageal squamous cell carcinoma, especially within the group of patients with a PD-L1 tumour proportion score of at least 5%.
status were assessed using the G8 screening tool along with the Deficit Accumulation Frailty Index (DAFI). Patients with a G8 score greater than 14 (non-frail patients) were recruited as fit. If the G8 score was 14 or lower, the DAFI questionnaire was administered. DAFI scores of 0·20–0·34 (less-fit category) allowed the investigator to decide on a patient’s eligibility for the trial whereas a score of 0·35 or greater prevented enrolment in the trial. When the DAFI could not be administered in a timely manner, the investigator could decide whether the patient was sufficiently fit for the trial. Further key exclusion criteria were adenocarcinoma of the oesophagus, pre-treatment with immune checkpoint inhibitors, autoimmune disease, interstitial lung disease, active infection, other clinically significant cancer requiring treatment, or fewer than 5 years of disease-free interval. The trial protocol was approved by the institutional review board of the Mannheim Medical Faculty of Heidelberg University (2017-004F-MA) and is available online. At each site the ethics committee approved the trial. The trial was done in accordance with the Declaration of Helsinki and the International Conference on Harmonization Good Clinical Practice guidelines. Safety and efficacy were monitored by an independent data and safety monitoring committee. All patients provided written informed consent before enrolment.

**Procedures**

During the 4-week screening phase, patient eligibility was assessed, including a geriatric assessment. Drug dosing was based on previous reports by Kudo and colleagues and Hellmann and colleagues. A safety run-in phase was initiated for all patients with nivolumab (240 mg fixed dose once every 2 weeks, intravenously). After three cycles of nivolumab therapy, the functionality and comorbidity of all patients were assessed. Safety assessment after 6 weeks included analysis of cardiac functional reserve by echocardiography, confirmation of no clinical evidence of significant abnormal troponin or ECG, and a positive investigator judgement that the patients will potentially benefit from treatment escalation. Patients then received combination therapy with nivolumab (240 mg fixed dose once every 2 weeks, intravenously) and ipilimumab (1 mg/kg once every 6 weeks, intravenously). With safety concerns, patients could continue nivolumab monotherapy. In case of adverse events, treatment was interrupted or delayed and resumed according to protocol-predefined criteria. Dose reductions were not allowed. Treatment continued until disease progression, unacceptable toxicity, withdrawal of consent, or death. The first radiographic assessment of response by CT or MRI was done after 12 weeks, thereafter further monitoring was done every 8 weeks. Immune therapy could be continued beyond disease progression, based on the confirmed clinical benefit as assessed by the investigator. Laboratory monitoring was done every 2 weeks at patient visit, with a routine assessment of blood counts, assessment of organ function (liver and kidney), including thyroid function every 4 weeks.

**Outcomes**

The primary endpoint was overall survival of all included patients irrespective of the administered treatment in the intention-to-treat population. The key secondary endpoint was time to quality-of-life (QOL) deterioration, defined as a reduction of at least 10 points in the European Organisation for the Research and Treatment of Cancer quality-of-life questionnaire (EORTC QLQ-C30) global health status QOL subscale, compared with baseline score. Additional secondary endpoints were progression-free survival; objective response, defined as achieving a best overall response of partial or complete remission before first progression; disease control, defined as achieving a best overall response of stable disease or better before first progression; long-term progression-free survival, defined as having a progression-free survival time greater than or equal to the third quartile in all dosed patients (5·95 months), when patients with progression-free survival censored before this threshold were assigned a missing value; duration of response; and duration of treatment. Patient monitoring throughout the trial was as described previously. In brief, QOL and geriatric assessments were done after three cycles of nivolumab and after treatment discontinuation. Response to immunotherapy was monitored every 8 weeks, and tumour response was assessed according to Response Evaluation Criteria in Solid Tumors (RECIST) (version 1.1) and immune-related response criteria (modified RECIST). Pre-treatment and treatment biosamples were taken for molecular analysis. Prespecified, exploratory subgroup analysis included the assessment of overall survival, progression-free survival, and response in relation to clinical and pathological parameters (sex, age, type of pretreatment, treatment line, treatment with ipilimumab, presence of metastases, geriatric status, Karnofsky score, PD-L1 status according to combined positive score (≥1% and ≥5%) and tumour proportion score (≥1% and ≥5%), neutrophil-to-lymphocyte ratio, lactate dehydrogenase concentration, γ-glutamyltransferase, and alkaline phosphatase). Baseline geriatric assessment was also used as a subgroup discriminator (fit vs less fit; patients without assessment were excluded from analysis) for exploratory efficacy analysis. Translational research included the predictive value of PD-L1 expression, tumour mutational burden, and microbiome analysis (appendix pp 6–9). Adverse events were assessed by the investigators and graded according to the Common Terminology Criteria for Adverse Events (version 4.0).

**Statistical analysis**

Our trial was designed as a therapeutic exploratory study to compare double-checkpoint inhibition as a new
treatment strategy with standard chemotherapy regimens in second-line treatment of oesophageal squamous cell carcinoma. However, at the time of the trial planning, historical reference data for second-line treatments were scarce. The largest study reporting efficacy data in second-line treatment of oesophageal squamous cell carcinoma was published by Jin and colleagues from China. This study enrolled 46 patients (median age 55 years [range 28–73]) and reported a median overall survival of 5·9 months (95% CI 3·9–7·8). For the prespecified primary efficacy analysis we hypothesised that an immunotherapy approach consisting of nivolumab monotherapy along with a safety-guided treatment escalation to a combined regimen of nivolumab and ipilimumab would lead to increased overall survival time compared with this historical control with standard chemotherapy with a median overall survival time of 5·9 months. Following the protocol-specified sample size calculation, this analysis and the associated median overall survival time estimate and 95% CI are based on overall survival follow-up until the time of the 45th death (Aug 27, 2020). The hypothesis was tested via a one-sided (α=0·05), one-sample, log-rank test of overall survival time against an exponential survival curve with median survival time 5·9 months (equivalent to a 12-month overall survival rate of 24·4%). This test included all patients who received any study treatment (nivolumab or ipilimumab); all analysis populations as defined in the protocol (section 11.2). The original sample size calculation (protocol section 11.1) was based on 12-month survival rates of 17% in the control group and 30% in the treatment group. However, in the primary hypothesis test, the null hypothesis survival time distribution was defined using the median survival time reported for the historical control group in Jin and colleagues, which better reflected that group’s survival distribution. The resulting 12-month overall survival rate of 24·4% implies a more stringent hypothesis test than was originally planned for in the sample size calculation. The protocol was amended twice because of difficulties with participant accrual. The first amendment increased the duration of accrual and modified the eligibility criteria to permit enrolment of patients who were either ineligible for conventional chemotherapy-based first-line therapy or rejected chemotherapy. Following the approval of the second amendment the trial was prematurely terminated when the required number of events based on the prespecified sample calculation had been reached. Because the required number of events for the initial primary hypothesis test could not be reached, the stringency of hypothesis testing was reduced by decreasing the power of the test from 90% to 80%, which required fewer events. Neither the null hypothesis nor the alternative hypothesis of the prespecified test were changed. All substantial amendments were reviewed and approved by the competent authority and institutional review boards. All other reported results are based on all data available in the database as of June 1, 2021. Kaplan-Meier analysis was used to estimate the duration of overall survival and progression-free survival, including duration of response. We ran exploratory Cox proportional hazard and logistic regression models to assess the effects of clinically relevant characteristics on overall survival, progression-free survival, and tumour-response endpoints. We calculated HRs and odds ratios (ORs) with two-sided 95% CIs on the basis of these models. For descriptive summaries of binary endpoints, we used the Clopper-Pearson method to calculate 95% exact two-sided CIs. We used SAS (version 9.4) for all statistical analyses. For prediction of PD-L1 status, we used the DeepMed software, which was developed in-house (appendix p 8). This study is registered with ClinicalTrials.gov, NCT03416244.

Role of the funding source
The funding source had no role in study design, data collection, data analysis, data interpretation, or writing of the report.

Results
Between March 2, 2018, and Aug 20, 2020, we screened 75 patients with advanced oesophageal squamous cell

Figure 1: Trial profile

75 patients assessed for eligibility
- 9 excluded
  - 7 screening failures
  - 1 consent withdrawn
  - 1 died before treatment
- 66 enrolled
- 9 safety run-in assessment not completed
  - 7 started nivolumab but safety run-in assessment not completed
  - 2 received nivolumab but stopped after safety run-in assessment because of progressive disease
- 57 completed safety run-in assessment
  - 45 allocated to nivolumab and ipilimumab combination
  - 12 allocated to nivolumab only
  - 1 discontinued before ipilimumab treatment
- 44 treated with nivolumab and ipilimumab
  - 22 treated with nivolumab only
  - 1 patient dropped out after safety run-in assessment
- 66 included in intention-to-treat analysis
carcinoma (figure 1). Patients were excluded for screening failures (n=7), withdrawal of consent (n=1), and one patient died before treatment was started. We enrolled 66 patients and all patients received at least one dose of study treatment (median age 70.5 years [IQR 67·0–76·0]; table 1). The treatment population (50 [76%] men and 16 [24%] women) were treated with at least one nivolumab dose (table 1). 22 (33%) of 66 patients received only nivolumab, whereas 44 (67%) received nivolumab and ipilimumab combination therapy (table 1; figure 1; appendix pp 1, 10). Median follow-up was 6·8 months (3·4–15·4).

Baseline geriatric assessment showed 41 (62%) patients to be fit and 25 (38%) patients to be less fit. PD-L1 expression was assessed in 55 (83%) patients (table 1). 20 (36%) patients had a tumour proportion score of at least 1% and 13 (20%) had a tumour proportion score of at least 5%. 37 (56%) patients had a combined positive score of at least 1 and 23 (35%) had a combined positive score of at least 5. Lactate dehydrogenase concentrations above the upper limit of normal occurred in 19 (29%) patients. At baseline, absolute neutrophil count (≥7·500/μl; n=5) and neutrophil to lymphocyte ratio (≥3; n=49) were assessed.

At the time of data cutoff for the primary efficacy analysis (Aug 27, 2020), median overall survival was 7·2 months (95% CI 5·7–12·4) and significantly higher than the median overall survival of 5·9 months in the historical control group (p=0·0063; figure 2). 12-month overall survival rate based on this data cutoff was 39·1% (95% CI 26·4–51·5; figure 2).

Using data until June 1, 2021, median overall survival was 6·9 months (95% CI 5·7–12·4), the 12-month overall survival rate was 38·3% (26·2–50·2), median progression-free survival was 2·7 months (2·5–2·9), and the 12-month progression-free survival rate was 8·7% (3·3–17·5; figure 2). Partial responses were observed in 12 of 66 patients.

For more details about the study, please refer to the full text.
The median duration of response in responders was 5.3 months (range 4.2–12.5), 13 patients showed stable disease (19.7% [10.9–31.3]), and 25 patients had overall disease control (37.9% [26.2–50.7]). Exploratory univariate Cox models of overall survival and progression-free survival with clinically relevant characteristics (appendix pp 11–12) indicated that treatment with ipilimumab was significantly associated with improved progression-free survival (HR 0.55 [95% CI 0.32–0.94]; p=0.030). Tumour proportion score of at
least 5% at baseline (OR 6·35 [95% CI 1·26–31·97]; p=0.025) and study treatment including any ipilimumab dose (OR 4·40 [1·02–18·89]; p=0.046) were highly predictive of disease control in multivariate analyses (table 2). Because several patients had sustained prolonged response, we defined a new group of patients with long-term progression-free survival to characterise this population. Long-term progression-free survival was reached in 15 of 66 patients (22·7% [13·3–34·7]). A baseline tumour proportion score of at least 5% was predictive of long-term progression-free survival (OR 6·27 [1·66–23·62; p=0.0067; table 3).

Of all 66 patients assessed in the safety analysis, treatment-related adverse events occurred in 42 patients (64%; table 4), with the most frequent being fatigue (12 [29%]), nausea (11 [26%]), and diarrhoea (ten [24%], including six [60%] who received ipilimumab; appendix p 13). Adverse events grade of 3 or worse occurred in 54 (82%) of 66 patients, and serious adverse events in 45 (68%) patients (table 4). Grade 3–5 treatment-related adverse events occurred in 13 (20%) patients. Overall, we found no difference in the proportion of grade 3–5 treatment-related adverse events between patients who received nivolumab monotherapy (five [23%] of 22) or combination therapy (eight [18%] of 44). The most frequent grade 3–5 treatment-related adverse events included pneumonitis (n=2, both received ipilimumab), colitis or diarrhoea (n=2), general physical health deterioration (n=2), and increased γ-glutamyltransferase (n=2). Among the treatment-related serious adverse events noted in 12 patients, pneumonitis was the most frequent (n=4, all received ipilimumab). Treatment-related death occurred in one patient with bronchiolitis obliterans while on nivolumab and ipilimumab treatment. Treatment-related adverse events leading to permanent treatment discontinuation occurred in three of 22 patients who received nivolumab monotherapy (14% [95% CI 3–35]) and four of 44 who received combination therapy (9% [3–22; table 4]). Overall, we found no significant difference in the frequency of treatment-related adverse events and treatment-related adverse events leading to treatment discontinuation between fit and less-fit patients (data not shown).

QOL assessed at baseline was stable in most patients after 3-month immunotherapy, EORTC-QLQ30 questionnaire was completed by 64 (99%) of 65 patients at screening, 41 (48%) after 3 months of immunotherapy, and 18 (28%) after 6 months of immunotherapy. Mean global health status was 47·8% (SD 23·9) at baseline, 55·6% (20·5) at 3 months, and 57·4% (21·2) at 6 months. The median time to deterioration in QOL (reduction of >10 points) was 5·3 months (95% CI 3·3–9·5).

In the less-fit group, 19 (29%) of 25 were eligible for the trial according to DAFI score. One patient with a DAFI score 0·35 or greater was ineligible; although five (8%) patients had no DAFI score, the local principal investigator classified them as eligible. These patients were not included in the subsequent correlation analysis of the association between geriatric assessment and efficacy. The average treatment duration was 180·5 days (95% CI 1·0–642·0) for fit (n=41) patients, 136·1 days (1·0–754·0) for less fit patients, and 125·8 days (10·4 months [5·5–14·0] vs 2·6 months [2·4–2·8] vs 2·8 months [1·8–5·4]; and median overall survival (10·4 months [5·5–14·0] vs 6·3 months [3·0–7·9]; HR 1·56 [0·87–2·80]; p=0·14).

### Table 2: Predictors of disease control in treated patients

| Demographics                                      | Univariate models | Multivariate model |
|---------------------------------------------------|-------------------|--------------------|
|                                                   | Odds ratio (95% CI) | p value | Odds ratio (95% CI) | p value |
| Gender                                            |                   |         |                   |         |
| Female                                            | 1·68 (0·51–5·51)  | 0·39    |                   |         |
| Age at screening ≥70 years                        |                   |         |                   |         |
| Stage 1 and 2                                     | 0·72 (0·25–2·05)  | 0·54    |                   |         |
| Stage 3 and 4                                     | 1·04 (0·34–3·19)  | 0·95    |                   |         |
| Health status                                     |                   |         |                   |         |
| Any distant metastases documented at screening    | 0·59 (0·17–2·06)  | 0·41    |                   |         |
| Genitourinary status at baseline worse than fit   | 2·01 (0·67–6·05)  | 0·22    |                   |         |
| Karnofsky score at screening ≤80                  | 0·41 (0·10–1·74)  | 0·23    |                   |         |
| Laboratory parameters                             |                   |         |                   |         |
| Neutrophil to lymphocyte ratio at baseline known to be ≥3 | 0·60 (0·18–1·95)  | 0·39    |                   |         |
| Lactate dehydrogenase at baseline known to be above upper limit of normal range | 1·69 (0·54–5·29)  | 0·37    |                   |         |
| γ-glutamyltransferase at baseline known to be above upper limit of normal range | 1·58 (0·55–4·55)  | 0·39    |                   |         |
| Alkaline phosphatase at baseline known to be above upper limit of normal range | 1·35 (0·34–5·30)  | 0·67    |                   |         |
| PD-L1                                             |                   |         |                   |         |
| Combined positive score at baseline known to be ≥x1 | 1·12 (0·39–3·21)  | 0·83    |                   |         |
| Combined positive score at baseline known to be ≥x5 | 1·47 (0·49–4·38)  | 0·49    |                   |         |
| Tumour proportion score at baseline known to be ≥x1% | 2·81 (0·89–8·88)  | 0·079   |                   |         |
| Tumour proportion score at baseline known to be ≥x5% | 4·55 (1·06–19·50) | 0·041   | 6·35 (1·26–31·97) | 0·025   |
| Previous treatment                                 |                   |         |                   |         |
| Any previous documented chemoradiotherapy          | 1·21 (0·30–4·86)  | 0·79    |                   |         |
| Any previous documented tumour surgery            | 0·90 (0·30–2·73)  | 0·85    |                   |         |
| Current study treatment known to be third-line or later | 0·57 (0·15–2·17)  | 0·41    |                   |         |
| Study treatment                                    |                   |         |                   |         |
| Any ipilimumab administered                       | 3·15 (0·87–11·41) | 0·080   | 4·40 (1·02–18·89) | 0·046   |

An odds ratio greater than 1 corresponds to increased probability of absence of progression, compared with patients in analysis set where the respective condition does not apply. Criterion for inclusion into the multivariate model was p<0.10. Number of observations used to fit all models. The wording “known to be” and “documented” indicate that “yes” responses on the respective predictor were collapsed with missing values into a single reference category against which “yes” responses were compared.
### Table 3: Predictors of achievement of long-term progression-free survival in treated patients

| Demographics | Univariate models | Multivariate model |
|--------------|-------------------|-------------------|
|              | Odds ratio (95% CI) | p value | Odds ratio (95% CI) | p value |
| Female       | 1.12 (0.30–4.18)   | 0.87    | ...                 | ...     |
| Age at screening ≥70 years | 0.71 (0.22–2.27)   | 0.57    | ...                 | ...     |
| Age at screening ≥75 years | 1.13 (0.33–3.89)   | 0.84    | ...                 | ...     |
| Health status |                   |         | ...                 | ...     |
| Any ipilimumab administered 1.60 (0.44–5.76) 0.48 | ... | ... |

#### Laboratory parameters

| Variable | Univariate models | Multivariate model |
|----------|-------------------|-------------------|
| Neutrophil to lymphocyte ratio at baseline known to be ≥3 | 0.65 (0.18–2.28) | 0.50 |
| Lactate dehydrogenase at baseline known to be above upper limit of normal range | 1.85 (0.55–6.20) | 0.32 |
| γ-glutamyltranspeptidase at baseline known to be above upper limit of normal range | 1.07 (0.34–3.43) | 0.90 |
| Alkaline phosphatase at baseline known to be above upper limit of normal range | 1.42 (0.37–5.41) | 0.61 |

#### PD-L1

| Variable | Univariate models | Multivariate model |
|----------|-------------------|-------------------|
| Combined positive score at baseline known to be ≥1 | 0.86 (0.27–2.74) | 0.80 |
| Combined positive score at baseline known to be ≥5 | 1.98 (0.61–6.47) | 0.26 |
| Tumour proportion score at baseline known to be ≥1% | 2.42 (0.73–8.02) | 0.15 |
| Tumour proportion score at baseline known to be ≥5% | 6.27 (1.66–23.62) | 0.0067 |

#### Previous treatment

| Variable | Univariate models | Multivariate model |
|----------|-------------------|-------------------|
| Any previous documented chemotherapy | 1.88 (0.37–9.63) | 0.45 |
| Any previous documented tumour surgery | 1.03 (0.30–3.52) | 0.96 |
| Current study treatment known to be third-line or later | 0.43 (0.08–2.15) | 0.30 |

#### Study treatment

| Variable | Odds ratio | p value |
|----------|------------|---------|
| Any ipilimumab administered | 1.60 (0.44–5.76) | 0.48 |

Overall, we found no significant differences in overall survival, progression-free survival, long-term progression-free survival, or response with regard to genetic or molecular alterations (appendix pp 2–4, 14–16). However, RNA sequencing from peripheral blood mononuclear cells revealed significant differences in the expression of FAM154B, ACO24257.1, and FLVCR1 regarding response to immunotherapy (appendix p 16). In patients with stable disease and partial response (n=9), ACO24257.1 (gene with unknown function) was upregulated compared with in patients with progressive disease (n=3); log2 fold change −1.95; p=0.0034), whereas FAM154B (also known as SAXO2, which encodes for proteins related to microtubule binding) was downregulated (log2 fold change 2.86; p=0.0092). Patients with long-term progression-free survival (n=5) expressed significantly lower levels of the heme transporter, FLVCR1, compared with patients without long-term progression-free survival (n=7; log2 fold change −1.76; p=0.0020).

Deep learning can predict clinically relevant features, such as molecular subtypes and genetic alterations, directly from histopathology images stained with haematoxylin and eosin.13,15 We found that PD-L1 expression was predictable from haematoxylin and eosin staining alone, reaching cross-validated area under the receiving operating characteristic curves (AUROCs) of 0.769 (SD 0.270) and 0.930 (SD 0.144), respectively. p values were 0.411, and 0.069, respectively (appendix p 5).

### Discussion

In the RAMONA trial, we addressed the clinical need for immune therapy in patients with oesophageal squamous cell carcinoma in Germany aged at least 65 years. Overall survival was significantly increased in our trial compared with that of a historical control group treated with chemotherapy.1 However, our trial with older and more vulnerable patients did not meet the overall survival range observed in previous phase 3 trials (appendix p 17).6–8 We speculate that not all our patients underwent subsequent treatment lines following immuno-oncology (I/O) combination therapy because of their increased vulnerability. This is supported by the observation that progression-free survival was longer in our trial, whereas overall survival was not increased compared with that in the phase 3 trials, which generally enrolled younger patients with a better performance status. Furthermore, the RATIONALE-302 and ATTRACTION-3 trials primarily enrolled Asian patients and reported a higher degree of efficacy of I/O therapies in oesophageal squamous cell carcinoma.7,8 Finally, an overall lower efficacy of I/O therapy due to immunosenescence has been reported in older patients.9

In our trial, 44 patients were treated with nivolumab and ipilimumab. In the univariate and multivariate analyses, the administration of any dose of ipilimumab significantly increased progression-free survival and disease control rate. However, the efficacy of ipilimumab needs to be regarded with caution. We cannot exclude a potential bias in that the efficacy of ipilimumab was associated with a better performance status in these patients. Nonetheless, clinical characteristics at baseline, such as geriatric status or Karnofsky score, were not associated with progression-free survival in these patients. Overall survival and progression-free survival results in this study were substantially better than those with combination of nivolumab and ipilimumab in patients with gastroesophageal adenocarcinoma, including oesophageal adenocarcinoma: in the CheckMate 032 trial, overall survival was 4–8 months and progression-free survival was 1–6 months, and the overall response rate was only 8%.9 Together, these data indicate that this I/O combination is far more active in oesophageal squamous...
cell carcinoma than in oesophageal adenocarcinoma, which underscores the activity of the I/O combination therapy as second-line therapy for this cancer even in older, Asian, and European patients.

Geriatric assessment might help in appropriate selection of patients and in controlling the risk of severe toxicity in older patients undergoing immune therapies. However, geriatric assessment has so far only been used in the ELDERS study,19 which assessed the safety and risk of immune-related adverse events in the treatment of non-small cell lung cancer and melanoma using pembrolizumab. Overall, in the ELDERS study the rate of immune-related adverse events of grade 3 or worse was not significantly different in the cohort of patients aged at least 70 years (13 [19%] of 70) versus patients younger than 70 years (nine [13%] of 70), and no differences were found in the rate of discontinuation between these two groups. We also used a brief G8 screening and DAFI geriatric assessment in our trial. Overall, between the fit versus less-fit groups, we found no differences in response rates and progression-free survival; however, we found non-significantly increased median overall survival in the fit group, which needs validation in larger patient populations. Treatment-related adverse events were observed in 42 (64%) of 66 patients. The most frequent treatment-related adverse events were fatigue, nausea, and diarrhoea. Grade 3–5 treatment-related adverse events were, however, noted in only 13 (20%) patients, which is similar to the proportions in the large phase 3 PD-1 inhibitor treatment trials (18–19%) and even less than that in the combination group with nivolumab and ipilimumab in the CheckMate-032 trial (14 [27%] of 52 patients).6,8,18 We observed no difference in the proportion of treatment-related adverse events between patients who received nivolumab monotherapy or combination therapy. The rate of treatment-related adverse events leading to permanent treatment discontinuation in our patient population was similar to that in the phase 3 trials.6,8,18 In the PD-1 monotherapy trials, this rate varied between 2% and 9%, whereas the combination with ipilimumab in the CheckMate-032 trial increased the rate to 13%.6,8,18

The low termination rates due to treatment-related adverse events support our concept that upon careful selection of patients this combination therapy can be administered safely to an older patient population. Because of the vulnerability and multimorbidity of our patient population, special focus was also given to potential immune-related adverse events. In line with previous reports,19 we also observed colitis, pneumonitis, and various endocrine disorders in our patients. Interestingly, pneumonitis occurred in five patients, who all had treatment with ipilimumab. This finding is consistent with previous reports that observed an increased frequency of interstitial lung disease in the ATTRACTION-1 trial and was the most common reason for treatment discontinuation in the ATTRACTION-3 trial.15 Because of the frequency and severity of inflammatory lung conditions in an older patient population, this adverse event requires close monitoring in patients undergoing I/O treatment.

Several tumour-related and host-related factors have been reported to predict sensitivity or resistance to checkpoint inhibitors.20 Regarding oesophageal squamous cell carcinoma, few data are available on the predictive biomarkers for checkpoint inhibitor treatment. In the KEYNOTE-181 trial, a PD-L1 combined positive score of at least 10 showed significant association with survival benefit in patients receiving pembrolizumab therapy (HR 0·64), which was less pronounced (HR 0·88) in patients with a combined positive score of less than 10.21 In the RAMONA trial, response and survival were also
independent of microsatellite instability, tumour mutational burden, gene variants, and microbiota composition. A potential predictive role of blood-based markers in the response to I/O therapy was also not confirmed in our trial. However, we observed a highly significant association between tumour proportion score of at least 5% and disease control in univariate and multivariate analyses. Next, we specifically addressed and defined the long-term progression-free survival patients to characterise this clinically relevant population more closely. In this study population with extended response, the baseline tumour proportion score of at least 5% was highly predictive of long-term progression-free survival in univariate and multivariate analyses. To facilitate PD-L1 analysis and quantification in combined positive score and tumour proportion score we applied our DeepMed pipeline and were able to predict tumour proportion score and combined positive score directly from haematoxylin and eosin with high performance. Our exploratory study resulted in high AUROCs; however, the number of patients was lower than in previous studies and p values were greater than 0-05. Thus, our findings indicate a strong trend but need validation in larger cohorts.

Our trial has several limitations. Historical reference data for second-line therapy were scarce at the time of trial planning. Apart from a small study in 16 patients from Europe, Jin and colleagues reported the largest dataset in a Chinese trial with 46 patients. Median overall survival was 5-9 months. We are aware that the comparability between Asian and European patients is limited. However, these data were similar to data in the study by Conroy and colleagues. Since older European patients with oesophageal squamous cell carcinoma frequently show worse prognosis compared with younger and Asian patients, testing against this historical control was regarded as a valid option. Because of the unknown toxicity profile of a combined nivolumab and ipilimumab treatment in vulnerable older patients, we decided to start with a safety run-in phase with nivolumab only. After passing the safety assessment after three cycles of nivolumab monotherapy, patients could continue with the combination therapy. The rate of treatment-related adverse events leading to treatment discontinuation was similar to that in other trials. Overall treatment-related adverse events might, however, be more frequent and should be carefully monitored in an older patient group. Also, because of the design of our trial, assessing tolerability of this combination is largely restricted to patients aged 65 years or older, who were safely pre-treated with nivolumab. Furthermore, although we observed improved response in ipilimumab-treated patients, this result is also based on the preselection of patients pre-treated with nivolumab and should, therefore, be interpreted with caution. Our results warrant validation in a randomised trial. However, because of the success of immune oncology strategies in first-line and second-line therapy of oesophageal squamous cell carcinoma, it will be difficult to design a randomised trial with a double checkpoint inhibitor treatment as second-line treatment for oesophageal squamous cell carcinoma, especially in an older patient population. Therefore, we regard our data as potentially valuable for the selection of the nivolumab and ipilimumab combination in pre-treated European patients with oesophageal squamous cell carcinoma, aged at least 65 years, especially in the subgroup of patients with a tumour proportion score of at least 5%. Pre-treatment might include first-line chemotherapy or chemoradiation, and potentially a checkpoint inhibitor and chemotherapy combination. Interpretation of the key secondary end-point of QOL deterioration in our trial is difficult because of attrition bias and a potential survivorship bias. However, QOL data indicate that patients who respond to immunotherapy are stable in their QOL in the long term. Finally, predicting response in this vulnerable patient group is important; however, because of the small sample size our translational studies are largely exploratory. All findings should, therefore, be interpreted with caution and need to be validated in larger cohorts.

In conclusion, the combined nivolumab and ipilimumab therapy led to improved overall survival as the second-line therapy of oesophageal squamous cell carcinoma in older German patients. PD-L1 expression with a tumour proportion score of at least 5% was associated with long-term progression-free survival and disease control. Using this criterion, nivolumab and ipilimumab might present a novel treatment option for older patients with oesophageal squamous cell carcinoma beyond the first-line therapy.

Contributors
MPE and NH designed the trial. MM, TGu, JB, NS, TZ, PW, and EB made substantial contributions to the conception and design of the trial. MPE, NH, NMB, TGu, JB, NS, TZ, PW, RH, AV, SA, CB, MfW, RJ, MK, GS, PTP, and JNK were involved in recruitment and treatment of the patients within the trial. MPE, NH, NMB, TGu, JB, NS, TZ, PW, RH, AV, SA, CB, MfW, RJ, MK, GS, PTP, and JNK contributed to data and sample collection. MPE, NH, MM, and TL performed the data analysis. MPE, NH, NMM-B, EB, TGu, and TGa designed and led the translational sample analysis. MPE, NH, MM, TL, TGu, NMB, and TGu contributed to interpretation of data from the translational programme. MM and TL conducted statistical analysis. MPE, NH, and NMB interpreted all data with assistance of MM and TL and the study team and wrote the manuscript. MPE, NH, MM, NMM-B, TGu, and TL accessed and verified the data. MPE, NH, NMB, TGu, MM, and TL vouch for the integrity of the data and adherence to the study protocol.

Declaration of interests
MPE reports receiving funding to conduct the trial from AIO Studien, the regulatory sponsor of the trial, and serving as an advisor with Bristol Myers Squibb. NMB reports receiving research funding and receipt of equipment from Deutsche Forschungsgemeinschaft. RH reports serving as an advisor with Amgen, Astra Zeneca, Bristol Myers Squibb, Boehringer, Daichi, Lilly, Merck, MSD, Pierre Fabre, Roche, and Servier; honoraria for lectures and presentations from Amgen, AstraZeneca, Bayer, Bristol Myers Squibb, Boehringer, Daichi, Lilly, medac, Merck, MSD, Pierre Fabre, Roche, Salaxad, Sanofi, and Servier; and consulting fees from Amgen, Astra Zeneca, Bayer, BMS, Boehringer, Daichi, Lilly, medac, Merck, MSD, Pierre Fabre, Roche, Salaxad, Sanofi, and Servier. AV reports serving as an advisor with Roche, Bayer, Bristol Myers Squibb, Lilly, Eissi, AstraZeneca, IPSEN, MSD, Sirtex, BTG, Servier, Terumo, and Imaging Equipment; and consulting fees and honoraria for lectures from Roche, Bayer, Bristol Myers Squibb, Lilly, Eissi, Astra Zeneca, IPSEN, MSD, Sirtex, BTG, ...
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