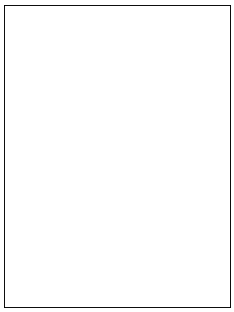
Article:
Arriola, E., Wheater, M., Galea, I. et al. (13 more authors) (2016) Outcome and biomarker analysis from a multi-centre phase 2 study of ipilimumab in combination with carboplatin and etoposide (ICE) as first line therapy for extensive stage small cell lung cancer. Journal of Thoracic Oncology, 11 (9). pp. 1511-1521. ISSN 1556-0864

https://doi.org/10.1016/j.jtho.2016.05.028

Accepted Manuscript



Outcome and biomarker analysis from a multi-centre phase 2 study of ipilimumab in combination with carboplatin and etoposide (ICE) as first line therapy for extensive stage small cell lung cancer

E. Arriola, MD, PhD, M. Wheater, FRCP, I. Galea, PhD, N. Cross, B.Med.Sci, T. Maishman, MSc, D. Hamid, BAPPA, L. Stanton, MSc, J. Cave, FRCP, T. Geldart, BSc, MB BS, FRCP, DM, C. Mulatero, MB, V. Potter, PhD, S. Danson, PhD, P.J. Woll, FRCP, R. Griffiths, FRCP, L. Nolan, PhD, C. Ottensmeier, MD, PhD

PII: S1556-0864(16)30503-2

DOI: 10.1016/j.jtho.2016.05.028

Reference: JTHO 223

To appear in: *Journal of Thoracic Oncology*

Received Date: 25 April 2016

Revised Date: 23 May 2016

Accepted Date: 23 May 2016

Please cite this article as: Arriola E, Wheater M, Galea I, Cross N, Maishman T, Hamid D, Stanton L, Cave J, Geldart T, Mulatero C, Potter V, Danson S, Woll PJ, Griffiths R, Nolan L, Ottensmeier C, Outcome and biomarker analysis from a multi-centre phase 2 study of ipilimumab in combination with carboplatin and etoposide (ICE) as first line therapy for extensive stage small cell lung cancer, *Journal of Thoracic Oncology* (2016), doi: 10.1016/j.jtho.2016.05.028.

Title: Outcome and biomarker analysis from a multi-centre phase 2 study of ipilimumab in combination with carboplatin and etoposide (ICE) as first line therapy for extensive stage small cell lung cancer

Authors: E. Arriola MD, PhD[1*], M. Wheater FRCP[1b], I. Galea PhD[1c], N. Cross B.Med.Sci[2], T.Maishman MSc[2], D.Hamid BAPPA[2], L. Stanton MSc [2], J. Cave FRCP [1b], T. Geldart BSc, MB BS, FRCP, DM [3], C. Mulatero MB[4], V. Potter PhD[5], S. Danson PhD [6], P. J. Woll FRCP[6], R. Griffiths FRCP [7], L. Nolan PhD[1b], C. Ottensmeier MD, PhD[1*]

Affiliations:

1. University of Southampton, Tremona Road, Southampton, SO16 6YD, UK

1b.University Hospital Southampton, Tremona Road, Southampton SO16 6YD, UK

1c. Clinical Neurosciences, Clinical and Experimental Sciences, Faculty of Medicine, Tremona Road, Southampton, SO16 6YD, UK

2. Southampton Clinical Trials Unit, University of Southampton, Tremona Road, Southampton, SO16 6YD, UK

3. Royal Bournemouth and Christchurch Hospitals NHS Trust, Castle Ln E, Bournemouth BH7 7DW, UK,

4. St James' University Hospital, Beckett St, Leeds, West Yorkshire LS9 7TF, UK

5. Nottingham University Hospitals NHS Trust, Hucknall Rd, Nottingham NG5 1PB UK

6. Sheffield Experimental Cancer Medicine Centre, Weston Park Hospital, Whitham Road, Sheffield, S10 2SJ, UK

7. The Clatterbridge Cancer Centre NHS Foundation Trust, Clatterbridge Health Park, Clatterbridge Rd, Wirral, Merseyside CH63 4JY, UK

Corresponding authors*:

Edurne Arriola

Southampton NIHR Experimental Cancer Medicine Centre, Faculty of Medicine, University of Southampton

University Hospital Southampton NHS Foundation Trust

Tremona Road, Southampton SO16 6YD, United Kingdom

Telephone number: 00442381205097

Email address: e.arriola-aperribay@soton.ac.uk

Christian Ottensmeier

Southampton NIHR Experimental Cancer Medicine Centre, Faculty of Medicine, University of Southampton

University Hospital Southampton NHS Foundation Trust

Tremona Road, Southampton SO16 6YD, United Kingdom

Telephone number: 00442381205097

Email address: C.H.Ottensmeier@soton.ac.uk

Authors' contributions:

C.O, M.W, L.N contributed to the study design. C.O, I.G, P.J.W, N.C, T.M, S.S, D.H, J.C, T.G, C.M, V.P, S.D, R.G, L.N contributed to data collection. E.A, I.G, T.M, D.H, N.C, L.S, L.N and C.O contributed to data analysis and interpretation. E.A, M.W, I.G, N.C, T.M, D.H, L.S, J.C, T.G, C.M, V.P, S.D, P.J.W, R.G, L.N, C.O contributed to manuscript writing and review.

Funding sources: Bristol-Myers Squibb and Cancer Research UK (grant number C491/A12135). EA is supported by PI13/00140/FEDER.

Abstract

Background: To evaluate safety and efficacy of ipilimumab combined with standard first-line chemotherapy for patients with extensive stage SCLC.

Methods: Chemotherapy-naïve extensive stage SCLC patients were treated with carboplatin and etoposide up to six cycles. Ipilimumab 10 mg/kg was given on day 1 of cycles 3-6 and every 12 weeks. Response was assessed by RECIST v1.0 and immune related response criteria (irRC). The primary endpoint was 1-year progression-free survival (PFS) according to RECIST. Secondary endpoints included PFS by irRC (irPFS) and overall survival (OS). Autoantibody serum levels were evaluated and correlated with clinical outcomes.

Results: 42 patients were enrolled between September 2011-April 2014, 39 evaluable for safety and 38 for efficacy. 6/38 patients (15.8% [95% CI: 7.4%-30.4%]) were alive and progression-free at 1-year by RECIST. Median PFS was 6.9 months (95%CI: 5.5-7.9). Median irPFS was 7.3 months (95% CI: 5.5-8.8). Median OS was 17.0 months (95% CI: 7.9-24.3). In patients evaluable for response, 21/29 patients (72.4%) achieved an objective response by RECIST and 28/33 (84.8%) by irRC. All patients experienced at least one adverse event; 35/39 (89.7%) patients developed at least one toxicity ≥ Grade 3; in 27 (69.2%) this was related to ipilimumab. Five deaths were reported to be related to ipilimumab. The positivity of an autoimmune profile at baseline was associated with improved outcomes and severe neurological toxicity.

Conclusion: Ipilimumab in combination with carboplatin and etoposide might benefit a subgroup of patients with advanced SCLC. Autoantibody analysis correlates with treatment benefit and toxicity and warrants further investigation.

Introduction

Small Cell Lung Cancer (SCLC) accounts for around 15-20% of all lung cancers. Despite the high percentage of initial responses to chemotherapy, overall prognosis remains dismal, with median survival times of 9.5 months for extensive stage disease [1]. No therapeutic strategy except for the addition of radiotherapy to chemotherapy has produced improvements in survival [2-7].

Harnessing the immune response to attack tumor cells with antibodies directed against checkpoint molecules has had dramatic impact in the treatment of melanoma [8, 9] and other solid tumors [10, 11].

Clinical evidence supports immune recognition of SCLC, in the form of paraneoplastic immune mediated syndromes (PNS). PNS are associated with the cross reactivity of immune responses with self-antigens, frequently neuronal antigens, physiologically expressed by the normal nervous system and ectopically by cancer cells [12] but the T-cell based mechanisms for PN events remains poorly understood [13]. The presence of autoimmune disease seems to be associated with better outcomes [14, 15]. These findings suggest that the effective antitumor immune responses are linked to autoimmune manifestations[16].

Cytotoxic T-lymphocyte antigen-4 (CTLA-4) is expressed by lymphocytes early in the adaptive immune response, and binds to B7 expressed in antigen presenting cells to downregulate T-cell responses [17]. Additionally, CTLA-4 is highly expressed on regulatory T-cells and antibody binding to CTLA-4 leads to their removal by antibody dependent cytotoxicity [18]. Release of these “brakes” with anti-CTLA-4 antibodies has been successfully tested in several tumors.

Ipilimumab is an anti-CTLA-4 antibody approved for the treatment of metastatic melanoma [8, 19, 20]. It is however unclear, how effective ipilimumab is in rapidly progressing tumors, such as SCLC. However, chemotherapy for SCLC is effective in killing tumor cells and cell death will release tumor antigen [21]. It is therefore possible that in the context of immune modulation with ipilimumab, recognition of these antigens might induce clinically useful anti-tumor immunity.

In 2013, a study was published assessing ipilimumab added to carboplatin and paclitaxel randomizing patients with extensive stage SCLC to only chemotherapy, or chemotherapy with concurrent or

phased ipilimumab [22]. This study suggested that phased ipilimumab after two cycles of chemotherapy was a promising strategy.

The current study enrolled patients with extensive stage SCLC treated with standard carboplatin and etoposide in the first line setting and aimed to evaluate the safety and efficacy of ipilimumab added to this combination and explore predictive biomarkers (ClinicalTrials.gov Identifier:NCT01331525).

Patients and methods

Patient population

Eligible patients were men and women aged ≥18 who had a histological or cytological diagnosis of SCLC, no previous systemic therapy for SCLC, an Eastern Cooperative Oncology Group performance status of 0 or 1, adequate baseline laboratory tests and no active or chronic infection with HIV, Hepatitis B, or Hepatitis C. Exclusion criteria included: limited stage SCLC appropriate for radical treatment with chemoirradiation, symptomatic CNS metastases, autoimmune disease, live vaccines (for up to 1 month before or after any dose of ipilimumab), a history of prior treatment with a CD137 agonist or CTLA-4 inhibitor or agonist and concomitant therapy with any of the following: Interleukin-2, interferon, or other immunotherapy regimens; immunosuppressive agents; other investigational therapies; or chronic use of systemic corticosteroids.

Study design and treatment plan

This single stage non-randomized phase II study examined the efficacy and toxicity of ipilimumab (10 mg/kg) together with carboplatin AUC=6, IV on day 1 and etoposide 120 mg/m2 IV Day1, 100mg BD PO days 2 and 3, every 21 days (ICE). Patients could enroll the trial at any point up until cycle 3. Patients received carboplatin and etoposide up to 6 cycles. Chemotherapy was discontinued in the event of progressive disease (RECIST v1.0) or excessive toxicity. Ipilimumab 10 mg/kg was given IV

on day 1 of chemotherapy cycles 3-6 and then once every 12-weeks from week 30 until immune related disease progression or excessive toxicity.

Patients could be offered prophylactic cranial irradiation (PCI) after completion of induction chemoimmunotherapy.

The trial was conducted in accordance with good clinical practice and ethical approval was obtained (MREC 10/H0502/95; ISRCTN: 14095893); written informed consent was provided by all patients before enrolment.

Study assessments

Tumor assessments were conducted by computerized tomography (CT) 6-weekly for the first year (until week 54), then 12-weekly until disease progression by both RECIST v1.0 and immune response criteria (irRC)[23]. A baseline brain CT scan (not MRI) was performed for CNS disease evaluation if clinically indicated.

Patients who received at least one dose of ipilimumab were considered evaluable for safety, assessed using the National Cancer Institute (NCI) Common Terminology Criteria for Adverse Events (CTCAE), Version 4.0 (http://ctep.cancer.gov). As no safety data was available for the combination, a planned interim safety monitoring assessment was performed. Once, 9 patients had been treated with the combination for at least 6 weeks a first clinical safety assessment was performed to identify any early safety signals from ipilimumab given in combination with Carboplatin and Etoposide. In addition, a review of safety was triggered throughout the trial: if ≥ 40% of patients treated develop a ≥ Grade 3 toxicity thought to be related to the study drugs; or if ≥ 10% of patients experience an unexpected Ipilimumab -related ≥ Grade 3 toxicity that could not be alleviated or controlled by appropriate care and/or steroid and/or infliximab therapy within 14 days of the initiation of such therapy; or in response to any Ipilimumab-related deaths unless attributed to disease progression.

Data on adverse events (AEs) and immune related AEs (irAEs) were collected at each study visit and until 90 days after the last ipilimumab dose. irAE was defined as an AE that was treatment related and considered to be immune mediated.

irAEs were managed according to international guidelines and package inserts/product label. No dose reductions were allowed for ipilimumab. Dose modifications for carboplatin and etoposide were according to local practice.

Biomarker assessment

Detection of autoantibodies was performed at baseline and during follow up in cases where clinically indicated. Anti-VGCC and VGKC antibodies were determined with radio-immunoprecipitation assays[24, 25]. Antibodies against intracellular neuronal antigens were detected using indirect immunohistochemistry on primate cerebellum (NOVA Lite, Inova, Werfen, Warrginton, UK), immunoblotting (Ravo PNS Blot, ravo Diagnostika, Freiberg, Germany) and a semi-automated ELISA[26]. Interpretation was done according to protocol instructions.

Statistical analysis

The sample size was based on A'Hern's single stage phase II design, with two-sided significance level of 0.05, 80% power, p0 (clinically uninteresting true PFS according to RECIST v1.0)=10%; and p1 (sufficiently promising true PFS according to RECIST v1.0)=25%. The design required recruiting 40 evaluable patients, and the efficacy of the treatment was considered to be worth developing further if eight or more patients were alive and progression-free at 1-year.

The intention-to-treat (ITT) population consisted of all registered patients. Toxicity was assessed using the safety population which excluded patients who did not receive any ipilimumab. Baseline, treatment and efficacy information was performed on the efficacy analysis population that consisted of all eligible patients included in the safety population.

Outcome analysis

The primary endpoint was 1-year progression-free survival (PFS) according to RECIST v1.0. PFS was defined as the time from day 1 of the 1st cycle of chemotherapy to the date of progression or death from any cause.

Secondary endpoints included PFS; PFS by irRC (irPFS); overall survival (OS), defined as the time from the date of day 1, cycle 1 of chemotherapy to the date of death from any cause; best overall response, defined as the maximum response by RECIST v1.0 compared to the baseline scan at study entry; duration of response, defined as the time from first response by RECIST v1.0 to disease progression or death from any cause; duration of response by irRC; and toxicity assessment according to NCI CTCAE v4.0.

Patients who had not died or progressed were censored for survival endpoints at the last documented clinical review. Survival analyses were performed using Kaplan-Meier estimates, and 95% confidence intervals (CIs) for proportions were calculated using the Wilson Interval as recommended for small n by Brown et al.[27] Summary statistics and plots were used to examine other secondary endpoints and to characterize response rates.

Immunological data was recorded for each patient. *Ad-hoc* exploratory analysis was carried out to assess associations between antibody positivity and clinical outcomes (irRC, irPFS, OS and toxicity occurrence).

Results

Patients and treatment

Forty-two patients with no previous systemic therapy for SCLC were registered into this study between September 2011 and April 2014 at six sites in the UK (Figure 1 CONSORT diagram). Three patients withdrew from the trial prior to receiving ipilimumab and one patient was retrospectively diagnosed with atypical carcinoid. Baseline demographics and disease characteristics are shown in Table 1 for the evaluable population (n=38). The majority of patients were male (66%), with a

performance status (PS) of 1 (66%) and involvement of the lung, lymph nodes and liver. The presence of autoantibodies was investigated at baseline in 38 patients (Table 2). Seventeen (45%) patients had at least one confirmed positive autoimmune antibody at baseline.

At the final database lock (03 November 2015) after a minimum follow up of 6.8 months (median 8.5 months) no patients were still receiving treatment.

The main reason for treatment discontinuation was toxicity (10/39, 26%).

Thirty-seven out of 38 patients started ipilimumab treatment on the third cycle of chemotherapy. The median number of cycles of the combination treatment for the efficacy analysis population (n=38) was six (range 3-6). Twenty-four patients (63%) completed the chemoimmunotherapy phase. Twenty-three patients (61%) had at least one chemotherapy dose delayed and 15 (40%) had dose modifications. Fifteen patients (40%) had at least one dose of ipilimumab delayed and 13 (34%) missed at least one dose during the combination phase. The number of patients who received at least one maintenance dose of ipilimumab was nine (24%) and one patient received treatment for 78 weeks.

Nine patients (24%) received PCI and eight (21%) radiotherapy to the chest.

Efficacy

Six out of 38 patients (efficacy analysis population) (15.8% [95% CI: 7.4%-30.4%]) were progression-free at 1-year by RECIST. Median PFS was 6.9 months (95%CI: 5.5-7.9) (Figure 2). Median irPFS was 7.3 months (95% CI: 5.5-8.8) with an irPFS at 1-year of 12.6% (95% CI: 4.0%-26.3%). Median OS was 17.0 months (95% CI: 7.9-24.3) (Figure 3). Response information by RECIST and irRC was available on 29 and 33 patients, respectively, of whom 21 (72.4%) achieved an objective response according to RECIST criteria and 28 (84.8%) according to irRC (Table 3). Supplementary Table 1 compares both patterns of response.

Patients receiving PCI had a numerically superior OS (median 18.5 vs 12.3 months respectively) but this difference did not reach statistical significance (p=0.447).

Safety

All toxicities are listed in Supplementary Table 2. Table 4 summarizes the incidence of treatment related grade 3 or higher AEs for the safety analysis population (n=39). All patients experienced at least one AE. Thirty-five (90%) patients developed at least one ≥ grade 3 toxicity; in 27 (69%) this toxicity was thought to be related to ipilimumab. Neurological AEs were reported for 19 patients (49%), although only four patients (10%) experienced 5 high grade AEs and three (8%) among these were related to ipilimumab. Two patients developed central neuropathies (described as mild encephalopathy and cerebellar syndromes, mimicking PNS) and one had severe headaches with deterioration of performance status. No association was observed between the occurrence of neurological toxicity and PCI treatment.

Other frequent AEs, probably irAEs were diarrhea in 28 patients (72%) and skin rash in 20 patients (51%), respectively. For 18 patients (46%) treatment delays were associated with ipilimumab related toxicity. Five deaths (13%) were reported to be related to ipilimumab. Two of the deaths happened while the patients where on treatment or shortly after (cardiac arrest, neutropenic sepsis) but the 3 remaining happened 4-5 months after the last treatment (pneumonia, autoimmune encephalitis and sepsis).

In an unplanned analysis, we evaluated if severity of irAEs was associated with outcome. Patients who had more severe (G3 or above) irAEs had numerically worse OS (Supplementary Figure 1), but this was not statistically significant. Moreover, 73% of patients with G1/2 irAEs were alive at 1-year when compared to 47% of patients with severe (G3 or above) irAEs.

Autoantibodies as predictive biomarkers

In an *ad-hoc* analysis, we explored the association between positivity of autoantibodies at baseline and clinical outcomes.

The most frequently detected antibodies were ANA in 10 patients and anti-SOX2 in 9 patients (Table 2). Twenty-three patients (60.5%) had at least one positive autoantibody detection. Antineuronal antibodies were more frequently positive (44.7%) than the rest of the autoantibodies (31.6%). We assessed the association between autoantibodies and response (irRC). We found that 0/14 patients with positive antineuronal antibodies vs 5/19 patients with no positivity showed irSD/irPD (p=0.049). Any autoantibody positivity showed a trend to association with response (p=0.066).We then evaluated the association between autoantibody positivity and irPFS. We observed that patients with any positive autoantibody detected at baseline experienced a significantly longer median irPFS (8.8 m (95%CI 5.1-10.7) vs 7.3 m (95%CI: 2.9-7.9; p=0.036) (Figure 2C). ANA positivity predicted for a significantly prolonged irPFS (10.2 m vs 6.9 m; p=0.032). Patients with any positive autoimmune antibody showed a trend to prolonged survival (18.5 m vs 17 m); p=0.144) (Figure 3B).

We assessed the correlation between autoantibodies and toxicity. We found that 3/15 patients with positivity for SOX2 and/or anti-Hu antibodies presented ipilimumab related G3 or above neurological toxicity, compared to 0/23 patients with negativity for these antibodies (p=0.054). One of these patients had more than one positive antineuronal autoantibody (anti-SOX2 and anti-Yo).

Discussion

In our trial, we observed substantial excess toxicity from the ICE combination, which made the delivery of the chemoimmunotherapy and the maintenance ipilimumab challenging. Delays were frequent as were interruptions of treatment due to toxicity.

Ipilimumab has a well-defined toxicity profile, and combination treatments have shown increased toxicity when compared to monotherapy. In the current study, the grade 3 or above toxicity rate is considerably higher (69%) (including five treatment related deaths). These figures are significantly higher than the toxicity reported in the randomised trial by Reck et al. [22], ranging from 43-50% (1 toxic death in the concurrent arm). This increased toxicity might be explained by the better tolerance of the chemotherapy regime used in that study and might also reflect excess toxicity from combining ipilimumab and etoposide. Combining a third drug (i.e. sunitinib, thalidomide) with the platinum and

etoposide doublet in advanced SCLC has been challenging due to increased toxic death rates[6, 7] and protocols have been amended to pursue a maintenance strategy[6]. Moreover, the dose used in this study (10mg/kg) was higher than the dose currently approved for melanoma (3mg/kg) and data suggests increased toxicity with higher doses [28]. Therefore, using ipilimumab at 3mg/kg might be more appropriate in combination as well as a sequential approach of immunotherapy after chemotherapy. Newer agents, such as anti-PD-1/PD-L1 drugs, with a more favorable toxicity profile might be easier to combine with chemotherapy. Moreover, ipilimumab in combination with the anti-PD-1 nivolumab seems to have an acceptable toxicity profile and adds clinical benefit in early phase testing in patients with SCLC [29].

In our study, G3 or above ipilimumab related neurological toxicity rate was 7.6%. A comprehensive study of the prevalence of neurological PNS in a similar population of SCLC observed that 9.1% of patients had a PNS by clinical evaluation [30], the majority (83%) having symptoms preceding the diagnosis of SCLC. Patients with clinical evidence of autoimmunity were however excluded in our study. As neurological toxicities developed after treatment initiation, they are most likely treatment-related. Autoimmunity to the intracellular antigens SOX2 and Hu has been associated with PNS in several publications [30-33]. Our exploratory analysis revealed an association between anti-SOX2 and Hu autoantibodies and severe neurological toxicities. Among patients with anti-SOX2 or anti-Hu antibodies at baseline we could not find differences (in antibody titers or subsequent antibody levels (Suppl Fig 2)) between those who developed neurological syndromes or not. The absence of anti-neuronal autoantibodies at diagnosis might therefore reflect a decreased likelihood of developing severe neurological toxicities triggered by ipilimumab. This suggests that careful monitoring of neurological symptoms in patients with anti-neuronal autoantibodies at baseline is important if immunotherapy is chosen as a strategy. We recognize that these findings need further validation and may additionally reflect the particular method of action of ipilimumab.

Our study is not randomized and therefore we cannot rule out that the neurological syndromes we clinically attributed to ipilimumab might have happened regardless of treatment with this drug. Of the 3 patients with severe neurological toxicity mimicking PNS, in 2 of the cases the onset of the

neurological syndrome preceded and perhaps therefore heralded disease progression. In the remaining case the progression was observed before the PNS. Thus, it remains possible that in spite of absence of PNS at primary diagnosis, the neurological syndrome post-treatment was caused by progression-related cross-reactive immune responses.

Markers of Treg function are lower in patients with autoantibodies and concomitant PNS as compared with those with no neurological syndromes [34]. Tregs express high levels of CTLA-4 and are downregulated or removed by ipilimumab, and this is a desirable effect to enhance immune response against the tumor [35, 36]. Our data are consistent with the hypothesis that downregulation of Tregs in patients with anti-neuronal autoantibodies by ipilimumab could promote development of autoimmune PNS.

The primary endpoint of the study was not met. Median PFS was 6.9 months. Interestingly, although irPFS seems to better reflect the efficacy of immunotherapeutic agents, in our study both parameters gave similar results (median irPFS of 7.3 months). These results are consistent with the 6.4 months median irPFS observed in the phased-ipilimumab arm in Reck et al. study [22]. Four patients with PD according to RECIST criteria were classified as responders or SD according to irRC. In other tumor types, patients with RECIST-defined PD but irRC-defined response or SD seem to have better outcome than those with progressive disease according to both parameters [37]. There is no previous assessment of this question in SCLC. Due to the low numbers, we were not able to compare survival of these patients to the RECIST responders.

A key secondary endpoint was OS. Although this is a relatively small cohort and cannot be directly compared with other studies, the median OS of 17 months exceeds the OS reported in other recent trials in this setting [6, 22] which is around 14 months. Interestingly, this happened despite the low rate (24%) of patients receiving PCI or thoracic radiotherapy. Fifty-six percent of the patients were alive at 1-year, 29% at 2-years and almost 10% at 3 years. This is consistent with findings in other studies where improved OS is the key benefit from ipilimumab [19]. More definitive data about the potential benefit of this combination will be available from the completed randomized trial (NCT 01450761).

To investigate potential biomarkers of benefit, we evaluated the association between autoimmunity and outcomes. We observed that a positive autoimmune profile at baseline predicted better response, irPFS with a trend to increased survival. The presence of autoantibodies at baseline has been linked to prognosis in this disease with conflicting results [31, 33, 38-40]. Overall, there is evidence of patients benefiting from naturally occurring tumor immunity with improved responses to tumor treatment or, in rare cases, complete eradication of tumor without tumor treatment [41-43].

This would be consistent with our results suggesting that a pre-existing immune response enhanced by ipilimumab could result in beneficial effect from this drug. Although interesting, these results are hypothesis generating and need further validation. Moreover, the lack of a control only-chemotherapy arm precludes us from demonstrating the predictive vs a merely prognostic role.

In conclusion, ipilimumab in combination with carboplatin and etoposide as first-line treatment for SCLC shows beneficial effects, particularly in patients with pre-existing autoimmunity. However, toxicity was significant, suggesting that sequential immunotherapy after chemotherapy might be a more feasible approach, maybe in combination with other immune modulators such as PD-1 or PD-L1 inhibitors. More work in needed to demonstrate if autoantibodies can serve as biomarkers for toxicity.

References

1. Oze I, Hotta K, Kiura K, et al. Twenty-seven years of phase III trials for patients with extensive disease small-cell lung cancer: disappointing results. *PloS one* 2009;4:e7835;10.1371/journal.pone.0007835.
2. Slotman BJ, van Tinteren H, Praag JO, et al. Use of thoracic radiotherapy for extensive stage small-cell lung cancer: a phase 3 randomised controlled trial. *Lancet* 2015;385:36-42;10.1016/S0140-6736(14)61085-0.
3. Ettinger DS, Finkelstein DM, Abeloff MD, et al. A randomized comparison of standard chemotherapy versus alternating chemotherapy and maintenance versus no maintenance therapy for extensive-stage small-cell lung cancer: a phase III study of the Eastern Cooperative Oncology Group. *Journal of clinical oncology : official journal of the American Society of Clinical Oncology* 1990;8:230-240
4. Arnold AM, Seymour L, Smylie M, et al. Phase II study of vandetanib or placebo in small-cell lung cancer patients after complete or partial response to induction chemotherapy with or without radiation therapy: National Cancer Institute of Canada Clinical Trials Group Study BR.20. *Journal of clinical oncology : official journal of the American Society of Clinical Oncology* 2007;25:4278-4284;10.1200/JCO.2007.12.3083.
5. Pujol JL, Breton JL, Gervais R, et al. Phase III double-blind, placebo-controlled study of thalidomide in extensive-disease small-cell lung cancer after response to chemotherapy: an intergroup study FNCLCC cleo04 IFCT 00-01. *Journal of clinical oncology : official journal of the American Society of Clinical Oncology* 2007;25:3945-3951;10.1200/JCO.2007.11.8109.
6. Ready NE, Pang HH, Gu L, et al. Chemotherapy With or Without Maintenance Sunitinib for Untreated Extensive-Stage Small-Cell Lung Cancer: A Randomized, Double-Blind, Placebo-Controlled Phase II Study-CALGB 30504 (Alliance). *Journal of clinical oncology : official journal of the American Society of Clinical Oncology* 2015;33:1660-1665;10.1200/JCO.2014.57.3105.
7. Lee SM, Woll PJ, Rudd R, et al. Anti-angiogenic therapy using thalidomide combined with chemotherapy in small cell lung cancer: a randomized, double-blind, placebo-controlled trial. *Journal of the National Cancer Institute* 2009;101:1049-1057;10.1093/jnci/djp200.
8. Schadendorf D, Hodi FS, Robert C, et al. Pooled Analysis of Long-Term Survival Data From Phase II and Phase III Trials of Ipilimumab in Unresectable or Metastatic Melanoma. *Journal of clinical oncology : official journal of the American Society of Clinical Oncology* 2015;33:1889-1894;10.1200/JCO.2014.56.2736.
9. Postow MA, Chesney J, Pavlick AC, et al. Nivolumab and ipilimumab versus ipilimumab in untreated melanoma. *The New England journal of medicine* 2015;372:2006-2017;10.1056/NEJMoa1414428.
10. Garon EB, Rizvi NA, Hui R, et al. Pembrolizumab for the treatment of non-small-cell lung cancer. *The New England journal of medicine* 2015;372:2018-2028;10.1056/NEJMoa1501824.
11. Borghaei H, Paz-Ares L, Horn L, et al. Nivolumab versus Docetaxel in Advanced Nonsquamous Non-Small-Cell Lung Cancer. *The New England journal of medicine* 2015;10.1056/NEJMoa1507643.
12. Darnell RB, Posner JB. Paraneoplastic syndromes involving the nervous system. *The New England journal of medicine* 2003;349:1543-1554;10.1056/NEJMra023009.
13. Roberts WK, Deluca IJ, Thomas A, et al. Patients with lung cancer and paraneoplastic Hu syndrome harbor HuD-specific type 2 CD8+ T cells. *The Journal of clinical investigation* 2009;119:2042-2051;10.1172/JCI36131.
14. Maddison P, Newsom-Davis J, Mills KR, et al. Favourable prognosis in Lambert-Eaton myasthenic syndrome and small-cell lung carcinoma. *Lancet* 1999;353:117-118;10.1016/S0140-6736(05)76153-5.

15. Wirtz PW, Lang B, Graus F, et al. P/Q-type calcium channel antibodies, Lambert-Eaton myasthenic syndrome and survival in small cell lung cancer. *Journal of neuroimmunology* 2005;164:161-165;10.1016/j.jneuroim.2005.04.001.
16. Pignolet BS, Gebauer CM, Liblau RS. Immunopathogenesis of paraneoplastic neurological syndromes associated with anti-Hu antibodies: A beneficial antitumor immune response going awry. *Oncoimmunology* 2013;2:e27384;10.4161/onci.27384.
17. Thompson CB, Allison JP. The emerging role of CTLA-4 as an immune attenuator. *Immunity* 1997;7:445-450
18. Romano E, Kusio-Kobialka M, Foukas PG, et al. Ipilimumab-dependent cell-mediated cytotoxicity of regulatory T cells ex vivo by nonclassical monocytes in melanoma patients. *Proceedings of the National Academy of Sciences of the United States of America* 2015;112:6140-6145;10.1073/pnas.1417320112.
19. Hodi FS, O'Day SJ, McDermott DF, et al. Improved survival with ipilimumab in patients with metastatic melanoma. *The New England journal of medicine* 2010;363:711-723;10.1056/NEJMoa1003466.
20. Maio M, Grob JJ, Aamdal S, et al. Five-year survival rates for treatment-naive patients with advanced melanoma who received ipilimumab plus dacarbazine in a phase III trial. *Journal of clinical oncology : official journal of the American Society of Clinical Oncology* 2015;33:1191-1196;10.1200/JCO.2014.56.6018.
21. Ma Y, Kepp O, Ghiringhelli F, et al. Chemotherapy and radiotherapy: cryptic anticancer vaccines. *Seminars in immunology* 2010;22:113-124;10.1016/j.smim.2010.03.001.
22. Reck M, Bondarenko I, Luft A, et al. Ipilimumab in combination with paclitaxel and carboplatin as first-line therapy in extensive-disease-small-cell lung cancer: results from a randomized, double-blind, multicenter phase 2 trial. *Annals of oncology : official journal of the European Society for Medical Oncology / ESMO* 2013;24:75-83;10.1093/annonc/mds213.
23. Wolchok JD, Hoos A, O'Day S, et al. Guidelines for the evaluation of immune therapy activity in solid tumors: immune-related response criteria. *Clinical cancer research : an official journal of the American Association for Cancer Research* 2009;15:7412-7420;10.1158/1078-0432.CCR-09-1624.
24. Mason WP, Graus F, Lang B, et al. Small-cell lung cancer, paraneoplastic cerebellar degeneration and the Lambert-Eaton myasthenic syndrome. *Brain : a journal of neurology* 1997;120 (Pt 8):1279-1300
25. Shillito P, Molenaar PC, Vincent A, et al. Acquired neuromyotonia: evidence for autoantibodies directed against K+ channels of peripheral nerves. *Annals of neurology* 1995;38:714-722;10.1002/ana.410380505.
26. Chapman CJ, Thorpe AJ, Murray A, et al. Immunobiomarkers in small cell lung cancer: potential early cancer signals. *Clinical cancer research : an official journal of the American Association for Cancer Research* 2011;17:1474-1480;10.1158/1078-0432.CCR-10-1363.
27. Lawrence D. Brown TTCaAD. Interval Estimation for a Binomial Proportion *Statistical Science* 2001;16: 101-117
28. Wolchok JD, Neyns B, Linette G, et al. Ipilimumab monotherapy in patients with pretreated advanced melanoma: a randomised, double-blind, multicentre, phase 2, dose-ranging study. *The Lancet Oncology* 2010;11:155-164;10.1016/S1470-2045(09)70334-1.
29. Antonia SJ, Bendel JC, Taylor MH, et al. Phase I/II study of nivolumab with or without ipilimumab for treatment of recurrent small cell lung cancer (SCLC): CA209-032. *ASCO Annual Meeting*. Chicago: J Clin Oncol 33, 2015 (suppl; abstr 7503).
30. Gozzard P, Woodhall M, Chapman C, et al. Paraneoplastic neurologic disorders in small cell lung carcinoma: A prospective study. *Neurology* 2015;85:235-239;10.1212/WNL.0000000000001721.
31. Titulaer MJ, Klooster R, Potman M, et al. SOX antibodies in small-cell lung cancer and Lambert-Eaton myasthenic syndrome: frequency and relation with survival. *Journal of clinical*

oncology : official journal of the American Society of Clinical Oncology 2009;27:4260-4267;10.1200/JCO.2008.20.6169.

32. Graus F, Keime-Guibert F, Rene R, et al. Anti-Hu-associated paraneoplastic encephalomyelitis: analysis of 200 patients. *Brain : a journal of neurology* 2001;124:1138-1148

33. Maddison P, Thorpe A, Silcocks P, et al. Autoimmunity to SOX2, clinical phenotype and survival in patients with small-cell lung cancer. *Lung cancer* 2010;70:335-339;10.1016/j.lungcan.2010.03.002.

34. Tani T, Tanaka K, Idezuka J, et al. Regulatory T cells in paraneoplastic neurological syndromes. *Journal of neuroimmunology* 2008;196:166-169;10.1016/j.jneuroim.2008.03.002.

35. Read S, Greenwald R, Izcue A, et al. Blockade of CTLA-4 on CD4+CD25+ regulatory T cells abrogates their function in vivo. *Journal of immunology* 2006;177:4376-4383

36. Selby MJ, Engelhardt JJ, Quigley M, et al. Anti-CTLA-4 antibodies of IgG2a isotype enhance antitumor activity through reduction of intratumoral regulatory T cells. *Cancer immunology research* 2013;1:32-42;10.1158/2326-6066.CIR-13-0013.

37. Chiou VL, Burotto M. Pseudoprogression and Immune-Related Response in Solid Tumors. *Journal of clinical oncology : official journal of the American Society of Clinical Oncology* 2015;33:3541-3543;10.1200/JCO.2015.61.6870.

38. Monstad SE, Drivsholm L, Storstein A, et al. Hu and voltage-gated calcium channel (VGCC) antibodies related to the prognosis of small-cell lung cancer. *Journal of clinical oncology : official journal of the American Society of Clinical Oncology* 2004;22:795-800;10.1200/JCO.2004.01.028.

39. Gozzard P, Chapman C, Vincent A, et al. Novel Humoral Prognostic Markers in Small-Cell Lung Carcinoma: A Prospective Study. *PloS one* 2015;10:e0143558;10.1371/journal.pone.0143558.

40. Vural B, Chen LC, Saip P, et al. Frequency of SOX Group B (SOX1, 2, 3) and ZIC2 antibodies in Turkish patients with small cell lung carcinoma and their correlation with clinical parameters. *Cancer* 2005;103:2575-2583;10.1002/cncr.21088.

41. Darnell RB, DeAngelis LM. Regression of small-cell lung carcinoma in patients with paraneoplastic neuronal antibodies. *Lancet* 1993;341:21-22

42. Darnell RB, Posner JB. Observing the invisible: successful tumor immunity in humans. *Nature immunology* 2003;4:201;10.1038/ni0303-201.

43. Graus F, Dalmou J, Rene R, et al. Anti-Hu antibodies in patients with small-cell lung cancer: association with complete response to therapy and improved survival. *Journal of clinical oncology : official journal of the American Society of Clinical Oncology* 1997;15:2866-2872

Conflict of interest statement:

I.G, L.S, N.C, T.M, D.H, and V.P declare no conflict of interest. E.A declares non-finantial support from Astra-Zeneca (AZ) and personal fees from Lilly outside the submitted work. CM reports personal fees from Clovis Oncology, PRMA consulting, Novartis, Lilly, Pfizer, GlaxoSmithKline, Ernst and Young, Amgen, Astra Zeneca, personal fees, non-financial support and other from Boehringer Ingelheim, personal fees and non-financial support from Bristol Myers Squibb, Pierre Fabre and Merck Sharp and Dome, personal fees and non-financial support from Roche, outside the submitted work; SD reports research funding from Astex Pharmaceuticals, Lilly, Plexicon, Bayer, Synta, Amgen, Boehringer Ingelheim and GlaxoSmithKline, non-financial support and other from Bristol Myers Squibb, non-financial support from Merck Sharp and Dohme, outside the submitted work; T.G declares personal fees from Bristol-Myers Squibb and Pfizer outside the submitted work. M.W declares personal fees and non-financial support from Bristol-Myers Squibb outside the submitted work. R.G. declares personal fees from Bristol-Myers Squibb outside the submitted work. J.C reports non-financial support from Novartis, Boehringer Ingelheim, and Lilly, outside the submitted work. P.J.W report non-financial support from Bristol-Myers Squibb, outside the submitted work. L.N declares personal fees from Bristol-Myers Squibb and Merck outside the submitted work. C.O reports grants from Bristol Myers Squibb, during the conduct of the study; personal fees from Transgene, Bristol Myers Squibb, Immatics, and Merck, other from Inovio, personal fees, non-financial support and other from Bristol Myers Squibb, and MSD, other from Verastem, Biontech AG, Serametrix, Touchlight genetics, personal fees and non-financial support from Roche, outside the submitted work .

Figure legends

Figure 1. CONSORT diagram showing the disposition of patients in the ICE study

Figure 2. Kaplan-Meier plots for progression free survival according to RECIST v1.0 criteria (A) and immune related response criteria (B) and according to autoantibody status at baseline (C)

Figure 3. Kaplan-Meier plots for overall survival (A) and according to autoantibody status at baseline (B)

Table 1. Baseline patient demographics and disease characteristics (efficacy population n=38)

Demographic or disease characteristics	n (%)
Age, years	
Median	63
Range	44-84
Sex	
Female	13 (34.2)
Male	25 (65.8)
ECOG PS	
0	11 (34.4)
1	21 (65.6)
Missing	*6*
Index and non-index lesions	
Lung	27 (71.1)
Lymph node	27 (71.1)
Liver	15 (39.5)
Bone	3 (7.9)
CNS	1 (2.6)
Effusion	2 (5.3)
Soft tissue	7 (18.4)
Other	13 (34.2)
LDH (IU/L)	
Median	398
Range	186-1252
Missing	*4*
IgG (g/L)	
Median	8.10
Range	0-18.00
Not performed/missing	*3*
IgA(g/L)	
Median	2.20
Range	0.70-4.20
Not performed/missing	*4*
IgM(g/L)	
Median	0.75
Range	0.20-2.60
Not performed/missing	*4*

IgG=Immunoglobulin G. IgA=Immunoglobulin A. IgM=Immunoglobulin M. LDH=Lactate Dehydrogenase. CNS=Central Nervous System.

Denominator is **non-missing** data for the analysis population for each test performed

Table 2. Autoantibody analysis at baseline (efficacy population n=38)

Autoantibody assays	N (%)
Anti-SOX2	
Positive	9 (23.7%)
Negative	29 (76.3%)
Not performed/missing	*0*
Anti-Hu	
Positive	6 (15.8%)
Negative	32 (84.2%)
Not performed/missing	*0*
Anti-Yo	
Positive	2 (6.5)
Negative	29 (93.5)
Not performed/missing	*7*
Anti VGCCA	
Positive	0
Negative	24 (100)
Not performed/missing	*14*
Anti VGPCA	
Positive	2 (8.3)
Negative	22 (91.7)
Not performed/missing	*14*
Thyroid peroxidase	
Positive	4 (16.0)
Negative	21 (84.0)
Not performed/missing	*13*
Rheumatoid factors	
Positive	3 (12.5)
Negative	21 (87.5)
Not performed/missing	*14*
Anti-muscle antibodies	
Positive	0
Negative	33 (100)
Not performed/missing	*5*
ANA	
Positive	10 (28.6)
Negative	25 (71.4)
Not performed/missing	*3*
ANCA	
Positive	2 (8.3)
Negative	22 (91.7)
Not performed/missing	*14*

VGCCA=Voltage-Gated Calcium Channel Antibody. VGPCA=Anti-Voltage Gated Potassium Channel Antibodies. ANA= anti-nuclear antibodies. ANCA= anti-neutrophil cytoplasmic antibodies

Denominator is **non-missing** data for the analysis population for each test performed

Table 3. Best overall tumour response (efficacy population n=38)

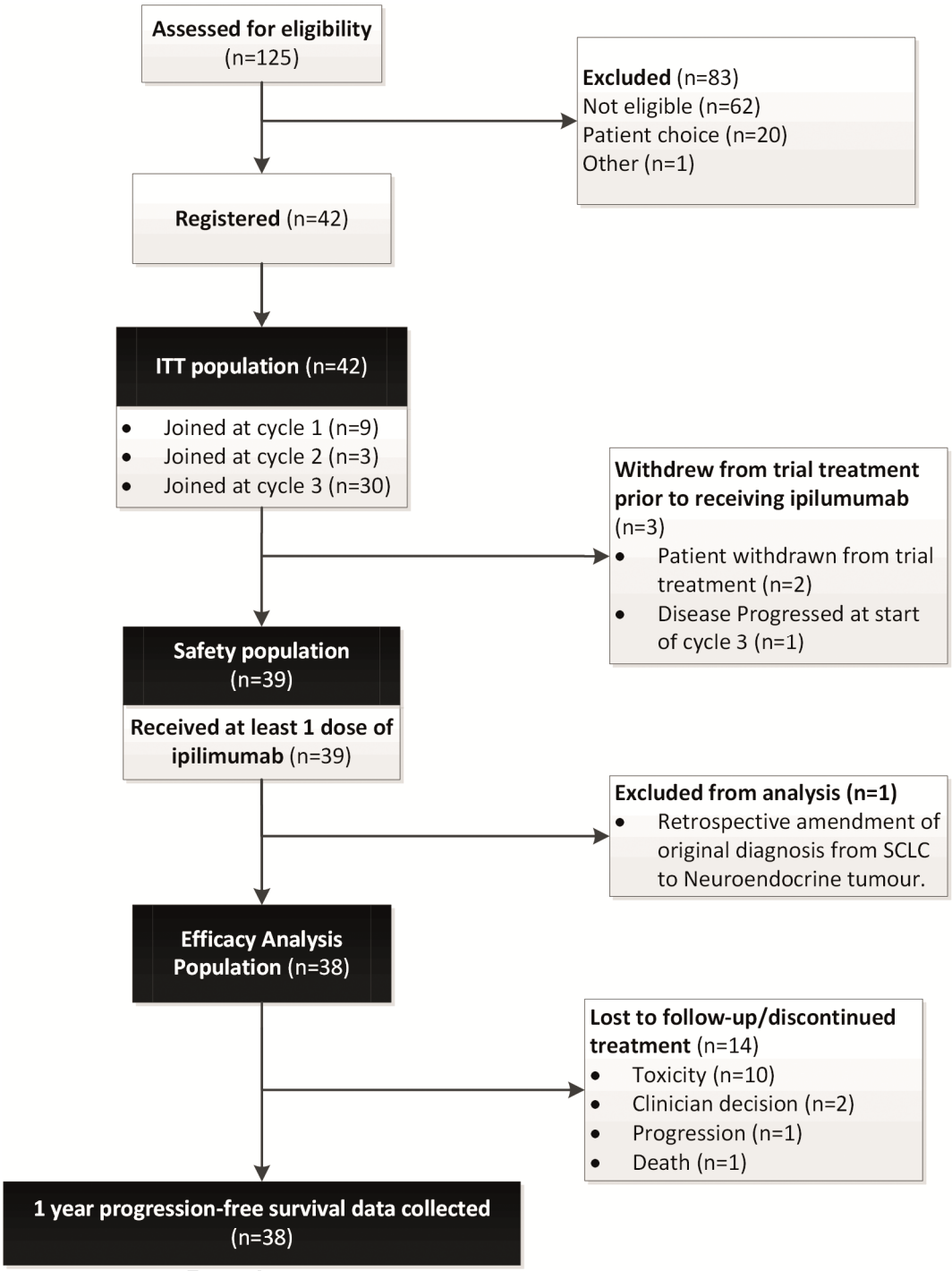
Tumour response	
RECIST v1.0	**n (%)**
Complete response	1 (3.4%)
Partial response	20 (69.0%)
Stable disease	3 (10.3%)
Progressive disease	5 (17.2%)
Not assessed/missing	*9*
Immune-related response criteria (irRC)	
Complete response	2 (6.1%)
Partial response	26 (78.8%)
Stable disease	5 (15.2%)
Progressive disease	0
Not assessed/missing	*5*

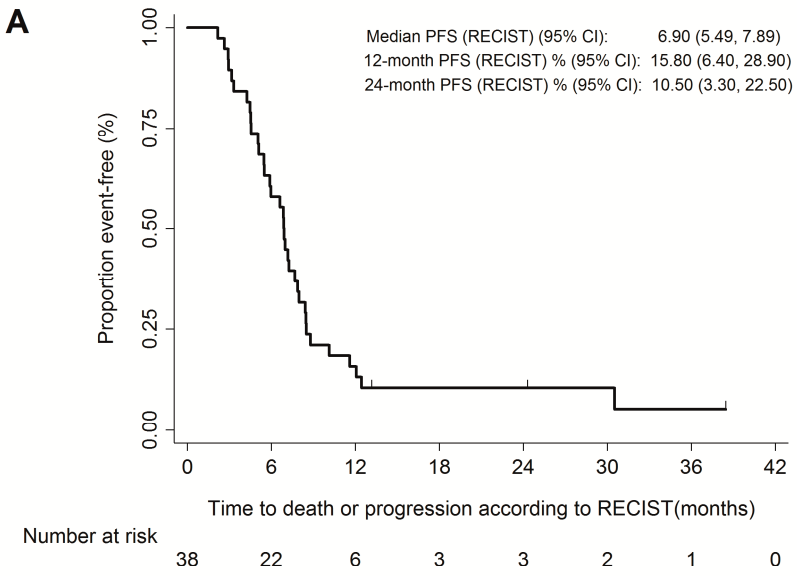
Table 4. Summary of grade 3 or above toxicities (patients receiving at least 1 cycle of ipilimumab, n=39)

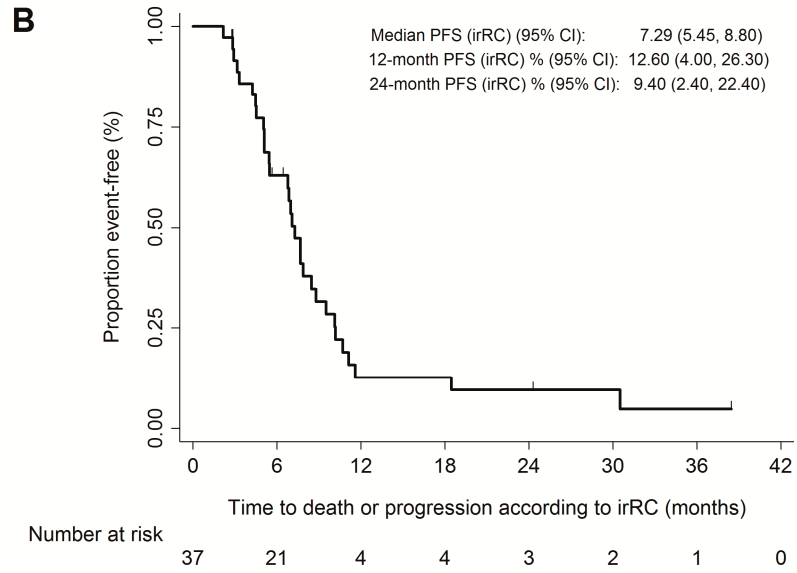
Toxicity	Total	Ipilimumab[1]	Carboplatin[1]	Etoposide[1]
Patients with at least one Grade 3 or above AE	35 (89.7%)	27 (69.2%)	25 (64.1%)	25(64.1%)
Neurological				
Generalised muscle weakness	1 (2.6%)	1 (2.6%)	0	0
Headache	1 (2.6%)	1 (2.6%)	0	0
Agitation	1 (2.6%)	1 (2.6%)	0	0
Nervous system disorder	1 (2.6%)	1 (2.6%)	0	0
Central neuropathy	1 (2.6%)	1 (2.6%)	0	0
Other immune related				
ALT increase/transaminitis	3 (7.7%)	3 (7.7%)	0	0
Alkalyne phosphatase increase	3 (7.7%)	3 (7.7%)	0	0
Autoimmune disorder	2 (5.1%)	2 (5.1%)	0	0
Colitis*/diarrhoea	19 (48.7%)	19 (48.7%)	6 (15.4%)	7 (18%)
Hyperglycemia	2 (5.1%)	1 (2.6%)	1 (2.6%)	1 (2.6%)
Lymphocyte count decrease	2 (5.1%)	0	1 (2.6%)	1 (2.6%)
Neutrophil count decrease	9 (23.1%)	2 (5.1%)	8 (20.5%)	8 (20.5%)
Rash	1 (2.6%)	1 (2.6%)	0	0
Thrombocytopenia	2 (5.1%)	1 (2.6%)	2 (5.1%)	2 (5.1%)
Other				
Anaemia	6 (15.4%)	0	6 (15.4%)	6 (15.4%)
Dyspnoea	3 (7.7%)	1 (2.6%)	1 (2.6%)	1 (2.6%)
Fatigue	3 (7.7%)	1 (2.6%)	2 (5.1%)	2 (5.1%)
Febrile neutropenia	3 (7.7%)	0	2 (5.1%)	2 (5.1%)
Hyponatraemia	3 (7.7%)	0	1 (2.6%)	0
Infection	11 (28.2%)	3 (7.7%)	7 (18%)	7 (18%)
Sepsis	4 (10.3%)	2 (5.1%)	2 (5.1%)	2 (5.1%)
Thromboembolic event	2 (5.1%)	2 (5.1%)	2 (5.1%)	2 (5.1%)

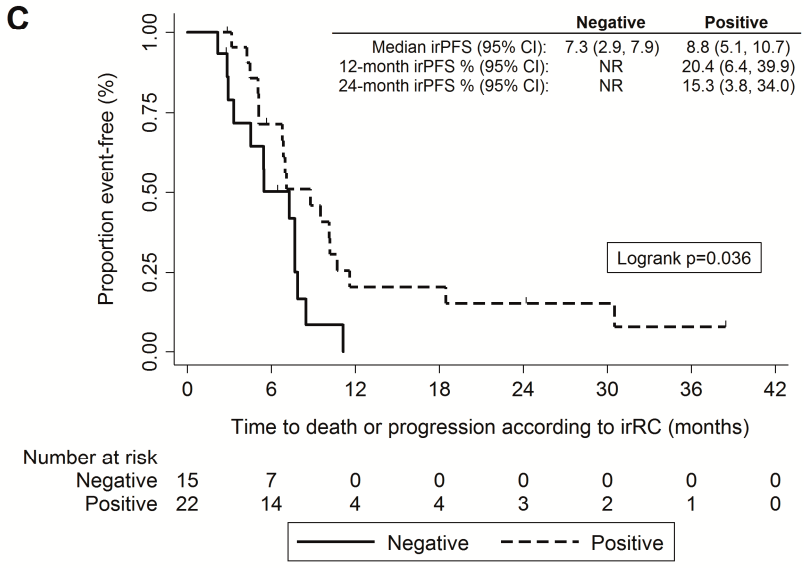
* One case of ileitis is included

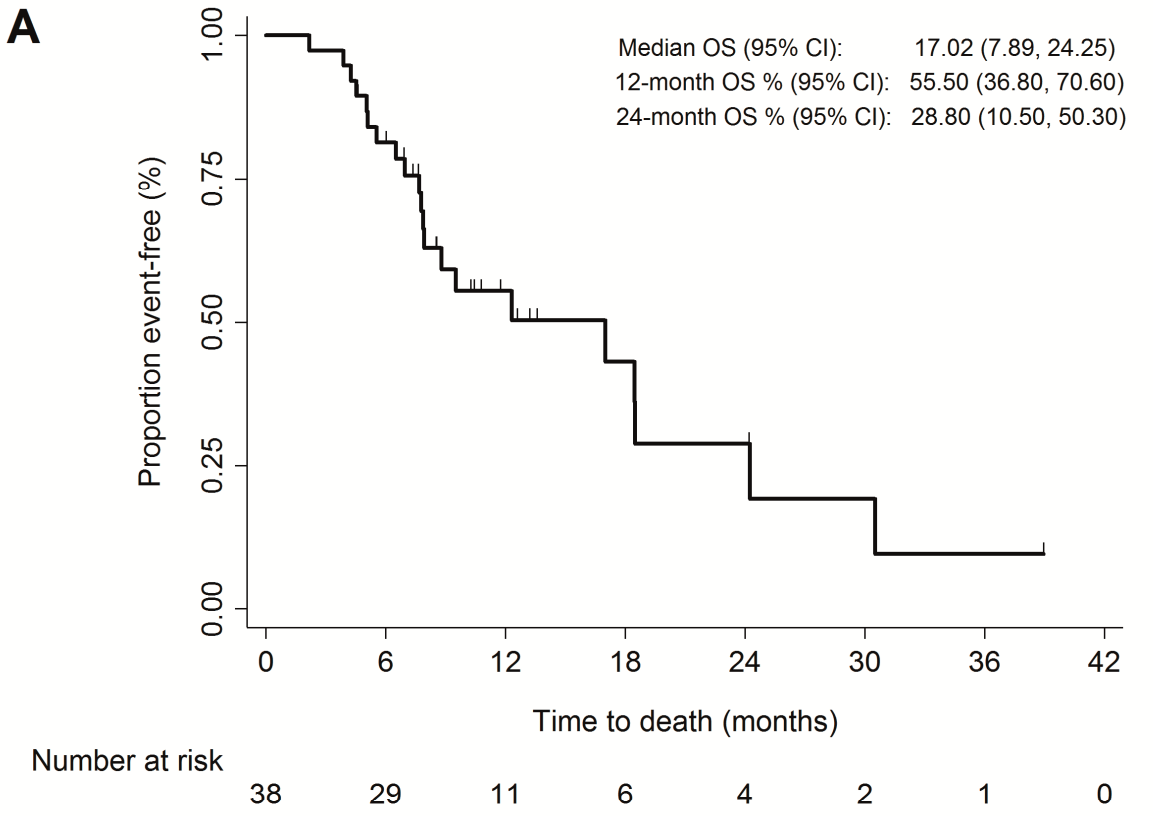
[1] Toxicities assessed by site principal investigator to be definitely, probably or possibly related to study drug

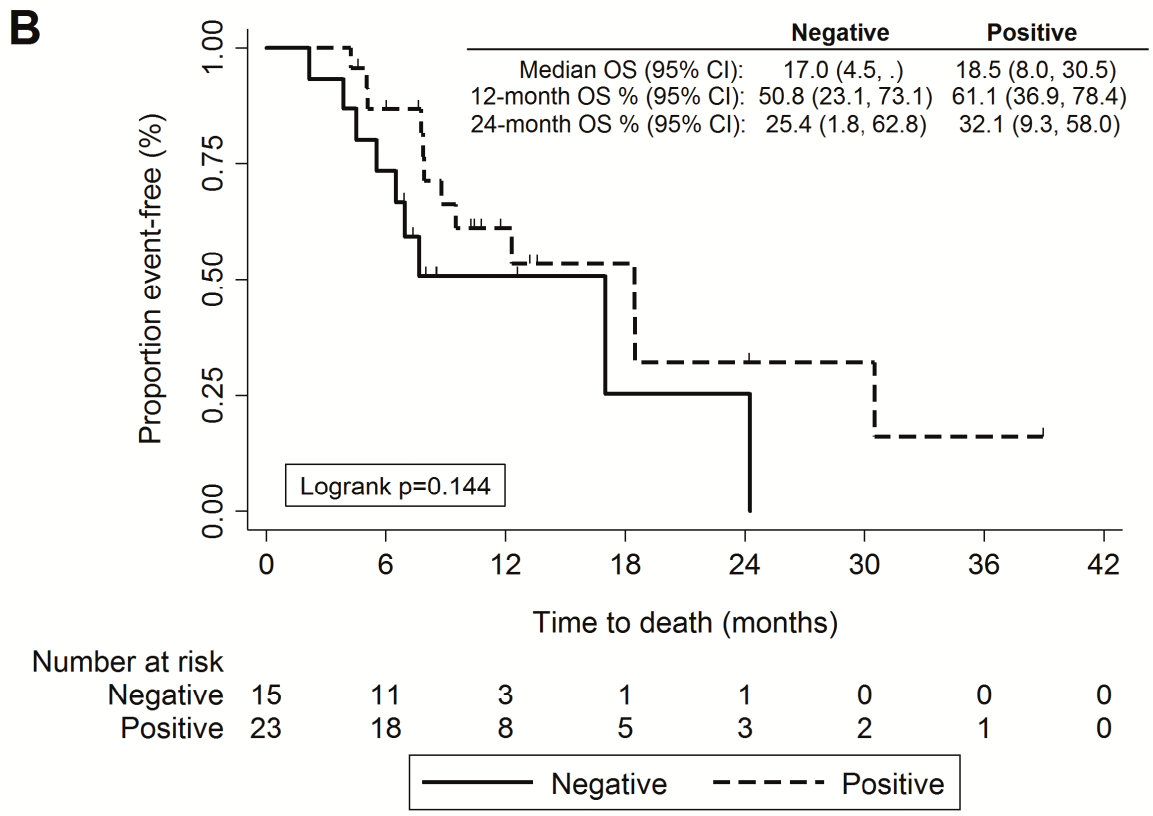

Assessed for eligibility (n=125)
Excluded (n=83)
Not eligible (n=62)
Patient choice (n=20)
Other (n=1)
Registered (n=42)
ITT population (n=42)
Joined at cycle 1 (n=9)
Joined at cycle 2 (n=3)
Joined at cycle 3 (n=30)
Withdrew from trial treatment prior to receiving ipilumumab (n=3)
Patient withdrawn from trial treatment (n=2)
Disease Progressed at start of cycle 3 (n=1)
Safety population (n=39)
Received at least 1 dose of ipilimumab (n=39)
Excluded from analysis (n=1)
Retrospective amendment of original diagnosis from SCLC to Neuroendocrine tumour.
Efficacy Analysis Population (n=38)
Lost to follow-up/discontinued treatment (n=14)
Toxicity (n=10)
Clinician decision (n=2)
Progression (n=1)
Death (n=1)
1 year progression-free survival data collected (n=38)


A
Median PFS (RECIST) (95% CI): 6.90 (5.49, 7.89)
12-month PFS (RECIST) % (95% CI): 15.80 (6.40, 28.90)
24-month PFS (RECIST) % (95% CI): 10.50 (3.30, 22.50)
Proportion event-free (%)
0.00
0.25
0.50
0.75
1.00
0
6
12
18
24
30
36
42
Time to death or progression according to RECIST(months)
Number at risk
38 22 6 3 3 2 1 0


B
Median PFS (irRC) (95% CI): 7.29 (5.45, 8.80)
12-month PFS (irRC) % (95% CI): 12.60 (4.00, 26.30)
24-month PFS (irRC) % (95% CI): 9.40 (2.40, 22.40)
Proportion event-free (%)
0.00
0.25
0.50
0.75
1.00
0
6
12
18
24
30
36
42
Time to death or progression according to irRC (months)
Number at risk
37 21 4 4 3 2 1 0


C
Negative
Positive
Median irPFS (95% CI): 7.3 (2.9, 7.9) 8.8 (5.1, 10.7)
12-month irPFS % (95% CI): NR 20.4 (6.4, 39.9)
24-month irPFS % (95% CI): NR 15.3 (3.8, 34.0)
Logrank p=0.036
Proportion event-free (%)
0.00
0.25
0.50
0.75
1.00
0
6
12
18
24
30
36
42
Time to death or progression according to irRC (months)
Number at risk
Negative 15 7 0 0 0 0 0 0
Positive 22 14 4 4 3 2 1 0
Negative
Positive


A
Median OS (95% CI): 17.02 (7.89, 24.25)
12-month OS % (95% CI): 55.50 (36.80, 70.60)
24-month OS % (95% CI): 28.80 (10.50, 50.30)
Proportion event-free (%)
0.00
0.25
0.50
0.75
1.00
0
6
12
18
24
30
36
42
Time to death (months)
Number at risk
38 29 11 6 4 2 1 0


B
Negative Positive
Median OS (95% CI): 17.0 (4.5, .) 18.5 (8.0, 30.5)
12-month OS % (95% CI): 50.8 (23.1, 73.1) 61.1 (36.9, 78.4)
24-month OS % (95% CI): 25.4 (1.8, 62.8) 32.1 (9.3, 58.0)
Logrank p=0.144
Proportion event-free (%)
0.00
0.25
0.50
0.75
1.00
0
6
12
18
24
30
36
42
Time to death (months)
Number at risk
Negative 15 11 3 1 1 0 0 0
Positive 23 18 8 5 3 2 1 0
Negative
Positive