Received: 6 August 2024 | Revised: 21 April 2025 | Accepted: 26 April 2025

DOI: 10.1002/cac2.70032

CANCER COMMUNICATIONS Open Access

ORIGINAL ARTICLE

First-line serplulimab plus chemotherapy in extensive-stage small-cell lung cancer: Updated results and biomarker analysis from the ASTRUM-005 randomized clinical trial

Ying Cheng[1] | **Shuang Zhang**[1] | **Liang Han**[2] | **Lin Wu**[3] | **Jun Chen**[4] | **Peiyan Zhao**[1] | **Hongmei Sun**[5] | **Guilan Wen**[6] | **Yinghua Ji**[7] | **Anastasia Zimina**[8] | **Jianhua Shi**[9] | **Zhijie Pan**[10] | **Jinsheng Shi**[11] | **Xicheng Wang**[12] | **Yuansong Bai**[13] | **Tamar Melkadze**[14] | **Yueyin Pan**[15] | **Xuhong Min**[16] | **Maksym Viguro**[17] | **Xingya Li**[18] | **Yanqiu Zhao**[19] | **Junquan Yang**[20] | **Tamta Makharadze**[21] | **Ekaterine Arkania**[22] | **Haoyu Yu**[23] | **Jing Li**[23] | **Fang Yang**[24] | **Xinyi Yang**[24] | **Chen Ling**[24] | **Qingyu Wang**[23] | **Yongqiang Shan**[24] | **Jun Zhu**[25] | **On behalf of the ASTRUM-005 Study Group**[#]

Correspondence
Ying Cheng, Department of Oncology, Jilin Cancer Hospital, 1066 Jinhu Road, High-tech Zone, Changchun 130000, Jilin, P. R. China.
Email: jl.cheng@163.com

[#]A complete list of the ASTRUM-005 Study Group members appears in Supplementary Table S1.

Funding information
Shanghai Henlius Biotech Inc.; National Natural Science Foundation of China, Grant/Award Number: 82473000

Abstract

Background: The ASTRUM-005 study previously demonstrated a significant overall survival (OS) benefit with serplulimab (a programmed death 1 inhibitor) plus chemotherapy versus chemotherapy alone in previously untreated extensive-stage small-cell lung cancer (ES-SCLC). Here, we report updated efficacy and safety results after an extended median follow-up of 19.8 months, along with the first report on findings from exploratory biomarker analyses.

Methods: A total of 585 patients were randomized in a 2:1 ratio to receive 4.5 mg/kg serplulimab (n = 389) or placebo (n = 196) intravenously every 3 weeks, together with carboplatin and etoposide. The primary endpoint was

Abbreviations: AE, Adverse event; AKT, Ak strain transforming; AUC, Area under the curve; CI, Confidence interval; COVID-19, Coronavirus disease 2019; CPS, Combined positive score; DAB, 3,3'-Diaminobenzidine tetrahydrochloride; DEP, Differentially expressed protein; DOR, Duration of response; ECOG, Eastern Cooperative Oncology Group; ENPP2, Ectonucleotide pyrophosphatase/phosphodiesterase family member 2; ES-SCLC, Extensive-stage small cell lung cancer; HR, Hazard ratio; irAE, Immune-related adverse event; iRECIST, Modified RECIST for immunotherapies; IRRC, Independent radiology review committee; LDH, Lactate dehydrogenase; MMP7, Matrilysin; MSI, Microsatellite instability; MSI-H, Microsatellite instability-high; MSI-L, Microsatellite instability-low; MSS, Microsatellite stable; NLR, Neutrophil-to-lymphocyte ratio; NSCLC, Non-small-cell lung cancer; ORR, Objective response rate; OS, Overall survival; PD-1, Programmed death 1; PD-L1, Programmed death-ligand 1; PFS, Progression-free survival; PI3K, Phosphoinositide 3-kinase; PLCB1, 1-phosphatidylinositol 4; PLR, Platelet-to-lymphocyte ratio; PRC1, Protein regulator of cytokinesis 1; *RB1*, Retinoblastoma-1; RECIST, Response evaluation criteria in solid tumors; ROC, Receiver-operating characteristic; SCLC, Small-cell lung cancer; SH3BGRL2, SH3 domain-binding glutamic acid-rich-like protein 2; SMC3, Structural maintenance of chromosomes protein 3; STAT3, Signal transducer and activator of the transcription 3; TEAE, Treatment-emergent adverse event; TME, Tumor microenvironment; *TP53*, Tumor protein 53; TPS, Tumor proportion score; TSPAN1, Tetraspanin-1; VCF, Variant call format.

Ying Cheng and Shuang Zhang contributed equally to this work.

OS. In addition, genomic profiling was performed to identify mutated genes, and quantitative serum proteome profiling was conducted to identify differentially expressed proteins (DEPs) between responders and non-responders of serplulimab plus chemotherapy. Regression analysis was subsequently used to construct a protein signature based on the DEPs. The associations between efficacy outcomes (objective response rate [ORR], OS, and progression-free survival [PFS]) and gene mutation status or DEP expression were also examined with regression analysis. Furthermore, the prognostic value of hematological parameters was evaluated.

Results: In the intent-to-treat population, the median OS was 15.8 months in the serplulimab group versus 11.1 months in the placebo group (hazard ratio, 0.62; 95% confidence interval, 0.50-0.76; $P < 0.001$). We identified 181 DEPs between responders and non-responders in the serplulimab group, from which a 15-protein signature was constructed. In the serplulimab group, patients with a higher 15-protein signature score were associated with significantly longer OS and PFS. Also, patients harboring tumor-suppressor retinoblastoma-1 (*RB1*) mutations or mutations in Notch pathway members showed improved ORR, OS, or PFS compared with their wild-type counterparts. Baseline neutrophil-to-lymphocyte ratio (NLR) and lactate dehydrogenase (LDH) level were independent prognosticators of patients with ES-SCLC.

Conclusions: First-line serplulimab provided a sustained clinical benefit over placebo in patients with ES-SCLC. A 15-protein signature and mutations in *RB1* or Notch pathway genes may serve as predictive biomarkers for benefits from serplulimab plus chemotherapy, while baseline NLR and LDH were independent prognosticators for ES-SCLC.

Trial registration: ClinicalTrials.gov, NCT04063163

KEYWORDS

Serplulimab, ES-SCLC, ASTRUM-005, phase 3

1 | BACKGROUND

Small-cell lung cancer (SCLC), which accounts for approximately 15% of lung cancer cases, is an aggressive cancer type [1]. Approximately two-thirds of patients with SCLC were diagnosed at the extensive stage, with a 5-year survival rate of 7% [1, 2]. Clinical trials evaluating novel treatment options, therefore, hold significant clinical value for patients with SCLC.

The addition of a programmed death-ligand 1 (PD-L1) inhibitor to chemotherapy represents an advancement in the treatment of extensive-stage SCLC (ES-SCLC) [3]. Phase 3 clinical trials, evaluating atezolizumab (IMpower133) [4] and durvalumab plus chemotherapy (CASPIAN) [5] as first-line treatment for ES-SCLC, showed statistically significant improvement in overall survival (OS). However, IMpower133 and CASPIAN reported a median OS prolongation ranging from 2.0 to 2.5 months, highlighting the need for further advancements in therapeutic strategies to achieve greater survival benefits.

The lack of established biomarkers for predicting clinical benefits from chemoimmunotherapy presents another knowledge gap in ES-SCLC management [6, 7]. PD-L1 showed potential as an efficacy predictor for therapies containing a programmed death 1 (PD-1) or PD-L1 inhibitor in several solid tumors; however, its expression is low in SCLC [7]. Several trials, including IMpower133 [4], CASPIAN [5], ASTRUM-005 [8], KEYNOTE-028 [9], and KENOTE-158 [9, 10], showed antitumor activity of the PD-1/PD-L1 inhibitor irrespective of PD-L1 expression. Conflicting results have been reported for other potential markers, including tumor mutation burden, which showed association with outcomes in patients treated with pembrolizumab in KEYNOTE-028 [11] and KEYNOTE-158

[12] but not in CAPSIAN [13]. A plethora of promising candidates, including transcription subtypes [14, 15], neoantigen load [16], immune cell infiltration [17], and so on [7], have been proposed based mostly on exploratory or retrospective analyses, awaiting further validation. To summarize, challenged by the inherent limitations of clinical data acquisition, robust predictive/prognostic biomarkers of ES-SCLC with high precision and sensitivity have yet to be identified.

Serplulimab is a fully humanized immunoglobulin G4 monoclonal antibody against PD-1. Serplulimab possesses a higher affinity to the human PD-1 than nivolumab and pembrolizumab in vitro [18]. The international phase 3 ASTRUM-005 trial was the first to demonstrate improved survival outcomes upon the addition of a PD-1 inhibitor to chemotherapy. Serplulimab plus chemotherapy (carboplatin combined with etoposide) significantly improved OS for 4.5 months in patients with previously untreated ES-SCLC compared to placebo plus chemotherapy at the prespecified interim analysis (median OS 15.4 vs. 10.9 months; hazard ratio [HR] = 0.63, 95% confidence interval [CI] = 0.49-0.82; $P < 0.001$) [8]. Nonetheless, the efficacy and safety outcomes with longer durations of follow-up remain to be revealed.

An updated efficacy and safety analysis was conducted after a prespecified number ($n = 342$) of OS events had occurred per the study protocol [8]. Here, we presented updated efficacy and safety results from this analysis. The median follow-up duration was 19.8 months, which had an additional median follow-up of 7.5 months since the previous (interim) analysis. Furthermore, we present findings from exploratory biomarker analyses, in which multiple candidates — including proteins (quantitative proteomics), genes (genomic profiling), and hematological parameters (routine blood tests) — were evaluated as potential predictive or prognostic biomarkers. These data provided further evidence supporting serplulimab plus chemotherapy as a first-line therapy for patients with ES-SCLC. In addition, insights into the biomarker predicting survival benefit from serplulimab plus chemotherapy or prognosis pave the way for further mechanistic and clinical research.

2 | METHODS

2.1 | Study design and patient recruitment

This was a multicenter, randomized, double-blind, placebo-controlled, phase 3 trial conducted in previously untreated patients with ES-SCLC (NCT04063163). Details of the study design and patient eligibility criteria, along with the study protocol, any amendments, and statistical analysis plan, have been previously reported [8]. Briefly, eligible patients were randomized (2:1) to receive serplulimab plus chemotherapy (serplulimab group) or placebo plus chemotherapy (placebo group). Major eligibility criteria included patients with no prior systemic therapy for histologically or cytologically confirmed ES-SCLC and Eastern Cooperative Oncology Group (ECOG) performance status of 0 or 1. This study was conducted in accordance with the principles of the Declaration of Helsinki, Good Clinical Practice guidelines, and local practice. The study was approved by the central or independent institutional review board/ethics committee (approval ID: 201903-014-01). All participants provided written informed consent.

2.2 | Randomization

Details of the randomization of this study have been reported previously [8]. Stratification factors for randomization included PD-L1 expression level (tumor proportion score [TPS] < 1%, ≥ 1%, or not evaluable/available) evaluated by immunohistochemistry, brain metastases (yes or no), and age (≥65 or <65 years).

2.3 | Procedures

Details of the study interventions have been reported previously [8]. Briefly, patients were given either serplulimab or a placebo at 4.5 mg/kg plus chemotherapy (carboplatin combined with etoposide) intravenously every 3 weeks. Chemotherapy was administered for up to 4 cycles.

Treatment-emergent adverse event (TEAE) was graded according to the National Cancer Institute Common Terminology Criteria for Adverse Events version 5.0 [19]. The investigator evaluated the causality between each study drug (serplulimab, placebo, carboplatin, and etoposide) and the TEAE, classifying it into one of five categories: related, possibly related, unlikely related, unrelated, or unknown. The criteria for classification are based on five factors and are detailed in Supplementary Table S2. TEAEs classified as related, possibly related, and unknown were recorded as related to the corresponding study drug. Deaths due to disease progression or coronavirus disease 2019 (COVID-19) were recorded as serious adverse events (AEs) and were counted towards the TEAEs leading to death. Microsatellite instability (MSI) status and PD-L1 expression (TPS and combined positive score [CPS]) were analyzed centrally. MSI status was analyzed using the Med1CDx™ Panel (MEDx Translational Medicine, Suzhou, Jiangsu, P. R. China), an in-house validated

next-generation sequencing-based method, on the Illumina platform (Illumina, San Diego, CA, USA).

Retrospective assessments were conducted on baseline samples for serum proteome, tumor tissue genomic profile, and hematological parameters to identify potential prognostic or predictive biomarkers.

2.4 | Proteomic profiling

Serums from 168 patients with available blood samples were used in this experiment. For each sample, 2.8 μL was used for protein expression assay using Olink® Explore 3072 (Cat. #BD0017, Olink Proteomics AB, Uppsala, Sweden) according to the manufacturer's instructions. The technology behind the Olink protocol was based on the Proximity Extension Assay, coupled with readout via next-generation sequencing. The assay enabled the detection of up to 2,925 proteins in 88 samples simultaneously, using only 2.8 μL of serum/plasma sample. In brief, pairs of oligonucleotide-labeled antibody probes designed for each protein bound to their target, bringing the complementary oligonucleotides in close proximity and allowing for their hybridization. The addition of a DNA polymerase led to the extension of the hybridized oligonucleotides, generating a unique protein identification "barcode." Next, library preparation added sample identification indexes and the required nucleotides for Illumina sequencing. Prior to sequencing using the Illumina NovaSeq 6000 system (Cat. #20012850, Illumina), libraries went through a bead-based purification step, and the quality was assessed using the Agilent 4200 TapeStation (Cat. #G2991BA, Agilent Technologies, Palo Alto, CA, USA). The raw output from the sequencer was converted into count data by bcl2counts (v2.2.0, Olink Proteomics AB) provided by Olink. Subsequently, the count data were imported into Olink NPX manager (v3.10.0, Olink Proteomics AB) to carry out quality control, normalization, and the conversion to Normalized Protein eXpression, Olink's proprietary unit of relative abundance. Data normalization was performed using an internal extension control and an external plate control to adjust for intra- and inter-run variation. All assay validation data (detection limits, intra- and inter-assay precision data, predefined values, etc.) were made available on the manufacturer's website (www.olink.com).

2.5 | DNA extraction

DNA from tumor samples from 305 ES-SCLC patients receiving serplulimab or placebo plus chemotherapy was extracted using the QIAamp DNA Formalin-Fixed Paraffin-Embedded Kit (Cat. #56404, Qiagen, Hilden, Germany). DNA concentration was quantified with the Qubit 3.0 (Cat. #Q33216, Thermo Fisher, Waltham, MA, USA), while purity was assessed with the Nanodrop 8000 (Cat. #ND-8000-GL, Thermo Fisher), and integrity was evaluated via agarose gel electrophoresis. Absorbance at 230 nm, 260 nm, and 280 nm was used to assess the purity of DNA. Samples with 260 nm/280 nm ratios of approximately 1.8 and 260 nm/230 nm ratios of 2.0 to 2.2 were accepted and used for subsequent analysis.

2.6 | Library preparation and DNA-targeted panel sequencing

The library for DNA-targeted sequencing was constructed using the NEBNext Ultra II DNA Library Prep Kit for Illumina (Cat. #E7645, New England Biolabs, Ipswich, MA, USA). DNA samples were fragmented using Covaris M220 (Cat. #500295, Covaris, Woburn, MA, USA), followed by end repair, A-addition, adapter ligation, and amplification. Targeted regions were captured using the Med1CDx™ Panel (Cat. #N020B01, MEDx Translational Medicine), which covers all exons, certain introns, and certain key hotspots across 601 genes. Sequencing was performed on the Illumina NovaSeq 6000 system (Cat. #20012850, Illumina), generating 150 bp paired-end reads.

2.7 | Bioinformatics processing on DNA sequencing data

The raw FASTQ files were processed by fastp (version 0.20.0) to trim adapters and remove low-quality reads [20]. The qualified reads were then mapped to the reference genome hg19 by using BWA (version 0.7.17) [21]. The BAM file was sorted by Samtools (version 1.9) [22] and marked duplicated reads by Picard (version 2.20, https://broadinstitute.github.io/picard/faq.html) [23]. Varscan (version 2.4.3) was used to call single nucleotide variants and insertions/deletions [24]. The ANNOVAR (release date: June 01, 2017) was then used to annotate the variant call format (VCF) file [25]. We defined non-synonymous single nucleotide variants or small (<50 base pairs) insertions/deletions as genetic mutations. Patients bearing wild-type variants of *NOTCH1, NOTCH2, NOTCH3*, and *NOTCH4* were considered wild-type in Notch signaling pathway members, while patients bearing a mutant variant of at least one of the genes were considered mutant in Notch signaling pathway members.

2.8 | MSI testing

The Med1CDx™ panel (MEDx Translational Medicine) was used to assess five microsatellite loci. MSI status

in tumor tissue samples was determined based on the sequencing data generated by the panel. Tumors with instability at two or more of these loci were interpreted as MSI-high (MSI-H), while those with instability at only one of the five recommended loci were interpreted as MSI-low (MSI-L). Tumors with no instability at any of the five loci were considered microsatellite stable (MSS).

2.9 | Principal components analysis

Genetic mutation data in the format of VCF file was converted to a binary matrix using "maftools" (version 2.14.0) in R (version 4.4.1) [26]. Then, principal components analysis was performed using the "prcomp" function of the "stats" R package. Samples with an absolute rotated value greater than 10 in the first or second component were identified as outliers and removed from the following analysis.

2.10 | PD-L1 expression evaluation

The PD-L1 IHC 22C3 kit (Cat. #SK00621-5, Agilent Technologies) and Autostainer Link 48 (Cat. #D64751, Agilent Technologies) were used for assessing the tumor tissue PD-L1 expression. Human specimens were sectioned into blocks of approximately 0.5 cm × 1 cm × 1 cm, fixed with 10% neutral buffered formalin, and embedded with paraffin. A 1:50 dilution of the EnVision™ FLEX low-pH target retrieval solution provided by the kit was prepared, and the pH was adjusted to 6.1 ± 0.2. This dilution was subsequently incubated with the sample blocks at 97°C for 20 minutes for heat-induced antigen retrieval. Following antigen retrieval, endogenous peroxidase activity was blocked by incubating the samples with FLEX peroxidase blocking reagent (ready-to-use, provided by the kit) for 5 minutes at 25°C. Sample blocks were then incubated at room temperature for 60 minutes with mouse monoclonal antibody anti-PD-L1, clone 22C3 (ready-to-use, provided by the kit), or with mouse monoclonal immunoglobulin G1 (ready-to-use, provided by the kit). Then, after incubation with EnVision™ FLEX + Mouse LINKER (as the secondary antibody, ready-to-use, provided by the kit) at room temperature for 30 minutes, samples were then incubated with visualization reagent (dextran coupled with peroxidase molecules and goat anti-rabbit and -mouse immunoglobulins; ready-to-use, provided by the kit). Then, the specimens were incubated with 3,3'-diaminobenzidine tetrahydrochloride (DAB) chromogen (ready-to-use, provided by the kit) and DAB enhancer (cupric sulfate; ready-to-use, provided by the kit). Afterward, the specimens were counterstained with hematoxylin and eosin and cover-slipped. The results were interpreted using a light microscope (Cat. #BX43, Olympus, Tokyo, Japan). PD - L1 positive cells were defined as viable cells showing partial or complete membrane staining at any intensity. The CPS was calculated using the following formula:

$$\text{CPS} = \frac{\text{Number of PD-L1 positive cells (including tumor cells, lymphocytes, macrophages)}}{\text{Total number of viable tumor cells}} \times 100\%$$

If the calculation result exceeds 100, the maximum score is defined as CPS 100. The TPS was calculated using the following formula:

$$\text{TPS} = \frac{\text{Number of PD-L1 positive tumor cells}}{\text{Number of PD-L1 positive cells} + \text{Number of PD-L1 negative cells}} \times 100\%$$

2.11 | Outcomes

The primary endpoint was OS. OS is defined as the time from randomization to death from any cause. Secondary endpoints included progression-free survival (PFS), objective response rate (ORR), duration of response (DOR), TEAEs, and the relationship between efficacy and PD-L1 expression or MSI status. PFS is defined as the time from randomization to the first disease progression or death from any cause. ORR is defined as the proportion of patients achieving complete response or partial response. DOR is defined as the time from the first complete response or partial response to disease progression or death from any cause. Tumor response was

assessed by the independent radiology review committee (IRRC) and investigators per Response Evaluation Criteria in Solid Tumors (RECIST) v1.1. PFS was additionally assessed by the investigators per modified RECIST for immunotherapies (iRECIST). Exploratory endpoints included the identification of serum proteins whose levels are strongly associated with ORR, OS or PFS of patients receiving serplulimab and chemotherapy, the relationship between efficacy and genetic mutation of tumor tissue, and the relationship between efficacy and baseline hematological parameters, including neutrophil-to-lymphocyte-ratio (NLR), platelet-to-lymphocyte ratio (PLR), and lactate dehydrogenase level (LDH). NLR was calculated using the following formula:

$$\text{NLR} = \frac{\text{Neutrophil count in peripheral blood}}{\text{Lymphocyte count in peripheral blood}}$$

PLR was calculated using the following formula:

$$\text{PLR} = \frac{\text{Platelet count in peripheral blood}}{\text{Lymphocyte counts in peripheral blood}}$$

2.12 | Exploratory analysis

Patients who had confirmed complete response or partial response were classified as responders, whereas those who had stable disease or progressive disease were classified as non-responders. For exploratory analysis, we conducted comparative analyses of genomic mutations and hematological parameters between responders and non-responders in the serplulimab group, respectively. We also conducted quantitative serum proteome profiling on samples from responders versus non-responders in both the serplulimab group and the placebobo group. The identified proteins meeting all the following criteria were selected as markedly differentially expressed proteins for further processing: (1) The protein was identified in both comparisons: responders vs. non-responders in the serplulimab group, and responders vs. non-responders in the placebo group; (2) In the serplulimab group, the protein showed a statistically significant difference ($P < 0.05$) in one-sample *t*-test comparing responders vs. non-responders; (3) In contrast, in the placebo group, the same protein did not show a statistically significant difference ($P \geq 0.05$) in the corresponding comparison. These characteristics were considered potential biomarker candidates. The optimal cutoffs for hematological parameters to predict OS were determined using X-tile (version 3.6.1) [27]. The HR and its 95% CI were estimated using the unstratified Cox proportional hazards model, and Efron's method was used to handle ties. The stepwise selection was used in the multivariable Cox regression model to identify independent factors.

2.13 | Construction of serum proteome-based predictive models for OS or PFS

Patients with available serum proteome profiles in the serplulimab group were divided into the training set ($n = 80$) and the validation set ($n = 48$), while patients with available profiles in the placebo group were used as the control validation set ($n = 40$). Regression analysis using a generalized linear model with 5-fold cross-validation was performed on proteome profiles of the training set. Based on the regression results, a composite score was constructed using the expression levels of proteins strongly associated with objective response to the serplulimab group. The protein levels were normalized by Normalized Protein eXpression, Olink's proprietary unit for relative abundance.

2.14 | Identification of the cutoff for patient subgrouping in survival analysis

The receiver-operating characteristic (ROC) curve was used to evaluate the performance of the constructed protein signature score in predicting the objective response of treatment with serplulimab plus chemotherapy in the training, validation, and control validation sets. Besides, ROC was used to evaluate the performance of tetraspanin-1 (TSPAN1) or 1-phosphatidylinositol 4,5-bisphosphate phosphodiesterase beta-1 (PLCB1) expression in predicting the objective response of treatment with serplulimab plus chemotherapy in the training set.

Subsequently, for each ROC curve of the 15-protein score, TSPAN1 expression, or PLCB1 expression derived from the training set, the cutoff for patient subgrouping was defined as the Normalized Protein eXpression value corresponding to the point with the greatest Youden's index. ORR analysis of subgroups by the 15-protein score was performed on a combined dataset consisting of patients in the validation and control validation sets, while ORR analysis of subgroups by TSPAN1 expression or PLCB1 expression was performed on a combined dataset consisting of patients in the training, validation, and control validation sets. Survival analysis of OS or PFS in subgroups stratified by the 15-protein score, TSPAN1 expression, or PLCB1 expression was performed on a combined dataset consisting of patients in the training, validation, and control validation sets.

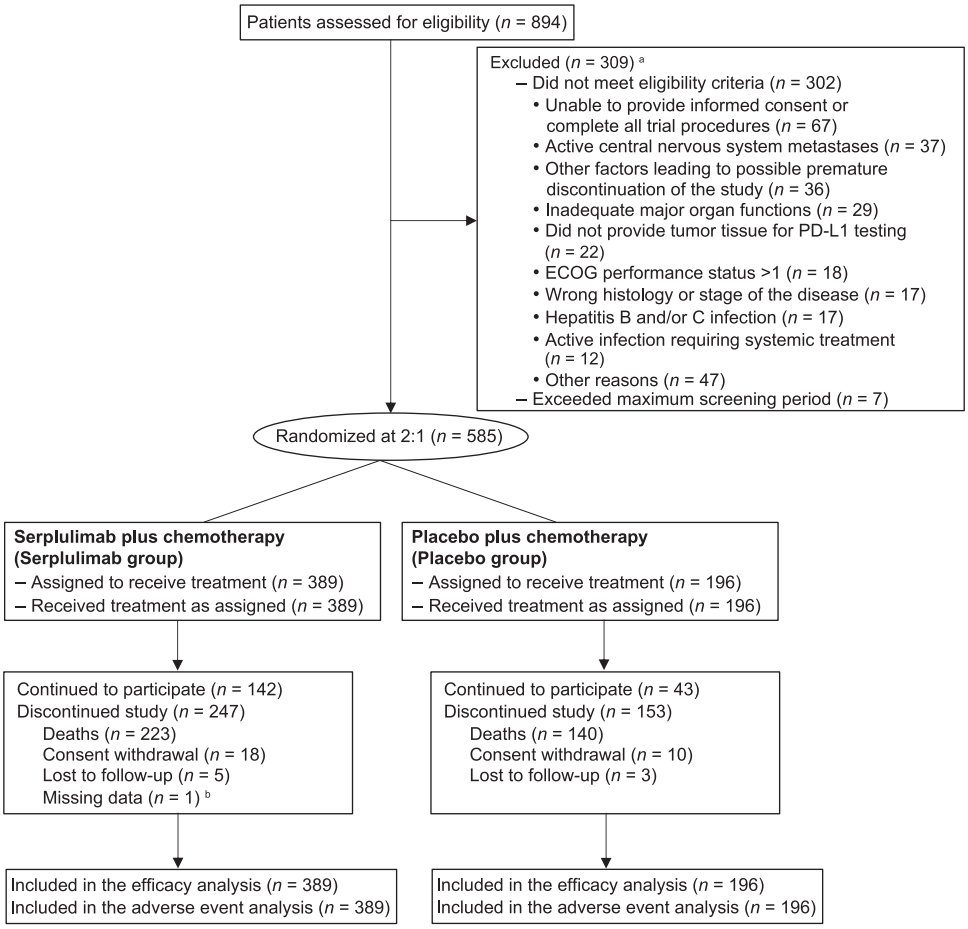


FIGURE 1 Consolidated Standards of Reporting Trials diagram of this study. [a] Full reasons for exclusion have been reported previously [8]. [b] The month of death was missing for 1 patient, who was excluded from overall survival analysis according to the statistical analysis plan. Abbreviations: ECOG, Eastern Cooperative Oncology Group; PD-L1, programmed death-ligand 1.

2.15 | Statistical analysis

Sample size calculation has been reported previously [8]. Briefly, it was estimated that 342 OS events would be needed to provide 85% power at a 2-sided α level of 0.05 to detect an HR for death of 0.7, assuming a median OS of 10 months in the placebo group and an entire study period lasting for 34 months with a 24-month enrollment period. The assumption of an HR for death of 0.7 was made on the basis of results from the IMpower133 trial [4]. Assuming a dropout rate of 20%, enrollment of 567 patients (378 in the serplulimab group and 189 in the placebo group) was needed.

The study met the primary endpoint at the prespecified interim analysis [8]. The updated analyses are considered descriptive in nature, and *P* values were not adjusted for multiplicity. Details of prespecified statistical analyses have been reported previously [8]. The statistical method for the exploratory analyses was performed according to Camp *et al.* [28]. Briefly, one-sample *t*-test was used to identify differentially expressed proteins (DEPs) between responders and non-responders to serplulimab or placebo plus chemotherapy. Principal component analysis was performed to identify outliers in genomic profiles. Regression analysis was performed to identify DEPs strongly associated with ORR. Association between clinical or molecular characteristics and OS or PFS were assessed with survival analysis. Survival curves were plotted using the Kaplan-Meier method. All statistical analyses were conducted using SAS version 9.4 software (SAS Institute Inc, Cary, NC, USA).

3 | RESULTS

3.1 | Patients and treatments

Between September 12, 2019 and April 27, 2021, 894 patients were screened, and 585 previously untreated ES-SCLC patients were randomly assigned in a 2:1 ratio to either the serplulimab group (n = 389) or the placebo group (n = 196; Figure 1). A total of 309 patients were

excluded due to not meeting eligibility criteria ($n = 302$) or exceeding the maximum screening period ($n = 7$). The comprehensive list of reasons for exclusion has been previously described [8]. Briefly, the most common reason was failure to provide informed consent or complete all study procedures ($n = 67$). Baseline characteristics, which have been reported previously [8], were balanced between the two groups. As of the data cutoff on June 13, 2022, the median follow-up was 19.8 months (range, 0.2-32.5 months). All patients received at least one dose of the study treatment, and all were included in the subsequent efficacy analysis and safety analysis. As of the data cutoff date, 247 (63.5%) patients in the serplulimab group and 153 (78.1%) in the placebo group had discontinued the study; the most common reason for study discontinuation was death. Subsequent anticancer treatments after the first progression are listed in Supplementary Table S3. Overall, 193 (49.6%) and 92 (46.9%) patients in the respective groups received post-progression therapies, including chemotherapy, immunotherapy, targeted therapy, or other anticancer therapies.

3.2 | Primary endpoint

As of the data cutoff date, 223 (57.3%) patients in the serplulimab group and 140 (71.4%) in the placebo group had died (Supplementary Table S4). Consistent with the interim analysis, serplulimab plus chemotherapy continued to show an OS benefit over placebo plus chemotherapy (median OS: 15.8 months versus 11.1 months; HR, 0.62 [95% CI, 0.50-0.76]; $P < 0.001$; Figure 2A). The estimated 1-year OS probabilities were 62.5% and 45.6% for the serplulimab and placebo group, respectively; the estimated 2-year OS probabilities were 31.7% and 18.7% for the serplulimab and placebo group, respectively.

3.3 | Secondary endpoints

Based on the assessments by the IRRC per RECIST v1.1, 248 (63.8%) patients in the serplulimab group and 164 (83.7%) in the placebo group had disease progression or died (Supplementary Table S4). The PFS benefit conferred by serplulimab plus chemotherapy was sustained (median PFS, 5.8 months versus 4.3 months; HR, 0.47 [95% CI, 0.38-0.58]; $P < 0.001$; Figure 2B), consistent with results at interim analysis [8]. The estimated 1-year PFS probabilities were 27.7% and 6.9% for the serplulimab and placebo group, respectively; the estimated 2-year PFS probabilities were 12.4% and 2.9% for the serplulimab and placebo group, respectively. Assessments by the investigator per RECIST v1.1 and iRECIST also consistently revealed prolonged median PFS in the serplulimab group than in the placebo group (Supplementary Figure S1).

The evaluations by the IRRC per RECIST v1.1 revealed a confirmed ORR of 68.9% for the serplulimab group and 58.7% for the placebo group, with 6 (1.5%) and 0 patients with complete response in the respective groups (Supplementary Table S5). Median DOR in the serplulimab group was longer than in the placebo group (6.5 months versus 4.2 months; HR, 0.45 [95% CI, 0.35-0.59]; $P < 0.001$). The serplulimab group also achieved higher estimated 1-year (32.1% versus 10.0%) and 2-year (17.2% versus 4.9%) DOR probabilities, compared to the placebo group. Consistently, the investigator-assessed ORR and DOR favored the serplulimab group (Supplementary Table S6).

3.4 | Prespecified subgroup analyses

A trend of OS benefit with serplulimab plus chemotherapy was observed across prespecified subgroups, including age, sex, race, ECOG performance status, and the presence of brain and liver metastases (Figure 3A; Supplementary Table S7).

For the Asian patients ($n = 401$), the median OS in the serplulimab and placebo group was 15.9 months and 11.1 months (HR, 0.63 [95% CI, 0.49-0.81]), respectively; for non-Asian patients ($n = 184$), it was 15.6 months and 11.2 months (HR, 0.56 [95% CI, 0.37-0.83]), respectively (Figure 3B-C). Among patients with liver metastasis ($n = 150$), the median OS in the serplulimab and placebo groups was 10.8 months and 7.8 months (HR, 0.58 [95% CI, 0.40-0.84]), respectively; among patients without liver metastasis ($n = 435$), it was 17.7 months and 12.2 months (HR, 0.62 [95% CI, 0.48-0.80]), respectively (Figure 3D-E).

PD-L1 TPS and CPS were evaluable for 565 (96.6%) and 562 (96.1%) patients, respectively. A total of 96 (16.4%) patients had a tumor PD-L1 TPS $\geq$ 1%, and 297 (50.8%) patients had a PD-L1 CPS $\geq$ 1. A trend was observed for the HR for death favoring the serplulimab group across PD-L1 subgroups defined by either TPS or CPS (Figure 3A).

MSI status was evaluable in 305 (52.1%) patients, including 282 (48.2%) with MSS/ MSI-L tumors and 23 (3.9%) with MSI-H tumors. Median OS in the serplulimab and placebo group was 15.1 months and 12.3 months in patients with MSS/MSI-L tumors (HR, 0.75 [95% CI, 0.55-1.01]), and 22.4 months and 11.1 months in those with MSI-H tumors (HR, 0.41 [95% CI, 0.13-1.26]), respectively (Figure 3A; Supplementary Table S7).

Subgroup analysis of PFS showed that the HR for disease progression or death favored serplulimab plus chemotherapy consistently across all subgroups except those with a relatively small number of patients, including those with

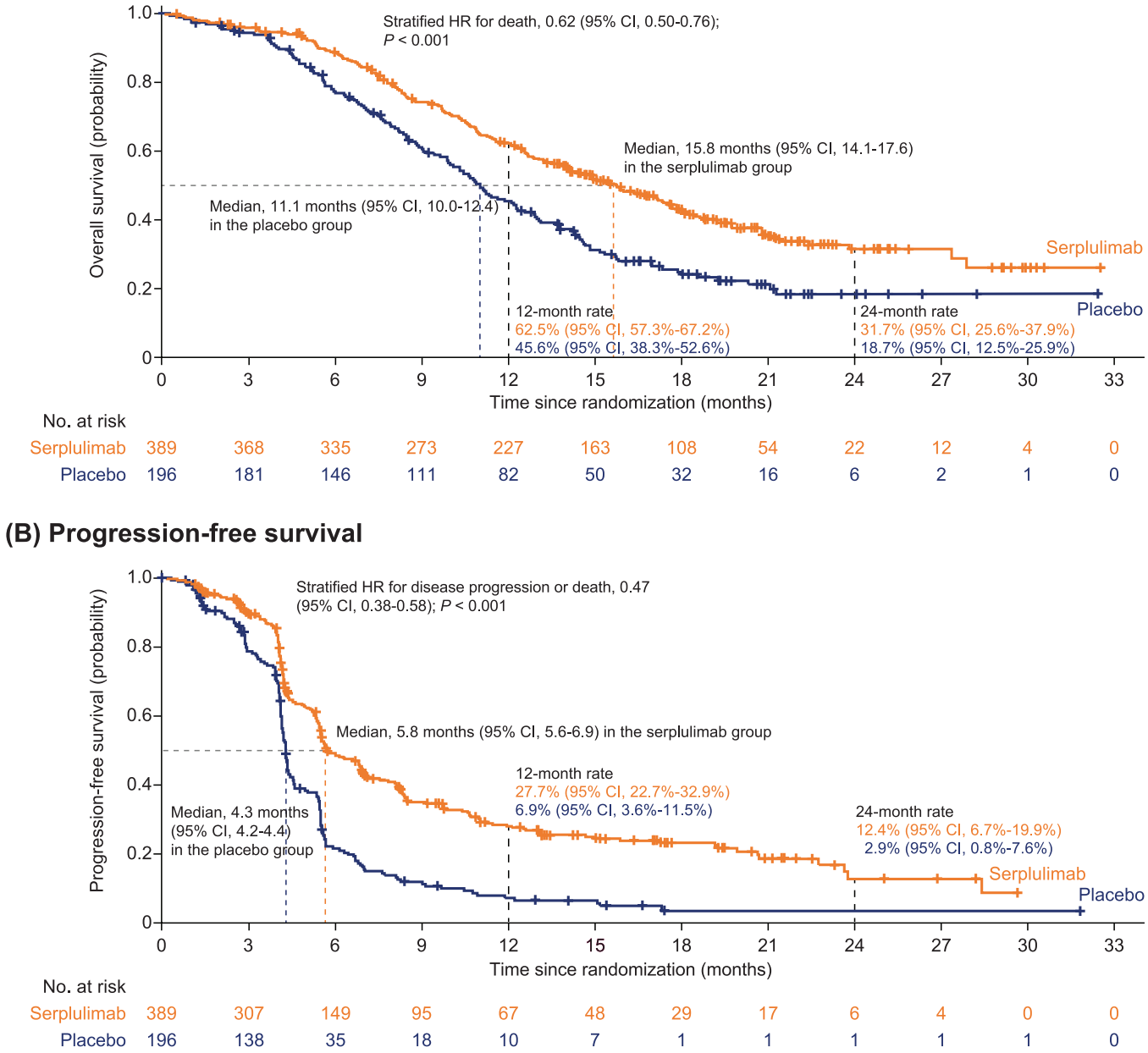


FIGURE 2 Survival outcomes of all 585 enrolled ES-SCLC patients treated with serplulimab or placebo plus chemotherapy. (A) Overall survival. (B) Progression-free survival according to IRRC assessments per RECIST v1.1. The tick marks indicate censored data. *P* values were for descriptive analysis and were not adjusted for multiplicity. Abbreviations: CI, confidence interval; ES-SCLC, ES-SCLC, extensive-stage small-cell lung cancer; HR, hazard ratio; IRRC, independent radiology review committee; RECIST, Response Evaluation Criteria in Solid Tumors.

TPS ≤ 1 and those with MSI-H tumors (Supplementary Figure S2; Supplementary Table S8).

3.5 | Exploratory biomarker analyses

Serum proteomic profiles were available for 168 patients, including 128 in the serplulimab group and 40 in the placebo group. The proteomes of the responders from the serplulimab group were quantitively compared with those of the non-responders from the serplulimab group. A total of 181 differentially expressed proteins were identified. Among these, the 15 most representative proteins—defined as those with a non-zero regression coefficient—were selected to construct a composite score for predicting treatment efficacy (Supplementary Table S9). The pre-

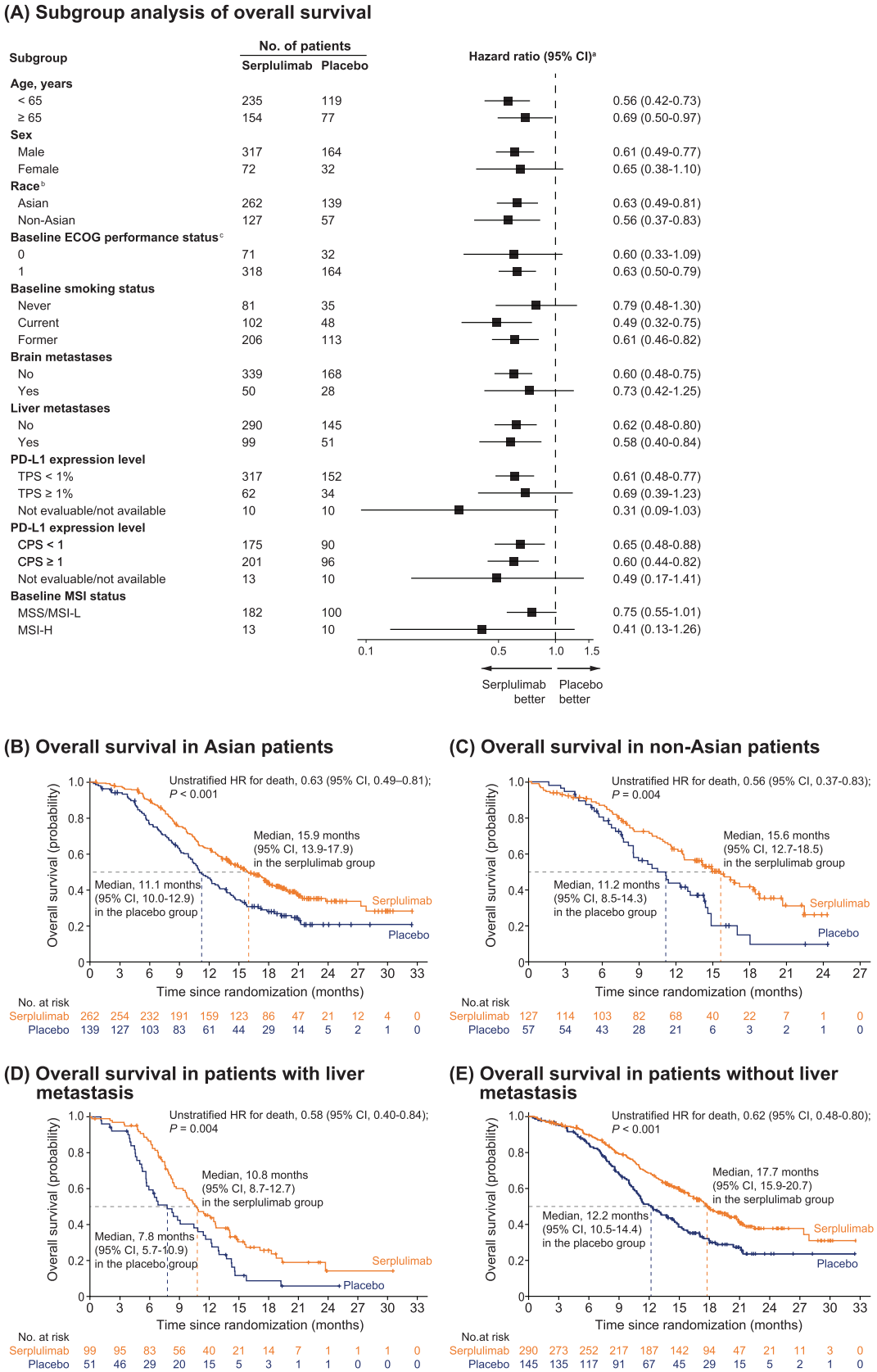


FIGURE 3 Subgroup analysis of OS in ES-SCLC patients treated with serplulimab or placebo plus chemotherapy. (A) Forest plot of OS in subgroups stratified by age, sex, race, baseline ECOG performance status, baseline smoking status, brain metastasis, liver metastasis, PD-L1 expression (TPS or CPS), and MSI status. (B-E) Kaplan-Meier curves of OS in Asian patients (B), in non-Asian patients (C), in patients with liver metastasis (D), and in patients without liver metastasis (E). *P* values were for descriptive analysis and were not adjusted for multiplicity.

dictive strength of the 15-protein signature for objective response was evaluated through ROC analysis. This signature score showed an area under the ROC of 0.982 for the training set, 0.918 for the validation set, and 0.545 for the control validation set (Supplementary Figure S3A-C). Notably, among the 15 proteins, TSPAN1 and PLCB1 showed the greatest potential in predicting response to serplulimab to chemotherapy, both with an area under the curve (AUC) of over 0.7 (Supplementary Table S10, Supplementary Figure S3D-E). The 15-protein signature, TSPAN1, and PLCB1 were further used to stratify patients for comparisons of ORR, OS and PFS, using cutoff values of -86.04, 0.12, and -0.01, respectively (Supplementary Figure S3).

Among the patients with high 15-protein scores, the confirmed ORR was 74.2% and 47.1% in the serplulimab and placebo group (odds ratio, 3.12 [95% CI, 0.95-10.22]; Supplementary Table S11), respectively. In patients with high 15-protein scores, the median PFS was 7.9 months and 4.2 months (HR, 0.36 [95% CI, 0.21-0.65]; $P < 0.001$; Figure 4A) in the serplulimab and placebo group, respectively, while the median OS was 17.2 months and 9.7 months (HR, 0.27 [95% CI, 0.15-0.49]; $P < 0.001$) in the respective groups (Figure 4B). These data showed trends suggesting that the addition of serplulimab to chemotherapy conferred benefits in ORR, PFS, and OS for patients with high signature scores, while there was no apparent benefit for those with low signature scores. In terms of the expression level of TSPAN1 or PLCB1, in patients with low TSPAN1 or PLCB1 expression, confirmed ORR was higher in the serplulimab group than in the placebo group (ORR in patients with low TSPAN1 expression: 80.8% [serplulimab group] *vs.* 45.8% [placebo group]; ORR in patients with low PLCB1 expression: 79.2% vs. 45.8%; Supplementary Table S11). By contrast, in patients with high TSPAN1 or PLCB1 expression, confirmed ORR was lower in the serplulimab group than in the placebo group (ORR in patients with high TSPAN1 expression: 43.4% vs. 56.3%; ORR in patients with high PLCB1 expression: 44.0% vs. 56.3%). Compared with patients with high TSPAN1 or PLCB1 expression, OS and PFS benefit from adding serplulimab to chemotherapy was also more pronounced in patients with low TSPAN1 or PLCB1 expression (Supplementary Table S12; Supplementary Figure S4). These data suggested a trend of association between low TSPAN1 or PLCB1 expression with more favorable survival outcomes following treatment with serplulimab plus chemotherapy.

Genetic mutation data were available for 305 patients, of whom 3 were outliers (Supplementary Figure S5), and the remaining 302 patients were included in subsequent analysis. A total of 38 genes with an overall mutation rate ≥ 5% were identified in these 302 patients, most commonly in tumor protein 53 (*TP53*; 90.1%) and tumor-suppressor retinoblastoma-1 (*RB1*; 68.2%; Supplementary Table S13). Compared to the placebo group, patients treated with serplulimab plus chemotherapy who harbored *RB1* mutations showed a trend toward a higher response rate, longer PFS, and longer OS (Supplementary Figure S6; Supplementary Table S14 and S15). Similarly, compared with the placebo group, patients in the serplulimab group harboring mutations in any of the members of the Notch signaling pathway (including *NOTCH1, NOTCH2, NOTCH3*, and *NOTCH4*) had a higher response rate (Supplementary Figure S6; Supplementary Table S14). In the serplulimab group, ORR was 68.5% (95% CI, 59.7%-76.3%) in the wild-type subgroup and 85.7% (95% CI, 74.6%-93.3%) in the mutant subgroup.

In terms of hematological parameters, 583 patients had available data for baseline NLR and PLR, and 582 patients had available data for baseline LDH. High baseline NLR (>4.6), PLR (>338.3), and LDH (defined as greater than the upper limit of normal) were associated with less favorable PFS and OS in both treatment groups (Supplementary Figure S7; Supplementary Table S16). Multivariate Cox regression further revealed that baseline NLR and LDH levels were independent prognostic biomarkers in this study ($P = 0.002$ and $P < 0.001$, respectively; Supplementary Table S17).

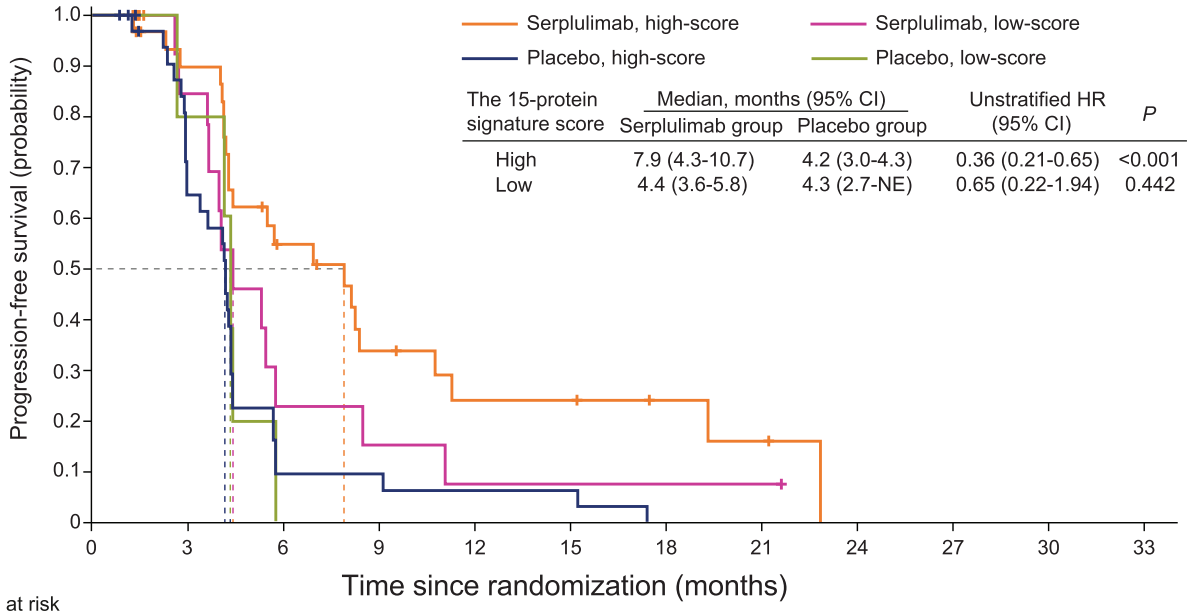
3.6 | Adverse events

The median duration of treatment was 22.0 weeks (range, 0.1-139.0 weeks) for the serplulimab group and 16.4 weeks (range, 0.1-139.0 weeks) for the placebo group. The median number of cycles was 8 (range, 1-42) for serplulimab and 6 (range, 1-46) for the placebo group (Supplementary Table S18).

TEAEs occurred in 373 (95.9%) patients in the serplulimab group and 191 (97.4%) in the placebo group,

[a]Hazard ratios were stratified for the overall population. [b]Self-reported by the patients by selecting 1 or more racial designations (American Indian or Alaska Native, Asian, Black or African American, Native Hawaiian or Pacific Islander, White, or Other) or based on identity information provided by the patients. All non-Asian patients were White. [c]ECOG performance status scores for patients in this study ranged from 0 to 1. A score of 0 indicates fully active; a score of 1 is restricted in physically strenuous activity but ambulatory. Abbreviations: CI, confidence interval; CPS, combined positive score. ECOG, Eastern Cooperative Oncology Group; ES-SCLC, extensive-stage small-cell lung cancer; HR, hazard ratio; MSI, microsatellite instability; MSI-H, MSI-high; MSI-L, MSI-low; MSS, microsatellite stable; No., number; OS, overall survival; PD-L1, programmed death-ligand 1; TPS, tumor proportion score.

(A) Progression-free survival by the 15-protein signature score

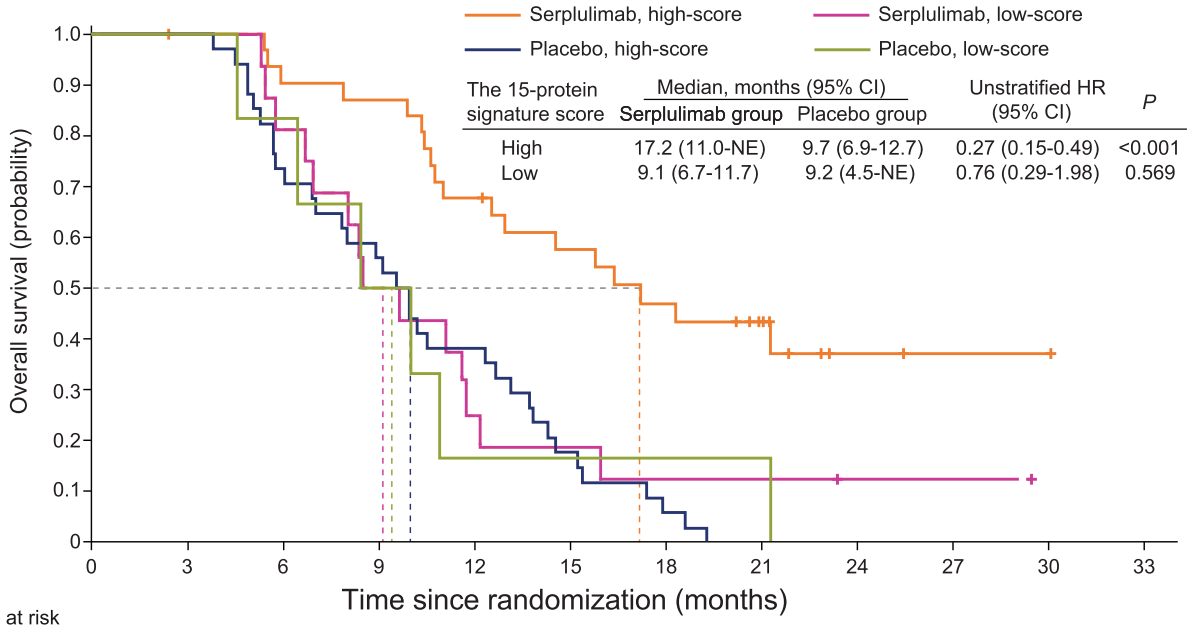


The 15-protein signature score	Median, months (95% CI)		Unstratified HR (95% CI)	*P*
	Serplulimab group	Placebo group		
High	7.9 (4.3-10.7)	4.2 (3.0-4.3)	0.36 (0.21-0.65)	<0.001
Low	4.4 (3.6-5.8)	4.3 (2.7-NE)	0.65 (0.22-1.94)	0.442

No. at risk

Serplulimab, high-score	31	26	14	8	5	5	3	2	0	0	0	0
Serplulimab, low-score	17	11	3	2	1	1	1	1	0	0	0	0
Placebo, high-score	34	20	3	3	2	2	0	0	0	0	0	0
Placebo, low-score	6	4	0	0	0	0	0	0	0	0	0	0

(B) Overall survival by the 15-protein signature score



The 15-protein signature score	Median, months (95% CI)		Unstratified HR (95% CI)	*P*
	Serplulimab group	Placebo group		
High	17.2 (11.0-NE)	9.7 (6.9-12.7)	0.27 (0.15-0.49)	<0.001
Low	9.1 (6.7-11.7)	9.2 (4.5-NE)	0.76 (0.29-1.98)	0.569

No. at risk

Serplulimab, high-score	31	31	28	27	21	17	13	9	2	1	1	0
Serplulimab, low-score	17	16	13	8	4	3	2	2	1	1	0	0
Placebo, high-score	34	34	25	19	13	6	2	0	0	0	0	0
Placebo, low-score	6	6	5	3	1	1	1	1	0	0	0	0

FIGURE 4 Survival outcomes of ES-SCLC patients treated with serplulimab or placebo plus chemotherapy, stratified by the 15-protein signature score. (A) Progression-free survival assessed by IRRC according to RECIST v1.1. (B) Overall survival assessed by IRRC assessments according to RECIST v1.1. The tick marks indicate censored data. *P* values were for descriptive analysis and were not adjusted for multiplicity. Abbreviations: CI, confidence interval; ES-SCLC, extensive-stage small-cell lung cancer; HR, hazard ratio; IRRC, independent radiology review committee; NE, not evaluable; RECIST, Response Evaluation Criteria in Solid Tumors.

TABLE 1 The summary of serplulimab- and placebo-related treatment-emergent adverse events

Adverse event	Serplulimab group ($n = 389$, %)		Placebo group ($n = 196$, %)	
	Any grade	Grade ≥3[a]	Any grade	Grade ≥3[a]
Any serplulimab- or placebo-related adverse events[b]	273 (70.2)	133 (34.2)	113 (57.7)	57 (29.1)
Anemia	85 (21.9)	21 (5.4)	37 (18.9)	11 (5.6)
White blood cell count decreased	79 (20.3)	33 (8.5)	33 (16.8)	17 (8.7)
Neutrophil count decreased	76 (19.5)	55 (14.1)	35 (17.9)	27 (13.8)
Platelet count decreased	61 (15.7)	24 (6.2)	36 (18.4)	16 (8.2)
Nausea	51 (13.1)	1 (0.3)	28 (14.3)	0 (0.0)
Alanine aminotransferase increased	48 (12.3)	4 (1.0)	19 (9.7)	1 (0.5)
Hypothyroidism	60 (15.4)	1 (0.3)	5 (2.6)	0 (0.0)
Aspartate aminotransferase increased	38 (9.8)	2 (0.5)	21 (10.7)	2 (1.0)
Decreased appetite	39 (10.0)	2 (0.5)	19 (9.7)	0 (0.0)
Hyperthyroidism	45 (11.6)	0 (0.0)	6 (3.1)	0 (0.0)
Neutropenia	26 (6.7)	17 (4.4)	10 (5.1)	9 (4.6)
Lymphocyte count decreased	27 (6.9)	8 (2.1)	8 (4.1)	2 (1.0)
Leukopenia	22 (5.7)	10 (2.6)	10 (5.1)	4 (2.0)
Hyperglycemia	25 (6.4)	8 (2.1)	6 (3.1)	0 (0.0)
Hyponatremia	18 (4.6)	10 (2.6)	5 (2.6)	2 (1.0)

[a]Adverse events were graded according to the National Cancer Institute Common Terminology Criteria for Adverse Events version 5.0.
[b]Grade ≥3 events that occurred in ≥2% of patients in any group, and events of any grade that occurred in ≥10% of patients in any group.

and grade ≥3 TEAEs occurred in 324 (83.3%) and 160 (81.6%) patients, respectively. Common TEAEs are shown in Supplementary Table S19. Serplulimab- or placebo-related TEAEs were reported in 273 (70.2%) patients in the serplulimab group and 113 (57.7%) in the placebo group (Table 1). Grade ≥3 serplulimab- or placebo-related TEAEs were reported in 133 (34.2%) and 57 (29.1%) patients, respectively; in both groups, these events were predominantly hematological toxicities such as neutrophil count decreased, white blood cell count decreased, platelet count decreased, anemia, and neutropenia.

Serious TEAEs occurred in 146 (37.5%) patients in the serplulimab group and 71 (36.2%) in the placebo group, and those that were serplulimab- or placebo-related occurred in 71 (18.3%) and 28 (14.3%) patients, respectively (Supplementary Table S20). A total of 38 (9.8%) patients in the serplulimab group and 18 (9.2%) in the placebo group discontinued treatment due to TEAEs; among these patients, 23 (5.9%) and 10 (5.1%), respectively, had serplulimab- or placebo-related TEAEs that led to treatment discontinuation. Deaths due to TEAEs occurred in 35 (9.0%) patients in the serplulimab group and 22 (11.2%) in the placebo group (Supplementary Table S20); among these patients, 5 (1.3%) were considered to be serplulimab-related (acute coronary syndrome, pyrexia, platelet count decreased, immune-mediated encephalitis, and immune-mediated lung disease each in 1 patient), while 1 (0.5%) was considered to be placebo-related (thrombocytopenia; Supplementary Table S21). Upon the exclusion of deaths resulting from disease progression, the incidence of TEAEs leading to death reduced to 6.2% in the serplulimab group and 7.7% in the placebo group. Furthermore, upon the exclusion of deaths resulting from disease progression or COVID-19, the incidence of TEAEs leading to death in our study further reduced to 5.4% and 6.6% in the respective groups (Supplementary Table S20).

Adverse events of special interest included infusion-related reactions and immune-related AEs (Supplementary Table S22). Infusion-related reactions remained infrequent (1.8% in the serplulimab group versus 0.5% in the placebo group). Immune-related AEs (irAEs) were reported in 147 (37.8%) patients in the serplulimab group and 38 (19.4%) in the placebo group, and most of these AEs, with the exception of hypothyroidism (11.8% versus 1.5%) and hyperthyroidism (9.3% versus 3.1%), occurred in less than 5% of patients in either group. Grade ≥3 irAEs were reported in 40 (10.3%) patients in the serplulimab group and 13 (6.6%) in the placebo group.

4 | DISCUSSION

ASTRUM-005 was the first study to show a significant OS improvement with a PD-1 inhibitor added to chemotherapy in patients with ES-SCLC. In this updated analysis, first-line treatment with serplulimab plus chemotherapy showed sustained clinical benefit over placebo plus

chemotherapy, supporting serplulimab plus chemotherapy as a first-line treatment in ES-SCLC.

The OS and PFS benefits in this updated analysis were consistent with those in the interim analysis [8]. A greater percentage of patients in the serplulimab group was estimated to be free from disease progression or death at 1 and 2 years compared to those of the placebo group. The PFS benefit has also been observed in the chemotherapy combined with atezolizumab [29] or pembrolizumab [30] but not with durvalumab [31]. The PFS improvement in this study was confirmed in accordance with RECIST v1.1 and iRECIST; therefore, pseudoprogression, a common phenomenon under the treatment of immunotherapy [32], could be excluded.

The present study included a larger proportion of Asian patients compared to previous international trials, such as the IMpower133 [33] and CASPIAN [34]. The Asian and non-Asian patients in this study benefited to a similar extent, suggesting that the OS improvement with serplulimab plus chemotherapy is generalizable to either population. Compared with data on smoking history in Asian and non-Asian patients from the IMpower133 [29] and CASPIAN trials [34], never-smokers were more common among Asian than non-Asian patients (24.9% versus 8.7%) in this study, possibly owing to the high prevalence of second-hand smoke exposure in P.R. China (adults exposed to second-hand smoke at workplace, 50.9%; adults exposed at home, 44.9%) [35]. Other major Chinese-only phase III studies in SCLC patients, including CAPSTONE-1 (percentage of never-smokers: 22.3%) [36], RATIONALE-312 (percentage of never-smokers: 24.5%) [37], and EXTENTORCH (percentage of never-smokers: 21.9%) [38] have also noted a greater proportion of never-smokers compared with reports from non-Asian patients [36, 39]. However, in this study, there was no notable effect of smoking status on the clinical benefit of serplulimab plus chemotherapy. Additionally, a previous study has shown poor prognosis associated with liver metastasis in SCLC [40]. Subgroup analyses in CASPIAN [34] and CAPSTONE-1 [36] both showed trends of less OS benefit in patients with liver metastasis compared to those without. In the present study, the HR for death was similar between patients with liver metastasis and those without. Moreover, OS and PFS benefits of patients with liver metastasis in this study were markedly greater than those observed in IMpower133 [29], CASPIAN [34], CAPSTONE-1 [36], and RATIONALE-312 studies [37]. These findings suggested that serplulimab plus chemotherapy may be an effective treatment for SCLC patients with liver metastasis; however, the results should be interpreted with caution due to the limited subgroup sample sizes.

CPS was widely used for calculating tumor tissue PD-L1 expression in previous trials conducted in ES-SCLC for immunotherapy, including CheckMate 331 [41] and KEYNOTE-604 [30]. No data on PD-L1 testing based on TPS in ES-SCLC was reported at the commencement of the ASTRUM-005 study except for CASPIAN [42], and this approach was later only adopted in the CAPSTONE-1 trial [36] as a stratification factor based on its performance as a biomarker for immunotherapy in non-small-cell lung cancer (NSCLC) [43, 44]. Both CPS and TPS were included in the subgroup analysis of this study for a comprehensive interpretation of our results. The improvement in OS was consistent for the PD-L1 expression subgroups defined by either TPS or CPS. In contrast to PD-L1 expression, patients with MSI-H tumors showed a trend of greater OS benefit than those with MSS/MSI-L tumors, although a definitive conclusion cannot be drawn because of the limited sample size.

As previously reported by Lekic, M. *et al.*, brain metastasis is common in SCLC, presenting in ∼20% of patients at the time of diagnosis [45]. In our study, only patients with asymptomatic brain metastases or those whose brain lesions had been stable for at least 2 months were recruited, rather than all SCLC patients with brain metastases. The rate of patients with brain metastases in this study was 13% (78/585). Nonetheless, this rate is slightly higher than those reported in several phase III clinical trials evaluating first-line immunotherapies in ES-SCLC, including IMpower133 (8.7%) [29], CASPIAN (10.2%) [34], and CAPSTONE-1 (2.2%) [36].

We have conducted preclinical research on the pathogenesis and tumor microenvironment (TME) of SCLC. The phosphoinositide 3-kinase (PI3K)/Ak strain transforming (AKT) pathway is a major downstream pathway through which proteins such as TSPAN1 and PLCB1 exert their biological functions [46, 47]. Our research in SCLC cell models confirmed that the signal transducer and activator of the transcription 3 (STAT3) pathway was regulated by PI3K/AKT and modulated SCLC phenotypic conversion [48], suggesting that proteins such as TSPAN1 and PLCB1 may as treatment efficacy factors in SCLC. Our research also revealed that fibroblasts and macrophages in the SCLC TME promoted differentiation into immunosuppressive SCLC phenotypes through the interleukin 6/Janus kinase 2/STAT3 axis and hypersialylation, respectively [49]. Both fibroblasts and macrophages are under regulation by matrilysin (MMP7), which, therefore, may promote SCLC metastasis and invasion. Another preclinical study showed that epigenetic regulation inhibited natural killer cell ligand natural-killer group 2, member D ligand and promoted an immunosuppressive microenvironment in SCLC [50], implicating epigenetic mechanisms in the formation of an immunosuppressive TME and epigenetic regulatory factors, such as protein regulator of cytokinesis 1 (PRC1) and structural maintenance of

chromosomes protein 3 (SMC3), as factors of response to immunotherapy plus chemotherapy. Based on the mechanistic insights from these studies, we conducted quantitative proteomic profiling on serum samples.

Using the Olink® Explore 3072 technology, we identified 15 serum protein biomarkers that are associated with eight hallmarks of cancer, including unlocking phenotypic plasticity (MMP7), deregulating cellular metabolism (5'-AMP-activated protein kinase subunit gamma-3), senescent cells (pappalysin-1), inducing or accessing vasculature (ectonucleotide pyrophosphatase/phosphodiesterase family member 2 [ENPP2]), evading growth suppressors (SH3 domain-binding glutamic acid-rich-like protein 2 [SH3BGRL2]), non-mutational epigenetic reprogramming (PRC1 and SMC3), avoiding immune destruction (galanin peptides), and activating invasion and metastasis (PLCB1, alpha-actinin-2, carcinoembryonic antigen-related cell adhesion molecule 20, coiled-coil domain-containing protein 50, Wiskott-Aldrich syndrome protein family member 3, versican core protein, and TSPAN1). Moreover, our study identified and validated the 15-protein signature as a predictive biomarker for clinical response to serplulimab and chemotherapy with better performance than PD-L1 (AUC = 0.743) based on area under the receiver-operating characteristic curve [51, 52]. Accordingly, patients with higher signature scores were more likely to benefit from the combination treatment than those with low scores. Our study validated the association between clinical efficacy and serum levels of the aforementioned hallmark-related proteins. Importantly, although MMP7 [53], ENPP2 [54], SH3BGRL2 [55], PRC1 [56], PLCB1 [57], and TSPAN1 [46] were previously suggested as prognosticators in tumor types such as hepatocellular carcinoma, clear cell renal cell carcinoma and glioblastoma, this study identified them as factors of OS or PFS outcomes in SCLC.

In addition to proteomics, genomic profiling is valuable in facilitating deeper insights into the genetic underpinnings of therapeutic response and resistance [58]. Consistent with previous studies [59, 60], our study found that *TP53* and *RB1* were the most frequently mutated genes in SCLC. *RB1* has dual biological effects in cell cycle regulation and immune function [61]. SCLC-bearing wild-type *RB1* is more frequently chemo-refractory; one previous study showed that mutated *RB1* was associated with more favorable OS and PFS in patients treated with first-line chemotherapy [62]. However, the effect of mutated *RB1* on immunotherapy sensitivity is controversial. Our study showed an association, albeit not statistically significant, between mutated *RB1* and better outcomes in patients treated with serplulimab plus chemotherapy, warranting further investigations.

Additionally, we found that patients harboring mutations in genes encoding for members of Notch signaling, including *NOTCH1, NOTCH2, NOTCH3*, and *NOTCH4*, tended to achieve greater response rate to serplulimab plus chemotherapy treatment, although no obvious OS or PFS benefit was observed. Notch signaling activation has been implicated in cell proliferation, neuroendocrine differentiation, chemoresistance, and modulation of the immune microenvironment [63]. Therapeutic agents targeting delta-like ligand 3, a Notch ligand, have been approved for the treatment of SCLC or are under active clinical development [64]. Moreover, there is evidence that the activation of Notch signaling boosted the intrinsic tumor immunity by suppressing neuroendocrine differentiation in a human SCLC cell line, suggesting Notch signaling is a potential determinant of response to PD-1/PD-L1 blockade [65]. However, it remains unclear as to whether and how genetic mutations in Notch signaling members regulate downstream biological processes and clinical therapeutic response. Further investigations on the characterization of the roles of Notch pathway mutations in immunotherapy sensitivity are needed.

In terms of hematological parameters, previous studies have shown that elevated NLR and PLR were associated with inferior outcomes in NSCLC cases treated with immunotherapy [66], and they predicted poor prognosis of limited-stage SCLC patients who underwent surgery [67]. The findings from this present study further support NLR as potential prognostic biomarkers in lung cancer.

In this updated analysis, no new safety signals were observed; the AE profile was consistent with that at the interim analysis [8] and that for each drug class [30]. The incidences of TEAEs of any grade or of grade ≥3 was largely comparable between groups. Immune-related hepatitis and immune-related pneumonitis were safety concerns with PD-1 or PD-L1 inhibitors when added to chemotherapy in patients with ES-SCLC [29, 34, 36–38, 68, 69]; these AEs occurred in a small number of patients in this study (2 and 1 patient, respectively; both in the serplulimab group). Compared with the data at interim analysis [8], there were an additional 5 and 2 deaths in the serplulimab and placebo groups, respectively, in this updated analysis; two of which were considered to be serplulimab-related (immune-mediated encephalitis and immune-mediated lung disease). Most TEAEs were transient as they were resolved, and patients had recovered without sequelae by data cutoff.

Compared with historical data [33, 34], the incidence of TEAEs leading to death appeared higher (9.0% versus 11.2%). In our study, deaths due to disease progression or COVID-19 were recorded as serious AEs and counted towards the TEAEs leading to death. Upon exclusion of

death caused by disease progression or COVID-19, the incidences of TEAEs leading to death (5.4% in the serplulimab group versus 6.6% in the placebo group) were similar to those reported in IMpower133 (2.0% in the atezolizumab plus chemotherapy arm versus 5.6% in the placebo plus chemotherapy arm) [29] and CASPIAN (5% in the durvalumab plus chemotherapy arm versus 6% in the chemotherapy alone arm) [34]. In terms of irAEs, nearly 13,000 cases of irAEs were reported up to 2018, among which more than two-thirds were related to immune checkpoint inhibitors [70]. In this study, higher rates of irAEs related to the immunological mechanism of action were observed in the serplulimab group (37.8%) than in the placebo group (19.4%). This observation was consistent with reports from other major phase III studies, including IMpower133 (atezolizumab plus chemotherapy, 39.9%; placebo plus chemotherapy, 24.5%) [29], CASPIAN (durvalumab plus chemotherapy, 20%; chemotherapy, 3%) [34], CAPSTONE-1 (adebrelimab plus chemotherapy, 28%; placebo plus chemotherapy, 17%) [36]. Taken together, the safety profile of serplulimab was comparable to that of the other anti-PD-1/L1 antibodies.

The limitations of the study have been reported previously and included a lack of head-to-head comparisons with approved PD-L1 inhibitors and the exclusion of cisplatin in the chemotherapy regimen [8]. Furthermore, the sample size was limited for the exploratory biomarker analyses on proteomic profiling and genomics. A phase III trial (NCT05468489) that evaluates serplulimab plus chemotherapy versus atezolizumab plus chemotherapy as first-line treatment for patients with ES-SCLC is ongoing and will no doubt provide new insights on the comparative advantage of serplulimab plus chemotherapy compared with a current standard of care [71].

5 | CONCLUSIONS

This updated analysis showed that serplulimab plus chemotherapy continued to confer OS and PFS benefits over placebo plus chemotherapy in patients with ES-SCLC, supporting this combination therapy as a first-line treatment option for ES-SCLC. Exploratory biomarker analyses revealed the predictive potential of a 15-protein signature for the efficacy of serplulimab plus chemotherapy, and baseline NLR and LDH level as poor prognostic factors for ES-SCLC, thereby warranting further investigations.

AUTHOR CONTRIBUTIONS

Dr. Cheng had full access to all of the data in the study and took responsibility for the integrity of the data and the accuracy of the data analysis. Concept and design: Ying Cheng, Qingyu Wang, Fang Yang. Acquisition, analysis, or interpretation of data: Ying Cheng, Shuang Zhang, Liang Han, Lin Wu, Jun Chen, Peiyan Zhao, Hongmei Sun, Guilan Wen, Yiinghua Ji, Anastasia Zimina, Jianhua Shi, Xicheng Wang, Jinsheng Shi, Yuansong Bai, Tamar Melkadze, Yueyin Pan, Xuhong Min, Maksym Viguro, Xingya Li, Yanqiu Zhao, Junquan Yang, Tamta Makharadze, Ekaterine Arkania, Haoyu Yu, Jing Li, Fang Yang, Xinyi Yang, Chen Ling, Qingyu Wang, Yongqiang Shan, Jun Zhu. Drafting of the manuscript: Ying Cheng, Qingyu Wang. Critical revision of the manuscript for important intellectual content: Ying Cheng, Shuang Zhang, Liang Han, Lin Wu, Jun Chen, Peiyan Zhao, Hongmei Sun, Guilan Wen, Yinghua Ji, Anastasia Zimina, Jianhua Shi, Zhijie Pan, Jinsheng Shi, Xicheng Wang, Yuansong Bai, Tamar Melkadze, Yueyin Pan, Xuhong Min, Maksym Viguro, Xingya Li, Yanqiu Zhao, Junquan Yang, Tamta Makharadze, Ekaterine Arkania, Haoyu Yu, Jing Li, Fang Yang, Xinyi Yang, Chen Ling, Qingyu Wang, Yongqiang Shan, Jun Zhu. Statistical analysis: Qingyu Wang, Jun Zhu. Obtained funding: Ying Cheng. Administrative, technical, or material support: Haoyu Yu, Jing Li, Fang Yang, Xinyi Yang, Chen Ling, Qingyu Wang, Shan, Jun Zhu. Supervision: Ying Cheng, Jun Zhu.

AFFILIATIONS

[1]Department of Oncology, Jilin Cancer Hospital, Changchun, Jilin, P. R. China

[2]Department of Oncology, Xuzhou Central Hospital, Xuzhou, Jiangsu, P. R. China

[3]Department of Thoracic Medical Oncology, Hunan Cancer Hospital, the Affiliated Cancer Hospital of Xiangya School of Medicine, Central South University, Changsha, Hunan, P. R. China

[4]Department of Lung Cancer Surgery, Tianjin Medical University General Hospital, Tianjin, P. R. China

[5]Department of Oncology, Jiamusi Cancer Hospital, Jiamusi, Heilongjiang, P. R. China

[6]Department of Respiratory Medicine, The First Affiliated Hospital of Nanchang University, Nanchang, Jiangxi, P. R. China

[7]Department of Oncology, The First Affiliated Hospital of Xinxiang Medical University, Xinxiang, Henan, P. R. China

[8]Department of Oncology, Budgetary Healthcare Institution of Omsk Region "Clinical Oncology Dispensary", Omsk, Russia

[9]Department of Oncology, Linyi Cancer Hospital, Linyi, Shandong, P. R. China

[10]Department of Respiratory Medicine, The First Affiliated Hospital of Zhejiang University School of Medicine, Hangzhou, Zhejiang, P. R. China

[11]Department of Oncology, Cangzhou People's Hospital, Cangzhou, Hebei, P. R. China

[12]Department of Oncology, The First Affiliated Hospital of Guangdong Pharmaceutical University, Guangzhou, Guangdong, P. R. China

[13]Department of Oncology and Hematology, China-Japan Union Hospital of Jilin University, Changchun, Jilin, P. R. China

[14]Academician Fridon Todua Medical Center–Research Institute of Clinical Medicine, Tbilisi, Georgia

[15]Department of Oncology, Anhui Provincial Hospital, Hefei, Anhui, P. R. China

[16]Department of Interventional Radiology, Anhui Chest Hospital, Hefei, Anhui, P. R. China

[17]Clinical Research Department, Medical Center "Mriya Med-Service", Kryvyi Rih, Ukraine

[18]Department of Oncology, The First Affiliated Hospital of Zhengzhou University, Zhengzhou, Henan, P. R. China

[19]Respiratory Department of Internal Medicine, The Affiliated Cancer Hospital of Zhengzhou University, Zhengzhou, Henan, P. R. China

[20]Department of Oncology, Tangshan People's Hospital, Tangshan, Hebei, P. R. China

[21]Department of Oncology-Endocrinology, High Technology Hospital MedCenter, Batumi, Georgia

[22]Department of Oncology, Israeli-Georgian Medical Research Clinic "Helsicore", Tbilisi, Georgia

[23]Department of Global Product Development, Shanghai Henlius Biotech, Inc., Shanghai, P. R. China

[24]Shanghai Innovation Center, Shanghai Henlius Biotech, Inc., Shanghai, P. R. China

[25]Chairperson of the Board Office, Shanghai Henlius Biotech, Inc., Shanghai, P. R. China

ACKNOWLEDGMENTS

We would like to express our sincere gratitude to the patients who participated in the trial, their families, the principal investigators, the clinicians, the study coordinators, and the nurses. We thank the clinical study team (clinical development: Fan Zhang and Xiaoli Hou; clinical operations: Dongqing Liu, Shan Tang, and Yun Li; biomarker operations: Hanyan Zhang; statistics: Qianhao Li, Dan Lu, Yuexiang Huang, Mengkai Chen, and Yi Zhu; data programming: Feng Qin); and Wenjie Zhang for their support in study execution, study design, data acquisition, and statistical analyses; all of these individuals received compensation as employees of Shanghai Henlius Biotech, Inc. Medical writing support was funded by Shanghai Henlius Biotech, Inc. and provided by Xinlei Yu of Parexel, and Shiqi Zhong, Chen Hu, Xiao Zou, and Zhi Hao Kwok of Shanghai Henlius Biotech, Inc. This study was supported by Shanghai Henlius Biotech Inc. and the National Natural Science Foundation of China (grant number: 82473000).

CONFLICT OF INTEREST STATEMENT

Haoyu Yu, Jing Li, Fang Yang, Xinyi Yang, Chen Ling, Qingyu Wang, Yongqiang Shan, and Jun Zhu have disclosed that they are employees of Shanghai Henlius Biotech, Inc. No other disclosures were reported.

DATA AVAILABILITY STATEMENT

The sequencing data analyzed in this study are publicly available in the Sequence Read Archive database under accession number PRJNA1243693 (https://www.ncbi.nlm.nih.gov/sra). Other data generated in this study are available from the corresponding author upon reasonable request.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

The study was approved by the central or independent institutional review board/ethics committee (approval number: 201903-014-01), and performed in accordance with the Declaration of Helsinki, Good Clinical Practice guidelines. Written informed consent was obtained from each subject or each subject's guardian before enrollment. This trial is registered on clinicaltrial.gov (NCT04063163).

ORCID

Ying Cheng https://orcid.org/0000-0001-9908-597X
Lin Wu https://orcid.org/0000-0001-7078-7767

REFERENCES

1. Rudin CM, Brambilla E, Faivre-Finn C, Sage J. Small-cell lung cancer. Nat Rev Dis Primers. 2021;7(1):3.
2. American Cancer Society. Cancer facts & figures. Published March 2021. Accessed October 28, 2022. https://www.cancer.org/content/dam/cancer-org/research/cancer-facts-and-statistics/annual-cancer-facts-and-figures/2021/cancer-facts-and-figures-2021.pdf
3. Zugazagoitia J, Paz-Ares L. Extensive-stage small-cell lung cancer: first-line and second-line treatment options. J Clin Oncol. 2022;40(6):671–80.
4. Horn L, Mansfield AS, Szczęsna A, Havel L, Krzakowski M, Hochmair MJ, et al. First-Line Atezolizumab plus Chemotherapy in Extensive-Stage Small-Cell Lung Cancer. N Engl J Med. 2018;379(23):2220–9.
5. Paz-Ares L, Dvorkin M, Chen Y, Reinmuth N, Hotta K, Trukhin D, et al. Durvalumab plus platinum-etoposide versus platinum-etoposide in first-line treatment of extensive-stage small-cell lung cancer (CASPIAN): a randomised, controlled, open-label, phase 3 trial. Lancet. 2019;394(10212):1929–39.
6. Mino-Kenudson M, Schalper K, Cooper W, Dacic S, Hirsch FR, Jain D, et al. Predictive Biomarkers for Immunotherapy in Lung Cancer: Perspective From the International Association for the Study of Lung Cancer Pathology Committee. J Thoracic Oncol. 2022;17(12):1335–54.
7. Li H, Zhao P, Tian L, Lu Y, Wang X, Shao W, et al. Advances in biomarkers for immunotherapy in small-cell lung cancer. Front Immunol. 2024;15:1490590.
8. Cheng Y, Han L, Wu L, Chen J, Sun H, Wen G, et al. Effect of First-Line Serplulimab vs Placebo Added to Chemotherapy on Survival in Patients With Extensive-Stage Small Cell Lung Cancer: The ASTRUM-005 Randomized Clinical Trial. JAMA. 2022;328(12):1223–32.
9. Chung HC, Piha-Paul SA, Lopez-Martin J, Schellens JHM, Kao S, Miller WH, et al. Pembrolizumab After Two or More Lines of Previous Therapy in Patients With Recurrent or Metastatic SCLC: Results From the KEYNOTE-028 and KEYNOTE-158 Studies. J Thorac Oncol. 2020;15(4):618–27.

10. Ott PA, Elez E, Hiret S, Kim D-W, Morosky A, Saraf S, et al. Pembrolizumab in Patients With Extensive-Stage Small-Cell Lung Cancer: Results From the Phase Ib KEYNOTE-028 Study. J Clin Oncol. 2017;35(34):3823–9.
11. Ott PA, Bang Y-J, Piha-Paul SA, Razak ARA, Bennouna J, Soria J-C, et al. T-Cell-Inflamed Gene-Expression Profile, Programmed Death Ligand 1 Expression, and Tumor Mutational Burden Predict Efficacy in Patients Treated With Pembrolizumab Across 20 Cancers: KEYNOTE-028. J Clin Oncol. 2019;37(4):318–27.
12. Marabelle A, Fakih M, Lopez J, Shah M, Shapira-Frommer R, Nakagawa K, et al. Association of tumour mutational burden with outcomes in patients with advanced solid tumours treated with pembrolizumab: prospective biomarker analysis of the multicohort, open-label, phase 2 KEYNOTE-158 study. Lancet Oncol. 2020;21(10):1353–65.
13. Goldman JW, Garassino MC, Chen Y, Reinmuth N, Hotta K, Poltoratskiy A, et al. LBA86 Durvalumab (D) ± tremelimumab (T) + platinum-etoposide (EP) in 1L ES-SCLC: Characterization of long-term clinical benefit and tumour mutational burden (TMB) in CASPIAN. Ann Oncol. 2020;31:S1212–S3.
14. Nabet BY, Hamidi H, Lee MC, Banchereau R, Morris S, Adler L, et al. Immune heterogeneity in small-cell lung cancer and vulnerability to immune checkpoint blockade. Cancer Cell. 2024;42(3):429–43.e4.
15. Xie M, Chugh P, Broadhurst H, Lai Z, Whitston D, Paz-Ares L, et al. Abstract CT024: Durvalumab (D) + platinum-etoposide (EP) in 1L extensive-stage small-cell lung cancer (ES-SCLC): Exploratory analysis of SCLC molecular subtypes in CASPIAN. Cancer Res. 2022;82(12_Supplement):CT024.
16. Shen L, Brown JR, Johnston SA, Altan M, Sykes KF. Predicting response and toxicity to immune checkpoint inhibitors in lung cancer using antibodies to frameshift neoantigens. J Transl Med. 2023;21(1):338.
17. Rudin CM, Kim HR, Navarro A, Gottfried M, Peters S, Csoszi T, et al. Exploratory biomarker analysis of the phase 3 KEYNOTE-604 study of pembrolizumab plus etoposide for extensive-stage SCLC. J Clin Oncol. 2023;41(16_suppl):8503.
18. Issafras H, Fan S, Tseng CL, Cheng Y, Lin P, Xiao L, et al. Structural basis of HLX10 PD-1 receptor recognition, a promising anti-PD-1 antibody clinical candidate for cancer immunotherapy. PLoS One. 2021;16(12):e0257972.
19. U.S. Department of Health and Human Services. Common Terminology Criteria for Adverse Events (CTCAE) Version 5.0. Published November 2017. Accessed January 1, 2019. https://ctep.cancer.gov/protocoldevelopment/electronic_applications/docs/CTCAE_v5_Quick_Reference_5X7.pdf
20. Chen S, Zhou Y, Chen Y, Gu J. fastp: an ultra-fast all-in-one FASTQ preprocessor. Bioinformatics. 2018;34(17):i884–i90.
21. Li H, Durbin R. Fast and accurate short read alignment with Burrows-Wheeler transform. Bioinformatics. 2009;25(14):1754–60.
22. Danecek P, Bonfield JK, Liddle J, Marshall J, Ohan V, Pollard MO, et al. Twelve years of SAMtools and BCFtools. GigaScience. 2021;10(2):giab008.
23. Broad Institute. Picard Tools - By Broad Institute. Published January 2010. Accessed June 13, 2022. https://broadinstitute.github.io/
24. Koboldt DC, Chen K, Wylie T, Larson DE, McLellan MD, Mardis ER, et al. VarScan: variant detection in massively parallel sequencing of individual and pooled samples. Bioinformatics. 2009;25(17):2283–5.
25. Wang K, Li M, Hakonarson H. ANNOVAR: functional annotation of genetic variants from high-throughput sequencing data. Nucleic Acids Res. 2010;38(16):e164.
26. Mayakonda A, Lin D-C, Assenov Y, Plass C, Koeffler HP. Maftools: efficient and comprehensive analysis of somatic variants in cancer. Genome Res. 2018;28(11):1747–56.
27. Camp RL, Dolled-Filhart M, Rimm DL. X-tile: a new bio-informatics tool for biomarker assessment and outcome-based cut-point optimization. Clin Cancer Res. 2004;10(21):7252–9.
28. Camp RL, Dolled-Filhart M, Rimm DL. X-tile: a new bio-informatics tool for biomarker assessment and outcome-based cut-point optimization. Clin Cancer Res. 2004;10(21):7252–9.
29. Liu SV, Reck M, Mansfield AS, Mok T, Scherpereel A, Reinmuth N, et al. Updated overall survival and PD-L1 subgroup analysis of patients with extensive-stage small-cell lung cancer treated with atezolizumab, carboplatin, and etoposide (IMpower133). J Clin Oncol. 2021;39(6):619–30.
30. Rudin CM, Awad MM, Navarro A, Gottfried M, Peters S, Csoszi T, et al. Pembrolizumab or placebo plus etoposide and platinum as first-line therapy for extensive-stage small-cell lung cancer: randomized, double-blind, phase III KEYNOTE-604 study. J Clin Oncol. 2020;38(21):2369–79.
31. Goldman JW, Dvorkin M, Chen Y, Reinmuth N, Hotta K, Trukhin D, et al. Durvalumab, with or without tremelimumab, plus platinum-etoposide versus platinum-etoposide alone in first-line treatment of extensive-stage small-cell lung cancer (CASPIAN): updated results from a randomised, controlled, open-label, phase 3 trial. Lancet Oncol. 2021;22(1):51–65.
32. Seymour L, Bogaerts J, Perrone A, Ford R, Schwartz LH, Mandrekar S, et al. iRECIST: guidelines for response criteria for use in trials testing immunotherapeutics. Lancet Oncol. 2017;18(3):e143–e52.
33. Liu SV, Reck M, Mansfield AS, Mok T, Scherpereel A, Reinmuth N, et al. Updated Overall Survival and PD-L1 Subgroup Analysis of Patients With Extensive-Stage Small-Cell Lung Cancer Treated With Atezolizumab, Carboplatin, and Etoposide (IMpower133). J Clin Oncol. 2021;39(6):619–30.
34. Paz-Ares L, Chen Y, Reinmuth N, Hotta K, Trukhin D, Statsenko G, et al. Durvalumab, with or without tremelimumab, plus platinum-etoposide in first-line treatment of extensive-stage small-cell lung cancer: 3-year overall survival update from CASPIAN. ESMO Open. 2022;7(2):100408.
35. Global adult tobacco survery. Fact sheet China 2018. Published January 2018. Accessed November 10, 2022. https://www.tobaccofreekids.org/assets/global/pdfs/en/GATS_China_2018_FactSheet.pdf
36. Wang J, Zhou C, Yao W, Wang Q, Min X, Chen G, et al. Adebrelimab or placebo plus carboplatin and etoposide as first-line treatment for extensive-stage small-cell lung cancer (CAPSTONE-1): a multicentre, randomised, double-blind, placebo-controlled, phase 3 trial. Lancet Oncol. 2022;23(6):739–47.
37. Cheng Y, Fan Y, Zhao Y, Huang D, Li X, Zhang P, et al. First-Line Chemotherapy With or Without Tislelizumab for Extensive-Stage Small Cell Lung Cancer: RATIONALE-312 Phase 3 Study. Report presentation at 2023 World Conference on Lung Cancer. 2023. Published September 2023. Accessed November 1,

2024. https://www.beigenemedical.com/CongressDocuments/Cheng_BGB-A317-312_WCLC_Presentation_2023.pdf
38. Cheng Y, Liu Y, Zhang W, Wu L, Zhou C, Wang D, et al. LBA93 EXTENTORCH: A randomized, phase III trial of toripalimab versus placebo, in combination with chemotherapy as a first-line therapy for patients with extensive stage small cell lung cancer (ES-SCLC). Ann Oncol. 2023;34:S1334.
39. Zhou F, Zhou C. Lung cancer in never smokers-the East Asian experience. Transl Lung Cancer Res. 2018;7(4):450–63.
40. Ren Y, Dai C, Zheng H, Zhou F, She Y, Jiang G, et al. Prognostic effect of liver metastasis in lung cancer patients with distant metastasis. Oncotarget. 2016;7(33):53245–53.
41. Spigel DR, Vicente D, Ciuleanu TE, Gettinger S, Peters S, Horn L, et al. Second-line nivolumab in relapsed small-cell lung cancer: CheckMate 331. Ann Oncol. 2021;32(5):631–41.
42. Paz-Ares L, Goldman JW, Garassino MC, Dvorkin M, Trukhin D, Statsenko G, et al. LBA89 - PD-L1 expression, patterns of progression and patient-reported outcomes (PROs) with durvalumab plus platinum-etoposide in ES-SCLC: results from CASPIAN. Ann Oncol. 2019;30:v928–v9.
43. Mok TSK, Wu YL, Kudaba I, Kowalski DM, Cho BC, Turna HZ, et al. Pembrolizumab versus chemotherapy for previously untreated, PD-L1-expressing, locally advanced or metastatic non-small-cell lung cancer (KEYNOTE-042): a randomised, open-label, controlled, phase 3 trial. Lancet. 2019;393(10183):1819–30.
44. Reck M, Rodriguez-Abreu D, Robinson AG, Hui R, Csoszi T, Fulop A, et al. Pembrolizumab versus chemotherapy for PD-L1-positive non-small-cell lung cancer. N Engl J Med. 2016;375(19):1823–33.
45. Lekic M, Kovac V, Triller N, Knez L, Sadikov A, Cufer T. Outcome of small cell lung cancer (SCLC) patients with brain metastases in a routine clinical setting. Radiol Oncol. 2012;46(1):54–9.
46. Wang Y, Liang Y, Yang G, Lan Y, Han J, Wang J, et al. Tetraspanin 1 promotes epithelial-to-mesenchymal transition and metastasis of cholangiocarcinoma via PI3K/AKT signaling. J Exp Clin Cancer Res. 2018;37(1):300.
47. Liang S, Guo H, Ma K, Li X, Wu D, Wang Y, et al. A PLCB1-PI3K-AKT signaling axis activates EMT to promote cholangiocarcinoma progression. Cancer Res. 2021;81(23):5889–903.
48. Li H, Zhong R, He C, Tang C, Cui H, Li R, et al. Colony-stimulating factor CSF2 mediates the phenotypic plasticity of small-cell lung cancer by regulating the p-STAT3/MYC pathway. Oncol Rep. 2022;48(1):122.
49. Tian L, Li H, Zhao P, Liu Y, Lu Y, Zhong R, et al. C-Myc-induced hypersialylation of small cell lung cancer facilitates pro-tumoral phenotypes of macrophages. IScience. 2023;26(10):107771.
50. Zhao P, Sun X, Li H, Liu Y, Cui Y, Tian L, et al. c-Myc Targets HDAC3 to Suppress NKG2DL Expression and Innate Immune Response in N-Type SCLC through Histone Deacetylation. Cancers. 2022;14(3):457.
51. Dolled-Filhart M, Roach C, Toland G, Stanforth D, Jansson M, Lubiniecki GM, et al. Development of a Companion Diagnostic for Pembrolizumab in Non-Small Cell Lung Cancer Using Immunohistochemistry for Programmed Death Ligand-1. Arch Pathol Lab Med. 2016;140(11):1243–9.
52. Garon EB, Rizvi NA, Hui R, Leighl N, Balmanoukian AS, Eder JP, et al. Pembrolizumab for the treatment of non-small-cell lung cancer. N Engl J Med. 2015;372(21):2018–28.
53. Sun Y, Chen Y, Li S, Lei Y, Xu D, Jiang N, et al. NanoVelcro-captured CTC number concomitant with enhanced serum levels of MMP7 and MMP9 enables accurate prediction of metastasis and poor prognosis in patients with lung adenocarcinoma. Int J Nanomed. 2017;12:6399–412.
54. Meng J, Ruan X, Wei F, Xue Q. High expression of ENPP2 is an independent predictor of poor prognosis in liver cancer. Medicine. 2023;102(31):e34480.
55. Yin L, Li W, Xu A, Shi H, Wang K, Yang H, et al. SH3BGRL2 inhibits growth and metastasis in clear cell renal cell carcinoma via activating hippo/TEAD1-Twist1 pathway. EBioMedicine. 2020;51:102596.
56. Zhu H, Zheng C, Liu H, Kong F, Kong S, Chen F, et al. Significance of macrophage infiltration in the prognosis of lung adenocarcinoma patients evaluated by scRNA and bulkRNA analysis. Front Immunol. 2022;13:1028440.
57. Wang Y, Tu Z, Zhao W, Wang L, Jiang J, Gu L, et al. PLCB1 Enhances Cell Migration and Invasion in Gastric Cancer Via Regulating Actin Cytoskeletal Remodeling and Epithelial-Mesenchymal Transition. Biochem Genet. 2023;61(6):2618–32.
58. Tang Z, Gu Y, Shi Z, Min L, Zhang Z, Zhou P, et al. Multiplex immune profiling reveals the role of serum immune proteomics in predicting response to preoperative chemotherapy of gastric cancer. Cell Rep Med. 2023;4(2):100931.
59. Liu J, Zhao Z, Wei S, Li B, Zhao Z. Genomic features of Chinese small cell lung cancer. BMC Med Genomics. 2022;15(1):117.
60. Peifer M, Fernández-Cuesta L, Sos ML, George J, Seidel D, Kasper LH, et al. Integrative genome analyses identify key somatic driver mutations of small-cell lung cancer. Nat Genet. 2012;44(10):1104–10.
61. Markey MP, Bergseid J, Bosco EE, Stengel K, Xu H, Mayhew CN, et al. Loss of the retinoblastoma tumor suppressor: differential action on transcriptional programs related to cell cycle control and immune function. Oncogene. 2007;26(43):6307–18.
62. Dowlati A, Lipka MB, McColl K, Dabir S, Behtaj M, Kresak A, et al. Clinical correlation of extensive-stage small-cell lung cancer genomics. Ann Oncol. 2016;27(4):642–7.
63. Shi Q, Xue C, Zeng Y, Yuan X, Chu Q, Jiang S, et al. Notch signaling pathway in cancer: from mechanistic insights to targeted therapies. Signal Transduct Target Ther. 2024;9(1):128.
64. Dhillon S. Tarlatamab: First Approval. Drugs. 2024;84(8):995–1003.
65. Roper N, Velez MJ, Chiappori A, Kim YS, Wei JS, Sindiri S, et al. Notch signaling and efficacy of PD-1/PD-L1 blockade in relapsed small cell lung cancer. Nature Commun. 2021;12(1):3880.
66. Russo A, Russano M, Franchina T, Migliorino MR, Aprile G, Mansueto G, et al. Neutrophil-to-lymphocyte ratio (nlr), platelet-to-lymphocyte ratio (PLR), and outcomes with nivolumab in pretreated non-small cell lung cancer (NSCLC): a large retrospective multicenter study. Adv Ther. 2020;37(3):1145–55.
67. Chen C, Yang H, Cai D, Xiang L, Fang W, Wang R. Preoperative peripheral blood neutrophil-to-lymphocyte ratios (NLR) and platelet-to-lymphocyte ratio (PLR) related nomograms predict

the survival of patients with limited-stage small-cell lung cancer. Transl Lung Cancer Res. 2021;10(2):866–77.
68. Atezolizumab prescribing information. U.S. Food and Drug Administration. Published October 2021. Accessed October 28, 2022. https://www.accessdata.fda.gov/drugsatfda_docs/label/2016/761041lbl.pdf
69. Durvalumab prescribing information. U.S. Food and Drug Administration. Published March 2020. Accessed October 28, 2022. https://www.accessdata.fda.gov/drugsatfda_docs/label/2017/761069s000lbl.pdf
70. Ramos-Casals M, Brahmer JR, Callahan MK, Flores-Chávez A, Keegan N, Khamashta MA, et al. Immune-related adverse events of checkpoint inhibitors. Nat Rev Dis Primers. 2020;6(1):38.
71. NCCN guidelines. Small cell lung cancer. Version 1.2023. Published November 2022. Accessed October 28, 2022. https://www.nccn.org/professionals/physician_gls/pdf/sclc.pdf

SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

How to cite this article: Cheng Y, Zhang S, Han L, Wu L, Chen J, Zhao P, et al. First-line serplulimab plus chemotherapy in extensive-stage small-cell lung cancer: Updated results and biomarker analysis from the ASTRUM-005 randomized clinical trial. Cancer Commun. 2025;1–20. https://doi.org/10.1002/cac2.70032