Evaluation of the efficacy of the combination of PD-L1 blockade by Durvalumab with Carboplatin plus Etoposide chemotherapy in metastatic pulmonary large-cell neuroendocrine carcinoma (LCNEC) – DUPLE study

- **BACKGROUND AND RATIONALE:**

Pulmonary large cell neuroendocrine carcinoma of the lung (LCNEC) is a rare and aggressive type of lung neuroendocrine tumor, which accounts for 2-3% of non-small cell lung cancers [1]. Given its rarity, there are no randomized trial available to guide treatment, so that therapeutic options mirror those of small-cell lung cancer (SCLC) to which LCNEC is similar on a genomic and transcriptomic level [2]. Among LCNECs, genomic and transcriptomic features identify at least two distinct molecularly-defined subtypes of LCNECs [2]. Type I LCNECs are characterized by mutations in *TP53*, *STK11* and *KEAP1* and a neuroendocrine-like expression profile (high *ASCL1* and *DLL3* expression, low *NOTCH1* expression), whereas type II LCNECs harbor mutations in both *TP53* and *RB1* and a reduced expression of neuroendocrine markers (low *ASCL1* and *DLL3* expression, high *NOTCH1* expression) but also an increased expression of immune response genes [2]. Whether these subgroups are associated with differential susceptibility to treatments is currently unknown. Even though LCNEC is diagnosed at an early stage more often than SCLC (about 25% vs 5%, respectively), median survival of advanced stage LCNEC patients is worse than those with NSCLC but not significantly different to extensive-disease (ED) SCLC patients, ranging from 10 to 16 months [1,3]. In particular, in a phase II trial of platinum-etoposide chemotherapy in 42 LCNEC, median survival was 7.7 months and 12 months survival rate was 27% [4]. However, in this study only 29 patients had confirmed LCNEC after pathology review: in those case, median survival was 8.0 months, but 12 months survival rate was no reported. In the randomized phase III placebo-controlled IMpower 133 trial comparing atezolizumab or placebo plus carboplatin and etoposide in ED-SCLC, both overall survival and progression-free survival (primary endpoints) were improved in the atezolizumab arm compared to the placebo arm: overall survival was 12.3 vs 10.3 months and PFS was 5.2 vs 4.3 months, in the atezolizumab and the placebo arm, respectively [5]. Proportion of patients alive at 1 year was 51.7% and 38.2% in atezolizumab and the placebo arm, respectively. In the randomized phase III open-label CASPIAN trial, 268 ED-SCLC patients received durvalumab plus platinum-based chemotherapy plus etoposide and 269 received platinum-based and etoposide chemotherapy alone [6]. Addition of durvalumab significantly improved overall survival, the primary endpoint, compared to chemotherapy alone. Overall survival was 13.0 (95%CI: 11.5-14.8) vs 10.3 months (95%CI: 9.3-11.2) in the durvalumab vs chemotherapy, respectively, with 12-month overall survival rate of 54% and 40%, respectively. Thus, combination of chemotherapy, namely carboplatin and etoposide, with a PD-1/PD-L1 immune checkpoint blocking agent improved outcomes in ED-SCLC and represents the new standard of treatment in these patients. However, whether these results are applicable to pulmonary LCNEC is currently unknown and specific trials of treatment strategies in this disease are urgently needed.

Preliminary data on durvalumab and immunotherapy in pulmonary large-cell neuroendocrine carcinoma

Lung cancer can evade immune surveillance using different immunosuppressive mechanisms, including “immune checkpoints” [7]. The correlation in non-small cell lung cancers between high mutation burden and higher likelihood of response to anti-PD- 1 treatments supports the rationale for immunotherapy in small-cell lung cancer [8,9]. Tumors with high mutation burden are inherently more likely to generate tumor-specific neoantigens—high mutation burden provides an increased

substrate of targets that may trigger an immune response through their presentation to T cells [8,10]. LCNEC genome, just like SCLC one, is notable for high mutation burden and genomic instability.

Durvalumab is a Fc-optimized human immunoglobulin G1 kappa (IgG1k) monoclonal antibody which can disrupt the interaction between PD-1 and PD-L1 leading to the recognition of cancer cells by cytotoxic T cells. Durvalumab as monotherapy is now approved by the US Food and Drug Administration (FDA) and by the European Medicines Agency (EMA) for the treatment of locally advanced, unresectable NSCLC in adults whose tumors express PD-L1 on ≥ 1% of tumor cells and whose disease has not progressed following platinum-based chemoradiation therapy. Data from the SCLC expansion cohort of a phase I/II study of durvalumab showed a response rate of 9.5% (N=2/21) with a duration of response of 14.6 and 29.5+ months, respectively, in unselected pretreated ED SCLC [11]. The median PFS was 1.5 months, the median OS was 4.8 months and the OS rate at 12 months was 27.6%. The CASPIAN study is a randomized open-label sponsor-blind multicenter phase III trial designed to evaluate durvalumab with and without tremelimumab, an anti-CTLA4 monoclonal antibody, plus carboplatin and etoposide in patients with extensive-stage small-cell lung cancer who had not previously received treatment. Patients were randomly assigned in a 1:1:1 ratio to receive platinum-based chemotherapy and etoposide alone or with either durvalumab or durvalumab + tremelimumab for up to six 21-day cycles (induction phase), followed by a maintenance phase during which they received durvalumab (in both the durvalumab monotherapy or the durvalumab + tremelimumab arm) until they had unacceptable toxic effects, disease progression according to Response Evaluation Criteria in Solid Tumors, version 1.1, or no additional clinical benefit. The primary endpoint was overall survival. Recently, interim analysis for the chemotherapy plus durvalumab arm vs. chemotherapy arm only were presented. A total of 268 patients were randomly assigned to the durvalumab group, and 269 patients to the chemotherapy only group.

Results of this trial showed that after 62.6% of survival events (N=336 across the durvalumab and the chemotherapy only arms), the median overall survival was 13.0 months in the durvalumab group and 10.3 months in the chemotherapy arm (hazard ratio for death, 0.73; 95% confidence interval [CI], 0.59 to 0.91; P=0.0047). The rate of patients alive at 12 months was 54% in the durvalumab arm and 40% in the chemotherapy arm. The median progression-free survival was 5.1 and 5.4 months, respectively (hazard ratio for disease progression or death, 0.78; 95% CI, 0.65 to 0.94). Overall response rate is 67.9% in the durvalumab arm compared to 57.6% in the chemotherapy only arm (odds ratio for disease response, 1.56; 95% CI, 1.09 to 2.22). The safety profile of durvalumab plus platinum-based chemotherapy and etoposide was consistent with the previously reported safety profile of the individual agents, with no new findings observed.
Specifically, no trial exists of immunotherapy in LCNEC patients. Overall, 16 cases of LCNEC patients receiving immune checkpoint inhibitors have been reported [12], with a response rate of 60% and a median PFS of 57 weeks in the larger series (N=10) [13].
We hypothesized that a combination strategy including durvalumab immunotherapy and platinum-based chemotherapy is feasible and effective in the treatment of patients with metastatic pulmonary LCNEC. To prove our hypothesis, we will design a phase II single-arm clinical trial of durvalumab combined with carboplatin and etoposide in treatment-naïve metastatic pulmonary LCNEC patients.

- **<u>OBJECTIVES OF THE STUDY</u>**

AIM #1: to evaluate the efficacy of the combination of durvalumab with carboplatin and etoposide chemotherapy in treatment-naïve metastatic pulmonary LCNEC patients.

Rationale: the addition of PD-(L)1 blockade to first-line platinum-based chemotherapy in ED-SCLC improved OS compared to chemotherapy alone in two randomized controlled phase III trials [5,6]. The proportion of patients alive at 1 year from enrollment was 51.7% and 54% in the immunotherapy

arms compared to 38.2% and 40% in the chemotherapy alone arms, in the IMpower133 and CASPIAN trial, respectively. We hypothesized that a similar efficacy can be obtained in pulmonary LCNEC.
Approach: A multicenter phase II single-arm clinical trial will be designed. Patients matching inclusion criteria will be enrolled in the study and treated with the following regimen:
Intravenous carboplatin (AUC 5 on day 1), etoposide (100 mg/sqm on days 1-3), and durvalumab (1500 mg on day 1) administered every three weeks for 4 courses (**induction phase**) until progression of disease, unacceptable toxicity, patient refusal or loss of clinical benefit (for durvalumab). Treatment with intravenous durvalumab (1500 mg on day 1) every 4 weeks (**maintenance phase**) will continue until disease progression, unacceptable toxicity, patient refusal, reaching of 2 years of treatment (24 maintenance courses, 28 total courses of durvalumab) or loss of clinical benefit. Efficacy will be assessed by measuring the percentage of patients alive at 1 year from the date of enrollment in the study (1yr-OS).

AIM #2: to evaluate safety of the combination of durvalumab with carboplatin and etoposide chemotherapy in treatment-naïve metastatic pulmonary LCNEC patients.

Rationale: the combination of different anticancer agents might potentially increase toxicity and pose harm to the safety of patients. In particular, PD-(L)1 blockade has been associated with immune-mediated adverse events. However, the most common reported immune-related adverse events are mild in severity and almost all of them reversible with drug discontinuation or temporary immunosuppressive treatment (e.g. corticosteroids).
Approach: This assessment will be based mainly on the frequency of adverse events and measurement of toxicity according to NCI Common Toxicity Criteria Adverse Event (CTCAE), version 4.03.

AIM #3: to characterize activity profile of the combination of durvalumab with carboplatin and etoposide chemotherapy in treatment-naïve metastatic pulmonary LCNEC patients.

Rationale: the addition of PD-(L)1 blockade to first-line platinum-based chemotherapy in ED-SCLC provided a PFS of 5.2 and 5.1 months in the immunotherapy arms compared to 4.3 and 5.4 months in the chemotherapy alone arms, in the IMpower133 and CASPIAN trial, respectively, [5,6].
Approach: the activity will be evaluated via overall response rate (ORR) and progression-Free Survival (PFS). ORR is defined as the sum of complete response (CR) + partial response (PR) evaluated according to standard RECIST v1.1 criteria (patients with no tumor assessment after baseline will be classified as non-responders). PFS will be calculated from the date of enrolment to the date of progressive disease evaluated according to standard RECIST v1.1 criteria, or death whichever occurs first.

AIM #4: to identify potential biomarkers of response to PD-(L)1 blockade combined with carboplatin and etoposide in LCNEC

Rationale: Increasing effort is being put to better distinguish pulmonary LCNEC from their large cell epithelial counterpart and to further characterize high-grade neuroendocrine tumors of the lung based on genomic and transcriptomic features. For the first point, in non-small cell lung cancers with no clear-cut evidence of neuroendocrine origin as opposite to an epithelial one, serum tumor markers may inform about the histology derivation of the tumor thus guiding treatment choice. The presence in serum of elevated levels of carcinoembryonic antigen (CEA) and cytokeratin 19 fragments (Cyfra 21-1) are in favor of an epithelial origin, while elevated neuron-specific enolase (NSE) levels are in favor of a neuroendocrine origin. Since susceptibility to specific treatment regimens differs between neuroendocrine and non-neuroendocrine tumors, identification of the tissue of origin is crucial to

maximize chances of response to treatment. In respect to the second point, SCLC, the most common high-grade neuroendocrine tumor of the lung, has been recently proposed to be classified in 4 molecular subtypes according to the expression of key transcription factors [14]. Similarly, genomic and transcriptomic features can be used to identify at least two distinct molecularly-defined subtypes of LCNECs [2]. Type I LCNECs are characterized by mutations in *TP53*, *STK11* and *KEAP1* and a neuroendocrine-like expression profile (high *ASCL1* and *DLL3* expression, low *NOTCH1* expression), whereas type II LCNECs harbor mutations in both TP53 and RB1 and a reduced expression of neuroendocrine markers (low *ASCL1* and *DLL3* expression, high *NOTCH1* expression) but also an increased expression of immune response genes [2]. Whether these subgroups are associated with differential susceptibility to treatments is currently unknown.
Approach: Efficacy (1yr-OS, see AIM #1) and activity (ORR and PFS, see AIM #3) measures will be compared between groups of patients defined by the type of serum tumor marker elevated (neuroendocrine vs non-neuroendocrine) and between the two LCNEC molecular subgroups defined by genomic and transcriptomic analysis (type I vs type II), for those patients with available tumor samples.

- **EXTIMATED STUDY TIMELINES:**

The minimum follow-up duration of each patient will be 12 months. Assuming an accrual rate equal to 26 patients/year across national 15 Centers, the study enrollment should be completed in 24 months. Thirty-six months will be needed from project start to completion of project. Additional 3 months will be needed to lock database, analyze data e produce first report.

- **STATISTICAL ANALYSIS PLAN:**

The primary endpoint of the study will be overall survival (OS), defined as the time interval between the date of enrolment and the date of death for any cause. The secondary endpoints of the study are: tumor response evaluated according to RECIST criteria version 1.1 [15]; progression-free survival (PFS) defined as the time interval between the date of enrolment and the date of progression or death whichever occurs first; incidence of serious and non-serious adverse events.
The 1-year cumulative probability of OS will be estimated with the Kaplan-Meier method. The two-sided 90% confidence interval of the crude estimate and the hypothesis test will be conducted according to Brookmeyer and Crowley.
Based on the results of IMpower 133 and CASPIAN trials (1yr-OS) 38-40% in the control arm and 51-54% in the immunotherapy arm) and of the chemotherapy phase II trial by Le Treut et al (1yr-OS 27%), a proportion of patients alive 1 year after the enrollment greater than 30% is considered valuable enough to pursue the treatment regimen in a phase III trial. The hypothesis is that the study regimen is associated with a probability of 1-year OS equal to 45%.
The null hypothesis that true 1-year probability of OS is <30% will be tested against a one-sided alternative. A total of approximately 45 patients, plus 4 patients to account for a lost-to-follow-up rate of 10%, will be accrued. This design yields a type I error rate of 10% and power of 80% when the true 1-year probability of OS is >45%.
The overall complexity and costs of the trial will justify such ambitious endpoint and this statistical design. Positive results will be considered achievable also based on the strict selection of eligible patients.
The minimum follow-up duration of each patient will be 12 months. Assuming an accrual rate equal to 26 patients/year across 15 National Centers, the study enrollment should be completed in 24 months.

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