Lung Cancer 118 (2018) 76–82

Contents lists available at ScienceDirect

Lung Cancer

journal homepage: www.elsevier.com/locate/lungcan

PD-L1 expression in large cell neuroendocrine carcinoma of the lung

F. Eichhorn[a,*], A. Harms[b], A. Warth[b,d], T. Muley[c,d], H. Winter[a,d], M.E. Eichhorn[a,d]

[a] Department of Thoracic Surgery, Thoraxklinik, Heidelberg University, Heidelberg, Germany
[b] Institute of Pathology, Heidelberg University, Heidelberg, Germany
[c] Section Translational Research (STF), Thoraxklinik, Heidelberg University, Heidelberg, Germany
[d] Translational Lung Research Center (TLRC), Heidelberg, Member of German Center for Lung Research (DZL), Germany

ARTICLE INFO

Keywords:
Lung cancer
Neuroendocrine carcinoma
LCNEC
PD-L1
PD-1

ABSTRACT

Objectives: Large cell neuroendocrine carcinoma of the lung (LCNEC) is associated with an unfavorable prognosis and only few patients are eligible for surgery. In most patients, chemotherapy is recommended alone or in addition to resection. Novel immunotherapies blocking the PD-L1 pathway have been introduced into therapeutic regimens for NSCLC with great success. In order to evaluate a possible efficacy of an anti-PD-L1 therapy, we analyzed the frequency of PD-L1 expression in LCNEC.

Material and methods: We retrospectively reviewed data from 76 patients with LCNEC treated in our institution between 1998 and 2010. The expression of PD-L1 was examined on the tumor cells and the tumor surrounding tissue by immunohistochemistry. An expression of > 1% was considered as positive. Statistical analysis was performed to determine significant predictors for survival.

Results: 56 of 76 patients with LCNEC were treated with a potentially therapeutic surgical approach. Tumor-specific survival (TSS) of the entire cohort was 29% at five years. 17 patients (22.3%) had PD-L1 positive tumors and 12 of these had no additional PD-L1 expression in the adjacent immune cell infiltrate. Tumor-flanking immune cells were found PD-L1 positive 28 patients; 16 of these had no additional expression on the tumor cells. The most considerable difference in survival was found when comparing patients with isolated PD-L1 expression on tumor cells and PD-L1 negative immune cell infiltrate to their counterpart (positive immune-cell infiltrate and PD-L1 negative tumor cell surface; 5-year TSS: 0% vs. 60%; $p < 0.017$).

Conclusion: PD-L1 expression in LCNEC was associated with poorer survival whereas PD-L1 expression in the tumor microenvironment seemed to have a beneficial effect. Therapeutic approaches have to be evaluated in future.

1. Introduction

Approximately 20% of all lung cancer patients suffer from neuroendocrine pulmonary tumors. These have been reclassified by the World health organization in 2015 into four major subtypes with distinct prognostic implications: typical (TC) and atypical carcinoids (AC) are associated with a better survival than poorly differentiated neuroendocrine tumors like large cell neuroendocrine carcinoma (LCNEC) and small cell carcinoma (SCLC) [1].

Regarding their different prognoses, treatment varies within the different entities: curative surgical concepts are well established for TC and AC [2]. Most patients with high proliferative SCLC are treated with a definitive cisplatin-based chemotherapy due to advanced disease at the time of the diagnosis. In contrast, only little is known about the biology of LCNEC, but it is supposed to share features of both SCLC and non-small cell lung cancer (NSCLC). Therefore, most authors recommend a combined treatment with surgery and chemotherapy (and radiotherapy, if necessary) according to current NSCLC guidelines. Nevertheless, high recurrence rates and poor survival have been reported [3–5].

In the last decade, the potential of targeted immunotherapies has raised special interest in the treatment of advanced non-small cell lung cancer. Especially so called regulatory immune-inhibitory pathways including the programmed death-receptor 1 (PD-1) and its ligand (PD-L1) have been focused recently. The PD-L1 is located on the tumor cell surface and its interaction with the PD-1 receptor, that is expressed on activated T-cells is known to suppress patients' immune-response mechanisms to the tumor [6,7]. Upregulated PD-L1 enhances tumor immune-escape and its overexpression has been reported to be associated with a high malignant potential and poor prognosis in several malignancies including lung cancer [8,9].

Antibody treatment targeting the PD-1/PD-L1 checkpoint has been

* Corresponding author at: Department of Thoracic Surgery, Thoraxklinik, Heidelberg University, Roentgenstraße 1, 69126 Heidelberg, Germany.
E-mail address: florian.eichhorn@med.uni-heidelberg.de (F. Eichhorn).

https://doi.org/10.1016/j.lungcan.2018.02.003
Received 7 December 2017; Received in revised form 14 January 2018; Accepted 5 February 2018

investigated in clinical trials with NSCLC patients and correlated with an increased survival compared to standard chemotherapy. This resulted in the approval of PD-1-Checkpoint-Inhibitors in the second line treatment for advanced, previously treated NSCLC. Lately, PD-1 antibodies were approved as first line therapy in NSCLC patients with a PD-L1 expression of more than 50% on the tumor surface [10]. Nevertheless, these studies have mainly examined patients with adenocarcinoma or squamous cell carcinoma. There is comparably poor information about the prognostic relevance of PD-L1 expression in neuroendocrine lung cancer. Contrary data exits about SCLC that show both positive and negative correlation between PD-L1 status and survival [11,12]. In addition, even less reports are available for LCNEC [13].

We hereby report on a large cohort of 76 patients with LCNEC either treated by surgery or definitive chemo- and/or radiotherapy. All patients were examined for their PD-L1 Status by immunochemistry. Data was analyzed for individual survival, tumor recurrence or disease progression as well as prognostic factors with special focus on the PD-L1 expression.

2. Material and methods

2.1. Patient selection

We retrospectively reviewed data from 76 patients with a diagnosis of LCNEC between 1998 and 2010. All patients were treated at our institution and all tumor samples were available for immunohistochemical staining and analysis. The study was approved by the institutional review board of the Heidelberg University (No. 080/2006).

All patients underwent complete radiological tumor staging using brain, chest and abdominal sectional imaging as well as bone scintigraphy prior to therapy to screen for distant metastases. Positron-electron-tomography (PET) was used in more recent cases for potential surgical candidates.

All patients were discussed in our interdisciplinary tumor-board. Primary surgery was recommended for physically eligible patients with a localized tumor and not more than single stage N2-level involved (Stages I-IIIA3 unilevel [14], 7th edition of the TNM-classification [15]). Patients with locally advanced but potentially curable disease received inductive treatment followed by surgery if possible. Adjuvant therapies including chemotherapy, radiotherapy or both were recommended by the institutional tumor board according to the actual guidelines, postoperative pathological tumor stage and individual recovery.

Patients with advanced mediastinal nodal involvement (> IIIA4), simultaneous distant metastases, technical or functional inoperability were planned for a definitive cisplatin-based doublet therapy and irradiation if recommended.

Tumor specimens of all patients were provided by the tissue bank of the National Center for Tumor Diseases (NCT, Heidelberg, Germany) in accordance with their regulations. All tissue was revisited by experienced lung pathologists (A.W. or A.H.). Both neuroendocrine morphology and expression of neuroendocrine markers were confirmed for all cases in line with the criteria of the World Health Organization Classification [1].

All specimens were analyzed for PD-L1 expression on the tumor cell surface (T) and neighboring tumor microenvironment/immune-cell-infiltrate (MI) by immunohistochemical staining (Antibody: Clone: SP263; Ventana Benchmark Ultra, Ventana Medical Systems, AZ 85755, USA) (Fig. 1). A minimum of 100 vital tumor cells were counted for the quantity of PD-L1 positive cells. Intensity of PD-L1 expression was scored using the Histoscore-method (H-score) involving a semi-quantitative assessment of the percentage of positive cells (0–100%) multiplied by the intensity of staining (0: no staining; 1: weak, 2: median; 3: strong)[16]. Scores ranged from 0 to 300 and positive PD-L1 expression was defined for a cut-off-score > 1. Follow up-examinations were scheduled quarterly including sectional imaging of the chest and abdomen. Documented recurrence or progressive disease by RECIST criteria [17] was clarified by sequential biopsy and resulted in whole body restaging followed by interdisciplinary discussion and individual treatment.

Data was analyzed using SPSS (v23, IBM Corporation, Armonk, NY). Tumor specific survival was defined as the time from the date of surgery or, in the non-surgical patients, date of biopsy to the date of tumor-related death or last follow-up in patients alive. Relapse free survival (RFS) was defined from end of initially administered therapy (surgical or non-surgical) to the date of first detection of disease relapse (local or distant, after curative intended surgery) of measurable progression of disease (after definitive radio- and/or chemotherapy). Cumulative survival was calculated using the Kaplan-Meier product method, the log rank-test was used to calculate differences. A p-value less than 0.05 was considered statistically significant.

3. Results

76 patients (54 male, 22 female; mean age 60.9 years) were diagnosed with LCNEC between 1998 and 2010 (Table 1). 56 patients (73.7%) underwent surgery with curative intent (surgical group). Within this group, main procedures were lobectomy (n = 42) and bilobectomies/pneumonectomies (n = 11). The remaining 20 patients (non-surgical group, 26.3%) either had a locally advanced (> stage IIIA3 unilevel, n = 9) or metastatic disease (stage IV, n = 11) at the time of diagnosis (non-curative group). 10 patients received a definitive platinum-based chemotherapy. A combined chemoradiotherapy was recommended for 7 patients. Local radiotherapy alone was administered to 3 patients.

Within the surgical cohort, pathological (p) nodal N0 status was observed in 26 patients, a pN1 status in 16 and a pN2 status in 14 patients.

Overall survival of the entire group was 29% at five years. 5-year survival in the curative group (41%) was significantly better than in the non-curative cohort (10%, $p < 0.005$). 17 patients were alive at the end of the follow up (April 2017). The 1-, 3- and 5-year tumor specific survival (TSS) was 73 %, 36%, and 32%, respectively. Advanced tumor stage and positive pathological nodal status were identified as independent factors predicting a poor survival (Fig. 2).

Tumors of 17 patients (22.4%) were found PD-L1 positive (T+). The tumor-adjacent immune-cell infiltrate was stained positive for PD-L1 in 28 patients (MI+; 36.8%). These cells were related to the tumor microenvironment and were morphologically identified as macrophages and leucocytes. Isolated PD-L1 expression on the tumor cell membrane (without PD-L1 expression in the MI, T+MI−) was found in 5 of the 17 patients, whereas 16 of 28 patients were PD-L1 positive only in the MI (tumor PD-L1 negative; T−MI+) (Table 2). A positive tumor PD-L1 status (T+) was associated with a poorer survival at five years (18% vs. 37%; p = 0.28; Fig. 3A), although it was not statistically significant. In contrast, positive PD-L1 in the tumor-adjacent immune cell infiltrate (MI+) tended towards a better five-year TSS (49.7% vs. 26.5%; p = 0.16; Fig. 3B). Interestingly, patients with only PD-L1 positive tumor cells but negative immune cell infiltrate (T+MI−) showed a significant poorer survival compared to those with PD-L1 positive immune-cell infiltrate but negative tumor cell surface (T−MI+; 0% vs. 60% at 2 years, p = 0.017) (Fig. 4). Patients with positive PD-L1 expression in both compartments (T+MI+; n = 12) showed no relevant difference in TSS compared to patients with both negative PD-L1 statuses (T−MI−; n = 31; 30% vs. 25%, p = 0.99).

Isolated PD-L1 expression on the tumor cell surface was not observed in any surgical patient but in 5 patients initially classified as stage IIIB (20%) or IV (80%).

RFS at five years was 26%. Intrathoracic recurrence (e.g. pleural carcinosis, mediastinal or pulmonary metastases) was found in 19