Histopathology

Histopathology 2023, **82**, 870–884. DOI: 10.1111/his.14873

Ascl1 and OTP tumour expressions are associated with disease-free survival in lung atypical carcinoids

Giovanni Centonze,[1,2] Patrick Maisonneuve,[3] Michele Simbolo,[4] Vincenzo Lagano,[1] Federica Grillo,[5] Natalie Prinzi,[6] Sara Pusceddu,[6] Loretta Missiato,[1] Marilena Colantuono,[1] Giovanna Sabella,[1] Luisa Bercich,[7] Alessandro Mangogna,[8] Luigi Rolli,[9] Salvatore Grisanti,[10] Mauro Roberto Benvenuti,[11] Ugo Pastorino,[9] Luca Roz,[2] Aldo Scarpa,[4,12] Alfredo Berruti,[10] Carlo Capella[13] & Massimo Milione[1]

[1]*1st Pathology Division, Department of Pathology and Laboratory Medicine,* [2]*Tumor Genomics Unit, Department of Research, Fondazione IRCCS Istituto Nazionale dei Tumori,* [3]*Division of Epidemiology and Biostatistics, IEO, European Institute of Oncology IRCCS, Milan,* [4]*Department of Diagnostics and Public Health, Section of Pathology, University of Verona, Verona,* [5]*Unit of Pathology, Department of Surgical Sciences and Integrated Diagnostics, University of Genoa and Ospedale Policlinico San Martino, Genoa,* [6]*Medical Oncology Department, Fondazione IRCCS Istituto Nazionale dei Tumori, Milan,* [7]*Department of Pathology, ASST Spedali Civili of Brescia, Brescia,* [8]*Institute for Maternal and Child Health, IRCCS Burlo Garofalo, Trieste,* [9]*Thoracic Surgery Unit, Fondazione IRCCS Istituto Nazionale Tumori, Milan,* [10]*Medical Oncology Unit, Department of Medical and Surgical Specialties, Radiological Sciences, and Public Health, University of Brescia, ASST Spedali Civili,* [11]*Thoracic Surgery Unit, Department of Medical and Surgical Specialties Radiological Sciences and Public Health, Medical Oncology, University of Brescia, ASST Spedali Civili of Brescia, Brescia,* [12]*ARC-NET Research Center for Applied Research on Cancer, and Department of Diagnostics and Public Health, Section of Pathology, University of Verona, Verona and* [13]*Unit of Pathology, Department of Medicine and Surgery, University of Insubria, Varese, Italy*

Date of submission 21 October 2022
Accepted for publication 28 January 2023
Published online *Article Accepted* 31 January 2023

Centonze G, Maisonneuve P, Simbolo M, Lagano V, Grillo F, Prinzi N, Pusceddu S, Missiato L, Colantuono M, Sabella G, Bercich L, Mangogna A, Rolli L, Grisanti S, Benvenuti M R, Pastorino U, Roz L, Scarpa A, Berruti A, Capella C & Milione M

(2023) *Histopathology* **82**, 870–884. https://doi.org/10.1111/his.14873

Ascl1 and OTP tumour expressions are associated with disease-free survival in lung atypical carcinoids

Aims: According to World Health Organization guidelines, atypical carcinoids (ACs) are well-differentiated lung neuroendocrine tumours with 2–10 mitoses/2 mm^2 and/or foci of necrosis (usually punctate). Besides morphological criteria, no further tools in predicting AC clinical outcomes are proposed. The aim of this work was to identify novel factors able to predict AC disease aggressiveness and progression.

Methods and results: Three hundred-seventy lung carcinoids were collected and centrally reviewed by two expert pathologists. Morphology and immunohistochemical markers (Ki-67, TTF-1, CD44, OTP,

Address for correspondence: M Milione, 1st Pathology Division, Department of Pathology and Laboratory Medicine, Fondazione IRCCS - Istituto Nazionale dei Tumori, Milan, Italy. e-mail: massimo.milione@istitutotumori.mi.it

This work is dedicated to the memory of Laura Salvaterra, a courageous woman who battled against cancer. This is an invitation to fight cancer every day in her name, even after she has left us.

List of abbreviations: AC, atypical carcinoid; Ascl1, mammalian achaete-scute homologue 1; CgA, chromogranin-A; CI, confidence interval; DFS, disease-free survival; HR, hazard ratio; H&E, haematoxylin–eosin; IHC, Immunohistochemical; MC, mitotic count; NEN, neuroendocrine neoplasm; NET, neuroendocrine tumour; OS, overall survival; OTP, orthopedia homeobox protein; Syn, synaptophysin; SSTR2A, somatostatin receptor 2A; STAS, spread through air spaces; TTF-1, thyroid transcription factor 1; TT, thoracic tumours; WHO, World Health Organization.

SSTR2A, Ascl1, p53, and Rb1) were studied and correlated with disease-free survival (DFS) and overall survival (OS). Fifty-eight of 370 tumours were defined as AC. Survival analysis showed that patients with Ascl1 + ACs and those with OTP-ACs had a significantly worse DFS than patients with Ascl1-ACs and OTP + ACs, respectively. Combining Ascl1 and OTP expressions, groups were formed reflecting the aggressiveness of disease ($P = 0.0005$). Ki-67 ≥10% patients had a significantly worse DFS than patients with Ki-67 <10%. At multivariable analysis, Ascl1 (present versus absent, hazard ratio [HR] = 3.42, 95% confidence interval [CI] 1.35–8.65, $P = 0.009$) and OTP (present versus absent, HR = 0.26, 95% CI 0.10–0.68, $P = 0.006$) were independently associated with DFS. The prognosis of patients with Ki-67 ≥10% tended to be worse compared to that with Ki-67 <10%. On the contrary, OTP (present versus absent, HR = 0.28, 95% CI 0.09–0.89, $P = 0.03$), tumour stage (III-IV versus I-II, HR = 4.25, 95% CI 1.42–12.73, $P = 0.01$) and increasing age (10-year increase, HR = 1.67, 95% CI 1.04–2.68, $P = 0.03$) were independently associated with OS.

Conclusion: This retrospective analysis of lung ACs showed that Ascl1 and OTP could be the main prognostic drivers of postoperative recurrence.

Keywords: Ascl1, atypical carcinoids, Ki-67 index, lung, OTP

Introduction

According to the WHO Classification of Thoracic Tumours (WHO-TT 2021), atypical carcinoids (ACs) are well-differentiated lung neuroendocrine neoplasms (NEN) classified according to the presence of necrosis and/or mitotic count.[1] In the spectrum of lung carcinoid tumours, ACs represent the rarest entities and account for only 0.2% of all lung neoplasms.[2] ACs have a greater chance of metastasizing compared to typical pulmonary carcinoids and a 5-year survival rate of 58–68%.[3] To date, no adjuvant therapy is recommended, despite their high recurrence rate,[4] and this is most probably secondary to the difficulty in data collection due to their rarity and biological heterogeneity.

Recently, different markers have been proposed as prognostic factors to further predict AC behaviour. Among others, mammalian achaete-scute homologue 1 (Ascl1) has been described as a neuroendocrine marker associated with a significantly shortened overall survival for SCLC patients,[5] suggesting the potential prognostic significance for all lung NENs. Furthermore, orthopedia homeobox protein (OTP) stands out as a promising marker to distinguish aggressive from indolent carcinoids[6]; nevertheless its prognostic value needs to be further evaluated to predict AC recurrence. Finally, the role of the proliferation index evaluated by Ki-67 staining has been discussed and still remains to be defined, although the evidence from the literature suggests a cutoff of 10% in order to provide a clinically meaningful stratification of ACs.[7]

The aim of this study was to identify novel factors able to predict AC disease aggressiveness and progression.

Materials and Methods

STUDY DESIGN AND CASE SELECTION

The surgical pathology and clinical databases of Fondazione IRCCS Istituto Nazionale dei Tumori - INT, Milan and ASST Spedali Civili di Brescia: Brescia, were retrospectively searched for one of the following histological diagnoses: “typical lung carcinoid”, “atypical lung carcinoid”, “lung carcinoid tumor”, “peripheral lung carcinoid”, and “bronchial carcinoid” during the period from 1988 to 2018. Exclusion criteria were: (i) cases which had not undergone surgical resection with curative intent; (ii) cases with only biopsy material available; (iii) cases with poorly differentiated neuroendocrine components; and (iv) cases of dubious primary.

Overall, a total of 370 candidate cases were identified. The study was performed according to the clinical standards of the 1975 and 1983 Declaration of Helsinki and was approved by the Ethics Committee of Fondazione IRCCS INT (No. INT 171/16).

The patients’ charts and tumour morphology were centrally and blindly reviewed by expert neuroendocrine tumour (NET) pathologists (M.M. and C.C.) prior to inclusion in the study. Carcinoid identification was based on the parallel investigation of at least three consecutive sections from representative formalin-fixed paraffin-embedded blocks, stained with

haematoxylin–eosin (H&E), and for synaptophysin (Syn) and chromogranin A (CgA). A total of 58 cases met the morphological criteria for ACs according to WHO-TT 2021 and were included in the study.[1]

HISTOLOGY AND IMMUNOHISTOCHEMISTRY

Morphologic analysis considered: (a) well-differentiated neuroendocrine morphology, (b) architectural pattern of the tumour registered as: (i) trabecular/nesting/organoid or (ii) insular/solid; (c) mitotic count (MC) counted in 2 mm^2; (d) Necrosis assessed as absent or present and present categorised as spot or extensive; (e) pathological tumour staging according to the Union for International Cancer Control/American Joint Committee on Cancer (UICC/AJCC) 8th edition; (f) vascular invasion (evaluated on H&E, and/or CD31-stained sections); (g) perineural invasion; (h) intra- and/or peritumoral lymphocyte infiltrate; (i) microscopic invasion of bronchial wall or pleura; or (j) tumour spread through air spaces (STAS).

The immunohistochemical (IHC) study included: Syn and CgA in order to confirm the diagnosis of lung NEN; Ki-67 labeling index calculation, using the MIB antibody, as a percentage of positive cells in 500–2000 tumour cells counted in areas of strongest nuclear labeling ("hot spots") as indicated in the WHO 2019 Digestive System Tumours[8]; thyroid transcription factor 1 (TTF-1), CD44, OTP, somatostatin receptor 2A (SSTR2A), Ascl1, menin, p53, and Rb1 using the antibodies listed in Supplementary Table S1.

To minimise assessment variability, with the exception of Rb1, p53, and SSTR2A, all markers were considered positive regardless of the number of positive cells. Rb1 was assessed adopting a scoring system with three levels: absent (no expression), heterogeneous (present: 1–50%), and overexpressed (present ≥50%). p53 was evaluated using four levels: absent (no expression), weak heterogeneous (scattered and weak staining in 1–20% of tumour cells), heterogeneous (variable expression in 21–60% of tumour cells), and overexpressed (strong p53 staining in more than 60% of tumour cells). Immunoreactivity and scores for SSTR2A were evaluated using a two-tiered system as suggested by Volante *et al.*[9]: negative for scores of 0 and 1 and positive for 2 and 3 positivity. For OTP, TTF-1, and Ascl1, only nuclear staining was considered, while for CD44 only membranous cytoplasmic staining was registered. For survival analysis, also the three-tiers grading system based on Ki67 index, mitotic count, and necrosis suggested by Rindi *et al.* was evaluated.[10]

STATISTICAL ANALYSIS

Data were analysed by descriptive statistics. Associations between demographic characteristics, clinicopathological features, and Ascl1 and OTP (absent versus present) and Ki-67 (<10 versus ≥10%), were assessed using the Fisher exact test for categorical variables and the nonparametric Wilcoxon test for continuous variables. Overall survival (OS) was assessed from the date of diagnosis to the date of death or last follow-up. Disease-free survival (DFS) was assessed from the date of diagnosis to the date of first relapse, death, or last follow-up, whichever occurred first. DFS was evaluated in stage I-II-III patients only. OS and DFS curves were drawn using the Kaplan–Meier method. The log-rank test was used to assess the survival difference between patient groups. Univariable and multivariable Cox proportional regression models were used to assess the association between clinicopathological characteristics and DFS and OS. Manual backward elimination was used to determine the best combination of predictors prioritizing the clinically relevant variables. Hazard ratios (HR) are presented with the respective 95% confidence interval (CI). Data analysis was performed using the R environment for statistical computing and graphics (R Foundation, Vienna, Austria, Version 4.0.3). All tests were two-sided and *P*-values <0.05 were considered statistically significant.

Results

CLINICOPATHOLOGICAL FEATURES AND TREATMENT

The main clinicopathological features of the 58 ACs included in the study are summarised in Table 1. The whole cohort comprised more females than males (56.9% versus 43.1%) with a median age of 61 years (range: 27–78 years). Current and former smokers (36.8% and 35.1%, respectively) were more represented than nonsmokers (28.1%). The series included 29 (50.0%) stage I, 12 (20.7%) stage II, 12 (20.7%) stage III, and five (8.6%) stage IV tumours. The median Ki-67 was 4.2% (range: 0.7–26) and, interestingly, eight cases (13.8%) showed a Ki-67 index ≥10% and were considered as ACs with elevated Ki-67 index (high Ki-67), including three cases with a Ki67 index >20%. All patients underwent surgical resection with curative intent, including 31 (53.4%) lobectomies, 13 (22.4%) segmentectomies or wedge resections, and 14 (24.2%) bilobectomies or pneumonectomies. Data on postoperative treatment were

Table 1. Characteristics of patients with AC tumours

	All patients	Female	Male
Total	58 (100)	33 (100.0)	25 (100.0)
Age			
Median [range]	61 [27–78]	59 [27–78]	64 [35–78]
Stage			
I	29 (50.0)	16 (48.5)	13 (52.0)
II	12 (20.7)	5 (15.2)	7 (28.0)
III	12 (20.7)	8 (24.2)	4 (16.0)
IV	5 (8.6)	4 (12.1)	1 (4.0)
Smoking status			
Never	16 (28.1)	10 (31.2)	6 (24.0)
Former	20 (35.1)	13 (40.6)	7 (28.0)
Smoker	21 (36.8)	9 (28.1)	12 (48.0)
Tumour site			
Upper lobe	18 (34.0)	10 (34.5)	8 (20.8)
Lower lobe	23 (43.4)	12 (41.4)	11 (45.8)
Hilum region	12 (22.6)	7 (24.1)	5 (33.3)
Surgery			
Lobectomy	31 (53.4)	14 (42.4)	17 (68.0)
Bilobectomy/pneumonectomy	14 (24.2)	11 (33.3)	3 (12.0)
Sublobar resection	13 (22.4)	8 (24.2)	5 (20.0)

available for 28 (48.3%) patients: two (7.1%) received somatostatin analogues, three (10.7%) chemotherapy, one (3.6%) radiotherapy, one (3.6%) combined chemo-radiotherapy, and 21 (75.0%) did not receive any treatment at all.

ASCL1, OTP, AND KI67 EXPRESSION

The associations between Ascl1, OTP, and Ki67 expression with main clinicopathological features and tumour biomarker expression are reported in Tables 2 and 3.

Ascl1 positive immunoreactivities were detected in 30 (51.7%) of all ACs. Specifically, Ascl1+ tumours were associated with the presence of necrosis (n = 12, 40.0%, P = 0.04), presence of STAS (n = 16, 61.5%, P = 0.0007), residual tumour (n = 6, 24.0%, P = 0.05), high Ki-67 index (n = 8 Ki-67 ≥10%, 26.7%, P = 0.005), positive immunoreactivity for TTF-1 (n = 18, 60.0% P < 0.0001), and negativity for SSTR2A (n = 22, 73.3%, P < 0.0001).

On the other hand, OTP expression was identified in 25 (43.1%) of all ACs. In particular, OTP+ tumours were significantly related to female sex (n = 19, 76.0%, P = 0.02), hilum/central region tumour site (n = 9, 37.5%, P = 0.02), expression of Rb1 (n = 25, 100%, P = 0.05), and of menin (n = 24, 96.0%, P = 0.001).

Finally, Ki-67 ≥10% was found in eight (13.8%) samples. Tumours with high Ki-67 were significantly associated with an increased number of mitoses (median 5 range 4–10, P = 0.0008), the presence of necrosis (n = 4, 50%, P = 0.01), peripheral location (n = 6, 75%, P = 0.01), the presence of peritumoral lymphocyte infiltrate (n = 7, 87.5%, P = 0.0005), expression of TTF-1 (n = 7, 87.5% P = 0.001), and

Table 2. Characteristics of patients with AC tumours according to Ascl1, OTP, and Ki-67 expression

	All patients *N* (%)	Ascl1 − *N* (%)	Ascl1 + *N* (%)	*P*-value*	OTP − *N* (%)	OTP + *N* (%)	*P*-value*	Ki-67 <10 *N* (%)	Ki-67 ≥10 *N* (%)	*P*-value*
Total	58 (100)	28 (100)	30 (100)		33 (100)	25 (100)		50 (100)	8 (100)	
Gender										
Female	33 (56.9)	15 (53.6)	18 (60.0)		14 (42.4)	19 (76.0)		31 (62.0)	2 (25.0)	
Male	25 (43.1)	13 (46.4)	12 (40.0)	0.8	19 (57.6)	6 (24.0)	**0.02**	19 (38.0)	6 (75.0)	0.06
Age										
Median [range]	61 [27–78]	58 [27–78]	64 [37–78]	0.2	64 [41–78]	59 [27–78]	0.2	61 [27–78]	66 [50–74]	0.2
Tumour stage										
I	29 (50.0)	17 (60.7)	12 (40.0)		14 (42.4)	15 (60.0)		27 (54.0)	2 (25.0)	
II	12 (20.7)	6 (21.4)	6 (20.0)		8 (24.2)	4 (16.0)		10 (20.0)	2 (25.0)	
III	12 (20.7)	5 (17.9)	7 (23.3)		8 (24.2)	4 (16.0)		9 (18.0)	3 (37.5)	
IV	5 (8.6)	0 (0.0)	5 (16.7)	0.1	3 (9.1)	2 (8.0)	0.6	4 (8.0)	1 (12.5)	0.4
Smoking status										
Never smoker	16 (28.1)	8 (28.6)	8 (27.6)		6 (18.8)	10 (40.0)		15 (30.6)	1 (12.5)	
Former smoker	20 (35.1)	8 (28.6)	12 (41.4)		12 (37.5)	8 (32.0)		17 (34.7)	3 (37.5)	
Current smoker	21 (36.8)	12 (42.9)	9 (31.0)	0.6	14 (43.8)	7 (28.0)	0.2	17 (34.7)	4 (50.0)	0.6
Mitoses										
Median [range]	3 [2–10]	3 [2–10]	3.5 [1–10]	0.3	4 [2–10]	3 [1–10]	0.4	3 [1–10]	5 [4–10]	**0.0008**
Necrosis										
Absent	43 (74.1)	25 (89.3)	18 (60.0)		23 (69.7)	20 (80.0)		39 (78.0)	4 (50.0)	
Spot	8 (12.1)	2 (7.1)	6 (20.0)		6 (18.2)	2 (8.0)		4 (14.0)	4 (50.0)	
Extensive	7 (13.8)	1 (3.6)	6 (20.0)	**0.04**	4 (12.1)	3 (12.0)	0.6	7 (8.0)	0 (0.0)	**0.01**
Location										
Central	38 (66.7)	18 (64.3)	20 (69.0)		23 (71.9)	15 (60.0)		36 (73.5)	2 (25.0)	
Peripheral	19 (33.3)	10 (35.7)	9 (31.0)	0.8	9 (28.1)	10 (40.0)	0.4	13 (26.5)	6 (75.0)	**0.01**

Table 2. (*Continued*)

	All patients *N* (%)	Ascl1 − *N* (%)	Ascl1 + *N* (%)	*P*-value*	OTP − *N* (%)	OTP + *N* (%)	*P*-value*	Ki-67 <10 *N* (%)	Ki-67 ≥10 *N* (%)	*P*-value*
Vascular Invasion										
Absent	31 (58.5)	17 (68.0)	14 (50.0)		20 (66.7)	11 (47.8)		26 (57.8)	5 (62.5)	
Present	22 (41.5)	8 (32.0)	14 (50.0)	0.3	10 (33.3)	12 (52.2)	0.3	19 (42.2)	3 (37.5)	1.0
Perineural Invasion										
Absent	46 (85.2)	22 (84.6)	24 (85.7)		27 (87.1)	19 (82.6)		39 (84.8)	7 (87.5)	
Present	8 (14.8)	4 (15.4)	4 (14.3)	1.0	4 (12.9)	4 (17.4)	0.7	7 (15.2)	1 (12.5)	1.0
Intratumoral lymphocyte infiltrate										
Absent	46 (86.8)	23 (95.8)	23 (79.3)		26 (86.7)	20 (87.0)		40 (88.9)	6 (75.0)	
Present	7 (13.2)	1 (4.2)	6 (20.7)	0.1	4 (13.3)	3 (13.0)	1.0	5 (11.1)	2 (25.0)	0.3
Peritumoral lymphocyte infiltrate										
Absent	37 (69.8)	19 (79.2)	18 (62.1)		19 (63.3)	18 (78.3)		36 (80.0)	1 (12.5)	
Present	16 (30.2)	5 (20.8)	11 (37.9)	0.2	11 (36.7)	5 (21.7)	0.4	9 (20.0)	7 (87.5)	**0.0005**
Microscopic infiltration										
Absent	4 (8.0)	4 (16.7)	0 (0.0)		3 (10.3)	1 (4.8)		4 (9.1)	0 (0.0)	
Positive STAS	19 (38.0)	3 (12.5)	16 (61.5)		14 (48.3)	5 (23.8)		14 (31.8)	5 (83.3)	
Bronchus	21 (42.0)	13 (54.2)	8 (30.8)		9 (31.0)	12 (57.1)		20 (45.5)	1 (16.7)	
Pleura	6 (12.0)	4 (16.6)	2 (7.7)	**0.0007**	3 (10.4)	3 (14.3)	0.2	6 (13.6)	0 (0.0)	0.2
Tumour site										
Upper lobe	18 (34.0)	9 (34.6)	9 (33.3)		14 (48.3)	4 (16.7)		15 (32.6)	3 (42.9)	
Lower lobe	23 (43.4)	13 (50.0)	10 (37.0)		12 (41.4)	11 (45.8)		20 (43.5)	3 (42.9)	
Hilum region	12 (22.6)	4 (15.4)	8 (29.6)	0.4	3 (10.3)	9 (37.5)	**0.02**	11 (23.9)	1 (14.2)	0.88
Morphological pattern										
Insular/solid	42 (73.7)	19 (67.9)	23 (79.3)		24 (75.0)	18 (72.0)		37 (74.0)	5 (71.4)	
Trabecular/nested/organoid	13 (22.8)	9 (32.1)	4 (13.8)		6 (18.8)	7 (28.0)		13 (26.0)	0 (0.0)	
Other	2 (3.5)	0 (0.0)	2 (6.9)	0.1	2 (6.2)	0 (0.0)	0.5	0 (0.0)	2 (28.6)	**0.005**

Table 2. (*Continued*)

	All patients *N* (%)	Ascl1 − *N* (%)	Ascl1 + *N* (%)	*P*-value*	OTP − *N* (%)	OTP + *N* (%)	*P*-value*	Ki-67 <10 *N* (%)	Ki-67 ≥10 *N* (%)	*P*-value*
Residual tumour										
R0	45 (86.5)	26 (96.3)	19 (76.0)		24 (85.7)	21 (87.5)		40 (87.0)	5 (83.3)	
R1-R2	7 (13.5)	1 (3.7)	6 (24.0)	**0.05**	4 (14.3)	3 (12.5)	1.0	6 (13.0)	1 (16.7)	1.0

Note: Statistically significant *P*-value are reported in bold.
Abbreviation: STAS, spread through air spaces.
**P*-value based on the Fisher's exact test for categorical variables and the Wilcoxon test for continuous variables.

p53 (n = 3, 37.5%, P = 0.02) and complete loss of SSTR2A expression (n = 8, 100%, P = 0.0004).

No other clinicopathological features and immunohistochemical expression were significantly associated with Ascl1, OTP, and Ki-67 expression.

DISEASE-FREE AND OVERALL SURVIVAL

The median DFS time for ACs was 65 months (95% CI 53–137). Survival analysis showed that patients with Ascl1 + ACs and those with OTP-ACs had significantly worse DFS than patients with Ascl1- and OTP + ACs, respectively (P = 0.001 and P = 0.02, Figure 1A,B). Furthermore, patients with high Ki-67 ACs had a significantly worse DFS than patients with low Ki-67 ACs (P = 0.0001; Figure 1D). Interestingly, combining Ascl1 and OTP expression, groups were formed reflecting the aggressiveness of disease (P = 0.0005, Figure 1C). Indeed OTP+/Ascl1- cases showed the best prognosis, the double negative or double positive intermediate prognosis and, finally, OTP-/Ascl1+ cases the worst prognosis. Of note was that high Ki-67 tumours were significantly more represented in the latter group (P = 0.01, Figure 2).

At univariate analysis (Table 4), significant clinicopathological predictors of poorer DFS among the cohort were: 10-year age increase (P = 0.02), lymph node involvement (P = 0.02), advanced tumour stage (P = 0.04), the presence of extensive necrosis (P = 0.02), Ki-67 ≥10% (P < 0.001), residual tumour (P = 0.03), and low or absent expression of SSTR2A (P = 0.006). In the entire cohort, the median OS was 102 months (95% CI 66-NA). Kaplan–Meier analysis showed that patients with stage III-IV and those with an absence of OTP expression had significantly worse OS than patients with stage I-II and OTP expression, respectively (log-rank P = 0.015 and P = 0.009; Figure 3A,B). Univariate analysis also showed that 10-year age increase (P = 0.006), expression of Ascl1 (P = 0.03, Figure 3C), and absent expression of SSTR2A (P = 0.003, Figure 3D) were associated with poor OS.

Results from multivariable Cox proportional regression analysis are reported in Table 5. After adjustment for center, stage, and period of diagnosis, Ascl1 (present versus absent, HR = 3.42, 95% CI 1.35–8.65, P = 0.009) and OTP (present versus absent, HR = 0.26, 95% CI 0.10–0.68, P = 0.006) were independently associated with DFS. The prognosis of patients with Ki-67 ≥10% tended to be worse compared to that with Ki-67 <10% (HR = 2.80, 95% CI 0.86–9.14, P = 0.09).

On the contrary, an increase of years in age (10-year increase, HR = 1.67, 95% CI 1.04–2.68,

Table 3. Association between selected tumour biomarkers with Ascl1, OTP, and Ki-67 expression in patients with AC tumours

	All patients *N* (%)	Ascl1− *N* (%)	Ascl1 + *N* (%)	*P*-value*	OTP − *N* (%)	OTP + *N* (%)	*P*-value*	Ki-67 <10 *N* (%)	Ki-67 ≥10 *N* (%)	*P*-value*
Total	58 (100)	28 (100)	30 (100)		33 (100)	25 (100)		50 (100)	8 (100)	
Ascl1										
Absent	28 (100)	—	—		—	—		—	—	
Present	30 (100)	—	—		—	—		—	—	
OTP										
Absent	33 (56.9)	17 (60.7)	16 (53.3)		—	—		—	—	
Present	25 (43.1)	11 (39.3)	14 (46.7)	0.6	—	—		—	—	
Ki-67										
<10	50 (86.2)	28 (100)	22 (73.3)		28 (84.8)	22 (88.0)		—	—	
≥10	8 (13.8)	0 (0.0)	8 (26.7)	**0.005**	5 (15.2)	3 (12.0)	1.0	—	—	
TTF-1										
Absent	39 (67.2)	27 (96.4)	12 (40.0)		25 (75.8)	14 (56.0)		38 (76.0)	1 (12.5)	
Present	19 (32.8)	1 (3.6)	18 (60.0)	**<0.0001**	8 (24.2)	11 (44.0)	0.2	12 (24.0)	7 (87.5)	**0.001**
CD44										
Absent	29 (50.0)	10 (35.7)	19 (63.3)		19 (57.6)	10 (40.0)		23 (46.0)	6 (75.0)	
Present	29 (50.0)	18 (64.3)	11 (36.7)	0.06	14 (42.4)	15 (60.0)	0.3	27 (54.0)	2 (25.0)	0.3
SSTR2A										
Absent	24 (41.4)	2 (7.1)	22 (73.3)		15 (45.5)	9 (36.0)		16 (32.0)	8 (100.0)	
Present	34 (58.6)	26 (92.9)	8 (26.7)	**<0.0001**	18 (54.5)	16 (64.0)	0.6	34 (68.0)	0 (0.0)	**0.0004**
Rb1										
Absent	4 (6.9)	1 (3.6)	3 (10.0)		4 (12.1)	0 (0.0)		3 (6.0)	1 (12.5)	
Heterogeneous	26 (44.8)	14 (50.0)	12 (40.0)		17 (51.5)	9 (36.0)		23 (46.0)	3 (37.5)	
Overexpressed	28 (48.3)	13 (46.4)	15 (50.0)	0.7	12 (36.4)	16 (64.0)	**0.05**	2 (48.0)	4 (50.0)	0.6
P53										
Absent	50 (87.7)	26 (92.9)	24 (82.8)		27 (84.4)	23 (92.0)		45 (91.8)	5 (62.5)	

Table 3. (*Continued*)

	All patients *N* (%)	Ascl1− *N* (%)	Ascl1 + *N* (%)	*P*-value*	OTP − *N* (%)	OTP + *N* (%)	*P*-value*	Ki-67 <10 *N* (%)	Ki-67 ≥10 *N* (%)	*P*-value*
Weak heterogeneous	6 (10.5)	2 (7.1)	4 (13.8)		4 (12.5)	2 (8.0)		4 (8.2)	2 (25.0)	
Heterogeneous	0 (0.0)	0 (0.0)	0 (0.0)		0 (0.0)	0 (0.0)		0 (0.0)	0 (0.0)	
Overexpressed	1 (1.8)	0 (0.0)	1 (3.4)	0.5	1 (3.1)	0 (0.0)	0.8	0 (0.0)	1 (12.5)	**0.02**
Menin										
Absent	13 (24.1)	9 (33.3)	4 (14.8)		12 (41.4)	1 (4.0)		12 (25.0)	1 (16.7)	
Present	41 (75.9)	18 (66.7)	23 (85.2)	0.2	17 (58.6)	24 (96.0)	**0.001**	36 (75.0)	5 (83.3)	1.0

Note: Statistically significant *P*-value are reported in bold.
Abbreviation: TTF-1, thyroid transcription factor 1; SSTR2A, somatostatin receptor 2A; OTP, orthopedia homeobox protein; Ascl1, mammalian achaete-scute homologue 1; Rb1, retinoblastoma protein.
**P*-value based on the Fisher's exact for categorical variables.

$P = 0.03$), advanced tumour stage (III-IV versus I-II, HR = 4.25, 95% CI 1.42–12.73, $P = 0.01$), and OTP (present versus absent, HR = 0.28, 95% CI 0.09–0.89, $P = 0.03$), were associated with OS.

Discussion

It is increasingly evident that lung ACs are more aggressive tumours and with a greater chance of metastasizing compared to typical carcinoids.[4] Nevertheless, to date no adjuvant therapy is recommended despite their high recurrence rate.[11] Apart from morphological classification, no further tools useful in predicting ACs clinical outcome have up till now been proposed, and this is probably due to their rarity, making case series with sufficient numerosity difficult to collect. The definition of specific markers of aggressiveness, therefore, represents an unmet clinical need.

In order to mine for new knowledge, a morphological and immunohistochemical characterisation of a cohort of ACs from two oncology centers was performed. Our study of a large well-characterised series ($n = 58$) of ACs demonstrates that Ascl1 and OTP expression could drive their clinical outcome. Specifically, Ascl1+/OTP- ACs appeared to be the most aggressive tumours, with a high Ki-67 proliferative index, strongly associated with postoperative recurrence while, on the contrary, Ascl1-/OTP+ ACs showed the best outcome.

Ascl1 regulates the expression of genes involved in cell cycle progression, including canonical cell cycle regulators and oncogenic transcription factors.[12] Some reports showed that it was highly specific for high-grade neuroendocrine carcinomas compared to carcinoids and other nonneuroendocrine neoplasms.[13,14] The recent integrative genomic characterisation of carcinoids identifying three novel molecular subtypes, with distinct clinical features, proves that Ascl1 is only expressed in the LC1 subgroup, associated with the worst patient outcome.[15] As Ascl1 represents a lineage-specific oncogene for high-grade neuroendocrine lung cancers,[16,17] its expression could predict carcinoids with a more aggressive clinical course. The present results, based on a substantial number of AC cases, showed that Ascl1 expression was found in 52% ($n = 30$) of ACs and strongly suggests, for the first time, that its expression is associated with a clinically aggressive course in terms of postoperative recurrence.

To understand and provide new insights into ACs aggressiveness, we also evaluated the well-known

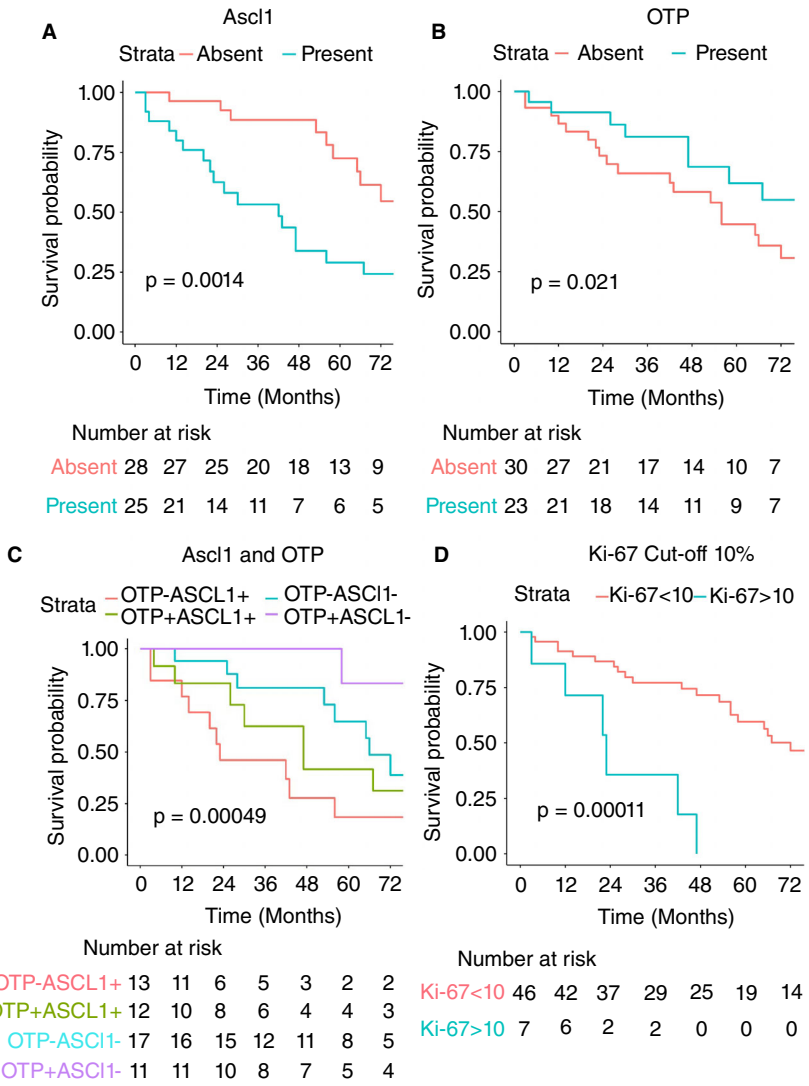

Figure 1. Disease-free survival in atypical carcinoids according to selected characteristics. (A) Ascl1 expression; (B) OTP expression; (C) Ascl1 and OTP expression; (D) Ki-67 cutoff 10%. Ascl1, mammalian achaete-scute homologue 1; OTP, orthopedia homeobox protein.

prognostic factor OTP. This is a transcription factor expressed almost exclusively in lung carcinoids but not in high-grade lung neuroendocrine carcinoma or in NETs of other organs.[6,18] Indeed, a multi-omics factor analysis of trascriptomes and methylomes of lung NENs proved that cases, initially classified as high-grade neuroendocrine carcinomas and harbouring high levels of OTP, were reclassified as carcinoids.[19] Independent studies showed that the absence of nuclear OTP expression was significantly associated with unfavourable disease outcome and an increased risk of metastasis for all pulmonary carcinoids.[18,20,21] In our study we observed OTP expression in 43.1% (n = 25) of ACs and we confirmed its independent strong prognostic value, proving that loss of expression was associated with an unfavourable prognosis in terms of OS and DFS. In agreement with the current literature,[22] we also reported that loss of OTP expression correlated with loss of menin nuclear immunostaining.

Proliferative analysis also showed a specific role of Ki-67 ≥10%, which was associated with reduced DFS. As previously proposed, Ki-67 could represent an independent prognostic marker for carcinoids.[23–26] A recent study carried out by Marchiò *et al.* showed a clinical role of Ki-67 10% cutoff for stratification of all lung carcinoid tumours and suggested that this cutoff is also reliable specifically for ACs identifying a

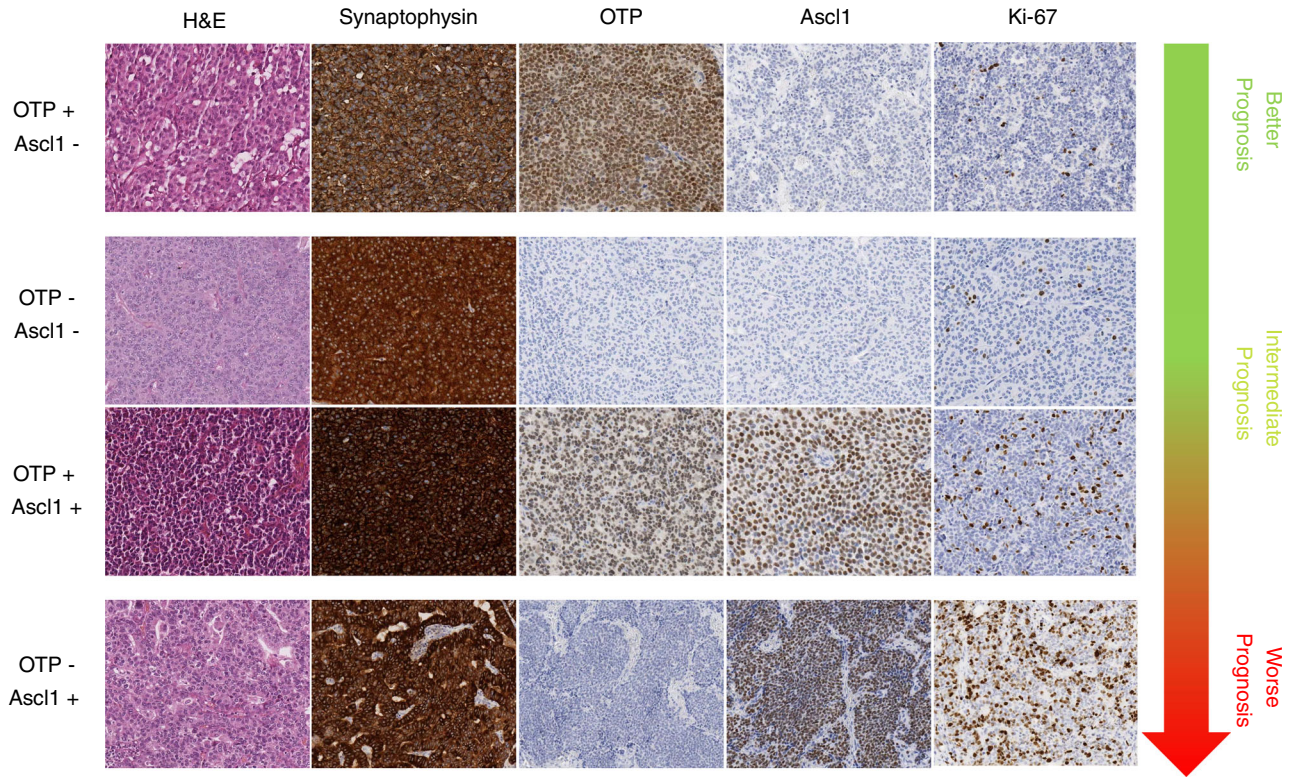

Figure 2. Atypical carcinoids morphological, immunohistochemical, and survival spectrum based on OTP and Ascl1 expression and– Ki-67 proliferative index. Ascl1, mammalian achaete-scute homologue 1; OTP, orthopedia homeobox protein.

subgroup with a dismal prognosis.[7] Our results underline that Ki-67 at 10% cutoff is a strong prognostic marker for ACs in univariate analysis, strongly associated with postoperative recurrence. However, in our study the prognostic impact of Ki-67 10% did not reach statistical significance when multiple risk factors were simultaneously assessed, probably due to the limited size of the cohort, with only eight cases with Ki-67 ≥10%. In addition, among cases with high Ki-67 we found three (5.2%) highly proliferative ACs with a Ki-67 ≥20%. Limited studies are focused on this rare entity: recently, Hermans *et al.* described seven of these cases suggesting that Rb1 staining might be helpful to predict prognosis.[27] One of our cases did not express Rb1 and was extensively positive for p53, while the other two cases had preserved Rb1 expression, while p53 was positive in a small number of cells in one case and negative in the other case. Interestingly, all our three cases expressed Ascl1 and were negative for both OTP and SSTR2A, highlighting their aggressiveness. Therefore, although rare, these cases could correspond to those classified as NET G3 in the digestive system,[28] but further studies are needed to better describe this group and elucidate their clinical relevance.

The present study had several limitations, including mainly its retrospective design. Due to the rarity of lung carcinoid tumours, the current series is relatively small and could also suffer from scarce information about adjuvant treatment. Although novel and informative results on ACs from two centers were reported, it does not allow drawing a definitive conclusion about the clinical relevance of Ascl1 and OTP and the best way to personalize the treatment of ACs patients. Therefore, it is necessary to investigate and validate the results of this study with prospective clinical studies.

In conclusion, this study shows that pulmonary ACs' clinical outcome could be described on the base of Ascl1 and OTP expression. These two markers, together with the Ki-67 proliferative index, could enable a more in-depth prognostic assessment of these rare tumours identifying patients at high risk of postsurgical relapse. Therefore, the evaluation of Ascl1 and OTP expression and the Ki-67 proliferative index could become a useful addition (if not a requirement) to routine pathology diagnostic workup.

Table 4. Univariate* analysis of disease-free survival and overall survival of patients with AC tumours

Variable	Comparison groups	Disease-free survival# HR (95% CI)	*P*-value*	Overall survival HR (95% CI)	*P*-value*
Sex	Male versus female	1.54 (0.70–3.43)	0.29	0.61 (0.24–1.55)	0.30
Age	10-year increase	1.49 (1.07–2.06)	**0.02**	1.85 (1.19–2.86)	**0.006**
Smoking status	Ex versus never	1.82 (0.60–5.57)	0.29	3.79 (0.91–15.76)	0.07
	Current versus never	1.06 (0.38–3.00)	0.91	1.14 (0.28–4.52)	0.86
T	2–3-4 versus 1	1.38 (0.62–3.06)	0.43	1.64 (0.63–4.28)	0.32
N	2–3 versus 0–1	2.69 (1.16–6.27)	**0.02**	2.94 (1.13–7.65)	**0.03**
Stage	III-IV versus I-II	2.48 (1.03–5.99)	**0.04**	4.58 (1.52–13.82)	**0.007**
Mitoses	1 mitosis increase	1.11 (0.90–1.36)	0.35	1.14 (0.88–1.46)	0.33
Necrosis	Spot versus absent	2.30 (0.57–9.30)	0.24	1.52 (0.42–5.50)	0.52
	Extensive versus absent	4.22 (1.21–14.72)	**0.02**	1.40 (0.36–5.51)	0.63
Ki-67	≥10 versus <10	6.10 (2.20–16.92)	**<0.001**	2.57 (0.89–7.42)	0.08
Vascular invasion	Present versus absent	0.86 (0.39–1.87)	0.70	0.70 (0.27–1.79)	0.46
Perineural invasion	Present versus absent	0.99 (0.31–3.13)	0.98	0.30 (0.06–1.52)	0.15
Intratumoral lymphocyte infiltrate	Present versus absent	1.55 (0.59–4.08)	0.38	1.10 (0.39–3.11)	0.86
Peritumoral lymphocyte infiltrate	Present versus absent	1.71 (0.71–4.10)	0.23	1.68 (0.66–4.25)	0.27
Location	Peripheral versus central	0.61 (0.25–1.48)	0.28	0.77 (0.27–2.24)	0.64
Microscopic infiltration	Positive STAS versus absent	3.68 (0.72–18.74)	0.12	2.71 (0.49–15.17)	0.26
	Bronchus versus absent	1.12 (0.22–5.66)	0.89	0.75 (0.13–4.47)	0.75
	Pleura versus absent	1.51 (0.13–17.99)	0.75	0.72 (0.06–8.17)	0.79
Tumour Site	Lower lobe versus hilum region	0.81 (0.24–2.79)	0.74	0.87 (0.21–3.56)	0.85
	Upper lobe versus hilum region	2.36 (0.76–7.37)	0.14	2.05 (0.55–7.64)	0.28
Rindi Grade	Grade 2–3 versus grade 1	1.52 (0.65–3.56)	0.34	1.73 (0.63–4.73)	0.29
Morphological pattern	Trabecular/nested/organoid versus insular/solid	0.99 (0.34–2.84)	0.98	0.91 (0.25–3.23)	0.88
Surgery	Lobectomy versus bilobectomy/pneumonectomy	2.02 (0.74–5.51)	0.17	2.37 (0.74–7.64)	0.14
	Sublobar resection versus bilobectomy/pneumonectomy	0.89 (0.20–3.95)	0.88	3.71 (0.96–14.33)	0.06
Residual tumour	R1/2 versus R0	3.31 (1.16–9.48)	**0.03**	1.75 (0.48–6.37)	0.40
TTF1	Present versus absent	1.63 (0.77–3.49)	0.20	1.37 (0.59–3.19)	0.46
CD44	Present versus absent	0.65 (0.31–1.37)	0.26	0.70 (0.30–1.64)	0.42
OTP	Present versus absent	0.33 (0.14–0.80)	**0.01**	0.25 (0.08–0.77)	**0.02**
SSTR2	Present versus absent	0.35 (0.17–0.74)	**0.006**	0.27 (0.11–0.65)	**0.003**
Ascl1	Present versus absent	3.30 (1.52–7.14)	**0.002**	2.73 (1.14–6.57)	**0.03**

Table 4. (*Continued*)

Variable	Comparison groups	Disease-free survival[#] HR (95% CI)	*P*-value*	Overall survival HR (95% CI)	*P*-value*
RB1	Heterogeneous or overexpressed versus absent	0.29 (0.06–1.41)	0.12	0.41 (0.12–1.41)	0.16
P53	Weak heterogeneous versus absent or overexpressed	1.76 (0.55–5.62)	0.34	0.67 (0.14–3.14)	0.61
Menin	Present versus absent	0.81 (0.30–2.20)	0.69	0.76 (0.29–2.00)	0.58

Note: Statistically significant *P*-value are reported in bold.
Abbreviation: STAS, spread through air spaces; TTF-1, thyroid transcription factor 1; SSTR-2A, somatostatin receptor 2A; OTP, orthopedia homeobox protein; Ascl1, mammalian achaete-scute homologue 1; Rb1, retinoblastoma protein.
*Adjusted for center and period of diagnosis categorised in decades (<1998, 1998–2007, 2008–2018).
[#]Evaluated on Stage I-II-III patients only.

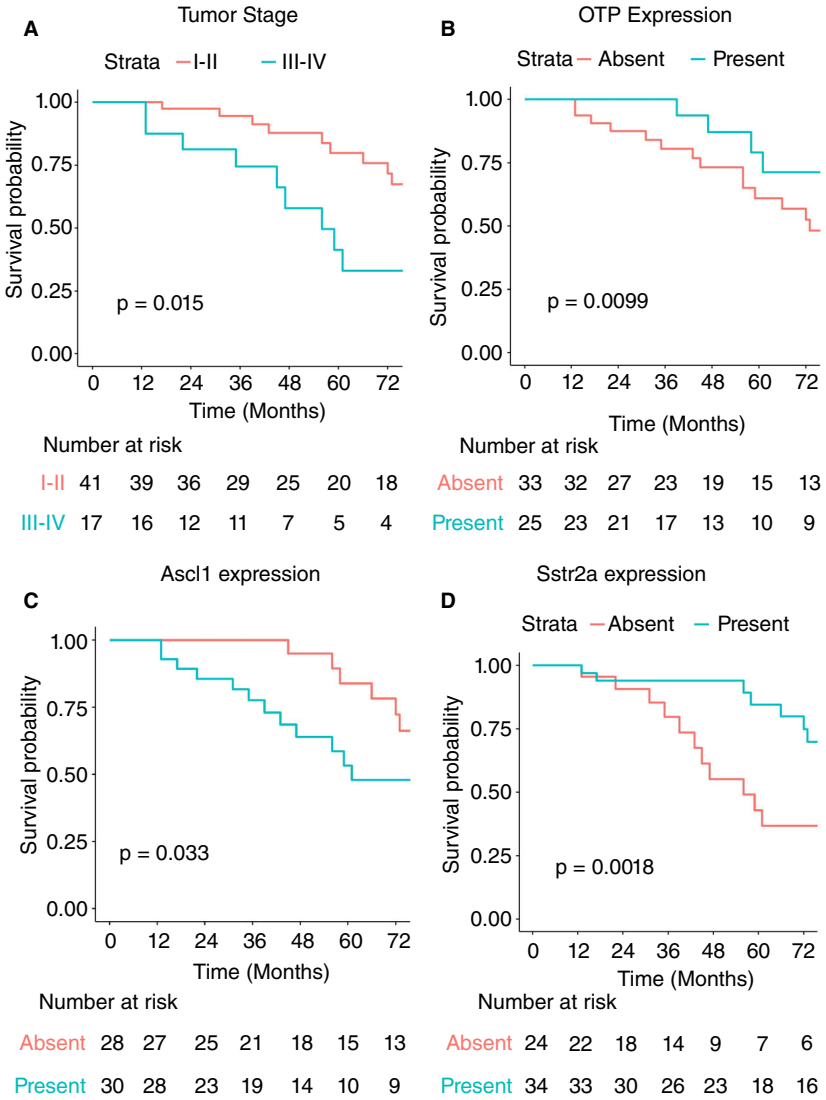

Figure 3. Overall survival in atypical carcinoids according to selected characteristics. (A) Tumour stage; (B) OTP expression; (C) Ascl1 expression; (D) SSTR2A expression. Ascl1, mammalian achaete-scute homologue 1; OTP, orthopedia homeobox protein; SSTR2A, somatostatin receptor 2A.

Table 5. Multivariable* models for disease-free survival and overall survival

Variable	HR (95% CI)	*P*-value*
DFS[#]		
Ascl1 (Present versus Absent)	3.42 (1.35–8.65)	**0.009**
OTP (Present versus Absent)	0.26 (0.10–0.68)	**0.006**
Stage (III versus I-II)	1.51 (0.55–4.13)	0.43
Ki-67 (≥10 versus <10)	2.80 (0.86–9.14)	0.09
OS		
Age (10-year increase)	1.67 (1.04–2.68)	**0.03**
Stage (III-IV versus I-II)	4.25 (1.42–12.73)	**0.01**
OTP (Present versus Absent)	0.28 (0.09–0.89)	**0.03**

Note: Statistically significant *P*-value are reported in bold.
*Adjusted for center and period of diagnosis categorised in decades (<1998, 1998–2007, 2008–2018).
[#]Evaluated on Stage I-II-III patients only.

Acknowledgement

Open access funding provided by BIBLIOSAN.

Author contributions

Study concept and design: Giovanni Centonze, Patrick Maisonneuve, Carlo Capella, Massimo Milione; Methodology: Michele Simbolo, Federica Grillo, Vincenzo Lagano, Loretta Missiato, Marilena Colantuono, Giovanna Sabella, Luisa Bercich, Luigi Rolli, Salvatore Grisanti; Analysis and interpretation of data: Giovanni Centonze, Patrick Maisonneuve, Federica Grillo, Luisa Bercich, Michele Simbolo, Giovanna Sabella, Alessandro Mangogna, Luca Roz, Aldo Scarpa, Carlo Capella, Massimo Milione; Drafting of article: Giovanni Centonze, Vincenzo Lagano; Critical revision of the article for important intellectual content: Patrick Maisonneuve, Michele Simbolo, Federica Grillo, Natalie Prinzi, Sara Pusceddu, Luisa Bercich, Luigi Rolli, Salvatore Grisanti, Mauro Roberto Benvenuti, Ugo Pastorino, Luca Roz, Aldo Scarpa, Alfredo Berruti, Carlo Capella, Massimo Milione; Statistical analysis: Giovanni Centonze, Patrick Maisonneuve; Study supervision: Patrick Maisonneuve, Carlo Capella, Massimo Milione. All authors have read and agreed to the published version of the article.

Conflict of interest

The authors have disclosed that they have no significant relationships with, or financial interest in, any commercial companies pertaining to this article.

Funding information

This work was supported by Italian Ministry of Health (ERP-2017-23671129 "PMTR-pNET" Project to M.M.); by 5 X 1000 Funds: 2014 MIUR: grant "Integrative Molecular Analysis of Pure and Combined Lung Large Cell Neuroendocrine Carcinoma (LCNEC)" (Project to M.M.); partially by the Italian Ministry of Health with Ricerca Corrente and 5 X 1000 funds (to P.M.). Giovanni Centonze was supported by a FIRC-AIRC fellowship for Italy.

Data availability statement

The data that support the findings of this study are available on request from the corresponding author.

References

1. World HO. *Thoracic tumors*. Lyon, France. Geneva, Switzerland: International Agency for Research on Cancer. Print copies are distributed by WHO Press, World Health Organization, 2021 xiii, 565 pages: illustrations (color, black and white).
2. Baudin E, Hayes AR, Scoazec JY *et al*. Unmet medical needs in pulmonary neuroendocrine (carcinoid) neoplasms. *Neuroendocrinology* 2019; **108**; 7–17.
3. Huang Y, Yang X, Lu T *et al*. Assessment of the prognostic factors in patients with pulmonary carcinoid tumor: a population-based study. *Cancer Med.* 2018; **7**; 2434–2441.
4. Caplin ME, Baudin E, Ferolla P *et al*. Pulmonary neuroendocrine (carcinoid) tumors: European neuroendocrine tumor society expert consensus and recommendations for best practice for typical and atypical pulmonary carcinoids. *Ann. Oncol.* 2015; **26**; 1604–1620.
5. Jiang SX, Kameya T, Asamura H *et al*. Hash1 expression is closely correlated with endocrine phenotype and differentiation extent in pulmonary neuroendocrine tumors. *Mod. Pathol.* 2004; **17**; 222–229.
6. Moonen L, Derks J, Dingemans AM, Speel EJ. Orthopedia homeobox (otp) in pulmonary neuroendocrine tumors: the diagnostic value and possible molecular interactions. *Cancers (Basel)* 2019; **1508**; 11.
7. Marchio C, Gatti G, Massa F *et al*. Distinctive pathological and clinical features of lung carcinoids with high proliferation index. *Virchows Arch.* 2017; **471**; 713–720.
8. Nagtegaal ID, Odze RD, Klimstra D *et al*. The 2019 who classification of tumours of the digestive system. *Histopathology* 2020; **76**; 182–188.
9. Volante M, Brizzi MP, Faggiano A *et al*. Somatostatin receptor type 2a immunohistochemistry in neuroendocrine tumors: a proposal of scoring system correlated with somatostatin receptor scintigraphy. *Mod. Pathol.* 2007; **20**; 1172–1182.
10. Rindi G, Klersy C, Inzani F *et al*. Grading the neuroendocrine tumors of the lung: an evidence-based proposal. *Endocr. Relat. Cancer* 2014; **21**; 1–16.

11. Prinzi N, Rossi RE, Proto C *et al*. Recent advances in the management of typical and atypical lung carcinoids. *Clin. Lung Cancer* 2021; **22**; 161–169.
12. Castro DS, Martynoga B, Parras C *et al*. A novel function of the proneural factor ascl1 in progenitor proliferation identified by genome-wide characterization of its targets. *Genes Dev.* 2011; **25**; 930–945.
13. Altree-Tacha D, Tyrrell J, Li F. Mash1 is highly specific for neuroendocrine carcinomas: an immunohistochemical evaluation on normal and various neoplastic tissues. *Arch. Pathol. Lab. Med.* 2017; **141**; 288–292.
14. Ye B, Cappel J, Findeis-Hosey J *et al*. Hash1 is a specific immunohistochemical marker for lung neuroendocrine tumors. *Hum. Pathol.* 2016; **48**; 142–147.
15. Laddha SV, da Silva EM, Robzyk K *et al*. Integrative genomic characterization identifies molecular subtypes of lung carcinoids. *Cancer Res.* 2019; **79**; 4339–4347.
16. Borromeo MD, Savage TK, Kollipara RK *et al*. Ascl1 and neurod1 reveal heterogeneity in pulmonary neuroendocrine tumors and regulate distinct genetic programs. *Cell Rep.* 2016; **16**; 1259–1272.
17. Baine MK, Hsieh MS, Lai WV *et al*. Sclc subtypes defined by ascl1, neurod1, pou2f3, and yap1: a comprehensive immunohistochemical and histopathologic characterization. *J. Thorac. Oncol.* 2020; **15**; 1823–1835.
18. Nonaka D, Papaxoinis G, Mansoor W. Diagnostic utility of orthopedia homeobox (otp) in pulmonary carcinoid tumors. *Am. J. Surg. Pathol.* 2016; **40**; 738–744.
19. Alcala N, Leblay N, Gabriel AAG *et al*. Integrative and comparative genomic analyses identify clinically relevant pulmonary carcinoid groups and unveil the supra-carcinoids. *Nat. Commun.* 2019; **10**; 3407.
20. Swarts DR, Henfling ME, Van Neste L *et al*. Cd44 and otp are strong prognostic markers for pulmonary carcinoids. *Clin. Cancer Res.* 2013; **19**; 2197–2207.
21. Papaxoinis G, Nonaka D, O'Brien C, Sanderson B, Krysiak P, Mansoor W. Prognostic significance of cd44 and orthopedia homeobox protein (otp) expression in pulmonary carcinoid tumours. *Endocr. Pathol.* 2017; **28**; 60–70.
22. Derks JL, Rijnsburger N, Hermans BCM *et al*. Clinical-pathologic challenges in the classification of pulmonary neuroendocrine neoplasms and targets on the horizon for future clinical practice. *J. Thorac. Oncol.* 2021; **16**; 1632–1646.
23. Clay V, Papaxoinis G, Sanderson B *et al*. Evaluation of diagnostic and prognostic significance of ki-67 index in pulmonary carcinoid tumours. *Clin. Transl. Oncol.* 2017; **19**; 579–586.
24. Oka N, Kasajima A, Konukiewitz B *et al*. Classification and prognostic stratification of bronchopulmonary neuroendocrine neoplasms. *Neuroendocrinology* 2020; **110**; 393–403.
25. Dermawan JKT, Farver CF. The role of histologic grading and ki-67 index in predicting outcomes in pulmonary carcinoid tumors. *Am. J. Surg. Pathol.* 2020; **44**; 224–231.
26. Centonze G, Maisonneuve P, Simbolo M *et al*. Lung carcinoid tumors: Histology and ki-67, the eternal rivalry. *Histopathology* 2022; **82**; 324–339.
27. Hermans BCM, Derks JL, Moonen L *et al*. Pulmonary neuroendocrine neoplasms with well differentiated morphology and high proliferative activity: Illustrated by a case series and review of the literature. *Lung Cancer* 2020; **150**; 152–158.
28. IARC. *World Health Organization classification of tumours 1. Digestive system tumours*. 1.1 ed. Lyon: IARC, 2019; 635.

Supporting Information

Additional Supporting Information may be found in the online version of this article:

Table S1. Antibody sources and dilutions.