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Prognostic Factors Across Poorly Differentiated Neuroendocrine Neoplasms: a Pooled Analysis

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Running header: Prognostic Factors Across Poorly Differentiated NECs

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Abstract

Introduction: Poorly differentiated neuroendocrine carcinomas (NECs) are characterized by aggressive clinical course and poor prognosis. No reliable prognostic markers have been validated to date; thus, the definition of a specific NEC prognostic algorithm represents a clinical need. This study aimed to analyze a large NEC case series to validate the specific prognostic factors identified in previous studies on gastro-entero-pancreatic (GEP) and lung NECs and to assess if further prognostic parameters can be isolated. **Methods:** A pooled analysis of four NECs retrospective studies was performed to evaluate: the prognostic role of Ki-67 cut-off, the overall survival according to primary cancer site, and further prognostic parameters using multivariable Cox proportional hazards model and machine-learning random survival forest (RSF). **Results**: 422 NECs were analyzed. The most represented tumor site was the colorectum (n=156, 37%), followed by the lungs (n=111, 26%), gastroesophageal site (n=83, 20%; 66 gastric, 79%). The Ki-67 index was the most relevant predictor, followed by morphology (pure or mixed/combined NECs), stage, and site. The predicted RSF response for survival at 1, 2, or 3 years showed decreasing survival with increasing Ki-67, pure NEC morphology, stage III–IV, and colorectal NEC disease. Patients with Ki-67 <55% and mixed/combined morphology had better survival than those with pure morphology. Morphology pure or mixed/combined became irrelevant in NECs survival when Ki-67 was ≥55%. The prognosis of metastatic patients who did not receive any treatment tended to be worse compared to that of the treated group. The prognostic impact of Rb1 immunolabeling appears to be limited when multiple risk factors are simultaneously assessed. **Conclusion**: The most effective parameters to predict OS for NEC patients could be Ki-67, pure or mixed/combined morphology, stage, and site.

Introduction

According to morphology and proliferation rate, neuroendocrine neoplasms (NENs) are classified into well-differentiated neuroendocrine tumors (NETs) and poorly differentiated neuroendocrine carcinomas (NECs) [1]. NECs, regardless of the tumor site, are high-grade cancers, mostly diagnosed at a late stage and characterized by aggressive clinical course and poor prognosis [2–5]. Due to their relative rarity, no reliable prognostic markers have been validated to date. The therapeutic indications assume they have a clinical behavior similar to small-cell lung cancer (SCLC). Therefore, NECs are usually treated with platinum–etoposide chemotherapy, but this approach does not give satisfactory results in all patients [6–9]. Consequently, the definition of a specific NEC prognostic algorithm represents a clinical need.

Overall, different studies defined further prognostic factors to enhance the classification of NECs [5,9–14]. Based on the genomic profile of lung cancers, large cell NECs (LCNECs) have been distinguished into non-SCLC (NSCLC)-like, characterized by alterations in *TP53* and *KRAS/STK11/KEAP1*, and SCLC-like, characterized by concurrent *TP53* and *RB1* inactivation, high proliferative rate, and shorter overall survival (OS) despite a high response rate to chemotherapy [12,13,15,16]. Gastro-entero-pancreatic (GEP) NECs have been distinguished into two categories according to the Ki-67 proliferation index, considering 55% as a threshold value: GEP-NECs with Ki-67 ≥55% are characterized by very poor survival despite a better response to platinum-based chemotherapy compared to GEP-NECs Ki-67 <55% (median OS: 5.3 vs 24.5 months) [5,10,11,17–20]. Our experience showed that 55% Ki-67 threshold as a prognostic factor is also shared with mixed GEP-NECs (MiNENs, mixed neuroendocrine neoplasms) neoplasms [19] and pure and combined LCNECs [21]. Similar to GEP-NEC, and especially in the colon–rectum, Ki-67 ≥55% in the neuroendocrine component is associated with the worst prognosis and shares morphological and molecular features with lung LCNECs [22]. Of note, *TP53* and *RB1* alterations are more frequent in GEP-NECs with Ki-67 ≥55% than with <55%.

The present study aims to analyze a large NECs case series to validate the specific prognostic factors identified in previous independent studies on GEP-NECs and lung LCNECs and assess if further prognostic parameters can be isolated. With this in mind, pooled data from four large studies on NECs patients were used to investigate the prognostic role of Ki-67 cut-off, the OS according to primary cancer site, and to define further prognostic parameters by multivariable Cox proportional hazards model and machine-learning random survival forest (RSF).

Methods

Study design

We performed a pooled analysis of four retrospective studies on Italian patients with NENs [10,18,19,21]. Those studies were selected since they are, to the best of our knowledge, the only studies comprehensively analyzing clinical, morphological, immunohistochemical and molecular data of patients with GEP and lung NECs. Qualified pathologists performed a central review of all specimens in common sessions, with the majority voting in odd groups for all studies. Information on clinical and therapeutic outcomes was collected consistently among all studies. All studies are published and were conducted according to the clinical standards of the Helsinki Declaration and approved by the relevant Ethics Committees.

Only patients with NEC were included in the present analysis; patients classified as NET G3 in the previous series were also excluded [21].

The pooled analysis considered: a) gender; b) age; c) pure neuroendocrine morphology or mixed/combined with non-neuroendocrine components (adenocarcinoma or squamous cell carcinoma or mucinous or signet ring cell); d) pathological tumor staging according to the Union for International Cancer Control/American Joint Committee on Cancer (AJCC/UICC) 8th edition for each tumor site; e) immunohistochemistry (IHC) in NEC component for Ki-67 (MIB-1), SSTR2A, p53 and rb1; and f) mutation for *TP53, RB1, KRAS* and *BRAF* genes. The outcome variable was OS, defined as the time in months from the date of diagnosis to death from any cause or last follow-up, whichever occurred first. SSTR2A IHC was evaluated using a four-tiered system with scores 0 and 1 considered negative according to Volante et al [23]. IHC for p53 and Rb1 were scored as absent or present to minimize assessment variability. Specifically, staining was evaluated as “absent” in the case of negative or low/moderate expression in a minority of cells, while overexpression in most cells was evaluated as “present.” Ki-67 labeling index was assessed as a percentage of positive cancer cells in areas of highest labeling.[1]

Statistical Analysis

Data were analyzed by descriptive statistics. Clinical and pathologic characteristics of patients were stratified according to the tumor site. OS curves were calculated using the Kaplan–Meier method, and the log-rank test was used to assess the survival difference between patient groups. Univariable and multivariable Cox proportional regression analyses were used to assess the association between clinicopathological characteristics and OS. Nonparametric machine learning RSF was used to build a risk prediction model in survival analysis and investigate the most important variables. Detailed information on statistical analysis is reported in Supplementary methods.

Results

Baseline characteristics

Overall, 422 patients were considered (Figure 1). Table 1 summarizes the clinicopathological features of included patients. Most patients were males (67%, n=283) and aged >60 years (n=291, 69%; 130 patients, 31%, were >70 years). The most represented tumor site was the colorectum (n=156, 37%), followed by lung LCNEC (n=111, 26%), gastroesophageal (n=83, 20%; 66 gastric, 79%) and pancreas (n=42, 10%). Most patients (n=346, 82%) had stage III–IV disease, and only 76 (18%) had stages I–II disease.

Pure neuroendocrine morphology (n=227, 54%) was slightly more represented than the mixed/combined (n=195, 46%), though more frequent in colorectal (59%), gastroesophageal (53%) and gallbladder/biliary (83%) compared to lung (31%) and pancreas (33%). All NECs in the small intestine (duodenum-ileum–cecum) had pure morphology. The median Ki-67 labeling index was 70% (range: 20–98%). Ki-67 was lower in lung LCNEC than in GEP sites (p=0.003). Overall, *TP53* was the most frequently mutated gene, with mutation reported in 78/169 (46%) patients, followed by *KRAS* (27/169, 16%), *RB1* (12/98, 12%), and *BRAF* (7/169, 4%).

Survival analysis

In the overall cohort, the median OS was 13 months (95% CI: 12–14) (Supplementary Figure 1). Ki-67 at 55% was the best prognostic factor for 3-year mortality. Using Kaplan–Meier analysis, patients with Ki-67 <55% had a significatively longer OS (median: 32 months; 95% CI: 28–39) than patients with Ki-67 ≥55% (median: 11 months; 95% CI: 9–12; log-rank p≤0.0001) (Figure 2). Patients with mixed/combined morphology had longer OS (median: 14 months; 95% CI: 13–16) versus those with pure NEC morphology (median: 12 months; 95% CI: 9–15; log-rank p=0.006) (Supplementary Figure 2). Interestingly, when merging morphology and Ki-67 proliferative groups, patients with mixed/combined morphology and Ki-67 <55% had longer OS (median 41 months; 95% CI: 36–68) when compared to both pure NECs morphology and Ki-67 <55% (median: 21 months; 95% CI: 17–30) and either mixed/combined or pure morphology and Ki-67 ≥55% (median: 12 months (95% CI: 11–13) and 9 (95% CI: 8–11), respectively) (Figure 3).

Survival by tumor site

Median OS was 11 months for colorectal, 13 months for gastroesophageal, 17 months for pancreas, 17 months for gallbladder/biliary, 6 months for ileum–cecum–duodenum, and 17 months for lung NEC. During Kaplan-Meier analysis, patients with colorectal NEC had significantly shorter OS than patients with pancreatic NEC and lung LCNEC (log-rank p=0.01 and p<0.001, respectively) (Figure 4). In addition, patients with gastroesophageal NEC had shorter OS than patients with pancreas NEC (p=0.09) and lung LCNEC (p=0.04).

Cox multivariable analysis

At multivariable cox model analysis, the variables stage, site, morphology and Ki-67 were significantly associated with survival (Table 2). Ki-67 ≥55 significantly increased the HR by a factor of 5.51 (95% CI: 3.98–7.63; p<0.0001), while pure NECs neuroendocrine morphology was associated with a 41% increase in HR for survival compared to mixed/combined (95% CI: 1.12–1.76; p=0.003). Stage III–IV disease significantly increased HR by 47% (95% CI: 1.01–2.14; p=0.04), while pancreatic disease was associated with a 42% decrease in HR (95% CI: 0.39–0.87; p=0.008) compared with patients with colorectal NEC. Furthermore, Cox multivariable analysis of the 169 patients with molecular data showed that pure NECs neuroendocrine morphology (HR: 1.70, 95% CI: 1.07-2.70; p=0.02) and Ki-67 ≥55 (HR: 6.87; 95% CI: 3.87-12.18; p<0.0001) were significantly associated with survival (Supplementary Table 1).

RSF and Evaluation of Survival Predictions

The variable importance of RFS showed that Ki-67 was the most important variable (wider blue bar), followed by morphology, stage, site, age, and Rb1 IHC (Figure 5). Predicted responses for survival at 1, 2, and 3 years showed decreasing survival with increasing Ki-67, pure neuroendocrine morphology, stage III–IV, and colorectal and

gastroesophageal NEC disease (Supplementary Figure 3). Furthermore, with Ki-67 55% cut-off, RSF showed that patients with Ki-67 <55% and mixed/combined morphology had longer survival than those with pure morphology. Morphology became irrelevant for survival when Ki-67 was ≥55% (Figure 6).

Medical therapy

Information on therapy was available for about half of the present cohort (*n*=240, 60%, Supplementary Table 2). Most patients (n=162/240, 67.5%) received platinum-based chemotherapies, 25 (10.4%) patients received other therapies, and 53 patients (22.1%) did not receive any treatment at all. This latter group comprised six patients at stage I, nine at stage II, 31 at stage III and seven at stage IV. Overall, focusing on the metastatic disease (n=86/240, 35.8%, Supplementary Table 3), Ki-67 index 55% cut-off was statistically confirmed for all the treated population (n=79/86, 91.9% Supplementary Figure 4) and also for the platinum-based chemotherapies group (n=70/86, 81.4% Supplementary Figure 5). The prognosis of the metastatic patients who did not receive any treatment tended to be worse compared to that of the treated group (HR 2.14, 95% CI: 0.97–4.72;p=0.06).

Discussion

The clinical management of NECs is controversial: the standard therapeutic approach is the same for all GEP-NECs, and no defined treatment regimen exists for lung LCNECs. Therapeutic indications are often derived from small and heterogeneous studies and are usually borrowed from the treatment of NSCLC and SCLC [6,9]. Furthermore, no reliable marker to predict clinical outcomes has been definitively validated to date.

We performed a pooled analysis of four retrospective studies of GEP-NECs and lung LCNECs to assess differences in OS according to different parameters. An extensive central review of a large series of cases (*n*=848) was performed; therefore, data obtained from the 422 included patients can be considered higher quality than previous studies based on a single series of raw data. Our data showed that the most relevant predictor identified by both Cox model and methods of selecting significant variables of machine learning RSF was Ki-67 followed by morphology, stage, and site. The predicted RSF response for survival at 1, 2, or 3 years showed decreasing survival with increasing Ki-67, pure NEC morphology, stage III–IV, and colorectal NEC disease. Colorectal and gastroesophageal NEC patients had shorter OS than pancreatic NEC and lung LCNEC. Furthermore, patients with Ki-67 <55% and mixed/combined morphology had better survival than those with pure morphology. Morphology became irrelevant in NENs when Ki-67 was ≥55%.

Several studies have explored the predictive value of Ki-67 index in NEC with conflicting results. In particular, Sorbye *et al.* initially reported that GEP NEC patients with Ki-67 <55% had longer survival than patients with Ki-67 >55% but were less responsive to platinum-based chemotherapy [5]. Other independent studies demonstrated the prognostic and therapeutic value of Ki-67 in NECs with a cut-off of 55% [10,19,20,24,25]. On the other hand, Elvebakken *et al.* confirmed that GEP NEC with Ki-67 ≥55% had a significantly better response rate (RR) to chemotherapy compared to NEC with Ki-67 <55%, but OS between these groups was equal: these findings could be probably explained by the inferior benefit of platinum/etoposide seen in NEC with Ki-67 <55% [11]. In addition, the authors reported no Ki-67 re-evaluation. No OS difference by applying a 55% Ki-67 cut-off was reported recently by Hadoux et al. on metastatic pure NECs from different origins treated first-line with platinum etoposide [26]. A study on a cohort of 77 lung LCNECs showed that neither OS nor disease-free survival significantly correlated with stratification by Ki-67% into six or two classes, whether ≥20% or ≥40% were used as cut-off points [27].

Results of this pooled analysis supported the key role of 55% cut-off for Ki-67 as a prognostic factor in NECs. These contrasting results may be related to the multiplicity of organs included in the studies with consequent morphological and therapeutic heterogeneity of cases, as well as the lack of standardized cytomorphological criteria and Ki-67 evaluation of these neoplasms in all the sites. Further prospective studies are needed to validate this cut-off in different anatomical origins.

We observed that colorectal and gastroesophageal NEC patients had shorter OS than pancreatic NEC and lung LCNEC. In previous studies, the primary site was already recognized as a prognostic factor in NECs, even with non-homogeneous results. In accordance with our results, Elvebakken *et al.* reported that pancreatic NEC has a superior progression-free survival and OS compared to colorectal NEC [11]. Instead, in a pure NEC metastatic patients' cohort of different primary including GEP, thoracic, gynecologic, head and neck, prostate and unknown origins, Hadoux *et al.* did not find any survival difference as per the primary location [26]. Dasari *et al.* analyzed 162,983 NENs from the SEER database and showed lung NECs had the worst prognosis [28]. However, this result should be considered with caution since a morphological review of cases by expert NEN pathologists was not performed, and the lung NEC

group was most constituted by SCLC (95.2%). Our pooled analysis comprised only lung LCNEC. Therefore, this discrepancy could be related to the cell type since our study is enriched by large-cell type as the most represented morphology in the digestive system, in addition to the treatment heterogeneity among different sites of origins.
The stage had a significant effect on patients' survival time, which is consistent with many studies showing that NEN with stages III and IV had a poor prognosis. Specifically, Alese *et al*., on a large series of GEP-NECs, showed that stage IV patients classified as such by the AJCC had the worst prognosis [29]. Zhou *et al*. showed that for the 126 lung LCNECs investigated, the 1-year OS rate was significantly poorer in patients with advanced stages [30]. In agreement with our data, patients with stage III–IV had the worst prognosis.
NECs stratified by proliferation rate demonstrated significant differences in response to chemotherapy and, therefore, pivotal in guiding therapeutic selection [5]. Unfortunately, in our analysis, even if it regards the largest cohort currently inquired, the response to first-line therapy treatment was scarce and fragmented; therefore, no adequate conclusion can be drawn. Subsequent studies for the analysis of this aspect are therefore warranted.
Recently, Rb1 has been described as a relevant candidate to drive therapeutic decisions, and therefore its prognostic impact on OS has been highlighted [15,31]. Hijioka *et al*. observed that loss of Rb immunolabeling was a predictive marker of response to platinum-based chemotherapy and an OS-independent prognostic factor [32]. The prognostic value of Rb1 has also been demonstrated by Tanaka *et al* on pancreatic NEC, proving that loss of expression is associated with unfavorable prognosis in univariate but not multivariate analysis [33]. Our pooled analysis, including different sites, confirms this observation: the prognostic impact of Rb1 does not appear to be independent when multiple risk factors are simultaneously assessed.
Despite the absence of molecular indicators in the predictive models, prognostic indications from our observations underline the importance of adequate pathological analysis. RFS alghoritm incorporates all univariate and multivariate effects automatically, so each predicted point is dependent on the full combination of all risk factors. Using VIMP (variable importance), a measure of how important is a variable, we showed the pivotal role of Ki-67 followed by morphology, stage and site in predicting survival. RSF has been complementary to the Cox model with similar performance for both models, as demonstrated by Chen et al. [34]. Therefore, the present data indicate that appropriate pathological diagnosis based on proliferation activity, morphology and disease stage by TNM classification are essential for diagnostic and therapeutic decision-making, as reported by ESMO Clinical Practice Guidelines [35]. Indeed, the easily accessible pathology analysis is sufficient for assessing the identified risk factors and for the consequent stratification of patients according to different prognostic categories, potentially resulting in therapeutic indications.
Nonetheless, intrapatient heterogeneity among lesions may be present [36], and tissue sampling of multiple lesions may be unfeasible. Therefore, additional and complementary imaging techniques may be used in patients affected by NECs. Particularly, positron emission tomography (PET)/computed tomography (CT) has been proven of value in disease staging [37] and risk stratification [38–40] using 2-(^{18}F)fluoro-2-deoxy-D-glucose ((^{18}F)FDG) and somatostatin targeting radiopharmaceuticals labelled with ^{68}Ga. Moreover, in a preliminary investigation, the ^{68}Ga-labelled 1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetic acid - Phe1-Tyr3-octreotide (DOTA-TOC)-PET/CT findings have been found to be correlated with loss of expression of tissue biomarkers (e.g. RB1 expression) [38].
The present study is a retrospective pooled analysis; therefore, several limitations should be acknowledged. First, it is a pooled analysis limited to retrospective studies. Second, heterogeneity between the cohorts with respect to data collection and the quality and detail of information available may exist. Third, the predominance of the large cell type could not fully describe NECs biological features. Fourth, fragmented and limited molecular alterations and the absence of treatment data in the predictive models may have been a source of bias.

Conclusion

The low prevalence, biological heterogeneity, and lack of information on the disease course hinder the definition of new prognostic indicators for NECs. Due to their rarity, pooled analysis of survival data may provide helpful information in understanding the course of this disease. Our results, obtained after an extensive central review of a large cohort, showed that NECs could display different survival depending on Ki-67 proliferation index, morphology (pure or mixed/combined NEC), stage, and site. Future integration of further clinical and molecular information on large, standardized datasets of NECs will likely result in a better understanding of their pathogenesis and possibly better therapy for this rare and deadly disease.

Statements

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- **Ethics statement**

This study protocol was reviewed and approved by the Ethical Committee of Fondazione IRCCS Istituto Nazionale dei Tumori (INT), approval number (INT 21/16). The study was performed according to the clinical standards of the 1975 and 1983 Declaration of Helsinki and the study protocol was reviewed and approved by the Ethical Committee of Fondazione IRCCS Istituto Nazionale dei Tumori (INT), approval number (INT 21/16). Written informed consent was obtained from all subjects involved in the study according the Ethical Committee of Fondazione IRCCS Istituto Nazionale dei Tumori (INT), approval number (INT 21/16).

- **Conflicts of interest statement**

The authors have declared no conflicts of interest.

- **Funding sources**

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- **Authors' contributions:**

Study concept and design: Massimo Milione, Carlo Capella, Giovanni Centonze and Patrick Maisonneuve. Analysis of data: Giovanni Centonze, Patrick Maisonneuve, Carlo Capella, Massimo Milione. Data collection and interpretation: Giovanni Centonze, Patrick Maisonneuve, Natalie Prinzi, Sara Pusceddu, Luca Albarello, Eleonora Pisa, Massimo Barberis, Alessandro Vanoli, Paola Spaggiari, Paola Bossi, Laura Cattaneo, Giovanna Sabella, Enrico Solcia, Stefano La Rosa, Federica Grillo, Giovanna Tagliabue, Aldo Scarpa, Mauro Papotti, Marco Volante, Alessandro Mangogna, Alessandro Del Gobbo, Stefano Ferrero, Luigi Rolli, Elisa Roca, Luisa Bercich, Mauro Benvenuti, Luca Messerini, Frediano Inzani, Giancarlo Pruneri, Adele Busico, Federica Perrone, Elena Tamborini, Alessio Pellegrinelli, Ketevani Kankava, Alfredo Berruti, Ugo Pastorino, Nicola Fazio, Fausto Sessa, Carlo Capella, Guido Rindi, Massimo Milione. Drafting of manuscript: Giovanni Centonze. Critical revision of the manuscript for important intellectual content: Guido Rindi, Stefano La Rosa, Mauro Papotti, Patrick Maisonneuve, Carlo Capella, Massimo Milione. Statistical analysis: Giovanni Centonze, Patrick Maisonneuve. Study supervision: Patrick Maisonneuve, Carlo Capella, Massimo Milione. Manuscript editing and approval to submit: Giovanni Centonze, Patrick Maisonneuve, Natalie Prinzi, Sara Pusceddu, Luca Albarello, Eleonora Pisa, Massimo Barberis, Alessandro Vanoli, Paola Spaggiari, Paola Bossi, Laura Cattaneo, Giovanna Sabella, Enrico Solcia, Stefano La Rosa, Federica Grillo, Giovanna Tagliabue, Aldo Scarpa, Mauro Papotti, Marco Volante, Alessandro Mangogna, Alessandro Del Gobbo, Stefano Ferrero, Luigi Rolli, Elisa Roca, Luisa Bercich, Mauro Benvenuti, Luca Messerini, Frediano Inzani, Giancarlo Pruneri, Adele Busico, Federica Perrone, Elena Tamborini, Alessio Pellegrinelli, Ketevani Kankava, Alfredo Berruti, Ugo Pastorino, Nicola Fazio, Fausto Sessa, Carlo Capella, Guido Rindi, Massimo Milione.

- **Data availability statement**

All data generated or analysed during this study are included in this article. Further enquiries can be directed to the corresponding author.

- **Authors’ note**

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.

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Figure Legends

Figure 1. Study flowchart. Source: original.

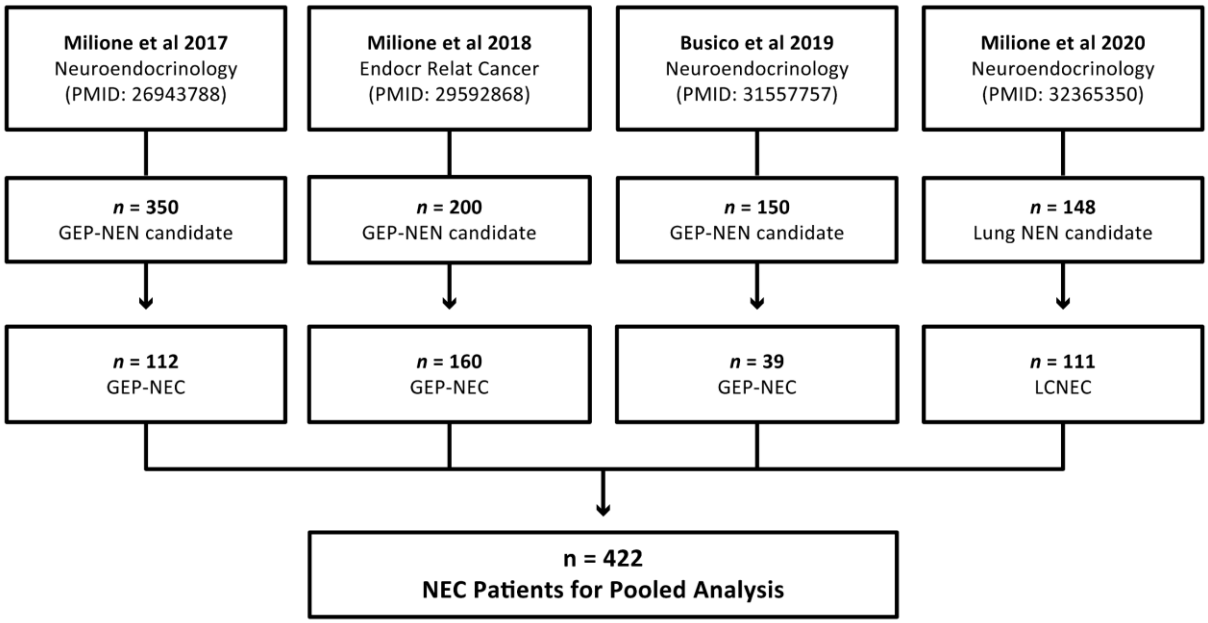
Figure 2. Overall survival in poorly differentiated neuroendocrine carcinoma according to Ki-67 <55 vs Ki-67 ≥55%; Top right: ROC and time-dependent area under the curve for the prediction of 3-year mortality according to Ki-67. Source: original.

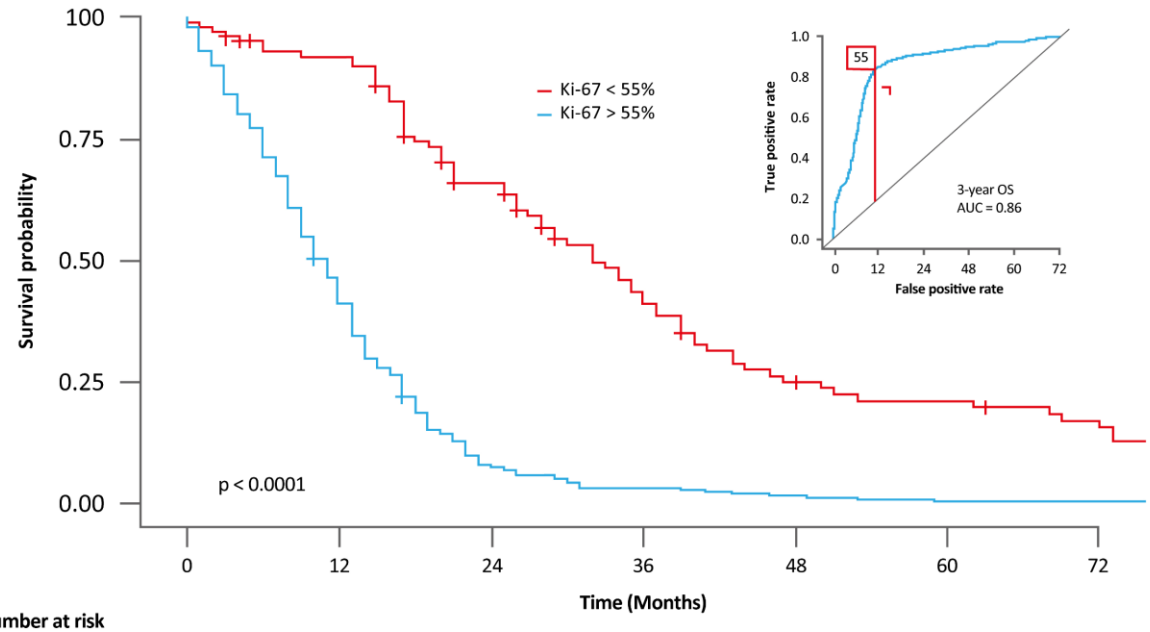
Figure 3. (A) Overall survival in poorly differentiated neuroendocrine carcinoma according to morphology and Ki-67 and **(B)** pairwise comparisons using Log-Rank test. Source: original.

Figure 4. (A) Overall survival of 422 poorly differentiated neuroendocrine carcinoma according to tumor sites and **(B)** pairwise comparisons using Log-Rank test. Source: original.

Figure 5. Variable importance (VIMP) of random survival forest. Blue bars indicate positive VIMP, red indicates negative VIMP. Importance is relative to positive length of bars. VIMP: Variable importance**.** Source: original.

Figure 6. Variable dependence coplot of survival at 1 **(A)**, 2 **(B)** and 3 **(C)** years of morphology group membership, conditional on Ki-67 55% cut-off. Symbols with red circles indicate censored cases and blue triangle indicate death events. Source: original.

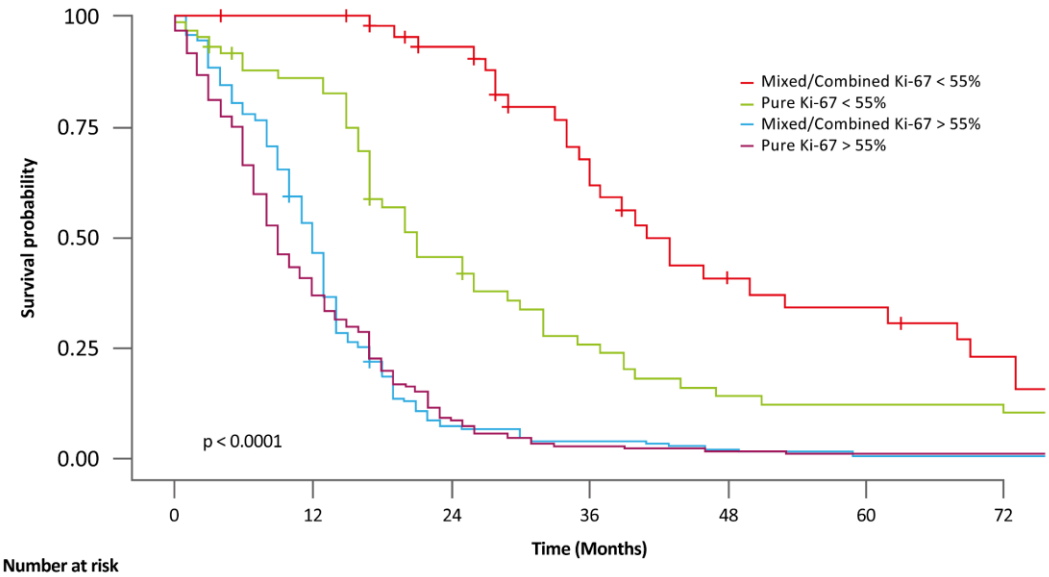




Number at risk

Ki-67 < 55%	101	90	60	36	20	16	12
Ki-67 > 55%	321	148	25	9	4	1	1

A



Number at risk							
Mixed/Combined Ki-67 < 55%	44	43	36	23	13	10	6
Pure Ki-67 < 55%	57	47	24	13	7	6	6
Mixed/Combined Ki-67 > 55%	151	79	10	5	2	0	0
Pure Ki-67 > 55%	170	69	15	4	2	1	1

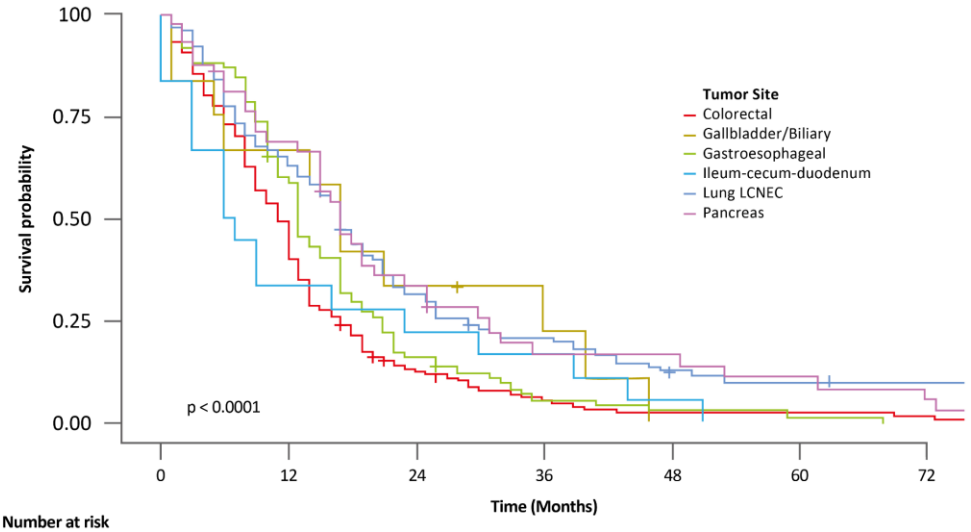
B

Pairwise comparisons using Log-Rank test

	Mixed/Combined Ki-67 < 55%	Pure Ki-67 < 55%	Mixed/Combined Ki-67 > 55%
Pure Ki-67 < 55%	<0.001	-	-
Mixed/Combined Ki-67 > 55%	<0.001	<0.001	-
Pure Ki-67 > 55%	<0.001	<0.001	0.40

P-value correction for multiple comparisons according to Benjamini-Hochberg

A



Number at risk

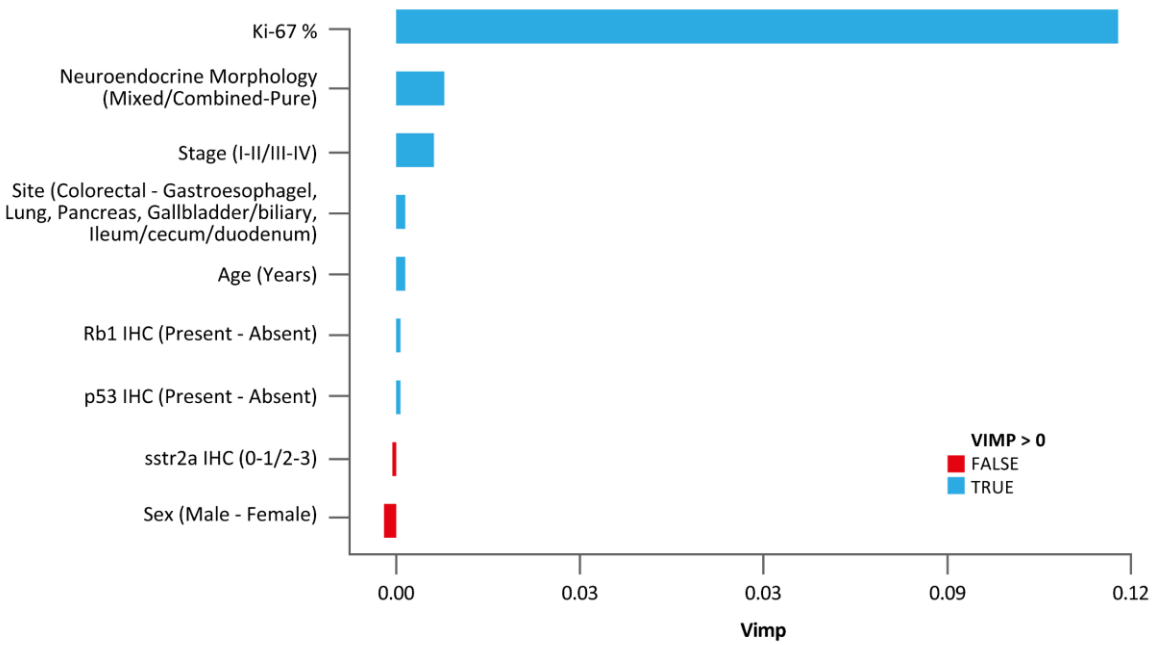
Colorectal	156	76	18	8	3	3	2
Gallbladder/Biliary	12	8	4	3	0	0	0
Gastroesophageal	83	49	13	4	2	1	0
Ileum-cecum-duodenum	18	6	4	3	12	0	0
Lung LCNEC	111	71	33	21	6	9	8
Pancreas	42	28	13	6		4	3

B

Pairwise comparisons across Tumor Site using Log-Rank test

	Colorectal	Gallbladder/Biliary	Gatroesophageal	Ileum-cecum-duodenum	Lung LCNEC
Gallbladder/Biliary	0.19	-	-	-	-
Gatroesophageal	0.22	0.43	-	-	-
Ileum-cecum-duodenum	0.88	0.72	0.74	-	-
Lung LCNEC	**<0.001**	0.86	**0.04**	0.14	-
Pancreas	**0.01**	0.86	0.09	0.14	0.88

P-value correction for multiple comparisons according to Benjamini-Hochberg



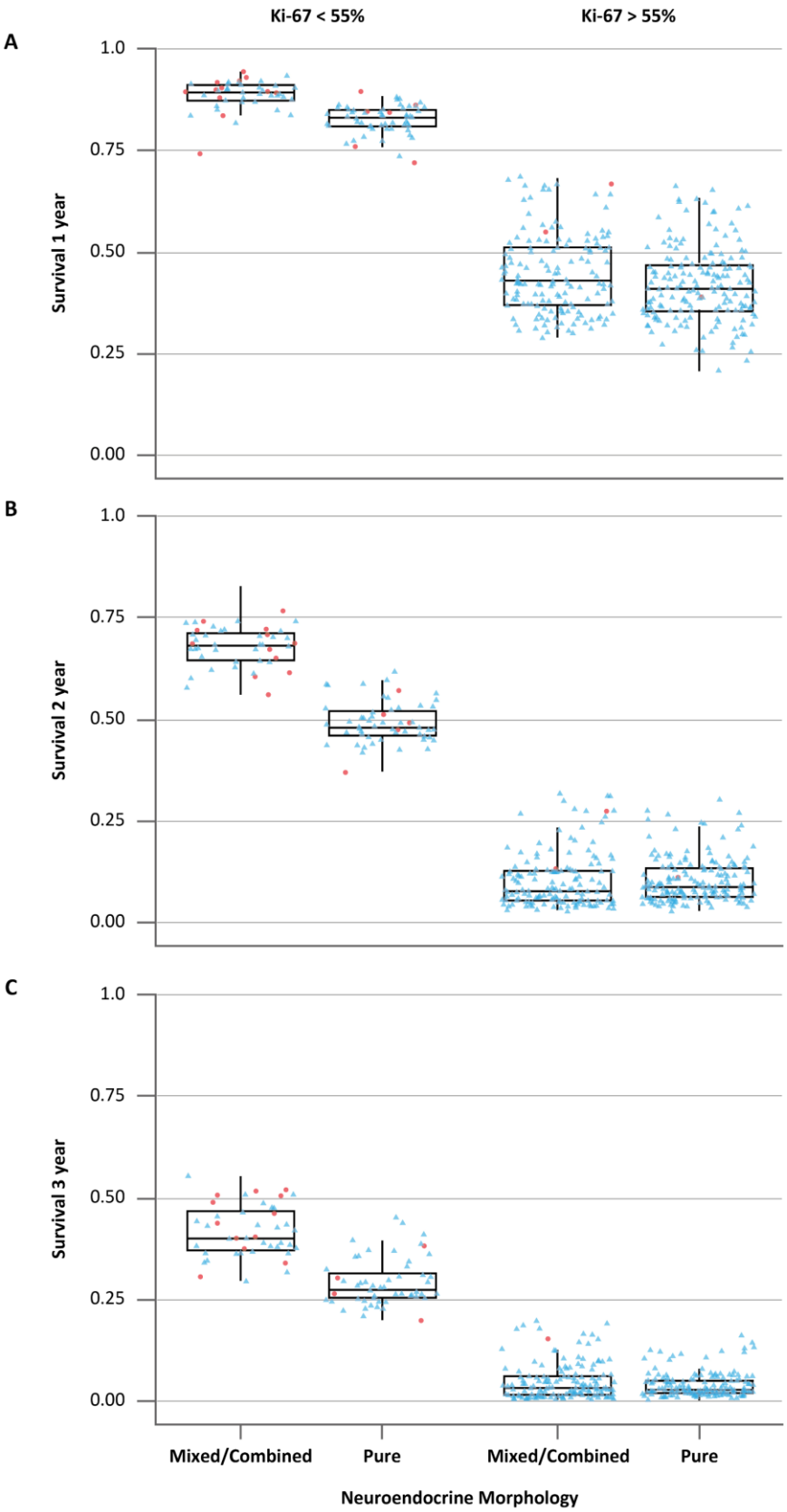


Table1. Characteristics of patients with poorly differentiated neuroendocrine carcinomas.

	All patients (n=422), n (%)	Colorectal (n=156), n (%)	Gastroesophageal (n=83), n (%)	Lung (n=111), n (%)	Pancreas (n=42), n (%)	Gallbladder/biliary (n=12), n (%)	Ileum–cecum–duodenum appendix (n=18), n (%)	p-value*
Sex:								
Male	283 (67.1)	97 (62.2)	67 (80.7)	74 (66.7)	27 (64.3)	8 (66.7)	10 (55.6)	
Female	139 (32.9)	59 (37.8)	16 (19.3)	37 (33.3)	15 (35.7)	4 (33.3)	8 (44.4)	0.08
Age (years):								
<50	56 (13.3)	20 (12.8)	11 (13.3)	13 (11.7)	9 (21.4)	0 (0.0)	3 (16.7)	
51–60	75 (17.8)	33 (21.2)	10 (12.0)	23 (20.7)	8 (19.0)	0 (0.0)	1 (5.5)	
61–70	161 (38.2)	58 (37.2)	34 (41.0)	40 (36.0)	16 (38.1)	6 (50.0)	7 (38.9)	
70+	130 (30.8)	45 (28.8)	28 (33.7)	35 (31.6)	9 (21.5)	6 (50.0)	7 (38.9)	0.45
Stage:								
I	35 (8.3)	1 (1.0)	1 (1.2)	33 (29.7)	0 (0.0)	0 (0.0)	0 (0.0)	
II	41 (9.7)	7 (4.5)	6 (7.2)	23 (20.7)	5 (11.9)	0 (0.0)	0 (0.0)	
III	202 (47.9)	91 (58.3)	52 (62.7)	30 (27.0)	16 (38.1)	8 (66.7)	5 (27.8)	
IV	144 (34.1)	57 (36.5)	24 (28.9)	25 (22.5)	21 (50.0)	4 (33.3)	13 (72.2)	<0.0001
Morphology:								
Pure	227 (53.8)	64 (41.0)	39 (47.0)	76 (68.5)	28 (66.7)	2 (16.7)	18 (100.0)	
Mixed/combined	195 (46.2)	92 (59.0)	44 (53.0)	35 (31.5)	14 (33.3)	10 (83.3)	0 (0.0)	<0.0001
Ki-67, median (range)	70 (20–98)	75 (25–98)	70 (25–95)	66 (20–95)	70 (24–95)	70 (30–98)	70 (23–90)	0.003

SSTR2A:								
Absent 0–1	246 (69.9)	95 (73.6)	46 (80.7)	69 (62.2)	24 (61.5)	6 (60.0)	6 (100.0)	
Present 2–3	106 (30.1)	34 (26.4)	11 (19.3)	42 (37.8)	15 (38.5)	4 (40.0)	0 (0.0)	0.04
p53 IHC:								
Absent	97 (31.6)	32 (28.6)	15 (27.3)	32 (28.8)	12 (60.0)	6 (66.7)	0 (0.0)	
Present	210 (68.4)	80 (71.4)	40 (72.7)	79 (71.2)	8 (40.0)	3 (33.3)	0 (0.0)	0.01
rb1 IHC:								
Absent	137 (46.3)	60 (56.1)	20 (39.2)	43 (38.7)	10 (52.6)	4 (50.0)	0 (0.0)	
Present	159 (53.7)	47 (43.9)	31 (60.8)	68 (61.3)	9 (47.4)	4 (50.0)	0 (0.0)	0.09
TP53:								
Wild-type	91 (53.8)	35 (59.3)	21 (60.0)	21 (35.6)	11 (84.6)	3 (100.0)	0 (0.0)	
Mutated	78 (46.2)	24 (40.7)	14 (40.0)	38 (64.4)	2 (15.4)	0 (0.0)	0 (0.0)	0.001
RB1:								
Wild-type	86 (87.8)	21 (95.5)	11 (100.0)	48 (81.4)	6 (100.0)	0 (0.0)	0 (0.0)	
Mutated	12 (12.2)	1 (4.5)	0 (0.0)	11 (18.6)	0 (0.0)	0 (0.0)	0 (0.0)	0.17
KRAS:								
Wild-type	142 (84.0)	45 (76.3)	34 (97.1)	48 (81.4)	12 (92.3)	3 (100.0)	0 (0.0)	
Mutated	27 (16.0)	14 (23.7)	1 (2.9)	11 (18.6)	1 (7.7)	0 (0.0)	0 (0.0)	0.05
BRAF:								
Wild-type	162 (95.9)	53 (89.8)	35 (100.0)	59 (100.0)	12 (92.3)	3 (100.0)	0 (0.0)	
Mutated	7 (4.1)	6 (10.2)	0 (0.0)	0 (0.0)	1 (7.7)	0 (0.0)	0 (0.0)	0.03
*p-value based on the Chi-squared test or Fisher's exact for categorical variables and the Kruskal-Wallis test for continuous variables.								

Table 2. Univariable* and multivariable* analysis of overall survival of patients with poorly differentiated neuroendocrine carcinoma

Variable	Univariate	P-value	Multivariable model HR (95% CI)	P-value
Sex (male vs female)	1.13 (0.91–1.40)	0.29	–	–
Age (increase of 10 years)	1.02 (0.93–1.13)	0.62	–	–
Site:				
Colorectal	1.00		1.00	
Ileum-cecum-duodenum	0.99 (0.58–1.70)	0.98		
Gallbladder/Biliary	0.70 (0.32–1.55)	0.38	0.90 (0.42–1.95)	0.80
Gastroesophageal	0.84 (0.63–1.10)	0.21	0.81 (0.62–1.07)	0.14
Lung LCNEC	0.50 (0.36–0.71)	**<0.0001**	0.69 (0.47–1.02)	0.06
Pancreas	0.51 (0.34–0.77)	**0.001**	0.58 (0.39–0.87)	**0.008**
Stage (III–IV vs I–II)	1.68 (1.19–2.37)	**0.003**	1.47 (1.01–2.14)	**0.04**
Morphology (pure vs combined)	1.42 (1.14–1.77)	**0.001**	1.41 (1.12–1.76)	**0.003**
Ki-67 (≥55 vs <55)	5.71 (4.13–7.89)	**<0.0001**	5.51 (3.98–7.63)	**<0.0001**
SSTR2A (2–3 vs 0–1)	0.95 (0.74–1.22)	0.68	–	–
p53 IHC (present vs absent)	1.19 (0.90–1.57)	0.22	–	–
rb1 IHC (present vs absent)	0.73 (0.56–0.95)	**0.02**	–	–

*Stratified for center; multivariable model includes all factors strongly associated with overall survival (p≤0.01) in univariate.