Lung Cancer 189 (2024) 107496

Contents lists available at ScienceDirect

Lung Cancer

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

Clinical management of NUT carcinoma (NC) in Germany: Analysis of survival, therapy response, tumor markers and tumor genome sequencing in 35 adult patients

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ARTICLE INFO

Keywords:
NUT carcinoma
BRD4-NUTM1
Fusion oncogene
Chemotherapy
Tumor genome sequencing

ABSTRACT

NUT carcinomas (NC) are very rare and highly aggressive tumors, molecularly defined by an aberrant gene fusion involving the *NUTM1* gene. NCs preferentially arise intrathoracically or in the head and neck region, having a highly adverse prognosis with almost no long-term survivors.

Here, we report on a cohort of 35 adult NC patients who were evaluated at University Hospital Tuebingen in an eight year time span, i.e. between 2016 and 2023. Primary objectives were overall survival (OS) and influence of primary tumor locations, fusion gene types and staging on OS. Secondary objectives were patient baseline characteristics, risk factors, tumor markers, treatment decisions and responses to therapy comparing thoracic vs non-thoracic origins. Further, data from tumor genome sequencing were analyzed.

In this monocentric German cohort, 54 % of patients had thoracic tumors and 65 % harbored a *BRD4-NUTM1* fusion gene. Median OS was 7.5 months, being significantly dependent on primary tumor location and nodal status. Initial misdiagnosis was a problem in 31 % of the cases. Surgery was the first treatment in most patients (46 %) and 80 % were treated with polychemotherapies, showing longer progression free survival (PFS) with ifosfamide-based than with platinum-based regimens. Patients treated with an immune checkpoint inhibitor (ICI) in addition to first-line chemotherapy tended to have longer OS. Initial LDH levels could be identified as a prognostic measure for survival prognosis. Sequencing data highlight aberrant *NUTM1* fusion genes as unique tumor driver genes.

This is the largest adult European cohort of this orphan tumor disease, showing epidemiologic and molecular features as well as relevant clinical data. Awareness to prevent misdiagnosis, fast contact to a specialized nation-wide center and referral to clinical studies are essential as long-term survival is rarely achieved with any of the current therapeutic regimes.

1. Introduction

NUT carcinoma (NC) is an aggressive solid tumor defined by a single gene fusion involving the *NUTM1* gene. Median age and overall survival (OS) prognosis have been reported 23.6 years and 6.5 months from a large cohort with 124 primarily US patients [1]. An incidence estimate based on US NGS data is a proportion of 0.06 % of all solid tumors, which would suggest 260 diagnosed NCs per year in Germany [2]. The true incidence can be estimated lower, given a larger proportion of NCs subjected to tumor genome sequencing (TGS) compared to all other solid tumors. So far, there are no known risk factors for developing NC and also no relevant biomarkers.

The established prognostic model from this US cohort divides NC patients in three groups, with (i) thoracic NC having the worst prognosis (4.4 months), (ii) non-thoracic NC with BRD4 as a fusion partner gene having an intermediate prognosis (10 months) and (iii) non-thoracic NC

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https://doi.org/10.1016/j.lungcan.2024.107496
Received 24 November 2023; Received in revised form 22 January 2024; Accepted 27 January 2024
Available online 29 January 2024

with non-*BRD4* fusion gene having the best prognosis (36.5 months) [1]. Further, no baseline metastases, surgery and radiation at any time, complete resection and initial complete response to treatment are predictors for improved OS [1]. In a review of 119 previously published NC cases, the usage of radiation and chemotherapy was found to predict longer OS, but not surgery [3].

Current regimens of care include surgery for resectable disease, chemotherapy with Ewing-sarcoma or platinum-doublet protocols and radiotherapy [4]. Targeted therapies such as HDACi (histone deacetylase inhibitors), BETi (bromodomain and external domain inhibitors) and immunotherapies including virotherapy are under clinical investigation, with reported treatment responses in case-based evidence [5–7]. Early clinical studies with BETi monotherapies did not show meaningful and only short-lasting treatment responses, warranting exploration of combinatorial therapies with BETi [8,9].

For pediatric patients, European standard clinical practice recommendations have been published recently [10].

On a molecular level, NC arises from a single aberrant fusion event as a somatic mutation, sometimes with features of chromoplexy resulting in complex genomic fusion breakpoints [11]. The NUT fusion protein works as an epigenetic super enhancer where NUT is bound to the DNA by its fusion partner and recruits the histone acetyltransferase p300 which forms nuclear hyperacetylated megadomains facilitating continuous expression of oncogenic transcription factors such as MYC, p63 and SOX2. This results in blockade of cellular differentiation and cell cycle progression [12–14].

In NC, the fusion oncogene is the sole tumor driver and siRNA mediated knockdown of BRD4-NUT, BRD3-NUT and NSD3-NUT results in immediate growth arrest and cellular differentiation [15,16]. Therefore, a low tumor mutational burden (TMB) is typical and no other known oncogenic mutations have been reported [1,17]. Of note, a recent *in vitro* study reported a frequent *RECQL5* mutation, being detactable in 75 % of NC cell lines (n = 12), but its relevance so far remains unclear *in vivo* as *RECQL5* is not included in generally used somatic cancer gene panels [18].

Baseline serum lactate dehydrogenase (LDH) level is a well-known prognostic parameter in a variety of cancers [19–22] and part of the International Prognostic Index (IPI) in non-Hodgkin's lymphoma [23]. Further, it was also shown to be a predictive parameter for evaluation of treatment responses in breast cancer and B-cell lymphoma [24,25]. Also, C-reactive protein levels at baseline were found to be of prognostic relevance in other tumor entities [26,27]. For NC, so far there is no knowledge on a possible benefit of these broadly used serum parameters.

2. Methods

2.1. Patient cohort

All patients aged 18 years or older who were evaluated at least once at University Hospital Tuebingen between 2016 and 2023 and had the confirmed diagnosis of NUT carcinoma in immunohistochemical staining, were included in the retrospective analysis. On this basis, a total of 35 NC patients were enrolled. Written and informed consent for data collection had been obtained from patients. The study was approved by the IRB of the University of Tuebingen (ethics committee of the Faculty of Medicine of University of Tuebingen, reference no. 609/2021BO2) and conducted according to the declaration of Helsinki.

2.2. Data collection

Data on symptoms, diagnosis, histology, treatment decisions and outcomes were collected from referrers and respective hospital information systems. List of data collected for the analysis: Date of diagnosis, defined as first radiological diagnosis of tumor. Sex. Date of birth. Age at diagnosis. Date of death. Fusion partner gene. Disease localization. Referring hospitals. Imaging modality for diagnosis. Duration of symptoms defined as time from first symptom to radiological diagnosis. Paraneoplastic syndromes. Pathological misdiagnosis defined as the final initial pathology report not reporting NC. Treatment modalities. Prior disease and chronic conditions. Medication. Family history of cancer. Ethnicity. Body mass index. Smoking status. Initial levels of LDH and CRP defined as the first available measures. All available LDH and CRP data from diagnosis. LDH change from baseline as the difference from the first to the last available LDH level. Initial histology including grading, immunohistochemical staining and PD-L1 status (tumor proportions score, TPS). Staging diagnostics with nodal status, metastases (for thoracic NC, extrathoracic nodal metastases were classified as metastases) and staging according to UICC 8 (Union for international cancer control). Development of metastases during treatment with localization. Surgical reports including resection status. Chemotherapy and radiotherapy protocols. Progression free survival defined as date from the first cycle of chemotherapy to radiological confirmation of tumor progression according to RECIST. Overall survival as the time from radiological diagnosis to death. Other systemic treatments such as targeted therapy, immunotherapy and virotherapy. Molecular diagnostic data with fusion panel sequencing and somatic tumor panel sequencing including tumor mutational burden (TMB), homologous recombination deficiency score as previously described [28,29], copy number variations (CNVs), single nucleotide variants (SNV) and microsatellite instability as predicted by tumor genome sequencing [30].

2.3. Statistical analysis

Analysis was performed via GraphPad Prism 9.4.1 (Dotmatics, Boston, MA, USA) and SPSS Statistics 29 (IBM, Armonk, NY, USA). For nominal variables, descriptive statistical analysis and chi-squared or Fisher's exact tests were employed to obtain p-values. For continuous variables, descriptive statistical analysis and Shapiro-Wilk test for normality were performed. For continuous gaussian parameters, unpaired, two-sided t-tests were used. For nonparametric parameters, Mann-Whitney-U tests and one sample Wilcoxon tests were conducted. For survival analysis, Kaplan-Meier analysis of progression free survival (PFS) and OS and Gehan-Breslow-Wilcoxon tests were performed. For LDH and CRP values, receiver-operating-characteristic (ROC) analysis was conducted, and Youden's J was calculated. P values ≤ 0.05 were considered statistically significant.

3. Results

3.1. Patient cohort

Between 2016 and 2023, 35 adult patients with NC from five European countries were identified and evaluated at University Hospital Tuebingen (Fig. 1); epidemiologic data are shown in Table 1.

Most of the NC patients (n = 19, 54 %) had thoracic tumors (Table 1). NC patients with extrathoracic tumor locations (n = 16) displayed tumor sites found generally in the ear-nose and throat (ENT) region, with eight of 16 in the nasal sinuses, three of 16 in the orbita, two of 16 in the salivary glands, and one intrahepatic NC, one intracranial suprasellar NC and one in the lingual region.

Most frequent symptoms were cough, dyspnea and thoracic pain in thoracic NC and local swelling, lymphadenopathy, and double vision in non-thoracic NC (Table 1). Eight of 35 had first-degree relatives with a history of malignant disease; no patient had a family history of reported NC. No accumulation of hereditary disorders could be found. Two patients with bone metastases had paraneoplastic hypercalcemia; besides, no other paraneoplastic syndromes could be detected. The median time from diagnosis to the contact with our specialized center was 100 days (Table 1).

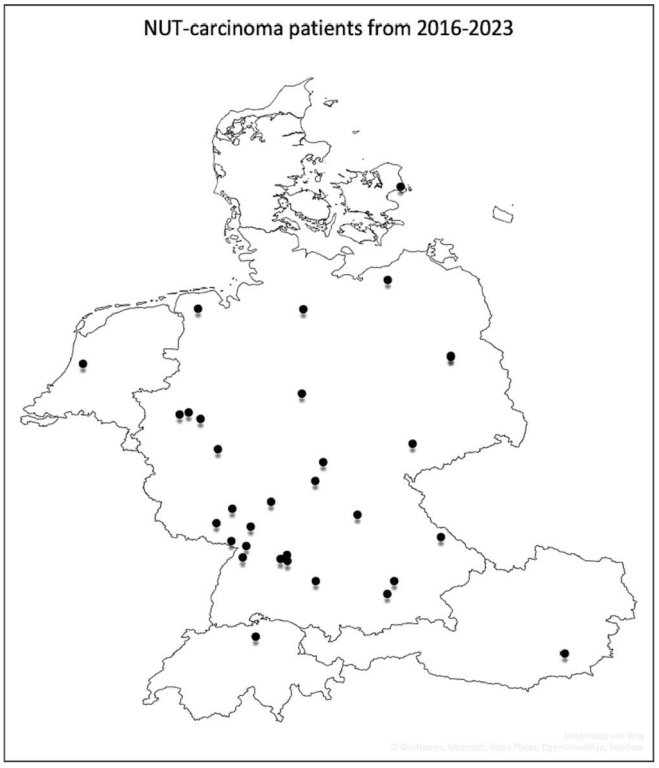

Fig. 1. Mapping of NUT carcinoma (NC) patients recruited from Germany, Austria, Denmark, Switzerland and the Netherlands.

3.2. Pathological misdiagnosis and PD-L1 status

Initial pathological misdiagnosis was made in 11 of 35 (31 %) cases. Misdiagnosis was more frequent in thoracic NC (47 %) than in non-thoracic NC (12 %, ns; Table 1). Frequent pathological misdiagnoses were small cell lung cancer (SCLC, n = 4), squamous cell carcinoma (n = 4), germ cell tumor (n = 1), primitive neuroectodermal tumor (n = 1) and lymphoma (n = 1). PD-L1 status was obtained in 17 patients, with a median tumor proportion score (TPS) of 0 % (range 0–70), seven patients with a TPS ≥ 1 % and one patient with a TPS ≥ 50 %. No significant differences between thoracic and non-thoracic NC were found, but a higher frequency of patients exhibiting a TPS ≥ 1 % was found in patients with non-thoracic NC (80 % vs. 25 %, Table 1).

3.3. Initial levels of LDH and CRP

Median initial LDH was 304 (U/l), being significantly higher than the ULN of 250 U/l (p = 0.005). Median initial LDH was 269 U/l in NC without metastases and 322 U/l in NC with metastases (ns). Median initial CRP was 1.48 mg/dl, differing significantly from the ULN of 0.5 mg/dl (p < 0.0001). Median initial CRP was found to be higher in metastatic (1.78 mg/dl) than in non-metastatic NCs (0.81 mg/dl). No relevant differences in initial LDH and CRP levels were found between thoracic and non-thoracic NCs (Table 1).

3.4. Clinical staging

At diagnosis, 27 patients (77 %) had involvement of regional lymph nodes and 22 (66 %) had distant metastases. All patients with thoracic NC showed involvement of regional lymph nodes at diagnosis (n = 19), whereas only 50 % of non-thoracic NCs displayed an involvement of regional lymph nodes (8 of 16, 50 %, p < 0.001; Table 1).

In thoracic NCs, two patients had initial surgically resectable stage IIIA. Staging for non-thoracic NC was done according to the location of

Table 1
Patient cohort, diagnostics and staging. P-values < 0.05 indicate significant differences between thoracic and non-thoracic NC. P-values were adjusted according to Bonferroni-Holm. PD-L1: Programmed death ligand 1. TPS: Tumor proportion score. LDH: Lactate dehydrogenase. ULN: Upper limit of normal. CRP: C-reactive protein.

Characteristics	All patients (No. or median)	Thoracic NC	non-Thoracic NC	p-value
Patients	35	19 (54 %)	16 (46 %)	
Age at diagnosis (y)	40 (range 18–67)	38 (18–65)	41 (20–67)	> 0.99
Sex				> 0.99
Male	23 (66 %)	15 (79 %)	8 (50 %)	
Female	12 (34 %)	4 (21 %)	8 (50 %)	
Year of diagnosis				
2016	1	0	1	
2017	2	2	0	
2018	7	3	4	
2019	5	2	3	
2020	10	7	3	
2021	3	1	2	
2022	7	4	3	
Duration of symptoms until tumor diagnosis (w)	8 (range 2–24)	6 (2–24)	8 (2–16)	> 0.99
Time from diagnosis to contact to specialized center (d)	100 (range 0–552)	100 (12–552)	115 (0–383)	> 0.99
Smoking history				> 0.99
Yes	9 (26 %)	7 (37 %)	2 (12 %)	
No	16 (46 %)	9 (47 %)	7 (44 %)	
Unknown	10 (28 %)	3 (16 %)	7 (44 %)	
Median py	0 (range 0–70)	0 (0–70)	0 (0–40)	
PD-L1 TPS				
Median TPS	0 (range 0–70, n = 17)	0 (0–70)	8 (0–20)	> 0.99
≥ 1 %	7 (41 %)	3 (25 %)	4 (80 %)	> 0.99
≥ 50 %	1 (6 %)	1 (8 %)	0 (0 %)	
Initial tumor markers				
LDH (U/l)	304 (range 149–4256)	304 (140–4256)	297 (149–1079)	> 0.99
LDH > ULN	20 (69 %)	12 (71 %)	8 (67 %)	> 0.99
CRP (mg/dl)	1.48 (range 0.01–20.3)	3.6 (0.01–15.2)	0.74 (0.01–20.3)	> 0.99
CRP > ULN	22 (76 %)	14 (82 %)	8 (67 %)	> 0.99
Initial histopathological misdiagnosis				0.35
Yes	11 (31 %)	9 (47 %)	2 (12 %)	
No	24 (69 %)	10 (53 %)	14 (88 %)	
Lymph node involvement at diagnosis				**< 0.01**
Yes	27 (77 %)	19 (100 %)	8 (50 %)	
No	8 (23 %)	0 (0 %)	8 (50 %)	
Metastases at diagnosis				**< 0.01**
Yes	22 (66 %)	17 (89 %)	5 (31 %)	
No	13 (34 %)	2 (11 %)	11 (69 %)	
Staging according to UICC8				
II	3 (9 %)	0 (0 %)	3 (19 %)	
III	8 (23 %)	8 (42 %)	0 (0 %)	
IIIA		2 (11 %)		
IIIB		3 (16 %)		
IIIC		3 (16 %)		
IV	22 (63 %)	11 (58 %)	11 (68 %)	
IVA		3 (16 %)		
IVB	3 (9 %)	8 (42 %)		

(continued on next page)

Table 1 (*continued*)

Characteristics	All patients (No. or median)	Thoracic NC	non-Thoracic NC	p-value
Location of metastases				> 0.99
Bone	22 (63 %)	13 (68 %)	9 (56 %)	
Distant lymph nodes	18 (51 %)	11 (58 %)	7 (44 %)	
Soft tissue	8 (23 %)	4 (21 %)	4 (25 %)	
Liver	7 (20 %)	4 (21 %)	3 (19 %)	
Lung	7 (20 %)	3 (16 %)	4 (25 %)	
Pleura	7 (20 %)	6 (32 %)	1 (6 %)	
Central nervous system	5 (14 %)	4 (21 %)	1 (6 %)	
Peritoneum	4 (11 %)	1 (5 %)	3 (19 %)	
Pericard	3 (9 %)	3 (16 %)	0 (0 %)	

the primary, in most cases staging for sinunasal carcinoma was applied. Although the majority did not have metastases at diagnosis, stage IV was apparent in 68 % of cases because of locally advanced tumors.

In 11 patients additional FDG-PET/CT imaging had been performed which changed metastasis status from M0 to M1 in six of 11 cases. At the time of death, distant metastases were apparent in 30 patients (86 %). Most frequently, metastases occurred in the bones (n = 22, 63 %) and distant lymph nodes (n = 18, 51 %) and soft tissues (n = 8, 23 %; Table 1). The localization of metastases did not differ between thoracic and non-thoracic NC. Pericardial involvement only occurred in thoracic NC, most likely *per continuitatem*.

3.5. Treatment decisions

In general, patients with thoracic NC received initial chemotherapy (74 %), whereas patients with non-thoracic NC were surgically resected (81 %, Table 2). The frequency of initial surgery and initial chemotherapy differed significantly between thoracic and non-thoracic NC (p = 0.001 and p < 0.001, Table 2).

Table 2
Treatment modalities in NC patients. P-values < 0.05 indicate significant differences between thoracic and non-thoracic NC. P-values were adjusted according to Bonferroni-Holm. ICI: Immune checkpoint inhibitor. VIDE: Vincristine, ifosfamide, doxorubicine, etoposide. IE: Ifosfamide, etoposide. VIP: Etoposide, ifosfamide, cisplatin, epirubicin. VIP-E: VIP + epirubicin.

Therapy	All patients (No.)	Thoracic NC	non-Thoracic NC	p-value
Initial treatment				
Surgery	16 (46 %)	3 (16 %)	13 (81 %)	**0.001**
Chemotherapy	15 (43 %)	14 (74 %)	1 (6 %)	**< 0.001**
Radiotherapy	4 (11 %)	2 (11 %)	2 (13 %)	> 0.99
Treatment modalities				
Surgery	19 (54 %)	5 (26 %)	14 (88 %)	**< 0.001**
Chemotherapy	28 (80 %)	17 (89 %)	11 (69 %)	> 0.99
Radiotherapy	29 (83 %)	16 (84 %)	13 (81 %)	> 0.99
Targeted therapy	15 (43 %)	10 (53 %)	5 (31 %)	> 0.99
Immunotherapy	10 (29 %)	8 (42 %)	2 (13 %)	0.42
Virotherapy	2 (6 %)	2 (11 %)	0 (0 %)	> 0.99
Chemotherapy regimens				**0.035**
Platinum doublet +/- ICI	15 (54 %)	12 (71 %)	3 (27 %)	
5-FU/Platinum/ Taxane	4 (14 %)	0 (0 %)	4 (36 %)	
VIDE	4 (14 %)	1 (6 %)	3 (27 %)	
IE	1 (4 %)	0 (0 %)	1 (9 %)	
VIP	1 (4 %)	1 (6 %)	0 (0 %)	
VIP-E	1 (4 %)	1 (6 %)	0 (0 %)	
GemOx/Paclitaxel	1 (4 %)	1 (6 %)	0 (0 %)	
Cisplatin/ Doxorubicine	1 (4 %)	1 (6 %)	0 (0 %)	

15 patients (43 %) had at least one targeted therapy, most frequently with the BET-inhibitor BI 894999 (n = 13, 37 %) or a different BET-inhibitor (n = 3), an aurorakinase inhibitor (n = 1) or the HDAC inhibitor vorinostat (n = 3; Table 2).

Ten patients (29 %) received immunotherapy with immune checkpoint inhibitors (ICIs), such as pembrolizumab, atezolizumab, nivolumab or ipilimumab. The use of ICIs was more frequent in patients with thoracic NC (n = 8) than in those with non-thoracic NC (n = 2, ns). Two patients (6 %) with thoracic NC had intratumoral virotherapy with the oncolytic virus (OV) talimogene laherparepvec (T-VEC).

The chemotherapy regimens employed also differed between thoracic and non-thoracic NC (p = 0.035; Table 2). Most patient with thoracic NC received platinum-based therapies with the addition of an ICI according to non-small cell lung cancer (NSCLC) guidelines (12 of 17). Overall, 5 patients did not receive platinum-based but ifosfamide-based treatment regimens (Table 2).

3.6. Overall survival outcomes

OS data were available from 30 patients, three patients were alive and censored at the point analyses. The Kaplan-Meier estimate for median OS was 7.5 months (228 d), the one-year survival rate was 27 % (8 of 30) and the two-year survival rate was 7 % (2 of 30; Fig. 2A). The longest survival time in our cohort was two and half years (938 d) in a patient with thoracic NC who underwent initial radical surgery with pneumonectomy and subsequent adjuvant radiochemotherapy.

Patients with non-thoracic NC had significantly improved median OS compared to patients with thoracic NC (415 d vs. 182 d, p = 0.048; Fig. 2B). Patients without initial nodal involvement and metastases (N0M0) showed significantly longer OS than patients with initial metastases (450 d vs. 195 d, p = 0.045; Fig. 2C). Of note, in our cohort, the respective fusion partner gene did not have a relevant impact on OS (Fig. 2D). Remarkably, initial nodal involvement without metastases (N1M0) resulted in the same adverse OS prognosis like with initial metastases (201 d vs. 195 d, ns; Fig. 2D).

3.7. Outcomes of systemic therapy

Most patients with thoracic NC had initial chemotherapy, whereas non-thoracic NCs were usually surgically resected (Fig. 3A). The Kaplan-Meier estimate for PFS during chemotherapy was two and half months (83 d), with the longest PFS of 168 d (Fig. S1). No significant differences in PFS were found between thoracic vs. non-thoracic NC, palliative vs. adjuvant, platinum and non-platinum, and ICI vs. no ICI therapy regimens or BRD4 vs. non-*BRD4* fusion genes when analyzing all systemic therapies, including first-line chemotherapies and chemotherapies after another initial treatment (Fig. 3B). The largest difference in median PFS was found between platinum and non-platinum (ifosfamide-based) containing treatment regimens (79 d vs. 142 d, ns; Fig. 3B and S1), showing a trend towards longer PFS with ifosfamide-based chemotherapies (VIDE or ifosfamide/etoposide protocols).

NC patients having undergone initial surgery tended to have improved OS compared to patients receiving initial chemotherapy or radiation (415 d vs. 198 d, ns; Fig. 3C), which is related to the survival benefit of non-thoracic NC as 11 of 13 initially resected patients had non-thoracic tumors. Addition of an ICI on top of chemotherapy regimens applied as a first-line treatment showed nominally longer OS without statistical significance. (167 d vs. 229 d, HR 0.65; p = 0.11, Fig. 3D). 14 of the 15 patients with upfront chemotherapy analyzed here had thoracic NC (Fig. 3 A).

3.8. Consecutive measurements of LDH and CRP levels

Measurements of serum parameters LDH and CRP showed only low correlation coefficients with OS ($R^2 = 0.05$ and $R^2 = 0.12$, respectively), indicating no relevant linear correlation between elevated levels of LDH

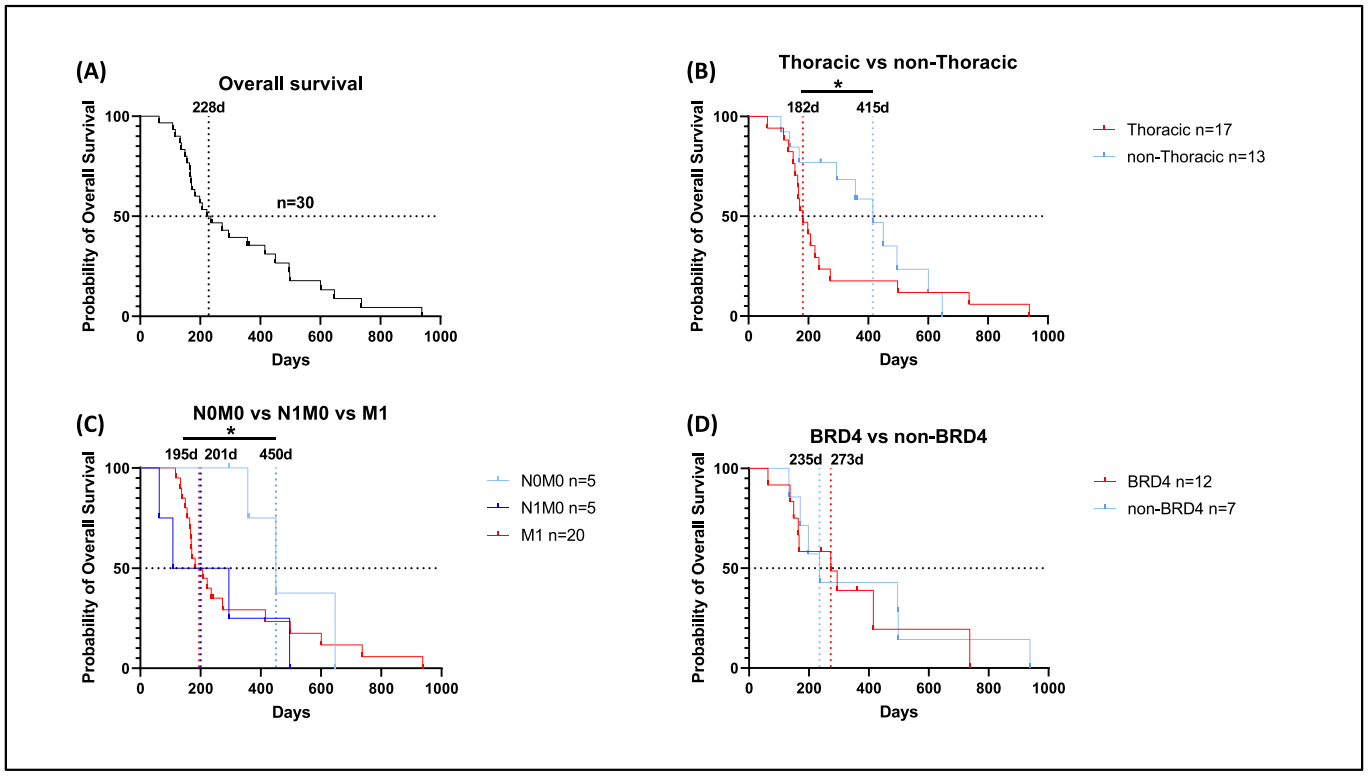

Fig. 2. Overall survival (OS) of patients with NC being influenced by location of initial tumor sites, extent of tumor disease and type of NUTM1 fusion genes. (A) Kaplan-Meier analysis of the 30 NC patients with accessible survival data shows a median OS of 228 d (7.5 months); 3 patients were alive at the time of analysis and censored at the respective points of time. (B) Non-thoracic NCs show a significantly improved median OS compared to thoracic NCs (median OS 415 d vs. 182 d; HR 0.59; 95 % CI 0.26–1.32, p = 0.048). (C) Patients without involvement of lymph nodes and metastases (N0M0) at the time point of diagnosis have a significantly improved OS compared to patients with metastases (M1) (median OS 450 d vs. 195 d; HR 0.49; 95 % CI 0.19–1.3, p = 0.045). Patients with lymph node involvement but without metastases (N1M0) exhibit almost the same OS as patients with metastases (M1) (median OS 201 d vs. 195 d; HR 0.64; 95 % CI 0.18–2.3, not significant). (D) The type of NUTM1 fusion partner gene (BRD4 vs. non-BRD4) does not have a relevant impact on OS (median OS 273 d vs. 235 d).

and CRP and survival prognosis (Fig. 4A & B). A receiver-operating characteristic (ROC) analysis for prediction of an OS below the median of 228 d revealed AUC of 0.85 and a Youden's J of 0.62 for the initial LDH level, pointing out the prognostic value of initial LDH for OS (Fig. 4C). A cut-off value of > 306 U/I predicted an OS below the median with 77 % sensitivity and 80 % specificity in our cohort. The AUC for initial CRP levels as a prognostic measure for OS below the median was 0.77, showing a poorer prognostic accuracy than initial LDH (Fig. 4D).

During the course of disease, LDH levels showed an increase in most NC patients with median increase of 492.5 U/l from first to last available LDH value (n = 26, p = 0.0001, Fig. S2A). LDH change from baseline was positive in 22 of 26 NC patients; only four patients showed a slight decrease in LDH over time (Fig. S2B). Further, most patients experienced steep increases in LDH levels shortly before death (Fig. S3).

8-week changes were positive in 15 and negative in nine patients and did not correlate with OS. OS differences between patients with positive and negative 8-week LDH change were not significant (276 d vs 384 d) but showed a trend towards favorable OS with negative change in LDH after 8-weeks.

3.9. *Tumor genome sequencing*

Molecular diagnostics were available in 18 NC patients, with at least a panel RNA sequencing for fusion gene diagnostics, revealing the respective fusion genes of *NUTM1* (Table 3). Somatic gene panel sequencing was performed in 15 cases, with a median of 708 sequenced genes. No actionable mutations were found; also, only very low numbers of single nucleotide variants (SNVs) and smaller copy number variants (CNVs) < 40 base pairs were detected. A list of all detected SNVs, CNVs and structural variants is given in Table S1.

The median TMB was 1.0 mutation per megabase, ranging from 0.5 to 3.1 mut/mb. No NC patient had a high TMB of > 10 mut/mb. No homologous repair deficiency (HRD) could be detected; the median HRD-score was nine (range 0 to 26) with levels over 30 indicating an HRD. Further, no microsatellite instability could be detected in any NC tumor. No germ line mutation or hereditary syndromes were found.

4. Discussion

NUT carcinoma (NC) is a rare, but upcoming diagnosis, as broader tumor genome sequencing approaches are increasingly applied. Importantly, the application of fusion panel sequencing as a standard diagnostic procedure in all cases of lung cancer reveals previously underdiagnosed cases of NC.

In this monocentric cohort of adult NC patients, OS outcomes show a median of 7.5 months with no long-term survivors > 3 years, which does not differ relevantly from the 6.5 months reported in the US cohort [1]. Patients with thoracic NC had a median OS of 6 months, which was slightly longer than the previously reported 4.4 months. In this study, the fusion oncogene did not have an impact on OS, as reported previously for thoracic NC. For non-thoracic NC, a separation between a non-*BRD4* and *BRD4* fusion subgroup was not applicable as there was only one patient with non-thoracic non-*BRD4* NC. The median OS for non-thoracic NC was 13.6 months, ranging between the intermediate and good prognostic subgroups for non-thoracic NC (10 and 36.5 months, respectively).

Patients without lymph node or distant metastases had a significantly improved prognosis in this cohort (14.8 months). Of note, the presence of nodal metastases massively worsened OS prognosis (6.6 months). Remarkably, FDG-PET/CT imaging revealed previously

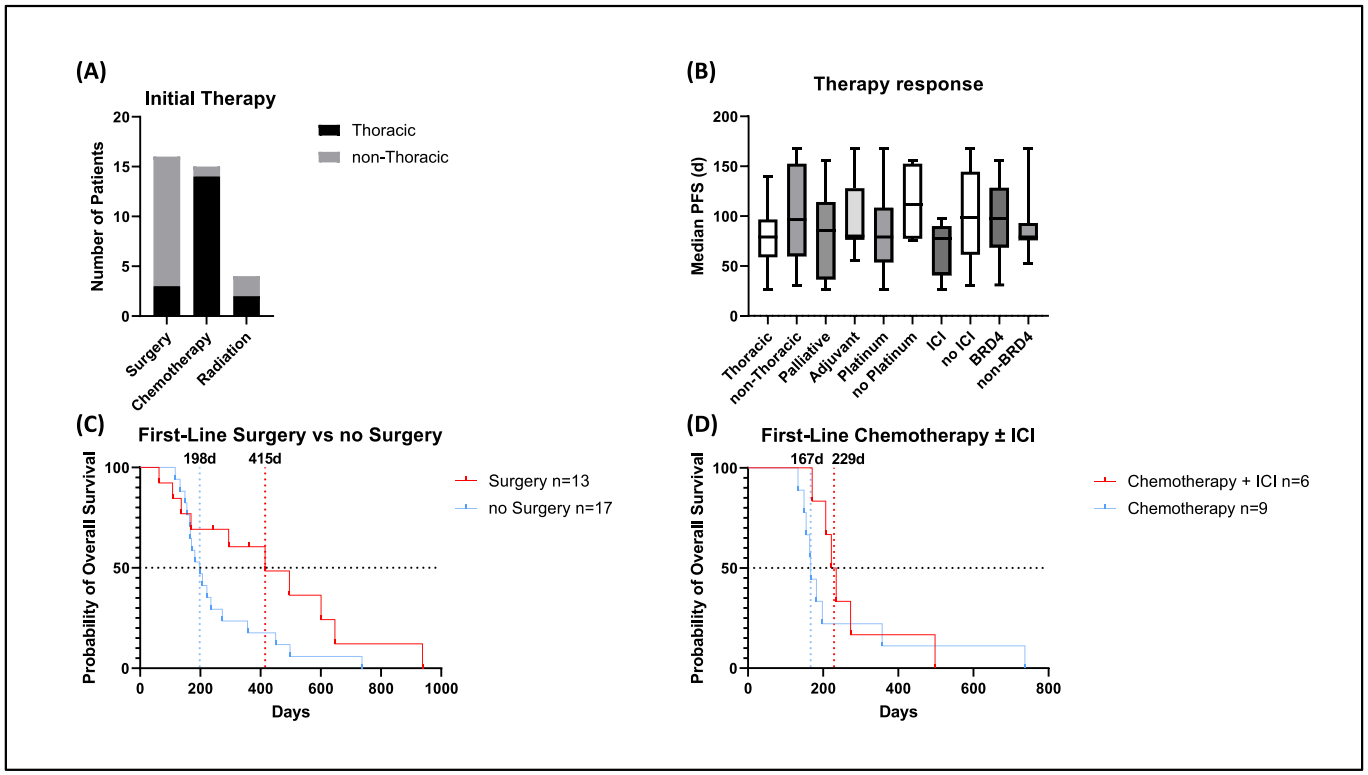

Fig. 3. Therapeutic decisions and outcomes in the Tuebingen cohort of NC patients. (A) 16 NC patients were initially resected, thereof 13 with non-thoracic disease. 15 NC patients received frontline chemotherapy, 14 of them had thoracic manifestations. 4 NC patients were primarily radiated, thereof 2 with thoracic manifestations and 2 with non-thoracic tumors. (B) Progression free survival (PFS) after the first chemotherapy, adjuvant and palliative therapies were included. Data from 20 NC patients were available, showing a median PFS of 82 d (2.7 months). The largest difference in median PFS is observed between platinum (n = 16) and non-platinum (n = 4)-based chemotherapy regimens (median PFS 79 d vs. 111 d; not significant). Boxes show mean, IQR and range. (C) NC patients who had resectable disease show longer OS; this is closely related to the significant difference in OS observed between NC patients exhibiting thoracic vs. only non-thoracic tumor manifestations, given the big overlap between those groups (11 of 13 initially resected patients had non-thoracic NC). Median OS 415 d vs. 198 d; HR 0.48; 95 % CI 0.22–1; not significant. (D) The 15 patients receiving first-line chemotherapy were analyzed. Addition of an immune checkpoint inhibitor to systemic therapy does not show an impact on OS (median OS 167 d vs. 229 d; not significant).

undetected metastases in 55 % of the cases, underscoring its importance in the staging procedure.

In this real-world setting, the fusion partner gene of *NUTM1* was only detected in 57 % patients. In the other cases, either detection of the fusion partner was not possible due to insufficient amounts of tumor materials or omitted, since IHC staining is sufficient for the diagnosis of NC.

Initial misdiagnosis occurred in 31 % of the cases and was significantly more frequent in thoracic NC. These results suggest that misdiagnosis is still a problem but recently becoming less frequent (earlier case analysis reported rates of misdiagnosis around 70 % [3]). The high occurrence of misdiagnosis in thoracic-NC (47 %) highlights the importance of fusion panel sequencing for all thoracic tumors.

Detection of the fusion partner gene is recommended in all NUT IHC positive cases, since some rare fusion partner genes such as CIC, MGA and MXD4 show a different clinical and molecular bromodomain independent behavior despite positive NUT IHC [2,31]. Further, the type of fusion partner gene was shown to be a relevant prognostic factor, importantly in non-thoracic NC [1].

Clinical management differed significantly between thoracic and non-thoracic NC in this cohort, with non-thoracic NCs undergoing more surgical resections and receiving more 5-fluoruracil and ifosfamide containing chemotherapy regimens, whereas thoracic NC was frequently treated with initial chemotherapy and platinum doublet therapy with an ICI, in accordance with current lung cancer guidelines.

As previously described, most patients received platinum-based combination chemotherapy with generally unsatisfactory outcomes (median PFS 79 d) [32]. In this cohort, 18 % received platinum-free, ifosfamide-based regimens, which showed a tendency towards improved PFS (142 d, ns). This finding is in one line with some case reports of complete or partial remission using ifosfamide-based therapy regimens [33–35].

Initial surgery had the highest impact on OS and was performed in almost all non-thoracic NCs in this cohort. Staging for thoracic NC was stage III in 8 cases and IV in 11 cases. The longest survivor suffered from thoracic NC (T2N2M0, stage IIIA) and possibly benefited from initial complete resection. This is in accordance with current case reports [36,37], concluding radical initial surgery can be a chance for long-term survival also in thoracic NC. Interestingly, the second patient with thoracic NC in stage IIIA (T2N2M0) had disease progression during an initial induction chemotherapy and deceased shortly after without surgery. For non-thoracic NC, the benefit from initial surgery has been described earlier [38] and the recommendations indicate surgery as the most effective therapy option [4].

Immunotherapy with ICIs did not have an impact on PFS or OS when analyzing all systemic therapies, but showed a trend towards improved OS when applied in a first-line therapy regimen. The predictive markers PD-L1 TPS and TMB were low (median 0 % and 1.0 mut/mb, respectively). A single patient with thoracic NC had a high TPS of 70 % but did not respond to atezolizumab. This is the largest NC cohort where PD-L1 was evaluated (n = 19), and in accordance with previous reports, predictive markers and response rates to immunotherapy were low. In a case series, median PD-L1 was reported to be 1 % TPS and no microsatellite instable tumors were found [17]. Median TMB was reported previously to be 1.7 mut/mb in cohort of 31 patients identified by tumor genome sequencing [39]. In contrast, in a review of published cases

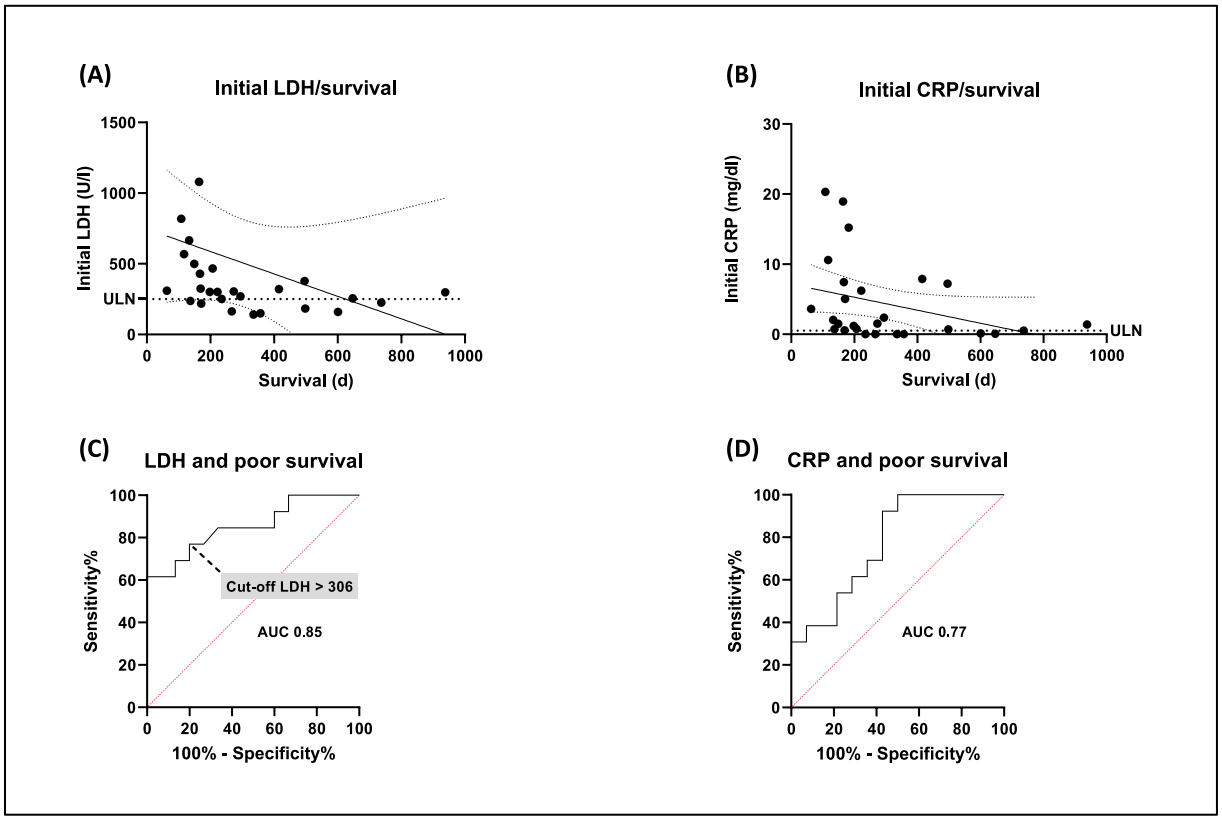

Fig. 4. Initial LDH and CRP values and overall survival (OS). (A) Linear regression of initial LDH values and survival time (OS) shows a weak correlation ($R^2 = 0.05$). Dotted lines show 95 % CI. Median initial LDH was 304 U/l (ULN 250 U/l, range 149–1079 U/l). Initial values were earliest available from tumor diagnosis. (B) Linear regression of initial CRP values and survival time (OS) shows a weak correlation ($R^2 = 0.12$). Dotted lines show 95 % CI. Median initial CRP was 1.48 mg/dl (ULN 0.5 mg/dl, range 0.01–20.3 mg/dl). (C) Receiver-operating characteristic (ROC) for initial LDH values as a prognostic value for poor survival (OS < 228 d). ROC analysis shows a sensitivity of 77 % and specificity of 80 % at an LDH cut-off value of > 306 U/l (AUC 0.85; 95 % CI 0.71–1.0; p = 0.0015). (D) Initial CRP value is a poorer predictor for poor survival (AUC 0.77; 95 % CI 0.6–0.95; p = 0.015).

Table 3
Molecular tumor diagnostics in NC patients. SNV: Single nucleotide variant. CNV: Copy number variant.

Diagnostic method	No. or median
Fusion panel sequencing	18
BRD4-NUTM1	13 (72 %)
BRD3-NUTM1	3 (17 %)
NSD3-NUTM1	4 (22 %)
Other gene fusions	0 (0 %)
Somatic gene panel sequencing	15
No. of genes	708 (range 8–766)
Actionable mutations	0
SNVs/CNVs < 40 bps	0 (range 0–5)
Structural variants	0 (range 0–2)
Tumor mutational burden/megabase	1 (range 0.5–3.1)
Homologous repair deficiency score	9 (range 0–26)
Microsatellite instability	
High	0 (0 %)
Low	11 (73 %)
Unknown	4 (27 %)

three of four thoracic NCs showed high TMB (>10 mut/mb), whereas no single NC with high TMB was identified in this cohort However, TMB is known to be higher in metastases and no data on the origin of the biopsy was reported [40,41]. Cases of responses to ICI therapy are described [6], but other predictive models such as neo-antigen affinity to MHC might be of interest in NC [39].

Regarding molecular data, no microsatellite instable NC case was detected. Data on homologous repair deficiency (HRD-Score) are reported for the first time, showing no signs of repair deficiency in any NC tested (median HRD-score 9, range 0–26, n = 8). Therefore SNVs, small CNVs and structural variants other than the fusion oncogene were rare, as reported previously in a cohort of 31 NC patients. Interestingly, a patient with an inactivating mutation in the histonacetyltransferase CREBBP could be identified in this cohort and in our cohort, raising the question whether this has consequences for tumor biology as CREBBP is a major target of the NUT fusion protein. Like in this cohort, there is no case of NC with an actionable mutation in the literature. One case reports an additional splice-site mutation in BRD4 in the NUTM1-BRD4 fusion gene, predicting an unusually good clinical response to a BETi [5].

Baseline serum LDH could be identified as relevant prognostic parameter for poor OS (<7.5 months, AUC 0.85, Youden's J 0.62). A threshold of > 306 U/l was identified to have a sensitivity of 77 % and a specificity of 80 %. So far, there is no data about LDH levels in NC in the literature.

This cohort underscores generally poor outcomes in management of the young NC patients. Besides pursuing new therapeutic options, the following conclusions to optimize the application of currently available treatment options should be made. (i) Allowing a quick diagnosis with fusion panel sequencing for every case of suspected lung cancer and NUT IHC staining for every atypical non-thoracic carcinoma, especially in young patients without risk factors and tumors in the ENT region. (ii) Early contact to a specialized center after diagnosis is of utmost importance as early surgery offers the best chance for long term survival. (iii) Risk stratification according to tumor location, nodal status, fusion oncogene and LDH level including initial FDG-PET-CT imaging. (iv) First-line surgical intervention if applicable, especially in stage IIIA according to lung cancer UICC staging. (v) If first-line chemotherapy is

necessary, ifosfamide-based chemotherapies and ICIs are recommended.

5. Conclusion

Our data represent the current clinical procedure of NC management in Germany. The established prognostic model could be confirmed; further, novel data on metastatic patterns, efficacy of chemotherapy regimens, PD-L1 expression, molecular data and serum LDH as a prognostic marker were presented. Although several innovative treatment options were applied beyond standard of care, no long-term survivors were identified in this cohort, underlining the urgent need for novel therapies in NC. Importantly, patients should be referred to clinical studies investigating BETi like NCT05019716, NCT05372640, NCT05488548 or to other basket trials for solid tumors if eligible to pursue clinical development of new therapeutic options in this rare cancer type.

Funding

LDK received funding by the Dres. Bayer Stiftung ("Württembergischer Krebspreis - Kategorie Nachwuchs").

CRediT authorship contribution statement

Linus D. Kloker: Writing – original draft, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Mirjana Sidiras:** Methodology, Data curation, Conceptualization. **Tim Flaadt:** Writing – review & editing, Conceptualization. **Ines B. Brecht:** Writing – review & editing. **Christoph K.W. Deinzer:** Writing – review & editing. **Thorben Groß:** Writing – review & editing. **Katrin Benzler:** Writing – review & editing. **Lars Zender:** Writing – review & editing, Project administration. **Ulrich M. Lauer:** Writing – original draft, Supervision, Project administration, Investigation, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

LDK is funded by the intramural *f*ortüne program of the Faculty of Medicine, University of Tuebingen (proposal number 3007-0-0).

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.lungcan.2024.107496.

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