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Alternative Lengthening of Telomeres Predicts Site of Origin in Neuroendocrine Tumor Liver Metastases

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Abstract

BACKGROUND—The determination of the primary tumor origin in patients with neuroendocrine tumor liver metastases (NELM) can pose a considerable management challenge. Recent studies have shown that the alternative lengthening of telomeres (ALT) is prevalent in some human tumors, including pancreatic neuroendocrine tumors (PanNET), and can be useful in predicting tumor biology. In this study, we aimed to evaluate the use of ALT as a biomarker in patients with NELM, in particular to predict the site of origin of metastases.

METHODS—Tissue microarrays (TMAs) were constructed using tumor tissue from NELM patients undergoing liver resection between 1998 and 2010. These included 43 PanNET and 47 gastrointestinal carcinoid tumors. The TMAs were tested for ALT using telomere-specific fluorescent in situ hybridization. The association between ALT positivity and clinicopathologic features and long-term outcomes was investigated.

RESULTS—Alternative lengthening of telomeres was positive (ALT+) in 26 (29%) of the 90 tumors included in the TMAs. Pancreatic neuroendocrine tumors were ALT+ in 56% of patients, compared with only 4% ALT+ among gastrointestinal carcinoid tumors ($p < 0.001$). The specificity of ALT for detecting pancreatic origin was 96% and the positive predictive value was 92%, and sensitivity was 56% and the negative predictive value was 70%. Additionally, ALT was associated with the pattern of metastatic disease: ALT + NELM were more likely to have

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Author Contributions

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oligometastases ($p = 0.001$) and less likely to be bilateral in distribution ($p = 0.05$) than were ALT tumors. In addition, ALT was associated with improved prognosis in the PanNET patient population.

CONCLUSIONS—Alternative lengthening of telomeres was found to be a useful biomarker in patients with NELM. This marker can be helpful in guiding therapy by identifying the site of origin in patients in whom the primary site is unknown.

Neuroendocrine tumors (NETs) consist of a diverse group of neoplasms that exhibit substantial variation in biological behavior and response to treatment. Specifically, NETs of unknown primary site account for 10% to 13% of all NETs.^{1,2} They often manifest as neuroendocrine liver metastases (NELM), discovered with nonspecific symptoms or incidentally during abdominal imaging performed for other indications. The diagnosis of NET of unknown primary is typically established after fine-needle aspiration biopsy of the liver metastases reveals neuroendocrine differentiation on light microscopy and immunohistochemical staining. In the majority of those cases, the primary NET will be either pancreatic neuroendocrine tumor (PanNET) or gastrointestinal (GI) carcinoid tumor.³⁻⁵ Extensive imaging with CT, MRI, somatostatin receptor scintigraphy, and PET is often used in an attempt to identify the primary tumor. When resectable, exploratory laparotomy combined with hepatectomy and search for the occult primary is generally recommended when preoperative evaluation fails to establish a site of origin.^{6,7} When unresectable, in the event that all attempts to identify the primary tumor fail, patients are usually treated empirically with hormonal or cytotoxic chemotherapy based on the liver metastases' histologic grade and the presence of symptoms. However, determination of the primary site is becoming increasingly important. Specifically, advances in the understanding of the genetics of PanNET have identified unique molecular features of these tumors, some of which, such as mammalian target of rapamycin pathway mutations, are potentially targetable.⁸ Newer targeted therapies have been shown to be effective in patients with PanNET, including everolimus and sunitinib.⁹⁻¹¹ Therefore, the benefit of identifying a molecular biomarker in NET that can help determine the primary tumor site from a biopsy of NELM would be clinically useful. Recent studies have demonstrated an uncommon but distinct pathway in which neoplastic cells can overcome the chromosome end replication problem and gain immortality. Although most neoplasms rely on the upregulation of telomerase to maintain telomere length, approximately 5% of human tumors appear to use a telomerase-independent pathway, called the alternative lengthening of telomeres (ALT) mechanism.¹² The ALT was originally described in human sarcomas and astrocytomas, but has since been found to be prevalent in a variety of human malignancies, including NET.¹³ Early studies have suggested that this biomarker can be useful in predicting tumor biology. Our aim in this study was to evaluate the use of ALT as a biomarker in patients with NELM. In particular, we aimed to determine if ALT can be useful in predicting the site of origin of the NET metastases.

METHODS

Identification of patients and construction of the tissue microarrays

All patients who underwent liver resection for NELM between 1998 and 2010 at the Johns Hopkins Hospital were identified after approval of the study by the Institutional Review Board. The pathology database was evaluated for the availability of formalin-fixed, paraffin-embedded tumor tissue blocks of their liver metastases. The available tissue blocks were reviewed and marked by a pathologist (MT) to confirm the presence of tumor tissue. Core biopsies were obtained from the tumor-containing area of the blocks and used to construct tissue microarrays (TMAs) to facilitate immunolabeling and scoring. Three core biopsies from each tissue block were used in the TMAs in an effort to minimize the sampling error. Controls of normal human tissue cores were also incorporated in the TMAs, in a ratio of 1 control for every 3 tumor cores.

Hybridization and scoring for alternative lengthening of telomeres

The methodology used for telomere-specific fluorescence in situ hybridization (FISH) of specimens was as described by Heaphy and colleagues.¹⁴ Briefly, deparaffinized TMAs slides were hydrated, steamed for 20 minutes in citrate buffer (catalog no. H-3300; Vector Laboratories), dehydrated, and hybridized with a Cy3-labeled peptide nucleic acid probe complementary to the mammalian telomere repeat sequence ([N-terminus to C-terminus] CCCTAACCCTAACCCTAA). As a positive control for hybridization efficiency, a fluorescein isothiocyanate-labeled peptide nucleic acid probe having specificity for human centromeric DNA repeats (ATTCGTTGGAAACGGGA; CENP-B binding sequence) was also included in the hybridization solution. After post-hybridization washes, slides were imaged with a Nikon 50i epifluorescence microscope equipped with X-Cite series 120 illuminator (EXFO Photonics Solutions Inc.) and appropriate fluorescence excitation/emission filters. Grayscale images were captured using Nikon NIS-Elements software and an attached Photometrics CoolsnapEZ digital camera, pseudo-colored and merged. Quantification from the digital images was conducted using Telometer, a custom software plugin created for the open source image analysis program ImageJ, freely available for download (<http://bui2.win.ad.jhu.edu/telometer/>).

The slides were scored by 2 independent reviewers (CH and AM) blinded to the tumor characteristics. In our study, NELM were classified as ALT+ if they met the following criteria: presence of ultra-bright, intra-nuclear foci of telomere FISH signals, with integrated total signal intensities for individual foci being >10-fold that of the per cell mean (integrated signal intensities for all telomeric signals in individual benign stromal cells within the same case) and 1% of neoplastic cells displaying ALT-associated telomeric DNA foci. Tumor samples lacking ALT-associated telomeric foci in which at least 500 cells were assessed were considered ALT negative. In all cases, necrotic foci were excluded from consideration.

Data acquisition and statistical analysis

Standard demographic data, as well as clinicopathologic characteristics of both the primary tumors and their NELM, were obtained through record review for the patients included in the TMAs. Functional tumors were defined as those for which both symptoms attributed to

hormonal secretion and biochemical evidence of hormonal secretion were documented. Synchronous NELM were defined as the synchronous discovery of the NET primary and the liver metastases. Curative intent surgery was defined as the ability of the surgeon to achieve a negative margin (R0) resection, and noncurative intent was defined as a surgery where the margins were either microscopically (R1) or macroscopically (R2) positive for tumor. Oligometastatic disease was defined ≤ 5 liver metastases and polymetastatic disease was >5 metastases. Minor hepatectomy was defined as the resection of ≤ 3 liver segments and major hepatectomy included >3 segments. Chi-square test was used for comparison of discrete variables and t-test and Kruskal-Wallis test were used for comparison of ordinal variables. The sensitivity, specificity, positive predictive value, negative predictive value, and positive likelihood ratio of ALT's ability to predict the pancreatic origin of NELM were calculated. Follow-up data were obtained from the patients' medical records, determining recurrence-free survival and overall survival (OS) using the Kaplan-Meier method. Univariate and multivariate survival analysis were performed with the Cox proportional hazards model. Statistical significance for all tests was set at $p < 0.05$. All statistical analyses were performed using the Stata version 11.2 software package (Stata Corp).

RESULTS

Patient and tumor characteristics

A total of 90 eligible patients and corresponding tumor blocks were identified and included in the TMAs. The location of the primary NET was known for all cases, determined either preoperatively or intraoperatively. They consisted of 43 (48%) metastases of PanNET origin and 47 (52%) metastases of GI carcinoid origin. A summary of patient demographics and primary tumor pathologic characteristics is presented in Table 1. Pancreatic NETs and GI carcinoids did not differ significantly in terms of patient demographics and primary tumor grade. However, GI carcinoid primary tumors were smaller in size ($p < 0.001$), more frequently involved the regional lymph nodes ($p = 0.029$), and more frequently were functional than were the PanNETs ($p = 0.014$). All 20 functional GI carcinoid tumors caused the carcinoid syndrome, and the 8 functional PanNETs consisted of 2 insulinomas, 2 glucagonomas, 1 VIPoma (vasoactive intestinal peptide), 1 gastrinoma, and 2 parathyroid hormone-related protein secreting tumors. A summary of liver disease pattern and liver operation details is presented in Table 2. Pancreatic NETs and GI carcinoid NELM were similar when considering the timing of the discovery of the liver metastases and the proportion undergoing surgery with curative intent. Additionally, the size of the largest liver metastasis did not differ between groups, nor did the extent of liver resection differ. However, NELM of GI carcinoid tumor origin were more likely to present with polymetastatic disease (>5 metastases) ($p < 0.001$), bilateral distribution ($p = 0.001$), and to undergo ablation combined with liver resection ($p < 0.001$).

Alternative lengthening of telomeres phenotype and association with patient and tumor characteristics

Alternative lengthening of telomeres was positive (ALT+) in 26 (29%) of the 90 NELM included in the TMAs. Specifically, PanNET liver metastases were ALT+ in 56% of cases (24 of 43), compared with only 4% ALT+ among GI carcinoid liver metastases (2 of 47) ($p <$

0.001) (Fig. 1). The specificity of ALT for detecting PanNET origin of NELM was 96% and the positive predictive value was 92%, and sensitivity was 56% and the negative predictive value was 70%. The positive likelihood ratio was 14. The association between ALT positivity and primary tumor characteristics is presented in Table 3, where it is shown that ALT was only associated with the location of the primary tumor. A breakdown of ALT phenotype's association with the pattern of liver disease and liver operation details is provided in Table 4. ALT+ NELM were less likely to present synchronously with the primary tumor ($p = 0.001$). However, curative intent was more likely to be achieved for ALT+ NELM ($p = 0.013$), as ALT+ NELM were more likely to present with oligometastatic disease (≤ 5 metastases) ($p = 0.001$) and with unilateral disease ($p = 0.045$) compared with ALT- NELM. The ALT- phenotype was not associated with the extent of liver resection or the use of ablation.

Alternative lengthening of telomeres phenotype and long-term outcomes

With a median follow-up of 51 months, 42 recurrences were observed, with a median recurrence-free survival of 26 months. The 23 patients in whom curative intent was not achieved were excluded from the recurrence analysis and therefore the association of ALT phenotype and recurrence-free survival cannot be accurately determined. In the remaining 67 patients in whom curative intent was achieved, ALT+ appeared to be associated with an increased risk of recurrence in univariate analysis (hazard ratio [HR] = 2.2; 95% CI, 1.2e4.1; $p = 0.014$). During the same period of follow-up, 26 deaths were observed, with a median OS of 132 months. ALT phenotype was not associated with OS ($p = 0.699$) in the entire cohort. However, when examining the tumors of PanNET origin alone, ALT+ was associated with increased OS (HR = 0.33; 95% CI, 0.12–0.97; $p = 0.043$) in univariate analysis (Fig. 2). Patients with an ALT+ PanNET had a median OS of 132 months vs 71 months for patients with an ALT- PanNET. The only other predictor of outcomes was tumor grade (HR = 5.3; 95% CI, 1.8e15.3; $p = 0.002$). In multivariate OS analysis, ALT phenotype approached the level of statistical significance, but failed to achieve it (HR = 0.22; 95% CI, 0.1–1.1; $p = 0.059$), and tumor grade remained statistically significant (HR = 4.8; 95% CI, 1.5–14.7; $p = 0.007$). A similar association between ALT phenotype and OS was not observed in the GI carcinoid tumors population ($p = 0.938$).

DISCUSSION

The wide spectrum of biologic behavior and varied primary sites of origin of NET can present considerable challenges when managing patients with NELM. In particular, patients often present with incidental or asymptomatic liver metastases in which the primary tumor is not evident. Although most often NELM are from GI origin, the primary site of origin cannot be identified in more than one third of patients.^{4,6} Even among those undergoing abdominal exploration for the surgical management of the NELM, the primary tumor cannot be identified in 13%.⁵

Beyond standard histologic and immunohistochemical assessment, the development of novel biomarkers in NET is needed to determine more accurately the biologic behavior, as well as ascertaining the site of origin. In our study, we demonstrated that the use of a recently

described molecular biomarker, ALT, can be of use in interrogating tissue specimens from NELM. Through the use of telomere-specific FISH from paraffin-embedded tissue using TMAs, we found that determination of the ALT phenotype is feasible and reproducible. In addition, we found that ALT assessment is useful in identifying pancreatic origin for NELM.

One way in which the cancer cell can circumvent mechanisms that limit cell proliferation is through the maintenance of telomere length, which usually occurs through the upregulation of the enzyme telomerase. However, approximately 5% of human cancers activate the ALT pathway, which relies on a telomerase-independent pathway of recombination-mediated elongation.^{13,15} This mechanism leads to significant heterogeneity of telomere length within ALT+ cells and large intra-nuclear foci of telomere DNA; a finding that can be ascertained with telomere-specific FISH. Although the exact mechanisms involved in ALT are unknown to date, mutational analyses have linked the ALT phenotype to the ATRX/DAXX/H3.3 chromatin-remodeling pathway.¹⁴ In addition, studies have demonstrated that these genes are frequently altered in PanNETs.^{8,14} Although ALT positivity has a relatively low sensitivity for PanNET, it is a very specific marker that also provides insight into the fundamental genetic alterations (*ATRX* and *DAXX* mutations) driving tumorigenesis in these tumors.

Determination of the site of origin of NELM is becoming increasingly important, as newer therapies have been shown to have varying benefit depending on primary site. The PROMID study (Placebo Controlled, Double Blind, Prospective, Randomized Study on the Effect of Octreotide LAR in the Control of Tumor Growth in Patients with Metastatic Neuroendocrine Midgut Tumors),¹⁶ which demonstrated benefit of somatostatin analogue in patients with nonfunctional advanced NET, included mostly carcinoid tumors, challenging the role of this hormone therapy in patients with PanNET.¹¹ More recently, randomized studies in NETs have resulted in the approval of 2 new drugs, everolimus and sunitinib, for the treatment of patients with well-differentiated PanNETs. Comparable trials did not show benefit in carcinoid NETs. In addition, for patients who are candidates for surgical therapy, identification of the primary site of disease preoperatively can increase the probability of identifying and managing the primary tumor during surgery.

Several investigators have examined various molecular biomarkers in an attempt to identify the site of origin of metastatic NET.¹⁷ Antibody staining for Cdx2 has been shown to be useful in identifying midgut carcinoid tumors.¹⁸ Other markers, including TTF-1 and Isl1, have been used to differentiate GI from pulmonary NET.¹⁹⁻²² Unlike these markers, our study specifically focused on a distinct single biomarker that describes a unique molecular pathway. As targeted therapies become identified in the future, the ability to easily identify alternations in specific molecular pathways that define the malignant phenotype will become increasingly important.

In our study, ALT phenotype was found to be significantly more prevalent in NELM of PanNET origin (59%) compared with those of GI carcinoid origin (4%). We attempted to extrapolate the clinical value of ALT for detecting the pancreatic origin of NELM and calculated its specificity at 96%, its positive predictive value at 92%, and its positive likelihood ratio at 14. These data suggest that an ALT+ NELM will almost certainly be of

pancreatic origin and therefore all pre- and intraoperative primary tumor localization efforts should be focused on the pancreas. Additionally, if the primary tumor remains clinically occult, our data could justify the use of biologic therapies directed at PanNET in ALT+ NELM. Conversely, the calculated sensitivity and negative predictive value of ALT in terms of predicting the pancreatic origin of NELM were only 56% and 70%, respectively. Although this would suggest that an ALT- NELM is more likely to be of GI carcinoid origin, it is insufficiently sensitive to rule out a pancreatic primary.

Surgical resection is generally recommended in patients with limited oligometastatic NELM.¹¹ However, preoperative imaging often fails to reveal the full extent of the liver disease compared with intraoperative evaluation and ultrasound imaging of the liver, frequently identifying unsuspected miliary liver metastases.⁵ In such cases, some patients might be subjected to nontherapeutic surgical exploration. In this study, we found that ALT determination can also be a useful marker for determining the extent and distribution of metastases. Specifically, ALT NELM were found more likely to be oligometastatic (< 5 metastases) and unilateral in distribution. Therefore, such patients might have a greater potential for surgical therapy. Curative intent surgery (R0 resection) was more likely among ALT+ NELMs.

This study also found that the ALT+ phenotype was associated with an increased risk of recurrence after surgical therapy and poorer OS. However, because only patients with complete therapy were included, we cannot draw conclusions about ALT status and outcomes among the entire cohort. Interestingly, among the PanNET subset of patients, ALT + tumors were associated with an improved OS, along with low tumor grade. Our findings reinforce those reported by Jiao and colleagues, who found that among patients with primary PanNET, those with ATRX or DAXX mutations exhibited prolonged survival compared with wild-type DAXX and ATRX genes.⁸ In contrast, Marinoni and colleagues reported findings that DAXX and ATRX mutations were associated with poorer outcomes.²³

CONCLUSIONS

The current study describes a unique pathologic biomarker that can be useful in the management of patients with NELM. Determination of the ALT phenotype is feasible and reproducible from histologic specimens and can be useful in determining NETs of pancreatic origin. Alternative lengthening of telomeres can also aid in the assessment of the pattern extent and distribution of metastases, potentially impacting the recommendation for surgical therapy. Finally, ALT can serve as a useful prognostic and predictive biomarker, potentially contributing to clinical decision making about locoregional and systemic therapies.

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Biography

DR BRYAN CLARY (Durham, NC): I very much appreciate the opportunity to discuss this paper from a very thoughtful and productive group of collaborators led by Dr Choti. In a world in which clinical research and interests are increasingly superspecialized, it's always a treat to take the opportunity to critically think about something outside of one's own silo.

My first question is that in your analysis of alanine transaminase (ALT) as a prognostic factor, you assessed ALT only as a binary variable and you mentioned that with respect to the images, specifically it being either positive or negative. In doing so, you were left with very small numbers that may have obscured a real effect. So my question is, did you assess either fluorescence as a continuous variable or potentially with different thresholds other than a 10-fold higher level than your background benign stromal cells?

From a clinical perspective, the negative predictive value seems to me to be the most important statistical outcome because it excludes patients from helpful and classically targeted therapeutics. The negative predictive value of 70% in your report here seems a bit low in this context. And I was wondering if you had tried using a higher fluorescence threshold or potentially, a higher number of cells surveyed in an attempt to improve on the 70% figure.

Because these studies involved specimens from as far back as 1998, can you comment on the influence of time on telomere stability in archived specimens?

Last, in the methods, tumors were determined to be ALT negative only after at least 5,000 cells were assessed. Can you explain to me how the imaging software distinguishes between the neoplastic cell and the non-neoplastic cells in looking at this?

DR MARTY HESLIN (Birmingham, AL): Again, I congratulate Dr Choti and his colleagues on an excellent study that uses translational research and its clinical applicability. I echo Dr Clary's comments about the choice of continuous vs categorical variable in the analysis. Secondly, you scored a tumor as positive if only 1% of cells were ALT positive, which is a very small number. Many other immunohistochemical studies use 5% or 10%, because I don't believe there is necessarily an industry standard. Could you comment on how that decision was made?

From a clinical perspective, how do you come to terms with no change in the recurrence-free survival for all tumors, but an improved outcome in pancreatic neuroendocrine tumors that were ALT positive?

Last, I would ask you to comment on the clinical applicability. There were 90 tumors in 12 years. Is this something that the clinical laboratories need to add to their armamentarium? Again to echo Dr Clary's comments, I wonder if being ALT negative was helpful in clinical decision making? For example, if a tumor was radiologically resectable but ALT negative, would you not treat that patient?

DR PHILIP BOUDREAUX (New Orleans, LA): Did you or do you use the commercially available biotheranostics to identify site of origin for tumor types?

DR MICHAEL A CHOTI: Several questions from Drs Clary and Heslin were related to the methodology of the analysis. Tumors that use this alternate pathway for telomere lengthening tend to be distinct, either present or absent, so a binary readout is clearer here. The specific choices of thresholds and controls are based on standard protocols for this assay as reported in other studies. With regard to the practicality of this fluorescence in situ hybridization (FISH) assay compared with immunohistochemistry of potentially associated gene products, we think this assay is more specific for this alteration and fairly feasible in most hospitals, with comparable cost to that of immunohistochemistry.

With regard to the negative predictive value, indeed it does appear as though a neuroendocrine tumor metastasis that is ALT negative can have variable sites of origin, including the pancreas.

A negative ALT would be less helpful in the management. In contrast, when ALT positive, it is almost always of pancreatic origin, potentially directing locoregional and systemic therapies. Finally, regarding other commercially available testing on these tumors, we do not have any experience with this different technique of studying these tumors.

Abbreviations and Acronyms

ALT	alternative lengthening of telomeres
FISH	fluorescence in situ hybridization
GI	gastrointestinal
HR	hazard ratio
NET	neuroendocrine tumor
NELM	neuroendocrine liver metastases
OS	overall survival
PanNET	pancreatic neuroendocrine tumor
TMA	tissue microarray

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Figure 1. Alternative lengthening of telomeres (ALT) status among 90 patients with neuroendocrine liver metastases (NELM), comparing pancreatic (PanNET) vs gastrointestinal (GI) carcinoid primary origin ($p < 0.001$).

Figure 2. Overall Kaplan-Meier survival of 43 patients with pancreatic neuroendocrine liver metastasis managed with resection, stratified by alternative lengthening of telomeres (ALT) status (hazard ratio = 0.33; $p = 0.043$).

Table 1**Primary Tumor Characteristics**

	All patients (n = 90)	PanNET (n = 43)	GI carcinoids (n = 47)	p Value
Sex				
Male	45	21	24	0.833
Female	45	22	23	
Age at operation, y, median (range)	56.5 (18–89)	55 (18–89)	58 (28–78)	0.203
Tumor size, cm, median (range)	2.55 (0.5–12.5)	4 (1.3–12.5)	2 (0.5–6.5)	<0.001
Tumor grade				
1	55	31	24	0.657
2	1	1	0	
3	4	2	2	
Unknown	30	9	21	
Nodal status				
Positive LN	66	27	39	0.029
Negative LN	17	12	5	
Unknown LN	7	4	3	
Functionality				
Functional	28	8	20	0.014
Nonfunctional	62	35	27	

GI, gastrointestinal; LN, lymph node; PanNET, pancreatic neuroendocrine tumor.

Table 2

Pattern of Liver Disease and Liver Operation Details

	All patient (n = 90)	PanNET (n = 43)	GI carcinoids (n = 47)	p Value
Synchronous NELM, n	66	28	38	0.092
Nonsynchronous NELM, n	24	15	9	
Curative intent, n	67	36	31	0.054
Noncurative intent	23	7	16	
Oligometastatic (≤ 5)	49	36	13	<0.001
Polymetastatic (>5)	41	7	34	
Bilateral distribution, n	56	19	37	0.001
Unilateral distribution, n	34	24	10	
Size of largest metastasis, cm, median (range)	3 (0.5–19)	2.8 (0.5–19)	3 (0.6–10.5)	0.231
Minor hepatectomy, n	67	30	37	0.331
Major hepatectomy, n	23	13	10	
Ablation, n	33	7	26	<0.001
No ablation, n	57	36	21	

GI, gastrointestinal; NELM, neuroendocrine tumor liver metastases; PanNET, pancreatic neuroendocrine tumor.

Table 3

Alternative Lengthening of Telomeres Phenotype and Primary Tumor Characteristics

	All patients (n = 90)	ALT+ (n = 26)	ALT- (n = 64)	p Value
Primary tumor site, n				
PanNET	43	24	19	<0.001
GI carcinoid	47	2	45	
Tumor grade, n				
1	55	17	38	0.131
2	1	1	0	
3	4	0	4	
Unknown	30	8	22	
Primary node status, n				
Positive LN	66	18	48	0.515
Negative LN	17	6	11	
Unknown LN	7	2	5	
Functionality, n				
Functional	28	7	21	0.584
Nonfunctional	62	19	43	

ALT, alternative lengthening of telomeres; GI, gastrointestinal; LN, lymph node; PanNET, pancreatic neuroendocrine tumor.

Table 4

Alternative Lengthening of Telomeres Phenotype and Pattern of Liver Disease and Liver Operation Details

	All patients (n = 90)	ALT+ (n = 26)	ALT- (n = 64)	p Value
Synchronous NELM, n	66	13	53	0.001
Nonsynchronous NELM, n	24	13	11	
Curative intent, n	67	24	43	0.013
Noncurative intent, n	23	2	21	
Oligometastatic (≤ 5), n	49	21	28	0.001
Polymetastatic (>5), n	41	5	36	
Bilateral distribution, n	56	12	44	0.045
Unilateral distribution	34	14	20	
Size of largest metastasis, cm, median (range)	3 (0.5–19)	3 (0.7–19)	2.6 (0.5–16)	0.626
Minor hepatectomy, n	67	17	50	0.209
Major hepatectomy, n	23	9	14	
Ablation, n	33	6	27	0.088
No ablation, n	57	20	37	

ALT, alternative lengthening of telomeres; NELM, neuroendocrine tumor liver metastases.