Molecular characterization of neuroendocrine neoplasms

Ph. D. Thesis

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List of full papers that served as the basis of the Ph.D. thesis

I. Kővári B, Turkevi-Nagy S, Báthori Á, Fekete Z, Krenács L. Syntaxin 1: A Novel Robust Immunophenotypic Marker of Neuroendocrine Tumors. Int J Mol Sci. 2020 Feb 12;21(4):1213. doi: 10.3390/ijms21041213. PMID: 32059362; PMCID: PMC7072745.
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II. Zombori T, Turkevi-Nagy S, Sejben A, Juhász-Nagy G, Cserni G, Furák J, Tiszlavicz L, Krenács L, Kővári B. The panel of syntaxin 1 and insulinoma-associated protein 1 outperforms classic neuroendocrine markers in pulmonary neuroendocrine neoplasms. APMIS. 2021 Apr;129(4):186-194. doi: 10.1111/apm.13113. Epub 2021 Jan 28. PMID: 33417719.
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III. Turkevi-Nagy S, Báthori Á, Böcz J, Krenács L, Cserni G, Kővári B. Syntaxin-1 and Insulinoma-Associated Protein 1 Expression in Breast Neoplasms with Neuroendocrine Features. Pathol Oncol Res. 2021 Oct 26;27:1610039. doi: 10.3389/pore.2021.1610039. PMID: 34764822; PMCID: PMC8575685.
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1. Introduction

1.1. Diagnosis of neuroendocrine neoplasms (NENs)

NENs comprise approximately 1% of malignant tumors. They can arise in any organ throughout the body, the most common sites of origin are the gastrointestinal (GI) tract and the lungs. Because of the unique therapy of NENs, an accurate pathology report is crucial. The diagnosis of low-grade neuroendocrine tumors (NETs) can be usually established based on histomorphology alone. However, especially in the case of high-grade NETs and large cell neuroendocrine carcinomas (LCNECs), additional methods are required to prove NE differentiation. For this purpose, the most widely applied diagnostic tool is immunohistochemistry (IHC). Numerous markers, such as CD56, chromogranin A (CHGA) and synaptophysin (SYP) are applied to prove NE differentiation. The diversity of currently used NE markers suggests that there is not a single molecule which is completely reliable.
To date, NE differentiation can only be reliably confirmed using a combinations of markers.

1.2. Novel NE markers

There is an ongoing search for more specific and sensitive NE markers. Insulinoma-associated protein 1 (INSM1), a recently reported, promising molecule is a transcription factor involved in cell cycle regulation and transcription of genes which are necessary for NE differentiation, therefore it is considered as a relatively sensitive and specific tool. Its expression in NE tissues and NENs has already been proven in several organs and anatomic regions including the lungs, central nervous system, head and neck, pancreas, prostate, and skin.
Syntaxins are proteins localized in the presynaptic plasma membranes and the membranes of the synaptic vesicles. Syntaxin-1 (STX1), as an essential component of the soluble NSF (N-ethylmaleimide-sensitive factor) attachment protein receptor (SNARE) complex, plays a crucial role in synaptic vesicle fusion and thus, exocytosis. Based on its function, it can be anticipated that SYX1 is expressed in neurons and NE cells. This assumption can be confirmed by in silico protein biomarker analysis; moreover, STX1 had previously been detected in various NE organs, namely the adrenal medulla and the endocrine pancreas. However, according to our knowledge, no comprehensive study has been made to assess STX1 as a general NE marker in diagnostic histopathology.

1.3. Pulmonary NENs

The second most common anatomical site of NENs is the respiratory tract. Their morphology and biological behavior are variable, from the indolent typical carcinoid (TC) to the small cell lung cancer (SCLC), one of the most aggressive human tumors.

Because of the unique therapeutic management, with the exception of SCLC, it is recommended to perform IHC reactions if NE differentiation is suspected. However, especially in the case of LCNEC, differential diagnosis can be challenging. Furthermore, necrotic or artificially damaged samples may show false positivity for NE markers, while immunoreactivity for CHGA in poorly-differentiated lesions may be weak, focal, dot-like, and difficult to evaluate. Similar limitations were also reported concerning SYP. INSM1 has been already reported as a promising molecule in IHC diagnostics of pulmonary NENs with excellent sensitivity.

1.4. Breast tumors with NE features

The classification of breast tumors exhibiting NE features has been rather controversial and obscure. Beside 'true' NENs, which are exceedingly rare in the breast, certain breast lesions, such as hypercellular (type B) mucinous carcinomas, solid papillary carcinomas, and even invasive carcinomas of no special type can display NE characteristics based on either histomorphology or IHC marker expression.

After many changes, currently, the classification of NENs had reached a relatively steady state. However, its application in practice, especially regarding the most common lesions (i.e., invasive carcinoma of no special type with NE differentiation), remained somewhat uncertain. This may explain the variable incidence rate reports (between 0.1-20%) of breast tumors with NE features as well as the unknown prognostic significance of NE differentiation in breast lesions.

2. Aims

1. To perform a retro- and prospective IHC study on a diverse cohort of benign and malignant tumors as well as non-neoplastic tissues, to establish the value of STX1 in the immunophenotyping of NENs.
2. To systematically assess the extent, intensity, and staining pattern of STX1 and INSM1 expression in pulmonary NENs.
3. To evaluate the characteristics of STX1 and INSM1 IHC expression in breast neoplasia showing NE features.

3. Materials and Methods

3.1. General considerations

All NENs were diagnosed and graded according to the volume of the WHO blue book series that corresponds to their anatomical location.

The IHC reactions were uniformly performed on FFPE sections. Tissue microarray (TMA) blocks were constructed with a manual TMA builder instrument. Each tumor case was represented by at least two cores (central and peripheral regions) of 2.2 mm.

Concerning staining intensity, three semiquantitative categories were applied: weak (1+), moderate (2+), and strong (3+).

The studies were performed in agreement with the guidelines of the Declaration of Helsinki for human medical research and was ethically approved by the Clinical Research Coordination Office of the University of Szeged (#4430/2018) on January 7th, 2019.

3.2. STX1 as a general NE marker

To study STX1 in non-neoplastic tissues, normal thyroid, parathyroid, skin, pancreas, appendix, adrenal gland, and brain tissue were evaluated. Hyperplastic NE lesions, such as linear and nodular enterochromaffin-like (ECL) cell hyperplasia in autoimmune metaplastic atrophic gastritis and one case of pancreatic nesidioblastosis were also included. In tumor samples, if present, peritumoral non-neoplastic NE cells were also assessed for STX1 expression.

To evaluate the specificity and sensitivity of STX1 in neoplastic conditions, altogether, 398 cases of non-NE and NE neoplasms were studied in either whole tissue sections or tissue microarrays (TMAs). Cases with potential diagnostic pitfalls were also included. Fisher's exact test was performed to compare the proportion of positive cases with the applied markers.

A tumor sample was considered STX1-positive if more than 50% of the neoplastic cells showed either membranous or cytoplasmic staining. The staining intensity was categorized as weak, moderate, or strong. In NENs investigated using the TMA technique, immunostainings for the most common NE markers, such as SYP, CHGA, and CD56, were also performed to compare the results with STX1.

3.3. STX1 and INSM1 IHC expression in pulmonary NENs

Lung specimens surgically resected between 2003 and 2019 were collected from the files of the Department of Pathology, University of Szeged, Hungary. SCLCs (n = 30), LCNECs (n = 17), TCs (n = 33), and atypical carcinoids (ACs) (n = 7) were included in our retrospective series of consecutive cases. In TCs, ACs, and LCNECs, at least 1 of the 3 classic NE markers (CHGA, SYP, CD56) had to be positive in association with a proper NE histomorphology to be included. To evaluate the specificity of STX1 for neuroendocrine differentiation, 20 pulmonary adenocarcinomas and 20 pulmonary squamous cell carcinomas were also included.

The extent of expression, intensity, cellular localization, and sensitivity of STX1, CHGA, SYP, CD56, and INSM1 were evaluated. Membranous or cytoplasmic staining for STX1 and CD56, cytoplasmic staining for CHGA and SYP, and nuclear staining for INSM1 in >5% of tumor cells were considered positive. Positivity was regarded diffuse if >75% of the tumor cells were labeled.

3.4. STX1 and INSM1 IHC expression in breast neoplasia

Altogether, 113 cases (79 from the archives of the Bács-Kiskun County Teaching Hospital, 34 from the University of Szeged) diagnosed between 2001-2019 were collected. Fifty-nine tumors from 55 patients (4 of them with bifocal lesions) demonstrated traditional NE marker positivity, whereas the remaining 54 were negative for these markers and formed a negative control group for our study. All lesions with NE marker positivity were diagnosed either as hypercellular (Type B) mucinous carcinoma, solid papillary carcinoma, invasive breast carcinoma of no special type with NE features or ductal carcinoma in situ, NE subtype. No tumor in this series fulfilled the criteria of NET or NEC.

For CHGA and SYP, cytoplasmic labeling in at least 1% of the tumor cells; for INSM1, at least 1% nuclear positivity; finally, for STX1 and CD56, at least 1% cytoplasmic and/or membranous staining were considered positive. The percentage of the labeled tumor cells, as well as the semiquantitative (0 to 3+, respectively) intensity of the staining was evaluated. Based on the frequently detected focal and weak INSM1 expression in a pilot series, INSM1-stained slides were also evaluated using two alternative practical definitions for positivity; 1) any nuclear staining of any intensity (referred to as high-power [HP] positivity), 2) nuclear staining obvious even at low-power view (referred to as low power [LP] positivity).

To assess the specificity of the novel markers, STX1 and INSM1 IHC reactions were performed on samples derived from other breast carcinomas proven to be negative for CHGA, SYP, and CD56.

4. Results

4.1. STX1 as a general NE marker

Virtually all normal NE cells and hyperplastic NE lesions showed strong membranous and weak to moderate cytoplasmic STX1 staining. Neuronal tissue in the brain and peripheral nerves revealed consistent STX1 expression.

All but one (99/100, 99%) cases of NENs, including metastatic NETs, proved to be STX1-positive. Regarding specific subsets of gastrointestinal NETs (i.e., EC cell NETs, ECL cell NETs, and rectal L cell NETs) were consistently positive. At least 50% of the tumor cells were labeled with a moderate to strong intensity, but in 92% of cases, the positivity rate was more than 85%. The STX1 staining intensity showed no correlation with the mitotic activity, Ki-67 labeling index, or tumor grade. The single STX1-negative case represented a grade 2 CHGA-positive NET of the major duodenal papilla. All NECs, including special types such as Merkel cell and medullary thyroid carcinomas were consistently STX1-positive, mostly with intense diffuse staining, regardless of the anatomical site.

The STX1 staining pattern varied considerably, from predominantly cytoplasmic to complete diffuse membranous. Membrane staining was typically complete in NETs, in some NECs, predominantly aberrant incomplete membrane staining was noticed. In comparison to common NE IHC markers, STX1 showed the highest sensitivity both in NETs (99%) and NECs (100%), which was followed by CHGA (98% and 91%), SYP (96% and 89%), and CD56 (70% and 93%), respectively. The four applied markers detected a significantly different proportion of positive cases regarding gastrointestinal NETs ($p < 0.001$), gastrointestinal NECs ($p = 0.01$), and NECs in general ($p = 0.007$).

STX1 expression was generally absent in conventional carcinomas. All 20 (100%) pituitary adenomas and 14/16 (88%) of pheochromocytomas showed STX1 positivity. Endocrine neoplasms were consistently STX1-negative.

All but one neuroectodermal/neuroepithelial tumor samples revealed strong diffuse STX1 positivity in neuroblasts and ganglion cells, while less than 10% of tumor cells were positive in the remaining one medulloblastoma. Where it was present, the neuropil component also showed a moderate expression. The Schwann cell component of the ganglioneuromas and all peripheral nerve sheet tumor cases proved to be STX1-negative.

4.2. STX1 and INSM1 IHC expression in pulmonary NENs

All NENs except 3 cases of SCLC showed STX1 expression. Diffuse positivity was detected in 80 of 87 cases (92%), and focal staining (range, 5%-75%) was present in 2 SCLCs, 1 LCNEC, and 1 TC. The median intensity of STX1 labeling was strong (3+) in ACs and SCLCs and moderate (2+) in TCs and LCNECs. Predominant membranous staining with weak cytoplasmic labeling was the most frequent pattern among TCs, ACs, and LCNECs. Cytoplasmic expression of STX1 was observed mostly in SCLCs and in a few LCNECs. Diffuse INSM1 positivity was registered in 68 of 87 cases (78%), and focal staining (range, 10%-75%) was observed in 8 SCLCs, 6 LCNECs, and 5 TCs. The median intensity of INSM1 positivity was strong in both carcinoids and high-grade NENs; CHGA and SYP tended to label carcinoids strongly and NECs moderately.

The sensitivity of STX1 was 93.6% (95% CI, 82-99) among high-grade pulmonary NENs and 100% (95% CI, 91-100) among pulmonary carcinoids. The overall sensitivity of STX1 for NE differentiation was 96.6% (95% CI, 90-99), while the sensitivity of the other evaluated NE markers was as follows: CHGA (85.2%), SYP (85.2%), CD56 (92.9%), and INSM1 (97.7%).

Regarding the pulmonary adenocarcinomas and pulmonary squamous cell carcinomas, nuclear INSM1 expression was detected in 2 of 20 (10%) adenocarcinomas and 5 of 18 (27.8%) squamous cell carcinomas. STX1 expression was absent in all investigated cases of pulmonary adenocarcinomas and pulmonary squamous cell carcinomas.

4.3. STX1 and INSM1 IHC expression in breast neoplasia

STX1 immunoreactivity was detected in 50/59 tumors. The labeling was diffuse in 37 (62.7%) of the 59 lesions. The median percentages of positive tumor cells were 85% and 55% for cytoplasmic or membranous staining patterns, respectively. INSM1 expression was noted in 53/59 lesions on HP and 51/59 on LP, with a uniform nuclear pattern. Independently of the applied threshold, positivity was diffuse in 28/59 (47.5%) lesions, while the median percentage of labeled tumor cells was 50%.

Regarding the classical NE markers, the ratios of the positive cases and the median percentages of positive tumor cells were 58/59 and 80% for SYP, 44/59 and 50% for CHGA, and 13/58 and 0% for CD56, respectively. Diffuse positivity was present in 69.5% (41/59) for SYP, 47.5% (28/59) for CHGA, and 5.2% (3/58) for CD56.

The overall sensitivities of the novel markers were 89.8% and 86.4% for INSM1 on HP and LP, respectively, and 84.7% for STX1. Concerning the classical NE molecules, the sensitivities

were 98.3% for SYP, 74.6% for CHGA, and 22.4% for CD56. The median intensity of staining was strong (3+) for each observed marker, except for CD56 (2+).

Regarding the specificity of the novel markers, only a single STX1 positive case was detected in the negative control group (1/54), resulting in a specificity of 98.1%. As for INSM1, applying the HP threshold, 23/54 cases were found to be positive with a specificity of 57.4%; however, using LP, the ratio of positive cases was only 6/54, increasing the specificity of INSM1 substantially, to 88.9%.

5. Discussion

5.1. STX1 as a general NE marker

In this study, we performed a comprehensive IHC analysis in an extensive series of benign and malignant tumors to evaluate STX1 as a NE marker. We found that STX1 represents a robust NE marker, with a sensitivity of 99% in NETs and 100% in NECs, outperforming other common NE markers, such as SYP (96% and 89%), CHGA (93% and 91%), and CD56 (70% and 93%), which was proven to be statistically significant regarding gastrointestinal NETs and NECs. As pulmonary, gastrointestinal, and pancreatic NETs and NECs showed similar frequencies of positivity and normal NE cells in different organs were uniformly positive, STX1 expression seems to be unrelated to the anatomical site. In contrast to the frequently negative CHGA staining in rectal and appendiceal L-cell NETs, STX1 was consistently positive in all those cases. Pheochromocytomas and paragangliomas were also almost consistently positive. Furthermore, STX1 was uniformly expressed in all NECs, regardless of the morphological subtype or special anatomic localization, including small and large cell NECs, as well as in Merkel cell carcinomas and medullary thyroid carcinomas. In contrast to the sometimes faint or dot-like cytoplasmic expression of SYP and CHGA, STX1 revealed crisp membranous and strong cytoplasmic staining in most cases, which makes the evaluation straightforward. Concerning the specificity of STX1, many endocrine tumors known to express either CHGA or SYP, such as parathyroid and adrenocortical neoplasms, were consistently negative for STX1. As further evidence of the excellent specificity of STX1, a broad spectrum of non-NE tumors, including various types of carcinomas, were consistently negative. Although STX1 was also expressed in neural tumors, considering the rather distinct presentation of these neoplasms, the differentiation appears to be straightforward.

5.2. STX1 and INSM1 IHC expression in pulmonary NENs

In our series, 84 of 87 (96.5%) pulmonary NENs were positive for STX1, and the positivity was diffuse in >90% of cases. INSM1 was positive in 83 of 85 (97.6%) pulmonary NENs. The degree of differentiation may influence the extent, distribution, and localization of protein expression in tumors. This is common among NE carcinomas, as the density of mature secretory granules is frequently decreased, resulting in only focal CHGA expression. However, we detected diffuse positivity for STX1 in >90% of SCLCs and LCNECs. Nevertheless, the 3 STX1-negative cases were SCLCs, and the extent of expression was also lower in NECs. It is worth emphasizing that 2 of the 3 SCLCs that were categorized negative for STX1 were diagnosed solely based on NE histomorphology. They were negative for classic NE markers

but showed STX1 expression in <5% of tumor cells. With a more permissive definition of positivity (e.g., 1% or “any stain”), these cases may be considered positive. Interestingly, the 2 cases that failed to express INSM1 were all ACs, whereas the SCLCs and LCNECs were all positive for this marker.

Regarding the pattern of expression, a strong membranous accentuation of STX1 positivity was characteristic of TCs, ACs, and most LCNECs. A cytoplasmic staining pattern was primarily observed in SCLCs and in a few LCNECs. The molecular alterations leading to this expression pattern change in NECs is not yet elucidated. However, the accumulated mutations in high-grade lesions may affect the STX1 protein and lead to an aberrant conformation or truncation that prevents the protein from reaching or binding to its physiological membrane-linked position. After comparing all tested NE markers, STX1 showed the smallest difference between carcinoids and NECs, regarding the extent of expression. INSM1 showed the smallest difference concerning the intensity of labeling.

Our findings suggest that the sensitivity of STX1 is 100% in pulmonary TCs, ACs, and LCNECs and 90.0% in cases of SCLC. Our results and literature review demonstrate that the overall sensitivity of STX1 (96.6%) and INSM1 (direct, 97.6%; indirect, 85.6%) was superior to the sensitivity of CHGA (direct, 89.3%; indirect, 75.1%), SYP (direct, 89.3%; indirect, 86.6%), and CD56 (direct, 95.2%; indirect, 92.7%).

Concerning the specificity of the novel NE markers, in keeping with the results other authors, we detected focal expression of INSM1 in 10% of pulmonary adenocarcinomas and 35% of pulmonary squamous cell carcinomas. STX1 expression was uniformly absent in all 20 pulmonary adenocarcinomas and 20 squamous cell carcinomas tested in the present study. It was also uniformly absent in the 8 adenocarcinomas and 5 squamous cell carcinomas evaluated previously by whole slide IHC. Overall, the specificity of STX1 has been found to be excellent (99.41%; 95% CI, 96-99), even somewhat better than that of INSM1 (96.3%; 95% CI, 92-98).

Altogether, STX1 and INSM1 have mutually promising sensitivity and specificity and should be recommended for routine diagnostic applications. One strength of these markers may be their possible expression in SCLCs negative for classic NE IHC markers. However, in our limited experience, STX1 labeling in such cases is very focal. This subgroup of SCLC poses a possible diagnostic pitfall and represents up to 10% of all SCLCs. Future analyses of more classic NE marker–negative SCLCs is warranted.

In our opinion, the nuclear expression of INSM1 and the frequently crisp membranous quality of STX1 labeling can be more consistently interpreted than the cytoplasmic staining for SYP and CHGA. Expression of the latter markers could be harder to distinguish from unspecific background staining in case with weak and focal positivity. Because of the limited amount of tissue in small biopsy specimens as well as financial reasons, the panel of NE markers could not be extended. Based on the presented data, a panel of STX1 and INSM1 could outperform the currently recommended CHGA, SYP, and CD56 panel. Owing to the different intracellular localizations of staining, STX1 and INSM1 can also be used in combination as double stained IHC reactions to spare biopsy material.

The limitations of this study are the relatively low case number and the use of the TMA method. The retrospective nature of our study is a potential source of selection bias. Moreover, the fact that the cases were originally diagnosed using the classic NE markers may have led to the overrepresentation of CHGA-, SYP-, and CD56-positive cases and the overinterpretation of the sensitivities of these markers.

5.3. STX1 and INSM1 IHC expression in breast neoplasia

The aim of this study was to assess the applicability of STX1 and INSM1 as NE markers of breast lesions, as well as to compare their performance with the traditional molecules used to assess NE differentiation (i.e., SYP, CHGA, and CD56). Similarly to results from other organs, STX1 proved to be a reliable marker for the diagnosis of breast lesions with NE features, with a sensitivity of 84.7%, characterized by a convincing, diffuse immunoreactivity in 62.7% of the cases included. A strong and easy-to-read membranous labeling pattern was also noted beside cytoplasmic staining in most of the lesions. Our experience was that the nuclear staining pattern of INSM1 (similarly to the membranous STX1-labeling) was more convenient to interpret than the cytoplasmic expression pattern of other markers. Another potential advantage is the different subcellular locations of INSM1 and STX1 labeling, which enable the use of a double IHC staining method in biopsy cases with limited neoplastic tissue. Furthermore, additionally to exhibiting great sensitivity, STX1 was also characterized by an excellent specificity (98.1%), with clear-cut negativity in all but one sample in the control group.

Concerning INSM1, sensitivity was excellent without significant difference between LP and HP definition for positivity (89.8% and 86.4%, respectively); but it worth to add that more than half of the evaluated lesions showed only focal positivity. Conversely, the results of the control cases were rather equivocal. If the more permissive LP definition was applied, the specificity of INSM1 was found to be only 57.4%; however, when the HP definition was used,

it increased to 88.9%. The fact that 17 tumors without NE features in our series would have been misclassified depending on the threshold, raises some concerns regarding the specificity of INSM1. However, given the excellent sensitivity, INSM1 is strongly recommended to be used in combination with other, more specific markers.

As concerns the traditional NE markers, the greatest sensitivity was achieved with SYP (98.3%), nevertheless, STX1 mildly outperformed it in the median percentage of labeled cells (STX1: 85% vs SYP: 80%). The remaining two classical NE molecules, CHGA and especially CD56 exhibited unexpectedly low sensitivities (74.6% for CHGA and 22.4% for CD56). Our experience with the latter marker is comparable to the findings of a recent publication, therefore CD56 should probably be decommissioned from the general NE marker arsenal, at least in the setting of breast tumors.

An obvious limitation of this study is its retrospective nature and the low number of included cases that may have biased our findings. Furthermore, an unexplained result was the fraction of investigated tumors that emerged as INSM1 positive (using the HP definition) without the expression of any other marker. This phenomenon, which was also observed by other authors, raises the concerns regarding the specificity of INSM1 even further. The isolated positivity for INSM1 without detectable expression of other components of the neurosecretory apparatus may be explained by an interrupted cascade of yet-to-be-found intermediate mediators, which transfer the signal of NE differentiation from the transcription factor. This would make INSM1 expression a necessary initial, but, on its own insufficient condition for the development of NE differentiation.

Another possible concern is the immanent focality of NE differentiation in the majority of breast tumors. Apart from intra-tumoral heterogeneity, these neoplasms also show considerable diversity in the same diagnostic category. Given these facts, despite multiple sampling, the TMA method may have led to false negative results. Nevertheless, NE markers are also needed in the routine reporting of core biopsies that are likewise subject to the same issues; thus, data obtained using the TMA technique may be used to extrapolate how these markers would perform in the core biopsy setting. Altogether, we believed that the few dozens of cases investigated were sufficient to validate the concept of STX1 and to a lesser extent of INSM1 as suitable NE markers of breast tumors, even if we were unable to formulate statements concerning the exceptionally rare primary mammary NETs or NECs.

6. Conclusions

In conclusion, we demonstrated that STX1 can outperform common NE IHC markers in various settings. Given its consistently near-perfect specificity and outstanding sensitivity in normal NE cells, NENs, and even in NECs, it appears to be the most advantageous immunophenotypic marker. We recommend STX1 to be added to the IHC panel of NE differentiation in routine diagnostic histopathology. Its consistent expression in all NENs, regardless of the anatomical site or subtype, makes it a reliable marker, even in the hands of non-subspecialized pathologists who are less experienced with NENs and unaware of certain site-specific expression patterns and possible pitfalls of classic NE markers.

After proving its applicability as a NE marker in general, our focused evaluation of pulmonary and breast neoplasms found STX1 to be a robust, easily interpretable, and reliable marker of NENs in both anatomic locations, with excellent sensitivity and specificity.

Therefore, along with INSM, we strongly recommend STX1 to be included in the routine diagnostic IHC panel of NE differentiation. Following further studies, STX1 and INSM1 may eventually replace the antibodies of the currently accepted panel of CHGA, SYP, and CD56.

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