**Letter to the Editor: Positivity rate of TTF-1 on immunohistochemistry in pancreatic neuroendocrine tumors.**

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**Running title:** TTF1 in pancreatic neuroendocrine tumor.

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To the Editor,

TTF-1 (thyroid transcription factor-1) is a homeodomain transcription factor, which is expressed in thyroid and lung epithelial cells, and their malignant counterparts. In lung cancers, its expression is found in about 60% adenocarcinomas (60%) and 90% of poorly differentiated neuroendocrine carcinomas (PD-NEC)/small cell lung carcinomas (SCLC), as well as 35% of well-differentiated neuroendocrine tumors (NET). However, positive TTF-1 immunostaining has been shown in a variety of other tumors, including adenocarcinomas of the kidney, breast, biliary tract, liver, ovary and uterus (ranging from 1-2%). With regards to NET, TTF-1 staining was also reported in those from the gastrointestinal tract, bladder, prostate and cervix uteri [1, 2]. However, in these studies, the authors did not find any TTF-1 positivity among the pancreatic tumors.

Here, we would like to highlight our observations regarding the positivity rates of TTF-1 in pancreatic well-differentiated NET. We studied a total of 57 pancreatic NET, resected between 1993 and 2005, retrieved from the Department of Histopathology, Hammersmith Hospital, London, United Kingdom. Ethics committee approval was obtained from the institutional review board of the Tissue Bank of The Imperial College Healthcare NHS Trust (ref: R1008). Histopathology reports and slides were first reviewed to confirm the diagnosis and select representative blocks for the study. All NET were positive for CD56, synaptophysin and chromogranin A. Next, the expression of TTF-1 was evaluated by immunohistochemistry, using sections from formalin-fixed, paraffin embedded tissue blocks. Two micron sections were cut from each block, and immunohistochemical staining and detection were performed using an automated Leica Bond 3 machine according to the manufacturer’s protocol. The primary anti-TTF-1 antibody used was (NCL-L-TTF-1, clone: SPT24, Leica, Germany, dilution 1:100). For positive controls, sections from normal lung were used. As a negative control for each case, the primary antibody was replaced by phosphate buffered saline.

We found that of the 57 tumors, 2 had strong nuclear positivity to TTF-1 in >80% of cells (Figure 1A). In addition, there were 2 other tumors with weak positivity in 10-20% of cells (Figure 1B). The rest of the tumors were uniformly negative for TTF-1. Therefore in our cohort of cases, 7% (4/57) of pancreatic NET were TTF-1 positive, with 3.5% (2/57) of the tumors strongly positive.

All 4 cases were well-differentiated NET with uniform cells with granular eosinophilic cytoplasm and coarse chromatin pattern, arranged either in nests or trabeculae. All had mitotic counts of =<1/10hpf (classified as Grade 1). However, the two cases with strong positive TTF1 staining were larger (6.5cm and 7cm in size) and distinguished by infiltrative borders and lymphovascular invasion. In one case, the patient also developed lymph node and liver metastases.

To our knowledge, this is the largest series of pancreatic NET studied using TTF-1. We therefore wish to highlight the findings that TTF-1 is not wholly specific for distinguishing between NET from the lung and the pancreas. This is important as these are among the two of the commonest primary sites of NET. Apart from determining if a pancreatic tumor is a localized primary or a metastatic tumor from a distant site (and therefore inoperable), pancreatic and lung NET are sensitive to different chemotherapeutic agents.

Since TTF1 is a transcription factor involved in the embryogenesis of thyroid and lung tissue, the significance of TTF1 positivity in pancreatic NET is not fully understood. Since it has been recognized that TTF1 positivity is higher in PD-NEC than NET, and PD-NET of other organs are recognized to express TTF1, it has been proposed that some extra-pulmonary PD-NEC, may develop characteristics of the pulmonary counterparts or share a pleuripotent cell origin [3, 4]. It may be speculated that this would also apply to NET, especially if they acquire more malignant traits, as in our TTF1 positive cases.

The second possibility is that the antibodies used in the detection of TTF1 is not wholly specific but may cross-reactive with other antigens. This may explain the difference seen in different TTF1 antibodies. In the study by Comperat *et al*, up to 10% of colorectal adenocarcinomas were TTF-1 positive using the SPT24 clone, with no positivity with the 8G7G3/1 clone [5]. This raises concern that the antibody clone SPT24 is less specific than clone 8G7G3/1. However, other studies which used both or either clone alone showed that both clones had similar positivity rates in non-lung tumors of up to around 5% [1, 2, 6]. In particular, Matoso *et al* showed similar TTF-1 positivity rates in non-lung cancers for both clones [6].

Apart from TTF-1, are there any other markers to help in elucidating the origin of a NET? While Napsin-A has been more recently used as a more specific marker than TTF-1 for lung epithelial cancers, its lack of expression in lung NET means that it would not be useful as a marker for distinguishing lung NET from other neuroendocrine tumors. While CDX2 and CK20 are useful for diagnosing gastrointestinal NET, they are often not expressed by pancreatic tumors [7]. Recent studies have shown that other markers may help; such as paired box gene-8 (PAX8), pancreatic and duodenal homeobox factor-1 (PDX-1) and neuroendocrine secretory protein-55 (NESP-55) expression in pancreatic NET) [7].

In conclusion, we show that TTF-1 can be expressed in pancreatic NET and that it cannot be relied on solely to determine the primary site of origin of a NET. Clinical, radiological and other immunohistochemical data should be correlated before arriving at the definitive diagnosis. Newer markers of specific to pancreatic NET, such as PDX-1 and NESP-55 should be evaluated further.

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**Conflict of interest statement:**

We declare that there is no actual or potential conflict of interest including any financial, personal or other relationships with other people or organizations regarding the material in the manuscript that could inappropriately influence (bias) our work. These include employment, consultancies, stock ownership, honoraria, paid expert testimony, patent applications/registrations and grants and any other funding.

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**Figure Legend:**

Figure 1A & B: Microphotograph on a pancreatic neuroendocrine tumor with immunohistochemistry showing strong positivity for TTF1 (X200).

Figure 1C & D: Microphotograph on a pancreatic neuroendocrine tumor with immunohistochemistry showing weak positivity for TTF1 (X200).