Supplementary Data

Primary non-functional pancreatic neuroendocrine tumor study cohort

The study cohort consisted of 561 patients with a non-syndromic and non-functional, solitary pancreatic neuroendocrine tumor (NF-PanNET) treated by pancreatectomy with curative intent. No synchronous distant metastases were identified at the time of surgical resection. Patients ranged in age from 20 to 93 years (mean, 56.9; median, 57.0 years) with a slight predominance in male gender (286 of 561, 51%). The tumors were predominantly located within the pancreatic body and tail (n = 325, 58%) and ranged in size from 0.6 to 18 cm (mean, 3.3 cm; median, 2.6 cm). Although all PanNETs were morphologically well-differentiated, on the basis of mitotic rate and Ki-67 proliferation index, PanNETs were classified into the following WHO grades: 362 (65%) grade 1 (G1), 189 (33%) grade 2 (G2), and 10 (2%) grade 3 (G3). Data regarding lymphovascular and perineural invasion was available for 424 (76%) cases and present for 142 (of 424, 33%) and 83 (20%) tumors, respectively. Using the AJCC prognostic staging system (eighth edition), the NF-PanNETs were classified into the following pathologic tumor (pT) stages: 179 (32%) pT1, 228 (41%) pT2, 147 (26%) pT3 and 7 (1%) pT4. Regional lymph nodes were submitted for histologic evaluation in 502 (89%) cases with involvement of 140 (of 502, 28%) cases. Follow-up information was available for all patients and postoperative (metachronous) distant metastases/recurrences were identified in 138 (25%) patients. The relapse-free survival (RFS) rates for this study cohort were 95% at 1 year, 72% at 5 years and 60% at 10 years. Of note, no statistically significant clinicopathologic differences were identified among patients from each of the submitting institutions (**Supplementary Tables 1, 2 and 3**).

In addition to immunohistochemistry for ARX and PDX1, and telomere-specific FISH for ALT, ATRX and DAXX immunolabeling were performed for all 561 NF-PanNETs. Loss of

nuclear expression for ATRX, DAXX or both was identified in 56 (10%), 73 (13%), and 14 (3%) NF-PanNETs, respectively. Intratumoral heterogeneity or heterogeneous staining was identified for 3 cases and was only detected among whole tumor sections. These cases consisted of 2 NF-PanNETs with loss of ATRX and 1 NF-PanNET with loss of DAXX. Heterogeneous staining was characterized by a clear presence of two distinct populations of tumor cells demonstrating preserved and loss of nuclear staining for the aforementioned protein. Based on the status of ATRX/DAXX, NF-PanNETs with loss of ATRX/DAXX correlated with a male predilection ($p = 0.026$), larger tumor size ($p < 0.001$), high WHO grade ($p < 0.001$), lymphovascular invasion ($p < 0.001$), perineural invasion ($p < 0.001$), advanced pathologic T-stage ($p < 0.001$), regional lymph node metastases ($p < 0.001$), metachronous (postoperative) distant metastases ($p < 0.001$) and the presence of ALT ($p < 0.001$) (**Supplementary Table 5**). Of note, among the 138 cases that developed metachronous distant metastases/recurrences, 81 (59%) patients had loss of ATRX/DAXX within their primary NF-PanNET. Similarly, 89 (65%) patients had ALT-positive primary NF-PanNETs (**Table 1**).

NF-PanNET metastases and non-pancreatic neuroendocrine tumor study cohort

Distant metastases from 107 patients with NF-PanNETs had sufficient pathologic material for further ancillary studies (**Table 3**). Fourteen (13%) patients had synchronous metastases, 48 (45%) had metachronous metastases and 45 (42%) had both. The sites of metastases varied and included 95 (89%) liver, 5 (5%) non-regional lymph nodes, 5 (5%) omentum, 1 (1%) diaphragm, and 1 (1%) ovary. At initial presentation, patients ranged in age from 31 to 85 years (mean, 60.2; median, 59.0 years) with a slight predominance in male gender (54 of 107, 51%). The corresponding primary tumors were predominantly located within the pancreatic body and tail (n

= 64, 60%) and ranged in size from 1.0 to 18 cm (mean, 5.3 cm; median, 4.5 cm). On the basis of mitotic rate and Ki-67 proliferation index, the primary tumors were classified into the following WHO grades: 29 (27%) grade 1 (G1), 72 (67%) grade 2 (G2), and 6 (6%) grade 3 (G3). In comparison, NF-PanNET metastases were categorized as: 20 (19%) G1, 79 (74%) G2 and 8 (7%) G3. Detailed follow-up information was available for all patients and deaths due to disease were documented for 63 (59%) patients. Disease-specific survival (DSS) was calculated from the date of primary diagnosis and the date of metastatic diagnosis to the date of death due to disease. DSS rates for this study cohort from the time of primary diagnosis were 95% at 1 year, 66% at 5 years and 39% at 10 years. In comparison, DSS rates for this study cohort from the time of metastasis were 91% at 1 year, 54% at 5 years and 32% at 10 years.

The expression of ARX, PDX1, and both proteins were identified in 71 (66%), 22 (21%), and 14 (13%) cases, respectively. Further, loss of ATRX, DAXX and both proteins were detected in 22 (21%), 34 (32%) and 10 (9%) NF-PanNET metastases. The presence of ALT by telomere-specific FISH was seen in 76 (71%) of NF-PanNET metastases. Based on ARX, PDX1 and ATRX/DAXX expression, DSS rates from the time of primary diagnosis and from the time of metastasis to death due to disease were not statistically significant ($p > 0.05$).

Primary non-pancreatic neuroendocrine tumors (NETs) were collected from 341 patients and included 48 lung typical/atypical carcinoid tumors, 32 gastric NETs, 41 duodenal NETs, 7 ampullary NETs, 35 jejunal NETs, 123 ileal NETs, 8 colonic NETs, 17 rectal NETs, 28 appendiceal NETs, and 2 gallbladder NETs (**Table 3**). None of the NETs were clinically functional, and, among 32 gastric NETs, 19 cases were associated with autoimmune metaplastic atrophic gastritis (AMAG). In addition to primary non-pancreatic NETs, 313 non-pancreatic NET metastases were assessed and included 28 cases from the lung (typical/atypical carcinoid), 10 from

the stomach, 32 from the duodenum, 71 from the jejunum, 151 from the ileum, 8 from the colon, 10 from the rectum and 3 from the gallbladder. None of the NET metastases were functional and none of the 10 gastric NET metastases arose in the setting of AMAG.

At initial presentation, patients ranged in age from 28 to 87 years (mean, 51.7; median, 55.0 years) with a slight male predominance (347 of 654, 53%). Among the 341 primary NETs, tumors ranged in size from 0.2 to 7 cm (mean, 4.7 cm; median, 4.1 cm). On the basis of mitotic rate and Ki-67 proliferation index, the 341 primary NETs and 313 NET metastases were classified into the following WHO grades: 510 (78%) grade 1 (G1), 124 (19%) grade 2 (G2), and 20 (3%) grade 3 (G3). Follow-up information was available for 571 of 654 (87%) patients and ranged from 2 months to 143 months. Among patients with 341 primary NETs, metachronous distant metastases/recurrences were documented for 98 (29%) patients.

Supplementary Figure Legend

Supplementary Figure 1. Kaplan-Meier curves comparing relapse-free survival (RFS) after surgical resection for patients with PDX1-positive/ARX-negative ($PDX1^+ARX^-$) NF-PanNETs versus the remaining cohort. (A) Patients with $PDX1^+ARX^-$ NF-PanNETs had improved RFS as compared to patients with other NF-PanNETs. However, (B) the prognostic significance between these two cohorts was dependent on ALT status. Among 401 ALT-negative (ALT^-) NF-PanNETs, (C) no statistically significant difference in RFS was identified between $PDX1^+ARX^-$ NF-PanNETs versus the remaining cohort. Interestingly, among 160 ALT-positive (ALT^+) NF-PanNETs, (D) a reverse association or shorter RFS was seen for patients with $PDX1^+ARX^-$ NF-PanNETs as compared to the remaining cohort. This is however likely due to a small number of $PDX1^+ARX^-ALT^+$ NF-PanNET patients.

Supplementary Figure 2. Kaplan-Meier curves of NF-PanNET patients with distant metastases comparing disease-specific survival (DSS) after primary diagnosis (A) and diagnosis of metastatic disease (B). In contrast to patients with a primary NF-PanNET, patients with ALT-positive metastatic NF-PanNETs had longer DSS as compared to patients with ALT-negative metastatic NF-PanNETs.

Supplementary Figure 3. Representative examples of non-pancreatic neuroendocrine tumors (NETs) that were immunolabeled for ARX and PDX1. The majority of gastric NETs (A) were ARX-positive (B) and PDX1-negative (C). Duodenal NETs (D) were more often either ARX-positive/PDX1-positive (E and F) or ARX-negative/PDX1-positive (not shown). In contrast, jejunal and ileal NETs (G) were consistently negative for ARX (H) and PDX1 (I). Finally, rectal NETs (J) were frequently ARX-positive (K) and PDX1-negative (L).

Supplementary Figure 4. Representative example of a (A) lung carcinoid with (B) loss of ATRX nuclear expression and (C) preserved nuclear expression for DAXX. Similar to NF-PanNETs, loss of ATRX and/or DAXX is associated with the presence of large, ultrabright intranuclear foci by telomere-specific FISH, (D) consistent with ALT.