Circulating Transcript Analysis (NETest) in GEP-NETs Treated With Somatostatin Analogs Defines Therapy

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Context: Early and precise delineation of therapeutic responses are key issues in neuroendocrine neoplasm/tumor management. Imaging is currently used but exhibits limitations in sensitivity and specificity. The utility of biomarkers is unclear.

Objective, Setting, and Design: This prospective cohort study (11 mo) sought to determine whether measurements of circulating neuroendocrine tumor transcripts (NETest) predict responses to somatostatin analogs (SSAs).

Patients: The test set consisted of 35 SSA-treated gastroenteropancreatic-NETs (RECISTevaluated). The prospective set consisted of 28 SSA-treated Grade 1–Grade 2 GEP-NETs.

Intervention(s): Whole blood for transcript analysis (NETest) and plasma for Chromogranin A (CgA) (baseline), were collected every 4 weeks (prior to SSA injection). Morphologic (multidetector computed tomography/MRI) and functional imaging (^{99m}Tc-[HYNIC, Tyr^3]-Octreotide) was undertaken at entry and 6-month intervals until progression (RECIST 1.0).

Main Outcome Measure(s): Treatment response.

Results: Test set: NETest (≥80%; scale, 0–100%) differentiated stable (SD) and progressive (PD) disease ($P < .0001$). Prospective set: 28 patients (26/28 SD) undergoing standard SSA. Grading: 12 G1, 16 G2. SSA Response: progression-free survival: 315 days: 14 (50%) SD, 14 (50%) PD. NETest: Twenty had elevated (≥80%) values; 14 developed PD; six, SD. CgA: Twelve of 28 exhibited elevated baseline values and/or subsequent >25% increase; eight developed PD; four, SD. NETest ($P = .002$) and grade ($P = .054$) were the only factors associated with treatment response. Multiple regression analysis established that the NETest could predict disease progression ($P = .0002$). NETest changes occurred significantly earlier (146 d prior to progression vs 56 d CgA; $P < .0001$; $\chi^2 = 19$) and in more patients (100 vs 57%; $P < .02$).

Conclusions: NETest values (80–100%) were more accurate and occurred at a significantly earlier time point than CgA and predicted SSA treatment response. **(*J Clin Endocrinol Metab* 100: E1437–E1445, 2015)**

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Abbreviations: AUC, area under the curve; CgA, Chromogranin A; CT, computed tomography; GEP, gastroenteropancreatic; MRI, magnetic resonance imaging; NEN, neuroendocrine neoplasm; NETest, NET transcripts; NET, neuroendocrine tumor; NPV, negative predictive value; PD, progressive disease; PFS, progression-free survival; PPV, positive predictive value; RECIST, Response Evaluation Criteria in Solid Tumors; ROC, receiver operating characteristic; SD, stable disease; SDev, standard deviation; SRS, somatostatin receptor scintigraphy; SSA, somatostatin analog; WHO, World Health Organization.

Multiple therapeutic strategies have been proposed for the management of gastroenteropancreatic (GEP) neuroendocrine neoplasms (NENs) (also NETs or carcinoids) (1, 2). Recommendations are based on the characteristics of a tumor and include clinical, pathological, biological, imaging, and biomarker assessments (3–6). The timely and early identification of disease progression and the identification of drug efficacy remain a key unresolved issue in management.

Disease status and treatment efficacy evaluations include clinical assessment, imaging analysis, and biomarker measurement (7). Clinical assessment is imprecise and generally reflects performance index and symptomatology, although the latter is of limited value in those without hormone overproduction (8). Imaging is a key determinant of disease status but its accuracy is observer dependent and limited by the indolent growth rates of some/most of the well- and moderately differentiated NEN/NETs. In addition, spatial resolution of both structural (computed tomography/magnetic resonance imaging [CT/MRI]: 2 mm) and functional imaging (positron emission tomography/CT: 4–5 mm; and single photon emission computed tomography/CT somatostatin receptor scintigraphy: 7–8 mm) approaches limits of tumor measurement. In addition, local confounders such as necrosis, hemorrhaging, or fibrosis may complicate assessment (9). The alternative strategy of disease assessment by biomarker measurement is also problematic (10). Monoanalytes such as insulin, gastrin, glucagons, and vasoactive intestinal polypeptide are accurate but of limited value given that they are only useful for specific tumor types that represent less than 2% of all NETs. Serotinin is a monoanalyte marker but is complex to measure in blood and is cumbersome, although accurate, in urine (11). Chromogranin A (CgA), although initially considered of value, has for numerous technical and biological reasons failed to meet the expectations of the clinical community (12–14). Elevated CgA (typically three consecutive increases in levels of ≥25%) is, however, considered to be an early and accurate marker (~85%) of disease recurrence (15).

Monoanalyte strategies for disease monitoring have been supplanted in other fields of cancer biology by the development of more sensitive and specific multianalyte strategies. ELISA/RIA has been replaced by QT-PCR and secretory products (serotonin/CgA) by circulating tumor transcript analysis. The latter capture the "cancer hallmarks" of a tumor and provides information regarding diagnosis, rate of progression, and ultimately, prognosis (16). In NET disease, measuring the transcript profile of blood is more sensitive and specific than CgA and other monoanalytes such as pancreastatin and neurokinin A (17). The performance metrics of the gene transcript measurement (NETest) meet the published acceptable standards for biomarker efficacy (18, 19) and this measure outperforms other biomarkers currently available for management and assessment of GEP-NETs (6, 17).

Somatostatin analogs (SSAs) are used to treat most NETs either for symptom relief or inhibition of tumor growth (20, 21). Given that clinical assessment and imaging are suboptimal methods for accurate objective assessment of treatment efficacy, the development of a biomarker strategy, to accurately demonstrate efficacy and/or measure disease progress/stabilization is necessary. The demonstration of the inhibitory effect Lanreotide on GEP-NET growth renders the issue of substantial clinical relevance (20).

To evaluate this issue, we examined the measurement of NET transcripts in blood compared with CgA for the identification of stable or progressive disease. Initially, a test set was used to develop cutoff levels for the NETest differentiating stable disease compared with those with progressive disease on SSA treatment. A prospective set of NET tumors receiving SSAs over a 7–11 month period was then studied.

Materials and Methods

Patients

Test set (set 1)

Thirty-five GEP-NETs were treated with SSAs with known disease status (Response Evaluation Criteria in Solid Tumors [RECIST] stable or progressive). The median age was 58 years (range, 33–82 y) with a male:female ratio of 12:23. The majority (n = 29, 83%) were small intestinal, 26 (~75%) had metastases, and all received SSAs (octreotide, n = 34; pasireotide, n = 1) with a median dose of 20 mg (range, 20–60 mg).

Prospective assessment (set 2)

Advanced GEP-NETs (n = 28), pathologically confirmed (well differentiated: Grade 1 or Grade 2), undergoing SSA therapy (octreotide, n = 14; lanreotide, n = 14) were consecutively enrolled. Subjects had radiologically measurable disease, ascertained within 6 months prior to study initiation, World Health Organization (WHO) performance status ≤1, and absence of poorly controlled diabetes or chronic treatment with corticosteroids. All patients were informed of the study aims and procedures and signed an informed consent. The study was authorized by the local ethics committee of University of Warmia and Masuria, Faculty of Medical Sciences as well as Yale University School of Medicine.

Blood sampling schedule

Whole blood (10 mL) for transcript analysis was collected at baseline, prior to SSA injection, then every 4 weeks (prior to SSA injection) for the duration of the study. Plasma for CgA analysis was obtained at the same time points.

Image analysis

CT/MRI examinations were analyzed by an experienced NET-specialized radiologist (A.S.) and somatostatin receptor scintigraphy (SRS) images by two nuclear medicine NET experts (J.B.C. and L.B.). Functional and structural imaging was used to evaluate patients at study entry and at appropriated time intervals until progression occurred. RECIST 1.0 criteria were used to assess the therapy response. CT or MRI findings were correlated with functional SRS using image fusion in each case. The consensus status for the therapeutic response as stable disease or disease progression during followup was confirmed by a NET interdisciplinary group (J.B.C., A.K.C., A.S., L.B., I.M.M.).

Somatostatin receptor scintigraphy

Somatostatin receptor scintigraphy (SRS) was performed using 600–700 MBq of ^{99m}Tc-[HYNIC,Tyr^3]-Octreotide [^{99m}Tc-Tyrosine 3-octreotide, Tektrotyd; National Center For Nuclear Research–Polatom, on a double-head camera (Symbia; Siemens) (22) to check for somatostatin receptors. Lesion uptake and extent of disease were graded (23).

Multidetector CT

Standard multidetector CT (GE) was used after iv contrast administration. Scanning parameters were adapted for size and weight. The lower neck, chest, abdomen, and pelvis were scanned during one examination before and after iv administration of 80–100 mL of nonionic contrast material (Ultravist 370 Bayer Schering Pharma) by a power injector (3.5–5 mL/s). Arterial phase images (30 s) were recorded were collected from lower chest to pelvis whereas portal-venous phase images (after 50 s), were collected from lower neck to pelvis (1-mm slice thickness; tube voltage, 80–120 kV; tube current, 165–210 mA).

MRI

In patients with renal impairment (n = 1) based on serum creatinine and estimated glomerular filtration rate measurement, and when deemed clinically useful, MRI of the abdomen and pelvis was performed on a 1.5-T system (Vantage Atlas Z, Toshiba) with a sensitivity encoding-phased array abdominal body coil approach as described (24).

Multianalyte algorithm analysis PCR-based test (NETest)

The NETest assesses NET biological activity using gene inference technology and cancer hallmark prediction (16). It measures expression of 51 NET marker genes in peripheral blood and includes measurements of biologically relevant genes that constitute the different "omes" (SSTRome, proliferome, metabolome, secretome, epigenome, and pluromes) that defines the "fingerprint" of a NET. Differential expression of these genes delineate progressive disease (PD) from stable disease (SD) (16). The NETest mathematically, therefore, provides a measure of disease activity risk on a 0–100% scale where minimal activity is less than 14%, low activity ranges from 14–47%, and high activity is greater than 47%. The specific set of circulating transcripts exhibits a high sensitivity (98%) and specificity (97%), is standardized and reproducible (inter and intra-assay coefficient of variation <2%), and outperforms other current GEP-NETs biomarkers (6, 17). The NETest is measured using a two-step protocol (RNA isolation, cDNA production, and PCR) as described (25, 26). Transcripts (mRNA) were isolated from EDTA-collected whole-blood samples and real-time PCR performed (25, 26). PCR values were normalized to housekeeping genes and expression was quantified against a population control (calibrator sample) (25). Four different learning algorithms were employed for categorization of samples into different groups using "majority vote" methodology (25). The 0–8 score (25, 26) was converted to an activity ranging from 0 (low activity) to 100% (high activity) (16). In this study, a value at least 80% (ie, NETest score of 80–100%) was used as indicative of highly active disease, based on analysis of data in the test set (see Results: Section 1).

CgA assay

CgA was measured using the NEOLISA CgA kit (Euro Diagnostics) (27, 28). A cutoff of 108 ng/mL defined the upper limit of the normal population. Values greater than 108 ng/mL signified an elevated CgA. In subjects with elevated CgA, an increase at least 25% between any two time points was used as a measure to predict disease progression (15).

Grading

Tumors were graded (G1 or G2) according to WHO classification, using the Ki-67 values obtained from the original histopathological reports (29).

Statistical analyses

Analyses included χ^2 (Fisher's, two tailed), nonparametric (Mann-Whitney *U* test, two tailed) measurements, logistic regression, multiple regression, receiver operating characteristic (ROC) analysis, Kaplan-Meier survival curves (progression-free survival [PFS]), and event curve analysis (based on K.M.). Both Prism 6.0 for Windows (GraphPad Software, www.graphpad.com) and MedCalc Statistical Software version 12.7.7 (MedCalc Software, http://www.medcalc.org; 2013) were used. The sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), and the area under the curve (AUC) were calculated (MedCalc) (30) for AUC comparison and derivation of the Z-statistic (31) (MedCalc). Data are presented as mean ± standard deviation (SDev) (set 1) and mean ± SEM or percentage correctly predicted (set 2).

Results

Test set (n = 35)

The NETest was positive in all patients. Twenty-five (71%) were classified as stable disease by imaging. NETest activity was significantly lower in stable disease than in progressive disease treated with SSAs (32 ± 19% vs 82 ± 12%, $P < .0001$) (Figure 1A). The upper limit of "stable" based on the standard of mean + 2 × SDev (of stable disease) was 70%, with three patients having values ranging from 57–80% (Figure 1A). ROC curve analysis identified that NETest activity significantly differentiated between stable and progressive disease status (AUC = 0.97 ± 0.023; 95% confidence interval, 0.93–1.02; $P < .0001$) (Figure 1B). A cutoff of at least 80% had sensitiv-

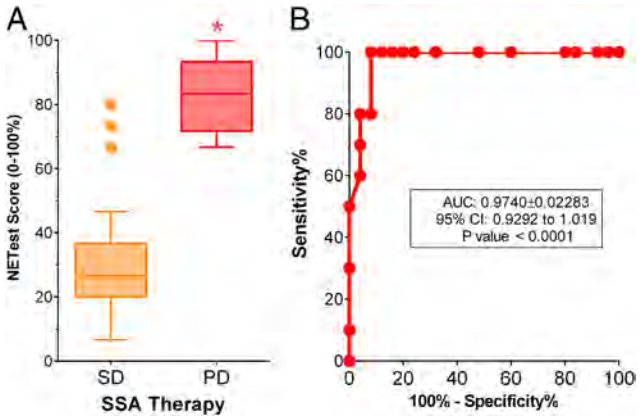

Figure 1. Performance metrics (A) and ROC analysis (B) of the NETest in the test set (n = 35) treated with SSAs. A, NETest was significantly increased (*, $P < .0001$) in the PD (n = 10) vs SD (n = 25) group. Box and whisker graph (Tukey's box plot). Outliers are identified by solid circles in the SD group. B, ROC analysis of the NETest data from panel A demonstrated an AUC of 0.97; $P < .0001$ for differentiating progressive from stable disease on somatostatin analogs.

ities and specificities of >80% and >95%, respectively as an indicator of progressive disease. We therefore chose this value to evaluate in the prospective group (set 2).

Prospective assessment set (n = 28)

Patient demographics

Twenty-eight patients (SD, 26/28; 93%) on treatment with SSAs were evaluated. Subjects had a median 55.5 months (range, 2–143 mo) history of disease and were predominantly stage IV (26/28), with minimal liver involvement (21/28 ≤10%), limited to moderate extent of disease (27/28), and a high somatostatin receptor density (26/28, grade 3–4) on SRS. All had been treated with SSA for 1–94 months (median, 43 mo). The baseline demographic and disease characteristics are provided (Table 1). At the end of a median 10-month (range, 7–11 mo) observation period, 14 patients (50%) were stable and 14 (50%) were in progression as defined by RECIST criteria 1.0 (Table 2).

Biomarker parameters

Chromogranin A

Elevated CgA (>108 ng/mL) was evident in 12 (43%) at baseline. Mean baseline levels were significantly higher (493 ± 144 vs 87 ± 28 ng/mL; $P = .006$) in the group that developed progressive disease during treatment. In 16 with normal CgAs, two exhibited abnormal values at the fifth month and at second and fifth month, respectively). One was associated with SD, the other with treatment failure. CgAs were normal throughout the observation period in nine (64%) stable subjects and in four (28%) who developed PD. The overall variance in CgA in those who remained stable was 89–118% and in those who

Table 1. Baseline Demographic and Disease Characteristics

Characteristic	Statistic
Patients	(n = 28)
Age, y	
Mean	60
Range (SD)	36–81 (9.8)
Men, n (%)	10 (36)
Time since diagnosis, mo	
Range	2–143
Mean (SD)	55.5 (37.1)
Median	55.5
Median time of enrollment, (range), mo	10 (7–11)
NET origin, n (%)	
Pancreas	9 (32)
Small bowel	13 (46)
Rectum	1 (11)
Stomach	1 (4)
Duodenum	1 (4)
Unknown	3 (11)
Tumor grade, n (%)[a]	
G1 (Ki-67 0–2%)	12 (43)
G2 (Ki-67 3–10%)	16 (57)
Initial clinical stage	
Stage IIIB n (%)	2 (7)
Stage IV n (%)	26 (93)
Hepatic tumor volume, n (%)	
0%	4 (14)
>0–10%	17 (60)
>10–25%	4 (14)
>25–50%	1 (4)
>50%	2 (8)
Extent of disease,[b] n (%)	
Limited	7 (25)
Moderate	20 (71)
Extensive	1 (4)
Intensity of uptake,[c] n (%)	
Grade 2	2 (7)
Grade 3	9 (32)
Grade 4	17 (61)
Previous therapy	
Primary tumor surgery, n (%)	25 (89)
Resection R0	16 (64)
Resection R1 or R2	3 (12)
Nonresectable (primary)	6 (24)
Somatostatin analogs	
Octreotide LAR 30 mg/4 wk	14 (50)
Lanreotide autogel 120 mg/4 wk	14 (50)
Prior nonsurgical treatment except SST, n (%)	16 (16)
PRRT	11 (39)
Chemotherapy	5 (19)
TACE	1 (4)

Abbreviations: LAR, long-acting release; PRRT, l TACE, trans arterial chemoembolization.

[a] ENETs Tumor Grade.

[b] Limited: up to five lesions in a single region of the body; moderate: multiple lesions in up to two regions; extensive: multiple lesions in more than two regions.

[c] Grade 2: uptake equal to the normal liver; grade 3: uptake higher than the normal liver; grade 4: uptake higher than normal spleen or kidneys.

Table 2. Performance Metrics of NETest, CgA, and Grading in Predicting Outcome

	NETest		CgA		Grading	
Tests	**NETest Increased**	**NETest Stable**	**CgA Increased**	**CgA Stable**	**G1 NETs**	**G2 NETs**
RECIST SD	6	8	4	10	9	5
RECIST PD	14	0	8	6	3	11
Sensitivity, %	100	100	57	57	79	79
Specificity, %	57	57	71	71	64	64
Accuracy, %	79	79	64	64	71	71
PPV, %	70	70	67	67	69	69
NPV, %	100	100	69	69	75	75

Abbreviations: NPV, negative predictive value; PPV, positive predictive value.

Disease status defined by RECIST on left compared with biomarker test results.

developed PD was 81–119%. In the twelve individuals with elevated CgAs at baseline, all 12 exhibited increases at least 25% in CgA levels (any time point); eight developed progressive disease and four remained stable. CgA levels were not significantly associated with outcome (Fishers exact probability: $P = .25$) (Figure 2). The metrics for CgA predicting disease were sensitivity, 57%; specificity, 71%; PPV, 67%; and NPV, 63% (Table 2).

NETest

The NETest was positive in all patients. An elevated NETest (80–100% activity) was identified in 3/28 patients (11%) at baseline, consistent with disease stability in the majority. Mean levels were, however, significantly higher in the group (57.5 ± 6% vs 41 ± 2%; $P = .02$) that subsequently developed PD. Fourteen (100%) with a NETest 80–100% during the course of treatment developed PD (Figure 2); six with elevated levels did not exhibit disease progression. All eight (100%) with activity levels less than 80%, remained stable. The overall variance in NETest was 18–50% (stable) and 28–74% (treatment failure). An elevated NETest (80–100%) during SSA treatment was significantly associated with failure of therapy (Fisher's exact probability, $P = .009$) (Figure 2). The metrics for elevated NETest (≥80%) in predicting treatment response (failure) was sensitivity, 100%; specificity, 57%; PPV, 70%; and NPV, 100% (Table 2).

Grading

Twelve (43%) were G1 and 16 were G2 at baseline. Nine (75%) G1 were stable and three developed PD. Eleven (68%) of G2 developed PD and five remained stable. Grade was associated with disease status (Fisher's exact probability, $P = .054$) (Figure 2). The metrics for grading in predicting disease were sensitivity, 79%; specificity, 64%; PPV, 69%; and NPV, 75% (Table 2).

Biomarkers and outcome prediction

Elevations in CgA levels (≥25%) may indicate PD (15); in our cohort, an elevation was evident in 12/28 subjects. Of these 12, eight (67%) developed PD and 4 were stable. The accuracy for CgA levels (changes <25% in SD or increased >25% in PD) to correctly identify disease status was 64% (18/28 cases). When using NETest activity, 22/28 patients were correctly identified (accuracy, 79%). Based on WHO histological grading, therapeutic response was correctly predicted in 20/28 cases (accuracy, 71%).

Logistic regression analyses identified an elevated NETest (80–100%) as associated with developing PD (odds ratio, 5.5×10^8); for grading the odds ratio was 3.5 and for CgA, it was 2.4. Multiple re-

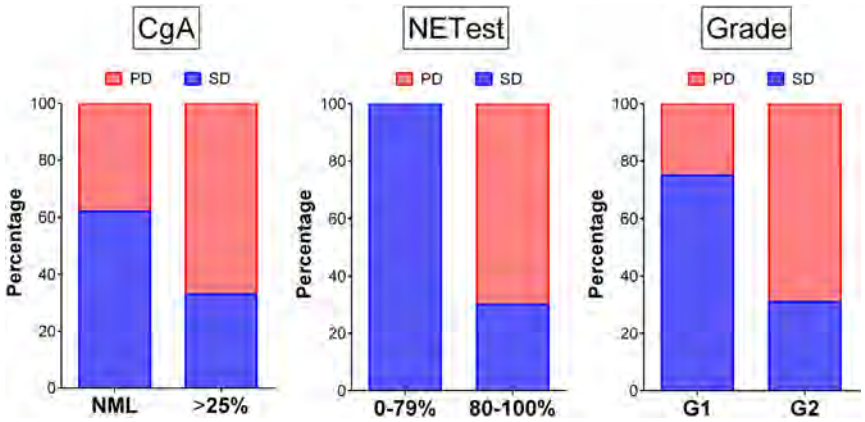

Figure 2. Relationship between markers and treatment response and the prospective set (n = 28) treated with somatostatin analogs. Alterations in CgA levels during SSA therapy were not significantly associated with either SD or PD. In contrast, both elevated NETest (80–100% activity; $P = .002$, measured anytime during therapy) as well as Grade ($P = .054$) were predictive of therapeutic responsiveness. Grading was defined according to ENETS/WHO 2010 classification (38), and was known for each patient. NML, normal (not elevated).

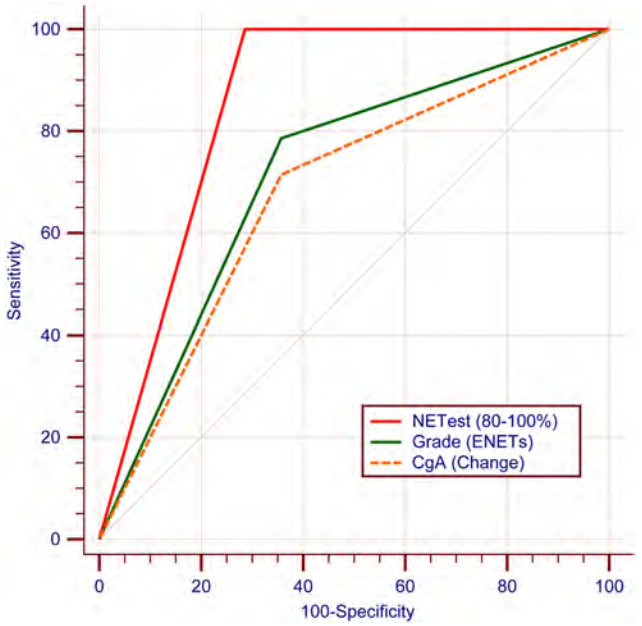

Figure 3. ROC for each biomarker as a predictor of disease status in the prospective set (n = 28). ROC analysis demonstrated the inferential order of AUCs to be NETest activity (AUC = 0.860 ± 08) >Grade (0.70 ± 0.10) >CgA (0.68 ± 0.10) ($P < .05$).

gression analysis identified that an elevated NETest was the only statistically valid variable predictive of disease progression (coefficient: 0.67 ± 0.16 $P = .0002$). For grading, the coefficient was 0.15 ± 0.14 ($P = .3$) and for CgA: 0.03 ± 0.13 ($P = .8$). Multiple correlation analyses determined that combinations of the NETest and grading had a significantly higher multiple correlation coefficient and F-ratio than CgA and grading as a combinatorial test (correlation: 0.76; F = 16.93; $P < .0001$ vs correlation: 0.51; F = 4.45; $P = .02$). ROC curve comparisons further confirmed the role for the NETest (AUC = 0.86 ± 0.08 vs 0.68 ± 0.1 for CgA, difference between AUCs: 0.2; z-statistic, 1.8, $P < .05$) (Figure 3).

Biomarkers and early prediction performance

The PFS of the cohort was 315 days (Figure 4A). This was not different between octreotide and lanreotide (data not shown). The predictive utility of each biomarker was assessed by identification of the time point at which significant changes occurred in biomarker expression prior to image evaluation indicating progressive disease. The mean time for these alterations (either 80–100% for NETest or >25% for CgA) was 105 days (range, 48–252 d) for the NETest and 70 days (range, 0–196 d) for CgA before the event (Figure 4B). The NETest was more informative, occurring at an earlier time ($P = .04$), and in more patients (high activity was noted in 14/14 patients) than CgA (8/14 exhibited >25% elevation; $P = .016$). An event curve analysis, based on the 14 patients for whom SSA therapy failed, identified that the elevation in NETest occurred at a significantly earlier time point than disease progression (Figure 4C). This was 94.5 compared with 241 days for disease progression ($P < .0001$, $\chi^2 = 19$). A similar analysis for CgA identified that this was not different to image-based assessment (Figure 4D; 185.5 vs 241 d). CgA alterations occurred significantly later than the NETest ($P = .002$; $\chi^2 = 13.6$).

Discussion

A timely appreciation of disease progression is a crucial issue in tumors such as NEN/NETs, which, in many instances, have a propensity for indolent growth (32). Biomarker assessment and imaging is one of the key elements of NET management (5). Specific monoanalytes eg, insulin or gastrin, which define the secretory status of a tumor, have proven useful in diagnosis but are disappointing in the assessment of disease progression given that evolving lesions may exhibit alterations in their secretory pattern during tumor progression (33). General biomarkers used to assess disease status include serotonin and CgA. There is general acceptance in the cancer community that advances in biomarker technology and clinical application thereof is required to enhance accuracy and clinical utility. Biomarkers have therefore been re-evaluated and are classified into three categories by the National Institutes of Health (19). Type 0 suggests the natural history of disease, type I reflects interventional effects, and type II are surrogate clinical endpoints. CgA is typically used as a type 0 biomarker (34) but it can be used as a type II biomarker. A 30% decrease in CgA (from pretreatment levels) may be predictive of a response to SSA (35), whereas an increase in three consecutive measures can anticipate relapse after radical surgery in midgut tumors (36). Furthermore, changes of at least 25% in CgA have been proposed to have high sensitivities (78–86%) and specificities (86–91%) for the prediction of disease events (37).

In the present study, we tested the predictive utility of CgA compared with the NETest and grading on therapeutic response (RECIST-defined disease status) in NETs treated with SSAs. In our initial evaluation (n = 35), we determined a cutoff value of 80% (activity scale, 0–100%) for the NETest to differentiate SSA responders from those who were treated but exhibited PD. In the independent, prospective group (n = 28), NETest elevation was significantly associated with outcome ($P = .002$) and was identified as the principal predictor of treatment failure (defined as disease progression; odds ratio = 5×10^8). Grading was also associated with outcome ($P = .054$) but changes (increase ≥25%) in CgA levels were not ($P = .25$). CgA exhibited limited clinical utility given that it was not

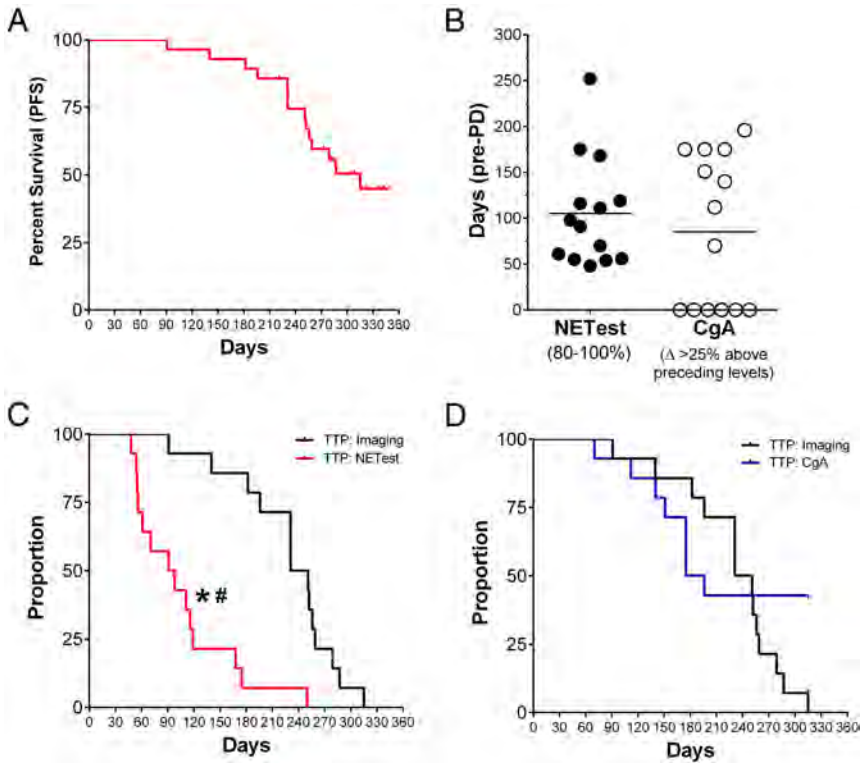

Figure 4. PFS and biomarker alteration relationship in the prospective set (n = 28). A, The PFS (measured as time to progression from treatment initiation) was 315 days for the cohort (n = 28). No difference was noted between Octreotide and Lanreotide. B, Time at which a significant biomarker change occurred (NETest 80–100%, or change in CgA > 25%) before clinically significant disease (PD) was detectable. This ranged from 48–252 days (mean, 105 d) for NETest and for 0–196 (mean, 70 d) for CgA ($P = .04$). The NETest was also informative in all 14 patients. CgA was informative in eight (57%; $P < .02$). C, Imaging and NETest event curve analysis identified that the elevation in NETest (80–100% score) occurred at a significantly earlier time (94.5 d) than image-identifiable disease progression (241 d) in the 14 patients (*, $P < .0001$; $\chi^2 = 19$). D, Event curve analysis identified that the elevation in CgA did not occur significantly earlier than clinical evidence of disease progression. This was 185.5 days. CgA alterations occurred significantly later than the NETest (#, $P = .002$; $\chi^2 = 13.6$; 4C).

elevated in 50%, whereas four (25%) of those who developed PD had normal levels. Jensen et al (37) identified a CgA increase (≥25%) was related to 88% of disease progression events in a retrospective analysis, as opposed to only 67% in our cohort. The 10-month time frame is shorter than their median of 35 months (range, 1–120 mo) (37) and it is possible that all patients with these changes (≥25% CgA in our study) could eventually exhibit progression. Nevertheless, the critical necessity in effective patient management is early detection of status change. Given that CgA alterations and disease progression timing (imaging) differ little (184.5 vs 241 d), it is not effective as an early predictive biomarker of disease. Welin et al (15) considered three consecutive increases in CgA to be an important prognostic for disease recurrence. Elevated expression was identified in 28 of 33 (85%) midgut patients, in whom three (11%) also exhibited concomitant radiological evidence of disease progression (15). In our prospective series, the 12 patients with greater than 25% change in CgA also exhibited at least three consecutive increases in CgA levels; however, only 67% were associated with disease progression. The Welin observations (15), when viewed within the context of a median followup of 32 months (range, 6–217 mo) and the fact that only 10% of patients exhibited radiological evidence (short term), suggest that alterations in CgA are not adequately sensitive to modify clinical decisions in a timely fashion. The differences between studies cannot be attributed to assay variations given that we used the same assay.

Decreases in CgA have been linked with treatment efficacy (35). A 30% decrease in CgA has been proposed [compared with the 25% decrease (37)] as evidence of response to SSAs (especially octreotide) (35). In our study, only four (14%) were associated with 5–29% decreases in CgA. Three patients who had stable disease exhibited decreased CgA levels of −5, −6, and −26%. One individual, who subsequently developed PD, exhibited the largest measureable decrease, −29%. Our data suggest no mathematical relationship between alterations in CgA and outcome on SSAs. An alternative explanation for the inconsistencies might reflect differences in study design (prospective vs retrospective), assay types (commercial vs in-house), as well as the differences in time between analog injection and CgA measurements.

Grading generally correlates with outcome and intuitively might be considered definitive as a prognostic factor given that Ki-67 levels provide much of the basis for therapeutic recommendations (2, 38). In our series, a greater proportion of G1 patients (9/12; 75%) remained stable on SSAs whereas a large proportion of G2 patients (11/16; 68%) progressed during the study period. It is noteworthy that a significantly larger analysis, which included 141 G1 and 61 G2 (with Ki-67 < 10%) patients, randomly assigned for lanreotide vs placebo (CLARINET), was not associated with a statistically significant difference in outcome based on grading (20). The PROMID study (octreotide) comprised 95% G1 tumors and was therefore noninformative in this regard (21). It is possible that the utility of grading in our study may be biased by the small study numbers. Nevertheless, grading exhibited metrics,

which could be clinically useful when combined with an accurate blood-based biomarker. Grading, however, is a one-time assessment of tumor biology. Furthermore, values differ in different locations of the lesion and the score is often different between metastases and primary (2, 38). Thus, grading alone fails to capture the dynamic events involved in tumor growth over time and during therapy. Repetitive tumor biopsy has obvious limitations compared with regular assessment of blood.

Metrics for predicting SSA therapeutic responses were significantly better for the NETest (79%) compared with either CgA (64%) or grading (71%) (Table 2). Multiple regression analysis identified that an elevated NETest was predictive of disease progression ($P = .0002$). Moreover, combining the NETest with grading identified an accuracy of 86% for predicting therapeutic responsiveness. CgA was noninformative in this regard and did not provide added information.

Study limitations included the relatively short followup (median, 10 mo) and the relatively small number of patients (n = 63 in both sets) but the analyses are substantiated by rigorous methodological and clinical points. Furthermore, the study is prospective, used a homogeneous protocol for the assessment of the disease status and represents a cross-sectional analysis of the clinical history of SSA treatment. The variability in month-to-month levels for each of the assays (CgA, 81–119%; NETest, 18–74%) suggests that circulating measurements capture biological fluxes in tumor behavior. Well-recognized paroxysmal secretory events as measured by changes in hormone production are reflected in the fluctuating CgA measurements (39). Similarly, fluctuations in tumor activity can be detected by the NETest; thus, threshold values commensurate with prediction of disease progression can be determined.

In conclusion, blood NET transcript analysis effectively defined the effect of SSAs in terms of identifying stable and progressive disease. Of particular clinical interest was the early predictive ability of the NETest to ascertain disease progression. Although Ki-67 has utility, it can rarely be obtained on more than one occasion and cannot monitor evolving disease biology. Integration of blood transcript analysis values with grading may, however, prove to be a highly effective predictor of therapeutic efficacy and outcome and should be further evaluated. Given that the blood transcript analysis can be easily repeated, the advantages of a real-time dynamic assessment of disease status and treatment efficacy have clinical utility in modifying management.

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