Association between neuroendocrine tumors biomarkers and primary tumor site and disease type based on total $^{68}$Ga-DOTATATE-Avid tumor volume measurements

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Abstract

Objective: To determine the association between neuroendocrine tumor (NET) biomarker levels and the extent of disease as assessed by $^{68}$Ga DOTATATE PET/CT imaging.

Design: A retrospective analysis of a prospective database of patients with NETs.

Methods: Fasting plasma chromogranin A (CgA), neuron-specific enolase (NSE), gastrin, glucagon, vasoactive intestinal peptide (VIP) and pancreatic polypeptide (PP), and 24-h urinary 5-hydroxyindoleacetic acid (5-HIAA) levels were measured. Correlation between biomarkers and total $^{68}$Ga-DOTATATE-avid tumor volume (TV) was analyzed.

Results: The analysis included 232 patients. In patients with pancreatic NETs ($n=112$), $^{68}$Ga-DOTATATE TV correlated with CgA ($r=0.6$, $P=0.001$, Spearman). In patients with multiple endocrine neoplasia type 1 ($n=39$), $^{68}$Ga-DOTATATE TV correlated with glucagon ($r=0.5$, $P=0.01$) and PP levels ($r=0.5$, $P=0.049$). In patients with von Hippel–Lindau ($n=24$), plasma VIP ($r=0.5$, $P=0.02$) and PP levels ($r=0.7$, $P<0.001$) correlated with $^{68}$Ga-DOTATATE TV. In patients with small intestine NET (SINET, $n=74$), $^{68}$Ga-DOTATATE TV correlated with CgA ($r=0.5$, $P=0.02$) and 5-HIAA levels ($r=0.7$, $P<0.001$), with 5-HIAA $\geq 8.1$ mg/24 h associated with metastatic disease with high positive (81.8%) and negative (85.7%) predictive values ($P=0.001$). $^{68}$Ga-DOTATATE TV in patients with NET of unknown primary ($n=16$) and those with NET of other primary location ($n=30$) correlated with 5-HIAA levels ($r=0.8$, $P=0.002$ and $r=0.7$, $P=0.02$ respectively).

Conclusions: Our data supports the use of specific NET biomarkers based on the site of the primary NET and the presence of hereditary syndrome-associated NET. High urinary 5-HIAA levels indicate the presence of metastatic disease in patients with SINET.
Introduction

Most neuroendocrine tumors (NETs) originate from endocrine cells spread throughout the respiratory and gastrointestinal tracts. Although these tumors tend to have an indolent natural course, NETs present with distant metastases in 40–50% of patients (1, 2, 3), usually to the liver and regional lymph nodes (LN), and less often to the lungs, brain, bones and peritoneal cavity (1). Tumor biomarkers are important for diagnosis and follow-up of patients with NETs, especially after treatment of locally advanced and/or metastatic disease. Several biomarkers are used for NET surveillance, including plasma levels of chromogranin A (CgA), pancreatic polypeptide (PP), neuron-specific enolase (NSE) and urinary 24-h 5-hydroxyindoleacetic acid (5-HIAA) levels depending on the primary tumor location (4, 5). The sensitivity and specificity of different biomarkers depend on the site of the primary tumor, with CgA mainly used for patients with pancreatic and midgut NET, NSE being most useful in patients with poorly differentiated NET and 5-HIAA in patients with carcinoid syndrome. The accuracy of biomarker measurement depends on other factors unrelated to the tumor, such as kidney function and presence of gastric atrophy for CgA and dietary factors and medical treatment for 5-HIAA (6). Furthermore, the determination of whether elevated NET biomarkers accurately reflect the presence of disease has been limited by the ‘gold standard test’ used for assessing the presence of disease; pathologic analysis and/or the type of imaging modality. As a result of these limitations, there is a lack of consensus on the utility of these biomarkers for estimating the total tumor burden in patients with NETs (7).

Well-differentiated NETs typically express somatostatin receptors (SSTRs); thus, radiolabeled somatostatin analogues have been used for diagnosis and follow-up of patients with NET (5). 111In-pentetetreotide (Octreoscan) has been widely used for NET imaging, but is limited by its low image resolution and long scan duration and relatively lower SSTRs affinity (8). In contrast, the 68Gallium (68Ga)-DOTA-compounds recently introduced into clinical practice (68Ga-DOTATATE, 68Ga-DOTANOC and 68Ga-DOTATOC) have been shown to have higher sensitivity for detecting NET than Octreoscan (9) and significantly influence the management of patients with NET (10, 11). To our knowledge, no study has yet assessed the utility of biochemical biomarkers of NETs and total 68Ga-DOTATATE-avid tumor volume (TV), as a measure of total tumor burden in patients with NET.

Subjects and methods

Study population

Patients known to have NETs based on imaging (CT, MRI and 18F-FDG PET), biochemical evidence of NETs and/or pathologically confirmed NET were included in this study. The study was performed under an investigational new drug approval from the United States Food and Drug Administration. The full study eligibility criteria were as previously reported (10). The study was approved by the National Cancer Institute Institutional Review Board and the National Institutes of Health Radiation Safety Committee (NCT01967537). Written informed consent was obtained from all study participants.

NETs were sub-grouped according to the primary tumor location based on conventional anatomic imaging, 68Ga-DOTATATE PET/CT and/or pathologic evaluation. Patients were subdivided into those with pancreatic NETs (PNETs) or small intestine NETs (SINETs). Subjects with metastatic NET and with pathologic 68Ga-DOTATATE uptake but no clear primary lesion were defined as NET of unknown primary (NEToUP, n=16). Thirty subjects with NET of other origins were grouped as NET of other primary location (NEToOL) due to their small number. Duodenal NETs were included in NEToOL due to their distinct characteristics compared with other SINET (14). Tumor grade was determined according to the 2010 World Health Organization (WHO) definitions as G1, G2 or G3 according to Ki-67 index (<3, 3–20 and >20%) and mitotic rate (<2, 2–20 and >20 per 10 high-power microscopic fields) in patients who had their tumor resected or biopsied respectively (15).

Biochemical and imaging evaluation

All patients underwent testing for fasting plasma CgA, PP, NSE, vasoactive intestinal peptide (VIP), gastrin and glucagon and 24-h 5-HIAA urinary collections. Thirty-one patients receiving chronic treatment with proton-pump inhibitors and one patient with a plasma creatinine >2 mg/dL were excluded from the analysis of plasma CgA levels, as they may have an increase in plasma CgA levels that does not reflect disease burden. Plasma glucagon
levels (LINCOplex Kit, Luminex 200, MO, USA), plasma VIP levels (Peninsula Laboratories, CA, USA) and plasma PP levels (Mayo Medical Laboratories, Rochester, MN, USA) were measured using an immunoradiometric assay, whereas immunochemiluminometric assays were used for measuring plasma chromogranin A (Mayo Medical Laboratories), gastrin (Innolite 2000, Siemens) and NSE levels (NSE Kryptor, BRAHMS, Germany). Urinary 5-HIAA levels were measured using liquid chromatography–tandem mass spectrometry (Mayo Medical Laboratories) (17). The cut-offs for positivity were determined by the kit manufacturer or by the laboratory performing the tests.

Both 68Ga-DOTATATE PET/CT scans and biomarkers level measurements were performed concurrently at study inclusion. The biochemical evaluation for patients with a clinical suspicion of insulinoma included measurements of fasting glucose, insulin, proinsulin and C-peptide during hypoglycemia. Supervised fasting test was conducted if necessary.

For 68Ga-DOTATATE PET/CT imaging, 185 MBq (5 mCi) 68Ga-DOTATATE was administered through a peripheral intravenous line. After approximately 60 min, the patient was positioned supine in a PET/CT scanner, and images were obtained from mid-thighs to the top of the skull. A low-dose, non-contrast-enhanced CT was used for attenuation correction and anatomic localization. Maximum standardized uptake values (SUVmax) of visualized lesions were calculated based on localization. Maximum standardized uptake values were obtained automatically. The sum of these volumes reflects the entire tumor tissue expressing somatostatin receptor type 2 and was defined as total volume (TV).

### Quantification of tumor volume by 68Ga-DOTATATE PET/CT imaging

Disease burden was assessed by quantifying 68Ga-DOTATATE uptake using the MIM Vista workstation (version 6.5.9). A VOI (volume of interest) encompassing the entire scan was drawn, and subsequently, a SUVmax threshold-based approach was applied to include all sites of non-physiologic uptake. The SUV threshold-based technique for segmentation is commonly used and was tested in many clinical studies (18). The software used in the current analysis enables automatic generation of separate VOIs encircling all areas above the SUVmax threshold set by the user. This software has been tested and reported before (19). Afterward, an experienced nuclear medicine physician who was blinded to the clinical and laboratory data manually excluded areas with physiologic 68Ga-DOTATATE uptake. The volumes of all lesions with a pathological 68Ga-DOTATATE uptake were obtained automatically. The sum of these volumes reflects the entire tumor tissue expressing somatostatin receptor type 2 and was defined as total volume (TV).

### Statistical analysis

Statistical analyses were performed using SPSS 20.0 software (SPSS). Results are expressed as mean ± standard deviation (S.D.) unless otherwise indicated. For group comparisons, the independent Student’s t-test was used to analyze the differences in continuous variables, and the chi-square test was used to analyze differences in categorical variables. The Pearson product was used for analysis of correlations.

### Table 1  Study cohort clinical characteristics (n = 232).

<table>
<thead>
<tr>
<th>Primary tumor n (%)</th>
<th>PNEN 112 (48.3%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>SINET 74 (31.9%)</td>
<td></td>
</tr>
<tr>
<td>Unknown primary 16 (6.9%)</td>
<td></td>
</tr>
<tr>
<td>Other* 30 (12.9%)</td>
<td></td>
</tr>
<tr>
<td>Genetic syndromes n (%)</td>
<td>MEN-1 39 (16.8%)</td>
</tr>
<tr>
<td>VHL 28 (12.1%)</td>
<td></td>
</tr>
<tr>
<td>NF-1 1 (0.4%)</td>
<td></td>
</tr>
<tr>
<td>Cowden 1 (0.4%)</td>
<td></td>
</tr>
<tr>
<td>Functional status n (%)</td>
<td>Nonfunctional 121 (52.2%)</td>
</tr>
<tr>
<td>Inulinoma 12 (5.2%)</td>
<td></td>
</tr>
<tr>
<td>Gastrinoma 16 (6.9%)</td>
<td></td>
</tr>
<tr>
<td>Carcinoid syndrome 79 (34.1%)</td>
<td></td>
</tr>
<tr>
<td>Other hormone production 4 (1.7%)</td>
<td></td>
</tr>
<tr>
<td>WHO tumor grade^</td>
<td>G1 51 (52.0%)</td>
</tr>
<tr>
<td>G2 42 (42.9%)</td>
<td></td>
</tr>
<tr>
<td>G3 5 (5.1%)</td>
<td></td>
</tr>
<tr>
<td>Metastases n (%)</td>
<td>Any 134 (57.8%)</td>
</tr>
<tr>
<td>Lymph nodes 84 (36.2%)</td>
<td></td>
</tr>
<tr>
<td>Liver 83 (35.8%)</td>
<td></td>
</tr>
<tr>
<td>Bones 32 (13.8%)</td>
<td></td>
</tr>
<tr>
<td>Lungs 12 (5.2%)</td>
<td></td>
</tr>
<tr>
<td>Treatment</td>
<td>SSA n (%) 79 (34.3%)</td>
</tr>
<tr>
<td>History of surgical resection of NETs before 68Ga-DOTATATE PET imaging 133 (57.3%)</td>
<td></td>
</tr>
<tr>
<td>Pathological uptake on 68Ga-DOTATATE PET 185 (79.7%)</td>
<td></td>
</tr>
</tbody>
</table>

*Consisted of gastric (n=6), duodenal (n=10), rectal (n=6), appendiceal (n=1), large intestine (n=2), bronchial (n=3), thymic (n=1) and bladder (n=1) NETs. ^Only a subset of patients had WHO tumor grade status available.

MEN-1, multiple endocrine neoplasia syndrome type 1; NF-1, neurofibromatosis type 1; PNEN, pancreatic neuroendocrine tumor; SINET, small-intestine neuroendocrine tumor; SSA, somatostatin analogues; VHL, von Hippel–Lindau disease.
between variables. Receiver-operating characteristic (ROC) curve analysis was performed to assess the accuracy of 24-h urinary 5-HIAA and plasma CgA levels for indicating metastatic SINET. Both TV and biochemical biomarker levels were logarithmically transformed to approximate normality. Non-parametric tests were used as appropriate, including Mann–Whitney U for continuous variables, and Fisher’s exact test for categorical variables. The P value for significance was set at less than 0.05.

Results

The study cohort consisted of 232 patients with a mean age of 54.3 ± 14.1 years, mean body mass index of 28.9 ± 6.8 kg/m² and 130 (56.0%) were females. The sites of NETs were PNET (n=112), SINET (n=74) and tumors of unknown primary (n=16). NEToOL consisted of gastric (n=6), duodenal (n=10), rectal (n=6), appendical (n=1), large intestine (n=2), bronchial (n=3), thymic (n=1) and bladder (n=1) NETs. The study cohort clinical characteristics are summarized in Table 1. Mean biomarkers levels, normal reference ranges and percentage of patients with elevated biomarkers levels are summarized in Table 2. Correlation analysis between ⁶⁸Ga-DOTATATE TV and biochemical biomarkers are summarized in Supplementary Table 1 (see section on supplementary data at the end of the article).

Tumor burden measurement by ⁶⁸Ga-DOTATATE TV

The mean ⁶⁸Ga-DOTATATE TV was 69.2 ± 152.1 mL, and mean SUVmax was 69.1 ± 50.9. ⁶⁸Ga-DOTATATE TV positively correlated with 24-h urinary 5-HIAA levels (r=0.5, P<0.001) and plasma CgA levels (r=0.4, P=0.001). Patients with metastatic disease had higher 24-h 5-HIAA levels (14.8 ± 29.7 vs 4.8 ± 2.2 mg/24 h, P<0.001), plasma gastrin levels (252 ± 863 vs 74 ± 120 pg/mL, P=0.02) and plasma CgA levels (382 ± 1030 vs 112 ± 255 ng/mL, P=0.002) compared to those in patients without metastatic disease respectively.

Tumor burden measurement in patients with PNET

Patients with PNETs (n=112) had mean ⁶⁸Ga-DOTATATE TV of 71.3 ± 167.5 mL and mean SUVmax of 85.5 ± 58.3. Sixty-two patients with PNETs (55.4%) had metastatic disease at evaluation. Among patients with PNETs, tumor burden as measured by ⁶⁸Ga-DOTATATE TV positively correlated with plasma CgA levels (r=0.6, P=0.001, Spearman’s rho), and plasma NSE levels had a similar trend (r=0.4, P=0.05). Similar trend was found for plasma NSE levels among patients with metastatic PNETs (r=0.5, P=0.07).

Among patients with multiple endocrine neoplasia type-1 (MEN-1) with PNETs, ⁶⁸Ga-DOTATATE TV positively correlated with plasma glucagon (r=0.5, P=0.01, Fig. 1A) and PP levels (r=0.5, P=0.049). In patients with von Hippel–Lindau (VHL), ⁶⁸Ga-DOTATATE TV positively correlated with plasma VIP (r=0.5, P=0.02, Fig. 1B) and PP levels (r=0.7, P<0.001, Fig. 1C).

Patients with WHO 2010 G1 PNETs had positive correlation between TV and plasma glucagon levels (n=22, r=0.5, P=0.02) that was not significant in patients with G2 PNETs (n=16). In 16 patients with gastrinomas of either pancreatic or duodenal location, ⁶⁸Ga-DOTATATE TV had strong positive correlation with plasma gastrin levels (r=0.8, P=0.001).

Tumor burden measurement in patients with SINET

In patients with SINET (n=74), the mean ⁶⁸Ga-DOTATATE TV and SUVmax were 74.1 ± 133.5 mL and 49.1 ± 25.9 respectively. 24-h urinary 5-HIAA (P<0.001) levels were higher among patients with distant metastases compared with those in patient without metastases.

Table 2 Neuroendocrine tumor biomarker levels distribution in study cohort.

<table>
<thead>
<tr>
<th>Biomarkers</th>
<th>Upper limit of normal range</th>
<th>n</th>
<th>Mean ± s.d.</th>
<th>n (%) of patients with elevated biomarkers*</th>
</tr>
</thead>
<tbody>
<tr>
<td>24-h urinary 5-HIAA levels</td>
<td>≤8 mg/24h</td>
<td>208</td>
<td>10.5 ± 22.9</td>
<td>48 (23.1%)</td>
</tr>
<tr>
<td>Plasma chromogranin A levels+</td>
<td>≤93 ng/mL</td>
<td>83</td>
<td>237 ± 712</td>
<td>24 (28.9%)</td>
</tr>
<tr>
<td>Plasma NSE levels</td>
<td>≤15 ng/mL</td>
<td>81</td>
<td>9.4 ± 1.9</td>
<td>6 (7.4%)</td>
</tr>
<tr>
<td>Plasma gastrin levels</td>
<td>≤100 pg/mL</td>
<td>219</td>
<td>176 ± 661</td>
<td>42 (19.2%)</td>
</tr>
<tr>
<td>Plasma glucagon levels</td>
<td>≤80 pg/mL</td>
<td>220</td>
<td>41.4 ± 42.0</td>
<td>25 (11.4%)</td>
</tr>
<tr>
<td>Plasma VIP levels</td>
<td>≤75 pg/mL</td>
<td>169</td>
<td>48.0 ± 117.0</td>
<td>8 (4.7%)</td>
</tr>
<tr>
<td>Plasma PP levels</td>
<td>≤291 pg/mL</td>
<td>199</td>
<td>228 ± 493</td>
<td>28 (14.1%)</td>
</tr>
</tbody>
</table>

* Based on upper limit of the reference range. † Patients with chronic kidney injury (n=1) and/or treatment with proton-pump inhibitors (n=31) were excluded from the analysis of chromogranin A.

S-HIAA, S-hydroxyindoleacetic acid; NSE, neuron-specific enolase; PP, pancreatic polypeptide; s.d., standard deviation; VIP, vasoactive intestinal peptide.
The burden of SINET as measured by $^{68}$Ga-DOTATATE TV correlated positively with plasma CgA levels ($r=0.5$, $P=0.02$; Fig. 2A) and with 24-h urinary 5-HIAA levels ($r=0.7$, $P<0.001$; Fig. 2B). Similarly, among the subgroup of patients with metastatic SINET, $^{68}$Ga-DOTATATE TV had positive correlation with CgA levels ($r=0.5$, $P=0.02$) and with 24-h urinary 5-HIAA levels ($r=0.7$, $P<0.001$). In patients with carcinoid syndrome ($n=32$), $^{68}$Ga-DOTATATE TV correlated with 24-h urinary 5-HIAA ($r=0.7$, $P<0.001$) and plasma CgA levels ($r=0.5$, $P=0.02$), whereas in patients without carcinoid syndrome, $^{68}$Ga-DOTATATE TV
correlated only with 24-h urinary 5-HIAA levels \((n = 15, r = 0.9, P < 0.001)\).

Urinary 5-HIAA levels \(\geq 8.1\) mg/24 h (upper limit of normal range, 8 mg/24 h) predicted metastatic disease based on \(^{68}\)Ga-DOTATATE PET/CT among subjects with SINET that were not treated with somatostatin analogues, with a sensitivity of 81.8% and a specificity of 85.7%, and positive and negative predictive values of 81.8% and 85.7% respectively \((P = 0.001, \text{ Fig. 3A})\). This was true also among patients treated with somatostatin analogues (Fig. 3B) and among all subjects with SINET (Fig. 3C). In contrast, ROC curve analysis revealed limited utility of plasma CgA levels for predicting metastatic SINET (area under the curve = 0.668, 95% confidence interval 0.548–0.788, \(P = 0.008\)).

**Tumor burden measurement in NEToUP and NEToOL**

Among sixteen patients with NEToUP, correlation analysis revealed strong correlation between \(^{68}\)Ga-DOTATATE TV and 24-h urinary 5-HIAA levels \((r = 0.8, P = 0.002)\). Subjects with NEToOL \((n = 30)\) had positive correlation between \(^{68}\)Ga-DOTATATE TV and 24-h 5-HIAA \((r = 0.7, \text{ } P = 0.02\), Spearman’s rho).

The analyses were repeated after excluding patients treated with somatostatin analogues with findings similar to those described for the whole study cohort analysis. Among subjects with NEToOL, excluding patients with somatostatin analogue treatment revealed a correlation between \(^{68}\)Ga-DOTATATE TV and plasma PP levels \((n = 7, r = 0.8, P = 0.03)\).

**Discussion**

In this study, we analyzed the utility of seven biomarkers for assessing NET burden using a novel tumor burden measurement approach based on \(^{68}\)Ga-DOTATATE TV in a large cohort of patients with NET. \(^{68}\)Ga-DOTATATE TV correlated strongly with urinary 5-HIAA and with plasma CgA among patients with SINETs. In addition, MEN-1 patients with PNETs had correlation between tumor burden and plasma glucagon levels, and subjects with VHL disease had correlation between \(^{68}\)Ga-DOTATATE TV and plasma PP and VIP levels. Finally, urinary 5-HIAA levels \(\geq 8.1\) mg/24 h were associated with metastatic disease in patients with SINET, independent of somatostatin analogue treatment.

Tumor biomarkers measurement are the cornerstone of NET diagnosis \((20)\) and are essential for surveillance after various treatment modalities are carried out. Plasma
CgA levels reflect tumor burden in non-functioning PNETs (21), and high urinary 5-HIAA was found mainly in patients with metastatic SINET (22) and is associated with carcinoid heart disease (23). Indeed, the current guidelines for NET management recommend the use of urinary 5-HIAA for SINET diagnosis, and the measurement of CgA and NSE for the diagnosis of PNET (4, 5). The current study provides data to support these recommendations of using specific NET biomarkers based on the primary tumor site and that elevated values of urinary 5-HIAA for SINET often indicate the presence of metastatic disease, which may not be seen by conventional anatomic imaging studies.

Somatostatin receptor-based PET/CT is a very sensitive imaging modality to detect NETs (10, 24, 25). 68Ga-labeled somatostatin analogue PET/CT is more sensitive for NET localization (5, 10), is faster and requires lower radiation dose (26) compared with Octreoscan. Hence, in the current study, disease extent was assessed by the quantification of whole-body 68Ga-DOTATATE pathological uptake. Thus, we believe our analysis of the levels of a comprehensive panel of NET biomarkers and NET disease burden more accurately reflects the diagnostic and surveillance clinical utility of biomarkers in patients with NET.

Total 68Ga-DOTATATE uptake in NETs was reported in the past in a series of patients, but not in specific subtypes of NET, and no correlation analysis with tumor biomarkers was reported (12, 13). Moreover, the correlations between tumor biomarkers and tumor burden reported in the past have been based on anatomical imaging (21), counting the number of lesions (10), the presence/absence of liver metastases (27) and not based on accurate total tumor volume measurements, as performed in the current analysis. The strong correlation between 24-h urinary 5-HIAA levels and 68Ga-DOTATATE TV in SINET is not surprising (22) and might explain the strong correlation between total volume measurements and 5-HIAA among patients with NEToUP, as these tumors arise mainly from the small intestine but are not detected (28).

Our findings have important clinical implications. First, baseline 5-HIAA levels ≥8.1 mg/24 h should prompt evaluation for metastatic disease in patients with SINET. Second, among patients with PNETs associated with hereditary syndromes, PP may be used as a biomarker in patients with MEN-1, while glucagon and VIP can be used as biomarkers in patients with VHL.

Our analysis has some limitations. First, the vast majority of the patients in the current study had gastroenteropancreatic (GEP) NETs, limiting the ability to make strong conclusions about biomarkers utility among patients with primary tumors at other sites. Second, although the analysis was performed by an experienced nuclear medicine specialist who was blinded to the clinical context, interobserver variation was not tested for in the current study and should be evaluated in future studies. Third, plasma CgA levels may be elevated in patients with atrophic gastritis and can lead to positive results, and every patient in the current study did not have endoscopy and biopsy to determine the presence of this. Finally, the biochemical biomarkers in the current analysis were performed using a single method per biomarker. As there might be a difference in the measured levels between different analytical methods, the study results may only apply to the methods used in our study and other analytical methods would need to be studied in the future.

In conclusion, our data support the use of specific NET markers based on the site of the primary NET and the presence of hereditary syndrome-associated NET. High levels of 24-h urinary 5-HIAA commonly indicate the presence of metastatic disease in patients with SINET. The heterogeneity of patients with NET necessitates careful matching of biochemical biomarker for each patient according to both tumor and patient characteristics and might justify personalized biomarker panel evaluation for tumor burden assessment.

Supplementary data
This is linked to the online version of the paper at http://dx.doi.org/10.1530/EJE-16-1079.

Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of this study.

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