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Consensus on biomarkers for neuroendocrine tumour disease

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Declaration of interests

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For more the **MammaPrint assay** see http://www.mammaprint.co.uk/

For more on the **Oncotype DX assay** see http://breast-cancer.oncotypedx.com/en-GB/Patient-Invasive

For more about the **MammoStrat assay** see http://www.clarient.com/mammostratoverview.aspx

Abstract

Management of neuroendocrine neoplasia represents a clinical challenge because of its late presentation, lack of treatment options, and limitations in present imaging modalities and biomarkers to guide management. Monoanalyte biomarkers have poor sensitivity, specificity, and predictive ability. A National Cancer Institute summit, held in 2007, on neuroendocrine tumours noted biomarker limitations to be a crucial unmet need in the management of neuroendocrine tumours. A multinational consensus meeting of multidisciplinary experts in neuroendocrine tumours assessed the use of current biomarkers and defined the perquisites for novel biomarkers via the Delphi method. Consensus (at >75%) was achieved for 88 (82%) of 107 assessment questions. The panel concluded that circulating multianalyte biomarkers provide the highest sensitivity and specificity necessary for minimum disease detection and that this type of biomarker had sufficient information to predict treatment effectiveness and prognosis. The panel also concluded that no monoanalyte biomarker of neuroendocrine tumours has yet fulfilled these criteria and there is insufficient information to support the clinical use of miRNA or circulating tumour cells as useful prognostic markers for this disease. The panel considered that trials measuring multianalytes (eg, neuroendocrine gene transcripts) should also identify how such information can optimise the management of patients with neuroendocrine tumours.

Introduction

Neoplasms arising from the diffuse neuroendocrine system, also known as neuroendocrine tumours, are relatively rare yet clinically challenging in their management. [1] Some patients present well-defined symptoms associated with the overproduction of circulating biologically active hormones, peptides, and amines—eg, insulinomas and gastrinomas. Others, such as the fore-gut carcinoids, produce various bioactive products, many of which are not fully characterised and can cause a complex clinical syndrome.[2] Many midgut neuroendocrine tumours (ie, small intestinal and pancreatic) are symptomatically related to bioactive product secretion and therefore are termed functional. Others, particularly pancreatic tumours, might have no identifiable clinical syndrome (termed non-functional), but can cause local symptoms (eg, obstruction and bleeding) and ultimately death from the local growth of the primary tumour or liver dysfunction due to hepatic metastases.

The number of patients with neuroendocrine tumours in the USA and worldwide has been increasing over the past several decades,[3] showing both an increase in the incidence and detection for this disease population. Management of patients with neuroendocrine tumours has been fragmented due to differences in disease management by individual centres and is often empiric with minimal advances in disease outcome. For these reasons, the US National Cancer Institute convened a summit meeting in 2007 about neuroendocrine tumours to review the challenges and clinical unmet needs associated with the management of these tumours, with the purpose to prioritise key research areas for development and to discuss key clinical issues with respect to this disease.[4]

Basis for consensus

Ten recommendations were made at this summit including the aim to develop tumour and plasma markers that could be used for early diagnosis and to monitor disease treatment.[4] The need for early diagnosis is predicated upon the delay in diagnosis of neuroendocrine tumours, which has typically been within the range of several years, despite development of modern imaging methods.[2] Late diagnosis restricts treatment effectiveness and the absence of adequately sensitive and specific biomarkers obviates accurate monitoring of disease progression.[5] Most neoplasia treatment is dependent on the assessment of many pathological criteria, whereas pathological inter pretation to predict disease aggressiveness in neuro endocrine tumours is limited as it is based on overlapping histological features in biologically diverse disease cohorts (figure 1). Prognostication based on pathological grading is dependent on the measurement of the proliferative index (mitotic rate or Ki-67 index),[6] which have manifold issues associated with its precise sensitivity and clinical use.[7] The variable prognosis of neuroendocrine tumours is related, in part, to tumour pathological heterogeneity and vagaries in the assessment of the proliferative activity, which can be illustrated by the differences in 5-year survival of between 15% and 95%, which is largely dependent on the primary tumour site, grade, and disease extent.[2,3,8]

Treatment options for patients with neuroendocrine tumours are diverse, including somatostatin receptor agonist blockade, targeted radionuclides, immunotherapy (interferon), cytotoxic chemotherapy, rationally designed targeted drugs, external radiation, interventional radiological approaches, and surgery (either for cure or palliative debulking).[9] This plethora of expensive and sometimes toxic treatment choices, usually selected empirically, highlights the need to monitor tumour responsiveness both in clinical trials and in routine practice.[10]

For most non-neuroendocrine neoplasms, tumour responsiveness is almost entirely assessed through imaging. However, neuroendocrine tumours are often indolent and the use of targeted drugs, although regularly used, rarely leads to objective remission but might produce disease stabilisation. Thus, this imaging strategy has obvious limitations in relation to the Response Evaluation Criteria in Solid Tumors (RECIST).[11] Present imaging modalities have great limitations to define persistent or progressive disease, monitor effectiveness of treatment, and predict aggressive tumour behaviour. Furthermore, cumulative radiation exposure and costs associated with repetitive imaging in the long-term follow-up of patients who might exceed a 10-year life expectancy, supports the need for accurate biomarkers that directly measure tumour-cell activity and provide real-time feedback to the clinician. In other cancers, such as breast cancer, the development of molecular markers, especially those based on multianalyte as opposed to monoanalyte delineation of neoplasia, has advanced disease management.[12,13] Such information informs clinical decision making with respect to the choice and the timing of therapy, assessment of effectiveness, and providing, in some instances, prognostic information.[14]

Biomarkers of individual tumour types, (eg, insulin for insulinoma, gastrin for gastrinoma, vasoactive intestinal peptide for vasoactive intestinal peptide tumour) are helpful serum indicators of tumour activity, but are useful in only a few neuroendocrine tumours with distinct clinical syndromes (table 1). Up to now, the most clinically useful neuroendocrine

tumour biomarker has been chromogranin A, a constitutive product of the neuroendocrine secretory granule, which can be measured in serum or plasma samples and has been correlated with both tumour mass and patient survival.[40,41] However, a biological chromogranin A standard does not exist and wide variations occur in assay measurements across different laboratories,[40] with results varying dependent on the different antibodies used in the assay. The sensitivity of a chromogranin A measurement is about 60–90% with a specificity of less than 50% due to raised chromogranin A concentrations in many other conditions—including renal failure, cardiac disease, non-neuroendocrine tumours, and the use of proton-pump inhibitors.[40] Several proprietary assays are represented by the chromogranin derivative pancreastatin, but these assays exhibit little advantage compared with chromogranin A except for the fact that they are not affected by the intake of proton-pump inhibitors.[42]

Serotonin and its metabolite 5-hydroxyindole acetic acid (5-HIAA) have been measured in blood and urine samples, respectively, as markers of carcinoid syndrome of predominantly midgut origin. Measurements of circulating serotonin are complex and whether taken from blood or platelet-poor or platelet-rich plasma, need careful consideration to ensure both accuracy and reproducibility.[43] Urine measurement of 5-HIAA needs adherence to a restricted diet because the test sensitivity is about 35% and provides poor prognostic information.[22] Several other potential biomarkers (eg, neuron-specific enolase, tachykinins, and pancreatic polypeptide) have also been found to be suboptimal in neuroendocrine tumour disease, though the progastrin-releasing peptide may be helpful as a marker of primary bronchopulmonary neuroendocrine tumours, as is the COOH-terminal fragment of chromogranin B in patients with renal insufficiency;[33,44] however, use of all these biomarkers in prognosis has great limitations.

On the basis of the need for improved biomarkers for neuroendocrine tumour disease, in addition to the emergence of some novel technologies and approaches to biomarker development in general, a meeting of multidisciplinary experts in the area of oncology was convened in Nashville (TN, USA) in October, 2014. The goal of this forum was to establish a consensus on the state of present art of biomarkers in neuroendocrine tumour disease and to define the specific needs for people with these tumours as novel biomarkers are developed (figure 2).

Methods

A group of 18 experts (including endo crinologists, oncologists, gastroenterologists, surgeons, experts in diagnostic imaging, pathologists, laboratory clinicians, and researchers) in the field of disease diagnosis and management of neuroendocrine tumours were selected from Europe and the USA. The Delphi method[45] was used to achieve consensus on 82 key questions and 25 subquestions, with a 75% agreement as the minimum basis for consensus. Questions were categorised into eight groups: back ground, diagnosis, bronchopulmonary neuroendocrine tumours, use, imaging, histopathology, circulating tumour cells, and novel biomarkers. The first iteration of the statements to be discussed was developed by a core group and distributed to all participants 8 weeks before the conference to eliminate inconsistencies or ambiguous statements. After feedback from all participants, a second list

of statements requiring yes or no answers was distributed electronically 1 month ahead of the consensus meeting, and all participants returned their answers (voting round one; table 2). Results of these statements were made available at the initiation of the meeting. At the meeting, any question with less than 75% previous agreement from round one was then reviewed and discussed by the entire panel and revoted (voting round two; table 3). Voting was anonymous (done by electronic touch pad), and ambiguous, controversial, or non-consensus (<75%) statements were reworded as necessary to attempt to achieve the 75% agreement threshold (voting rounds three to four). Experts in the diagnosis and management of neuroendocrine tumours then shared their assessment by answering specific questions during the conference. Guided by the moderator, the panel debated any conflicting viewpoints, followed by another opportunity to vote on the same question. This process continued with two further rounds of discussion and voting until the consensus threshold was met or it was apparent that no consensus could be achieved. Not all questions resulted in a consensus (tables 2 and 3).

Results

A total of 107 questions were posed and consensus was achieved in 53 (49%) of the 107 questions before the October, 2014, meeting. After statements or questions were reformulated and voting was repeated, final consensus was achieved on 88 (82%) of the 107 questions. Tables 2 and 3 list all these statements and the voting results. One participant was unable to attend the meeting and participate in the voting, therefore the final consensus includes input from this member at round two, but not at rounds three or four.

Discussion on clinical applications

Participants agreed that there was a crucial unmet need for a sensitive and specific neuroendocrine tumour biomarker with a sensitivity of at least 80%, specificity of at least 90%, and positive and negative predictive values of each at 80% or more. These parameters show the reported acceptable standards for biomarker effectiveness, [46,47] which were accepted by the panel of neuroendocrine tumour experts. Ease of use and safety were identified as important factors for biomarker collections, with venous collection indicated as being the most convenient method to acquire appropriate biomarker samples. The impracticability and limitations of serial tumour tissue acquisition to assess treatment responses were noted. Although the panellists agreed that a neuroendocrine tumour biomarker should not be developed for population screening, they did think that biomarkers should provide both diagnostic and prognostic information if possible. Type 0 biomarkers (defined as indicating the natural history of disease[47]) and type 1 biomarkers (showing interventional effects) were considered useful in the identification of neuroendocrine tumours, but type 2 biomarkers (regarded as surrogate clinical endpoint biomarkers) were thought to be a particularly important focus in assessing neuroendocrine tumours. Overall, a biomarker that encompasses all three types (0, 1, and 2) would be ideal.

However, the use of any neuroendocrine tumour biomarker associated with accurate diagnosis and the use of any neuroendocrine tumour biomarker to predict treatment effectiveness were officially agreed, as a consensus, to be of value. Added importance was

placed on the ability to quantify tumour burden in patients with low-volume disease. Multidimensionality (particularly the ability to provide information regarding the proliferative and metastatic capacity of a tumour), in addition to measures of aggressiveness or benignity, were identified as added value information that a neuroendocrine tumour biomarker should provide. However, meeting participants were in agreement that current biomarkers—including chromogranins A, B, and C, pancreastatin, and neurokinin A—were not able to provide this multidimensionality. Furthermore, the panel agreed that all conditions that resulted in false-positive or false-negative biomarker results should be established, as should all specific basic conditions for blood sampling and assaying of a biomarker.

Current biomarkers for diagnosis

Although circulating biomarkers were deemed useful to aid diagnosis, they were not considered mandatory to establish a neuroendocrine tumour diagnosis. Measurement of current biomarkers including chromogranin A, pancreastatin, neurokinin A, neuron-specific enolase, pre-progastrin, pancreatic polypeptide, serotonin, and urinary or plasma 5-HIAA were all considered to be of insufficient value to accurately identify the primary tumour site. This view led panellists to agree that a circulating biomarker should be specific to neuroendocrine tumours for diagnosis. Panellists also agreed that the measurement of circulating biomarkers that could differentiate between functional and non-functional tumours would be of benefit, particularly for patients with non-specific symptoms that might be suggestive of a neuroendocrine tumour-mediated clinical syndrome (eg, flushing in suspected carcinoid syndrome or diarrhoea, perhaps due to a vasoactive intestinal peptide tumour). Although no consensus could be reached on whether currently used biomarkers correlate with tumour burden, circulating biomarkers measurements were noted not to correlate with tumour grade and did not differentiate low-level malignancy from high-grade disease. The consensus of this group was that current, general neuroendocrine tumour monoanalyte biomarkers do not meet the standard of care.

Bronchopulmonary neuroendocrine tumours

Unanimous agreement was reached that biomarkers for gastroenteropancreatic neuroendocrine tumours were inadequate for bronchopulmonary neuroendocrine tumours. Additionally, panellists agreed that specific circulating biomarkers to bronchopulmonary neuroendocrine tumours were not available and that currently used markers (eg, chromogranin A) did not have the sensitivity or the specificity to diagnose bronchopulmonary neuroendocrine tumour disease.

Clinical use

The panel agreed that circulating biomarkers had many roles in neuroendocrine tumour disease and that they should be used for tumour diagnosis, disease follow-up, and identification of patients' medical treatment responses. Additionally, circulating biomarker measurements should be used in the definition of surgical effectiveness and the aggressiveness of remnant disease. Microscopic disease detection and definition of surgical cure were considered appropriate functions of circulating bio markers, as was the ability of a biomarker to predict disease relapse and to function as a prognostic indicator.

Imaging

Imaging was regarded as the best modality to measure treatment effectiveness; however, no consensus could be agreed as to the optimum imaging method to use in practice and in all different tumour types. Most panellists accepted that CT-MRI in conjunction with somatostatin receptor imaging was appropriate as a routine measure, which is consistent with standard of care for neuroendocrine tumour imaging. In centres of excellence or with specific expertise in treatment of this disease, PET-CT with ^{68}Ga-labelled somatostatin analogues or ^{18}F-DOPA was considered the best method of neuroendocrine tumour imaging. Furthermore, panellists acknowledged the variability in and between centres with respect to imaging protocols and quality, presenting difficulties in disease follow-up. The panellists were in agreement that the RECIST criteria,[48] developed to assess treatment responsiveness for clinical trials in solid tumours, were not appropriate for all neuroendocrine tumours. Consensus was made among the panellists that circulating biomarkers do provide a useful adjunct to imaging methods and that a combination of information from both imaging circulating biomarkers and radiographic images would be ideal. However, no imaging studies correlate well (>80%) with current circulating biomarkers.

Histopathology

Panellists agreed that immunohistochemistry for chromogranin A had value in the diagnosis of neuroendocrine tumours and that other tissue biomarkers were of use. However, both neuron-specific enolase and pancreastatin were not considered to be useful for immunohistochemistry. Although no consensus was made regarding the use of phosphohistone H3 as a marker for this disease, the panel agreed that the mitotic spindle marker might be better than mitotic counting in quantifying tumour proliferation; it has not yet been extensively evaluated in neuroendocrine tumours. There was disagreement about whether the measurement of Ki-67 as a proliferative marker was equivalent in the USA and Europe. All panellists agreed that Ki-67 measurements can vary among laboratories and that the interobserver and intraobserver variability were an issue in accurately defining proliferation rates. Most concluded that the Ki-67 index should be measured by digital image analysis, even though this technology is not universally available and has not been shown to be more accurate than manual counting. Use of so-called eyeballed estimates by pathologists of the Ki-67 proliferation was viewed as inaccurate. Panellists disagreed about the potential use of the mitotic index or Ki-67 as a proliferative marker, which was reflected in time and financial constraints in different geographical locations needed to undertake manual counting of many high-power fields, as per present guideline recommendations. All participants concurred that the Ki-67 index might not be uniform throughout a tumour, and that the highest Ki-67 level constituted a proliferation hot spot that defines the ultimate tumour grade.

Although no consensus could be achieved about whether more than one biopsy should be taken to assess proliferative activity in metastatic disease, all participants did agree that practicality dictates that it should be less than four. All participants also agreed that the Ki-67 index of a tumour could change during the clinical course of the disease. Consensus was achieved regarding the necessity of a standardised grading system. Consensus was achieved regarding the use of measurements for the proliferative potential of remnant

disease after surgical resection. Participants also agreed that the Ki-67 index could not predict micrometastases. Although consensus was achieved connecting recurrence prediction and a high Ki-67 index, the group noted that this was only relevant if measured in populations and was not useful in individuals. Overall, the group agreed that circulating biomarkers should be associated with proliferation, but current circulating biomarker levels (chromogranin A, pancreastatin, 5-HT [serotonin]) do not correlate with highly proliferative, aggressive disease. However, the group concluded that despite the limitations of Ki-67, it represented the best available index of tumour biology and should remain part of standard of care with respect to the pathological assessment of neuroendocrine tumour disease.

Circulating tumour cells

Although many different technologies are available for the enumeration and isolation of circulating tumour cells, the CellSearch system is the only circulating tumour cell test to have been approved by the US Food and Drug Administration. The CellSearch system enriches for circulating tumour cells by the use of epithelial adhesion molecules as a selection marker and is approved for monitoring breast cancer, prostate cancer, and colon cancer. A single large series[35] showed the potential use of circulating tumour cells in metastatic neuroendocrine tumour disease, but the panellists felt that further validation and corroboration would be necessary before accepting circulating tumour cells as a valid biomarker in neuroendocrine tumour disease. Consensus was established that current circulating tumour cell analyses are not reliable to detect all neuroendocrine tumours, that circulating tumour cells were not sensitive and specific as a diagnostic method for all neuroendocrine tumours, and that the sensitivity of circulating tumour cells was not the same to detect different types of neuroendocrine tumours or was specific for any subgroup of neuroendocrine tumour. No overall consensus was achieved about whether circulating tumour cells correlated with tumour burden, grade, or prognosis, and participants agreed that further studies on the use of circulating tumour cells as biomarkers are needed.

Novel biomarkers

Several new biomarkers for neuroendocrine tumours have been proposed, ie, many -omics approaches (figure 3). Some novel biomarkers of neuroendocrine tumours are in advanced clinical development, including a multianalyte whole blood RNA multigene signature with algorithmic analysis, specifically developed for gastroenteropancreatic neuroendocrine tumours.[23] Consensus was reached regarding all nine statements about novel biomarker development. Panellists agreed that new monoanalytes should be identified for neuroendocrine tumours, but agreed that these are more likely to be less effective compared with multianalytes, especially in view of the reported high sensitivity and specificity (>95%) of the multigene signature.[24] Agreement was unanimous that results from genomic technology should be used to identify novel biomarkers for neuroendocrine tumours and that circulating DNA should be assessed. miRNAs were considered potentially useful as circulating biomarkers and enthusiasm was noted for a metabolomic approach to identify novel circulating biomarkers.[49] Panellists agreed that some novel monoanalyte assays, including connective tissue growth factor for carcinoid heart disease (CCN2)[39] or paraneoplastic Ma antigen 2 (PNMA2) for small intestinal neuro endocrine tumours[50] were not available for use in clinical practice. Furthermore, general consensus was reached that

the absence of specific mutations and unique methylation patterns in neuroendocrine tumour disease would restrict the use of these markers in the development of a neuroendocrine tumour biomarker.

Discussion

Originally developed in the 1950s, the Delphi method[45] has been used extensively to develop consensus in health care. Although Delphi studies have varied from the original methodology, all use a form of consensus to develop a reliable agreement from a group of experts on a specific topic. Participants' individual responses are unknown to the rest of the group.[51] Consensus is reached in a Delphi study if a previously agreed percentage of participants have rated items similarly. In Delphi study literature, a flux exists as to what constitutes the correctly needed percentage of consensus; these can vary between 50% and 100%.[51] In our assessment, we used a consensus level of 75% as clear evidence of a majority opinion. Voting was anonymised (by the use of electronic touch pads) and followed by discussion if no consensus was reached. 17 (94%) of 18 participants completed all three rounds, which is similar to other Delphi-based studies[52,53] for neuroendocrine tumour disease. Overall, 88 (82%) of 107 statements and questions reached consensus by the end of our Delphic process, with only 19 (12%) questions remaining unresolved (no consensus achieved).

The major conclusion achieved by the panel regarding biomarkers for neuroendocrine tumours was that there is a crucial unmet need for high accuracy biomarkers for the diagnosis and management of the disease, and that multidimensionality would be an additional desired feature for a neuroendocrine biomarker to enable a clinician to gain information about diagnosis, prognosis, tumour bulk, and responsiveness to treatment. No biomarker yet fulfils these needs, with most biomarkers not even close to reaching the necessary diagnostic and prognostic requirements for effective disease management. Panellists expressed the opinion that despite the use of fairly sensitive imaging modalities for the follow-up of neuroendocrine tumour disease, an important role still exists for biomarkers in providing additional prognostic information to optimise disease management. Thought should be given to the development of a combinatorial system whereby imaging and circulating biomarkers used together provide additional information. The often indolent nature of many neuroendocrine tumours and the restricted ability of both imaging and pathology to provide ongoing real-time prognostic information are problematic. Thus, clinicians need more informative and accurate biomarkers than those available to predict aggressive disease emergence or loss of treatment effectiveness.

Overall, the panel deemed that current biomarkers exhibit many major limitations that need to be addressed. First, the scarcity of adequate performance metrics in providing sufficient sensitivity and specificity remains problematic. Second, there are serious limitations in the ability of monoanalytes to provide information about disease progression, drug efficacy, and tumour biological behaviour. Finally, linear correlations between tissue-derived data, imaging-derived evidence, and circulating biomarker information have not been established.

In light of the general dissatisfaction with current circulating biomarkers for neuroendocrine tumour disease, two major types of novel biomarkers were considered: multigene signatures and circulating tumour cells. Particularly, multigene signatures were thought to be of clinical use in multianalyte strategies to generate molecular data that could provide real-time information about tumour activity and treatment response. There was consensus among panellists that the measurement of circulating tumour cells is of potential interest if cells could not only be accurately identified, but also if their genomic information could be investigated. Data on the use of circulating tumour cells as prognostic markers are scarce and described from a single-centre study with a correlation with tumour burden in metastatic neuroendocrine tumour disease.[35,36] The panel of experts expressed the need to further investigate circulating tumour cells to corroborate these initial findings in addition to thinking about other applications and platforms associated with the biology of circulating tumour cells in the future. Although the technology and concept of circulating tumour cells are an attractive proposition, the present technology platform to detect circulating tumour cells needs further validation before it is accepted as an effective biomarker for neuroendocrine tumours.

A major issue that became apparent in the consideration of limitations with current biomarkers is evident in the emerging power provided by multianalyte algorithmic analysis in establishing the clinical use of biomarkers. So far, all biomarkers of circulating tumour cells have been monoanalyte and therefore severely restricted not only in sensitivity and specificity, but also in the paucity of information they provide. Because neuroendocrine tumours represent diverse neoplastic entities, monoanalyte tests have predictably not met stringent sensitivity and specificity criteria. An exception is if the monoanalyte is a secretory product of a specific tumour type, such as insulin or gastrin or if monoanalyte measurements (eg, chromogranin A and NT-pro-BNP) are combined in carcinoid heart disease.[38]

A new generation of tests called multianalyte assays with algorithmic analyses are based on correlating and normalising several sets of variables. These tests are increasingly being used and being applied to different diseases—eg, FibroSure (FibroTest) is a blood-based, biochemical, algorithmic test for liver damage used in the detection of hepatitis C.[54] Advantages of FibroSure include non-invasiveness, repeatability, and absence of discomfort or complication risk for patients compared with a biopsy.[55] Similarly, MammaPrint (Agendia, Irvine, CA, USA) is a 70 gene metastatic breast cancer assay that is one of three often used and commercially available tests (the other two are Oncotype DX [Genomic Health, Redwood City, CA, USA] and MammoStrat [Clarient Diagnostic Services, Aliso Viejo, CA, USA]). MammaPrint acts as a specific measure of metastatic and recurrent potential of breast cancer tumours.[56]

Multianalyte biomarkers exhibit difficulties since their technical complexity is often prohibitive and might need specific laboratory facilities in addition to the ability for sophisticated calculations. Algorithmic constructs and analysis necessary to interpret the results contribute to an increased complexity in deciphering the results of the multianalyte gene tests. In some instances, transport and handling of samples needs the application of rigorous standards. Development and application of multianalyte biomarkers is therefore much more interdisciplinary and complex than the development and application of single-

analyte biomarkers, necessitating a comprehensive statistical and systems approach for the identification and the use of new panels.[57] However, the multianalyte assays with algorithmic analyses strategy is well accepted for defining the complex biology of the disease, especially neoplasia that represents the future of biomarker development.[58] Overall, panellists were encouraged by developments in the past 5 years in nucleic acid-based technologies, but expressed reservations in relying on specific mutations or methylation patterns to understand the biology of neuroendocrine tumours. This concern reflected both the heterogeneity of neuroendocrine tumours and the paucity of information available regarding appropriate treatment options.[59]

Our panel considered microRNA profiling to have potential as a neuroendocrine tumour biomarker in view of their known dysregulation in neoplasia. The global miRNA profiles of pancreatic and small intestinal neuroendocrine tumours[60–63] are distinguished by non-overlapping expression in every type of tumour examined. Overall, a weak association between miRNA expression concentrations in serum and tissue has been reported. Both up and down regulation of miRNA expression concentrations has been noted in neuroendocrine tumours, suggesting that the use of this marker could be complex and might need algorithmic analysis to define its clinical use. In 2013, the American Association for Clinical Chemistry[64] noted that the detection and quantification of miRNA expression concentrations are “neither robust, rapid, simple, accurate, reproducible, nor inexpensive, and that weak correlations existed between different detection platforms or from the same platform using reagents from different vendors”. Additionally, data normalisation is regarded as problematic. Up to now, minimum clinical data are applicable to miRNA expression concentration measurements in neuroendocrine tumour disease.

Panellists gave the strongest support to the use of emerging biomarkers in multianalyte technologies based on neuroendocrine tumour genomics, particularly the NETest (Wren Laboratories, Branford, CT, USA)—a multianalyte qRT-PCR assay based on 51 marker genes with algorithmic analysis with a high sensitivity (>95%) and specificity (>95%) in the detection of all gastroenteropancreatic neuroendocrine tumours. This test provides a multidimensional (gene cluster analysis) assessment of disease status (eg, proliferome, metabolome, or secretome) and treatment effectiveness in patients with neuroendocrine tumour disease.[65,66] Further more, it is significantly more accurate than other monoanalytes used[23–26] and is not affected by the use of proton-pump inhibitors.[26] Panellists regarded the methodology for this multianalyte algorithmic test acceptable, but noted the need for information on whether the test was positive in non-gastrointestinal neuroendocrine tumours (eg, para gangliomas) or cancers with mixed epithelial or neuroendocrine phenotype cancers (eg, prostate cancer). The experts were of the opinion that such laboratory tests should be initially undertaken at a single central laboratory.

Data were not available to assess whether multianalyte assays with algorithmic analyses gene transcript analysis could differentiate neuroendocrine tumours from other hormonally driven (eg, thyroid and menopause) or anxiety and allergic disorders that mimic carcinoids (fauxnoids). A consideration was whether multianalyte assays with algorithmic analyses tests of this kind might provide adjunctive information for imaging or be useful in the stratification of patients at high risk or low risk of recurrence in conjunction with

histopathological information. Similarly, genomic multianalyte assays with algorithmic analyses tests were judged to have potential in accurately defining disease activity (by gene cluster analysis) in patients with residual tumours, thereby helping guide disease management. Thus, identification of high activity, low volume tumours might result in an increasingly aggressive intervention, including surgery and chemotherapy. Our panel thought that the NETest has a role in many situations including: identification of disease progression, definition of drug efficacy, and assessment of the completeness of primary tumour surgical resection, or decrease of metastatic tumour burden subsequent to hepatic resection or ablation procedures.

Conclusion

Significant consensus was reached among a group of experts in neuroendocrine tumour disease from different disciplines and countries that circulating biomarkers for neuroendocrine disease have substantial limitations and circulating biomarkers that accurately reflect disease activity and therapeutic efficacy are crucial requirements. Unanimous consensus was made by the panel that multianalyte measurement strategies of genomic indices are representative of the appropriate future direction of investigation (figure 4). Although tissue-based biomarker data can provide such information, blood-based analyses were preferred because of their easy accessibility of the compartment and the ability for repeated real-time point samplings. The NETest was regarded to have great potential for clinical use, but advances in circulating tumour cell technology (eg, genomic interrogation of cell content) and miRNAs specific to neuroendocrine tumours needed further investigation. Validation in different clinical studies of the multidimensionality of the multianalyte assays with algorithmic analyses class of tests is needed to confirm optimum areas of clinical use. Our panel of experts concluded that clinical trials using the blood gene transcript analysis should be actively pursued to identify and confirm how such information can best advise clinical management. Blood testing of patients with this disease once every 3 months was deemed advisable to confirm disease stability or monitor progression. Additionally, progressively raised values were noted to be of particular clinical relevance because they were consistent with advancing disease or they were evidence of ineffective treatment.

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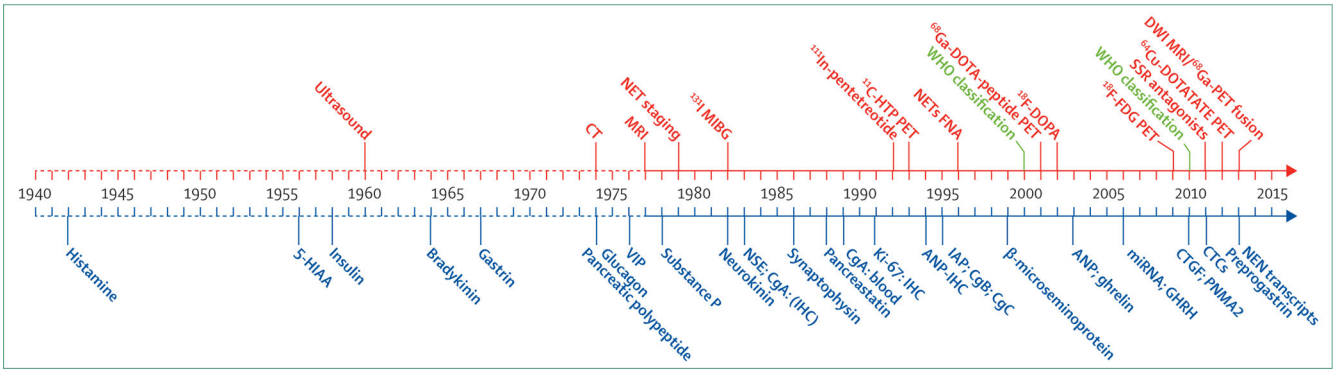

Figure 1. Timeline of diagnostic advances in neuroendocrine tumours

40 circulating monoanalytes of varying sensitivities and specificities have been developed since 1942 (only those of accepted clinical use are shown). More recent developments have focused on the use of novel technologies to quantify circulating tumour cells in addition to multianalyte-based molecular strategies (with miRNAs) and circulating neuroendocrine neoplasia (NEN) transcripts (NETest). Timing of the staging protocols (in 1979) and WHO classifications (2000 and 2010) are shown. Image-based modalities (anatomical and functional; red) are referenced to provide a framework to compare with biomarker development (blue). 5-HIAA=5-hydroxyindole acetic acid. NET=neuroendocrine tumour. VIP=vasoactive intestinal peptide. NSE=neuron-specific enolase. Cg=chromogranin. IHC=immunohistochemistry. CTGF=connective tissue growth factor. CTC=circulating tumour cell.

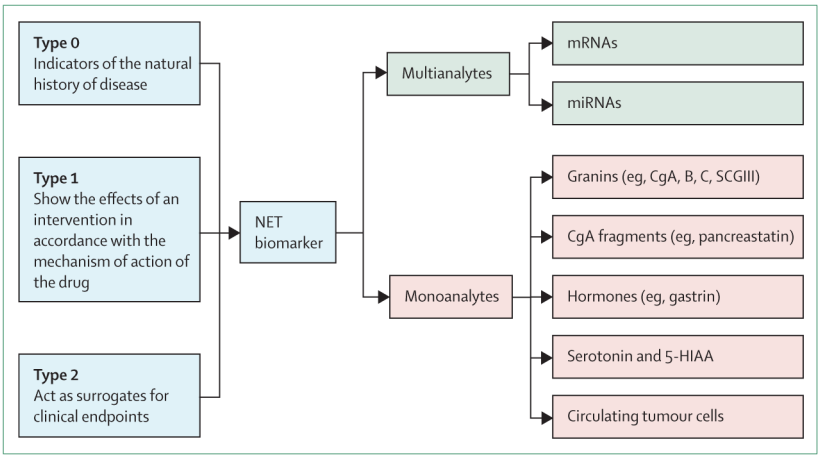

Figure 2. Categories of circulating neuroendocrine neoplasia biomarkers

The principle class of biomarkers is monoanalytes, which are generally quantitated using immunoassay except for circulating tumour cells that are assessed by epithelial antigen-dependent sorting and microscopy. The multianalyte class of biomarkers includes miRNAs and mRNA. miRNAs have not yet been shown to be of clinical use but circulating mRNA-based strategies have been shown to have a high sensitivity and specificity in initial clinical studies. NET=neuroendocrine tumour. Cg=chromogranin. SCG=secretogranin. 5-HIAA=5-hydroxyindole acetic acid.

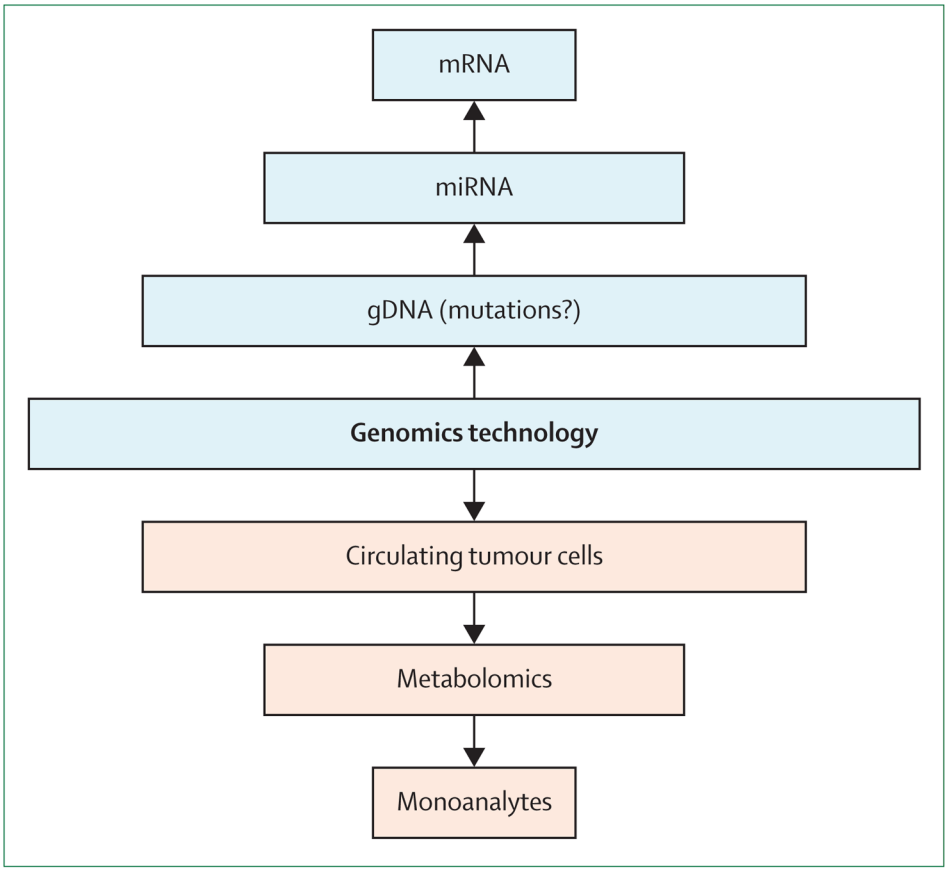

Figure 3. Overview of progress in biomarker indices of neuroendocrine tumours

Integration of genomics and advances in technology platforms have been instrumental by developing novel neuroendocrine neoplasia biomarkers, resulting in the development of novel monoanalyte assays, metabolomic screens, and advances in circulating tumour cell assessment. Similarly, microfluidic large-scale integration strategies, single-cell whole genomic analysis, and assessment of circulating DNA for informative mutations are methods that will advance the biomarker index of neuroendocrine neoplasia. Present methods under appraisal as diagnostic and prognostic testing platforms include multianalyte strategies—such as quantification of circulating neuroendocrine neoplasia transcripts and miRNA.

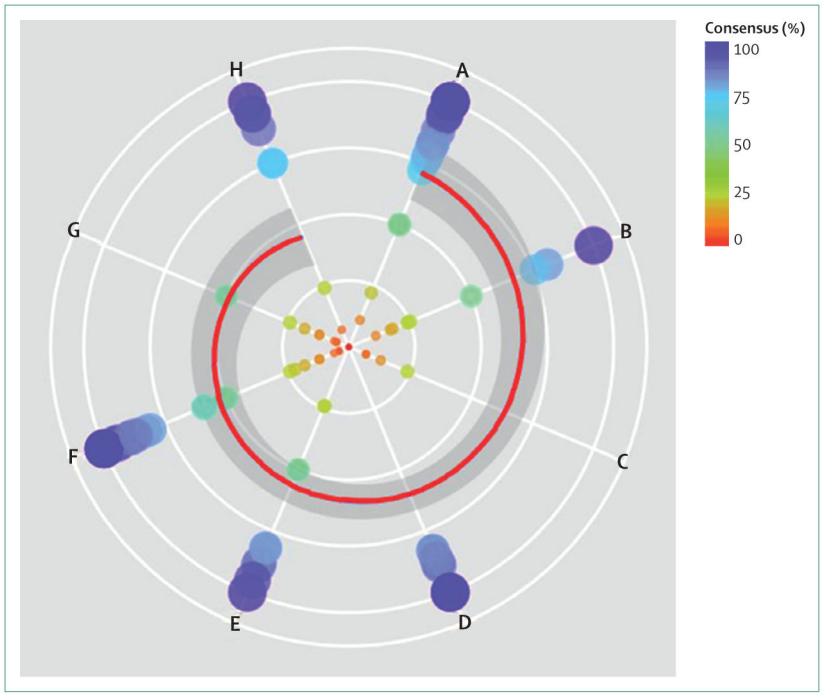

Figure 4. Radar chart of biomarker consensus

Multivariate data plot that includes information from every section of questions in addition to an assessment of the overall consensus achieved. Groups A–H refer to individual biomarker questionnaire groups. Heat map configuration shows the percentage of individual questions in every group. Consensus value for the individual questions is shown as individual coloured dots. The spiral red fit line with SD area (grey) provides a progressing appreciation of consensus as evidenced by the inwardly decreasing amplitude of the semicircular red line. Circular advance of developing consensus is represented by the circle as it moves from category A–G. Highest inward inflection (high consensus) achieved at group H, showing the need to focus on novel neuroendocrine tumour biomarkers. Group A=background. Group B=current biomarkers for diagnosis. Group C=bronchopulmonary neuroendocrine tumours. Group D=use. Group E=imaging. Group F=histopathology. Group G=circulating tumour cells. Group H=novel neuroendocrine tumour biomarkers.

Table 1

Neuroendocrine tumour biomarkers

	Type	Location of primary tumour	Sensitivity	Specificity
Chromogranin A[15–21]	0–2	All sites	43–100%	10–96%
u5-HIAA [*][22]	0–2	Midgut	35%	Up to 100%
NET-test[23–26]	0–2	All sites	90–98%	90–98%
Substance P [†][27]	0	Midgut	32%	85%
Pancreatic polypeptide[28–30]	0	Pancreas, midgut	31–63%	Up to 67%
Pancreastatin[15,16,21]	1	Pancreas, midgut	64%	58–100%
Neuron-specific enolase[22]	1	All sites	33%	Up to 100%
Neurokinin A [*][31]	1	Midgut	88%	No data
Chromogranin B[15,32]	1	All sites, colon	57–99%	Up to 100%
ProGRP[33,34]	1	Lung	24–99% (15–54% if lung excluded)	43%
CTCs[35,36]	0, 2	Pancreas, midgut	<40%	95%
Gastrin [‡][37]	0, 2	Stomach, duodenum, pancreas	Up to 100%	<20%
Insulin [§][37]	0, 2	Pancreas	Up to 100%	<20%
NT-proBNP [¶][38]	2	Midgut	87%	80%
CTGF [//][39]	2	Midgut	88%	69%

u5-HIAA=urinary 5-hydroxyindole acetic acid. NET=neuroendocrine tumour. CTC=circulating tumour cell. CTGF=connective tissue growth factor.

[*] Midgut NETs.

[†] An undecapeptide member of the tachykinin neuropeptide family.

[‡] In gastrinomas.

[§] In insulinomas.

[¶] In carcinoid heart disease.

[//] For right ventricular dysfunction.

Table 2

Responses to Delphi session one: routine molecular diagnostics

	N	Consensus agreed (%)
Background		
Is there a critical unmet need for a sensitive and specific NET biomarker?	17	95%
Should the sensitivity of a biomarker for detecting disease be ≥80%?	17	100%
Should the specificity of a biomarker for detecting disease be ≥90%?	14	78%
Is an acceptable positive predictive value ≥80%?	14	81%
Is an acceptable negative predictive value ≥80%?	13	76%
Is the ease and safety of biomarker collection an important consideration?	18	100%
Is venous collection the most convenient method?	16	88%
Should tumour tissue always be collected?	15	No consensus
Should a NET biomarker be developed for population screening?	17	No: 89%
Can circulating biomarkers provide several sets of information?	17	100%
Do type 0 markers indicate the natural history of disease and provide useful information for NET treatment?	17	100%
Are type 1 markers that capture the effects of an intervention in accordance with the mechanism of action of the drug, of use in NETs?	15	100%
Should type 2 markers, those that are used as surrogates for clinical endpoint reflecting patient health, functionality, and survival, be a focus in NETs?	15	83%
Is this type 2 marker more clinically useful than type 0 markers?		No consensus
Is this type 2 marker more clinically useful than type 1 markers?		No consensus
Is type 0, 1, or 2 biomarker the ideal type of circulating biomarker?	17	100%
Is the use of a NET biomarker linked to accurate diagnosis?	14	78%
Is a NET biomarker of value if it predicts therapeutic efficacy?	12	72%
Is a NET biomarker's ability to quantify tumour burden important?		No consensus
Should a circulating biomarker measurement be multidimensional (provide more than one set of information)?	15	83%
What additional information should a multidimensional biomarker provide?		
Tumour proliferation?	13	76%
Tumour secretion?		No consensus
Tumour metastasis?	14	78%
Aggressive tumour behaviour?	16	94%
Benign tumour behaviour?	15	83%
Do any of the present circulating biomarkers provide multidimensional data?	14	No: 78%
Should all conditions known to produce false-positive or false-negative outcomes of a biomarker be known and described?	17	95%
Should all basic conditions for blood sampling and assessment of a biomarker be known and described?	17	100%
Diagnosis		
Are measurements of circulating biomarkers necessary for NET diagnosis?	13	No: 75%
Are measurements of circulating biomarkers useful for NET diagnosis?	17	100%
Can measurement of circulating biomarkers be used to identify the primary tumour site?	15	No: 82%
Should circulating biomarkers be tumour-type specific?	15	81%
Are known circulating biomarkers useful in the diagnosis of all NETs?	13	No: 76%

	N	Consensus agreed (%)
Should the measurement of circulating biomarkers differentiate between functional and non-functional tumours?	13	76%
Do circulating biomarker measurements correlate with tumour burden?		No consensus
Do circulating biomarkers measurements correlate with tumour grade?	16	No: 89%
Do circulating biomarkers differentiate low-level malignancy from high-grade disease?	15	No: 83%
Bronchopulmonary NETs		
Are biomarkers for GEP NETs adequate for bronchopulmonary NETs?	13	No: 76%
Are specific circulating bronchpulmonary NET biomarkers available?	16	No: 87%
Is the sensitivity of the marker acceptable?	16	No: 93%
Is the specificity of the marker acceptable?	16	No: 93%

NET=neuroendocrine tumours. GEP=gastroenteropancreatic.

Table 3

Responses from Delphi session two: use of molecular diagnostics

	n	Consensus (%)
Use		
Should circulating biomarkers be used for tumour diagnosis?	16	88%
Should circulating biomarkers be used in disease follow-up?	17	100%
Are circulating biomarker measurements useful to identify therapeutic response?	15	83%
Should circulating biomarker measurements be useful to define surgical effectiveness?	15	83%
Should circulating biomarkers define aggressiveness of remnant disease?	16	89%
Should circulating biomarkers detect microscopic disease?	15	83%
Should circulating biomarkers be used to define a cure?	15	86%
Should circulating biomarkers predict disease relapse?	17	100%
Should circulating biomarkers be prognostic?	17	100%
Imaging		
Is imaging the best modality to measure treatment effectiveness?	16	89%
Is PET-CT the most useful?		No consensus
Is CT and MRI useful as a routine measure?	16	88%
Is PET-CT the most sensitive method for imaging tumours?	14	82%
Is imaging accurate and reproducible as a measure of treatment effectiveness?		No consensus
Is intraunit or centre variability in imaging an issue for disease follow-up?	14	82%
Are the RECIST criteria appropriate for NETs?	13	No: 76%
Is imaging more sensitive than circulating biomarkers?		No consensus
Are circulating biomarkers a useful adjunct to imaging?	17	100%
Does current circulating biomarker measurements correlate with imaging?	13	No: 76%
Is the ideal strategy a combination of a circulating biomarker and imaging information?	17	95%
Histopathology		
Is immunohistochemistry for CgA of value in diagnosing NETs?	17	95%
Are there other tissue biomarkers that have utility?	15	83%
Are other tissue markers useful for diagnosis?	17	100%
Are other tissue markers useful for prognosis?		No consensus
Are other tissue markers useful for targeted therapy?		No consensus
Does NSE immunohistochemistry have use?	15	No: 82%
Does pancreastatin immunohistochemistry have use?	16	No: 88%
Is PHH3 a better marker than mitotic counting to quantify tumour proliferation?		No consensus
Are Ki-67 measurements equivalent in the USA and in Europe?		No consensus
Does Ki-67 vary among laboratories?	16	94%
Is interobserver and intraobserver variability an issue to define proliferation?	16	89%
Should the Ki-67 index be determined by manual counting?	13	No: 76%
Should the Ki-67 index be determined by eyeballed estimate?	14	No: 78%
Should the Ki-67 index be determined by digital image analysis?	16	89%
Is the Ki-67 index uniform throughout a tumour?	18	No: 100%

	n	Consensus (%)
Does the highest Ki-67 level constitute a proliferation hot-spot?	18	100%
Should more than one biopsy be taken to determine the Ki-67 index of metastases?		No consensus
Should more than four biopsies be taken to determine the Ki-67 index of metastases?	17	No: 100%
Can the Ki-67 index of a tumour change over time?	18	100%
Is a standardised grading necessary?	18	100%
Are G1 and G2 NETs treated differently after complete resection?	16	No: 94%
Are G1 and G2 NETs treated differently in the metastatic setting?		No consensus
Is the G3 category homogeneous?	16	No: 94%
Should G3 be defined as Ki-67 index >20%?		No consensus
Are Ki-67 measurements important in the assessment of the proliferative potential of remnant disease?		No consensus
Can Ki-67 predict micrometastases?	16	No: 88%
Does the Ki-67 of a tumour predict disease recurrence in populations but not in individuals?	14	81%
Should circulating biomarkers correlate with low proliferative disease?	16	87%
Do circulating biomarkers correlate with high proliferative (aggressive) disease?	16	No: 94%
Circulating tumour cells		
Are current CTC analyses reliable for detecting all NETs?	17	No: 95%
Are CTCs sensitive and specific as a diagnostic for all NETs?	16	No: 88%
Is the sensitivity of CTCs the same for detecting different types of NETs?	17	No: 95%
Are CTCs sensitive and specific for a specific type of NET?	17	No: 100%
Does CTC analysis correlate with tumour burden?		No consensus
Do CTCs correlate with small intestinal NET burden?	16	No: 88%
Do CTCs correlate with pancreatic NET burden?	16	No: 94%
Does CTC analysis correlate with tumour grade?	13	No: 76%
Can CTC analysis be used as a prognostic?		No consensus
Can CTC analysis be used as a predictive biomarker?	15	No: 82%
Novel biomarkers		
Are novel monoanalyte assays available for use in clinical practice?	13	76%
Should new monoanalytes be identified for NETs?	17	95%
Are monoanalyte measurements less likely to be effective compared with multianalytes?	16	89%
Should results from genomics technology be used to identify novel NET biomarkers?	18	100%
Are sufficient specific mutations useful for the development of a NET biomarker?	16	No: 93%
Are changes in methylation patterns useful to define a NET?	17	No: 100%
Should circulating DNA be assessed?	13	75%
Would miRNAs be useful as a circulating biomarker?	13	75%
Would a metabolic approach be useful to identify novel circulating biomarkers?	17	95%

RECIST=Response Evaluation Criteria in Solid Tumors. NET=neuroendocrine tumour. CgA=chromogranin A. NSE=neuron-specific enolase. PHH3=phosphohistone H3. CTC=circulating tumour cell.