Discussion and Future Perspectives

DISCUSSION

Neuroendocrine tumors (NETs) are a group of neoplasms with highly variable presentation and prognosis. Therefore, the selection of biomarkers and treatment needs to be tailored in the individual patient. This thesis attempted to further elucidate the correct selection of biomarkers and therapy for NETs and highlight quality of care.

Diagnosis and prognosis

Currently, the final confirmation of the diagnosis of a NET is still based on histology, because as to date no circulating liquid biomarker has sufficient diagnostic and prognostic value.[1] The general biomarkers chromogranin A (CgA) and neuron-specific enolase (NSE) are most widely used. They are best validated for prognostic significance, but lack sensitivity in the diagnostic phase. As a diagnostic marker for NETs, CgA has an overall sensitivity of 73% and a specificity of 95%, but many studies exclude patients with interfering conditions and thus the marker is poorly validated in the general population.[2] NSE is mainly elevated in patients with poorly differentiated NETs and small-cell lung cancer. For the diagnosis of NETs in general, NSE has a sensitivity of only 39-43% and a specificity of 65-73%.[3,4] Therefore, both CgA and NSE lack the diagnostic power needed for diagnosing NETs, but they hold some merit in predicting prognosis. Strongly elevated CgA has been associated with a HR of 1.9-4.5 for death, but different cut-offs are used[5,6]. Also at follow-up, interfering conditions still hamper the prognostic value. Elevated NSE levels were associated with a hazard ratio (HR) of 2.0 for death in a single study [7]. But while an increase of NSE or CgA during follow-up can potentially indicate disease progression, it is not always associated with radiological progression and therefore, a biomarker increase alone will seldom lead to a decision change regarding treatment in real practice. As described in **chapter 2** of this thesis, addition of urinary 5-hydroxyindoleacitic acid (5-HIAA) excretion did not add to the prognostic value of this set of biomarkers. 5-HIAA is a metabolite of serotonin and can be measured in plasma and urine with equal reliability.[8] A very elevated urinary 5-HIAA excretion was associated with a shorter survival with a hazard ratio of 1.62 (95% CI: 1.09-2.39). This association with shorter survival has been confirmed in several studies.[9-13] However, after correction for stage, grading and other biomarkers, urinary 5-HIAA excretion was no longer a predictor for survival. Similar results were also reported in the only other study including CgA in the multivariate analysis.[5] **Chapter 3** confirmed this finding in pancreatic NET. In conclusion, CgA, NSE and 5-HIAA are predictive for prognosis, but lack the power to support clinical decision making.

But while the current available biomarkers have certain flaws, they cannot be abolished altogether. NETs can present with a variety of clinical syndromes associated with hypersecretion of hormones like serotonin, gastrin, glucagon, VIP and insulin. Especially these specific biomarkers are of great importance. It is essential that the clinician recognizes the symptoms of neuroendocrine syndromes for timely diagnosis and because they require specific treat-

ment. The diagnosis of neuroendocrine syndromes should be based on a combination of symptoms and an elevated biomarker. This requires the treating physician to know the test characteristics, as for example a randomly elevated insulin is not diagnostic for a insulinoma. When the specific biomarkers gastrin, glucagon, VIP and insulin are correctly used they have a high diagnostic value and this ensures correct treatment of the NET patient .

The need for new diagnostic and prognostic biomarkers remains. New assays using circulating tumor cells (CTC's), circulating tumor DNA or mRNA are currently being studied. The sensitivity of CTC's as a diagnostic test is limited, because only in about 50% of NET patients CTC's can be detected.[14] Several studies have shown promising results, using the "NETest" for the diagnosis or follow-up of NETs. The NETest measures circulating NET transcripts and uses an algorithm to produce a score reflecting the likelihood of a having a NET.[15] The first studies with this test showed very promising results with area's under the curve of more than 0.94.[16] However, there is no fixed cut-off at this time and the undisclosed algorithm limits reproducibility. Furthermore, the NETest performed poorly in a Dutch population with a specificity of only 56%.[17] With the limited performance of the different biomarkers, clinical decision making will probably remain to be based on radiology in the coming years.

Treatment

Chapters 5 to 7 of this thesis demonstrate that PRRT with ^{177}Lu-DOTATATE is effective in treating hypersecretory NETs by reducing the symptomatic burden and improving quality of life. In addition ^{177}Lu-DOTATATE has earlier been shown to increase progression-free survival in patients with low-grade metastatic small intestinal NET and high response rates have been reported in pancreatic NET patients.[18,19] While PRRT is already an effective therapy with relatively few side effects, further steps for optimization of this therapy remain necessary. Currently somatostatin analogues (SSAs) are advised as the first step in the treatment of low-grade NET in international guidelines.[20] They also have an anti-proliferative effect and reduce the secretion of hormones thereby reducing symptoms of the hormonal syndromes.[21-23] This effectivity combined with low toxicity rates have led to SSAs being regarded as the first-line treatment for low-grade NET. ^{177}Lu-DOTATATE has mainly been studied in patients with a progressive NET despite treatment with a SSA. Patients with functioning NETs should continue SSA after progression to continue to reduce hormone secretion, but the strategy for SSAs after PRRT in patients with non-functioning NET is not clear and requires further studies. Also, the timing of PRRT in the treatment of NETs needs further elucidation. There is limited experience with treatment with PRRT as a first line,[19] but a sequencing trial with SSAs has never been performed. Especially the high symptomatic response rates after PRRT for functioning pancreatic NETs could be an argument for PRRT as a first line treatment in these patients. Furthermore, the sequencing with other therapies like everolimus and sunitnib in relation to PRRT should be a topic of future studies.

A new strategy to increase effectivity of PRRT could be to combine ^{177}Lu-DOTATATE with other drugs. The first result of trials combining PRRT with chemotherapy are expected in the coming years. Secondly, the effect of combining PRRT with poly(ADP-ribose) polymerase-1 (PARP) inhibitors shows increases in cell cycle arrest and cell death in vitro.[24,25] As a third, immunotherapy has shown very promising results in various malignancies, like melanoma and lung cancer.[26,27] Currently expanding their clinical use are the immune-checkpoint inhibitors, which act on the interaction between T-cells and tumor cells. The immune checkpoints regulate the immune response to maintain self-tolerance, but tumor cells can also dysregulate the expression of immune-checkpoints as a way to avoid recognition by cytotoxic T-cells. The first immune-checkpoint to be targeted was cytotoxic T-lymphocyte-associated antigen 4 (CTLA4) which down modulates T-cell activation.[28] A second target is programmed death-1 (PD-1). The ligands of PD-1 (PD-L1) are found on the surface of antigen-presenting cells, non-lymphoid cells and cancerous cells. Binding of PD-1 to its ligand PD-L1 inhibits the activation of T-cells.[29] Based on the result of three randomized controlled trials the PD-L1 inhibitor pembrolizumab has become first-line treatment in metastatic non-small cell lung cancer with PD-L1 expression in more or equal than 50% of tumor cells.[30-32] In patient with non-small cell lung cancer with smaller percentages of PD-L1 expression, pembrolizumab did not significantly increase progression-free and overall survival.[31,32] A ≥50% expression of PL-L1 was demonstrated in only 14% of small intestinal NET, but seems to be higher in poorly differentiated NEN.[33,34] In a trial treating patients with small intestinal NET and pancreatic NET with pemprolizumab, less than 25% of patients were eligible for treatment based on PD-L1 positivity on histology. An objective response was seen in 6% of patients with a small intestinal NET and 12% of patients with a pancreatic NET resulting in a progression-free survival of respectively 5.6 and 4.5 months.[35] Higher response rates are seen with spartalizumab, mainly in lung NET and neuroendocrine carcinoma (*Yao J,* ESMO 2018). The relatively low response rates are probably caused by the low-immune response as NETs have a low mutational burden and will thus have limited neo-antigens as a recognition site for the immune system.[36] It can therefore be expected that immune therapy will likely be effective in high-grade NEN only or primary sites with higher mutational burden. In lung cancer with low PD-L1 expression pembrolizumab did become effective in combination with chemotherapy.[37] For NETs immune-checkpoint inhibitors could possibly be used in combination with other therapy, like PRRT. The cell damage caused by PRRT could potentially upregulate the immune response causing by increasing antigen presentation and this can be studied in future trials, but the especially in slow-growing NETs toxicity should be weighed against the potential benefit.

A change in radiopharmacon itself could potentially increase effectivity. In 2006 it was first demonstrated that somatostatin receptor (SSTR) antagonists labeled more receptor sites than agonists potentially increasing tumor uptake.[38] Thereafter the antagonist ^{111}In-DOTABASS (^{111}In-DOTA-pNO$_2$-Phe-c(D-Cys-Tyr-D-Trp-Lys-Thr-Cys)D-TyrNH$_2$) was tested to

four times higher tumor uptake than 111In-DTPA-octreotide.[39] But also SSTR antagonists labeled with PET radioisotopes have been developed. The main advantage of the SSTR antagonist is a lower uptake in normal organs resulting in a higher tumor to background.[40] The higher tumor-background ratio might also be useful for therapeutics PRRT with somatostatin antagonists, resulted higher tumor-to-kidney and tumor-to-bone marrow ratios than PRRT with octreotate with promising response.[41]

Lastly, the treatment with ^{177}Lu-DOTATATE could be expanded to other cancers. There is limited evidence for treating patients with medullary thyroid carcinoma and meningioma with ^{177}Lu-DOTATATE .[42](Beukhof CM, unpublished) **Chapter 5** of this thesis demonstrated a beneficial effect of PRRT with ^{177}Lu-DOTATATE for patients with a paraganglioma, following an earlier study also demonstrating considerable response rates.[43] Considering these two studies and in light of limited treatment options, PRRT with ^{177}Lu-DOTATATE can be recommend for the treatment of paragangliomas. However, the burden of evidence remains limited and would benefit from prospective trials. A randomized controlled trial will probably be hampered by the low incidence of paragangliomas, but a prospective trial with standardized inclusion criteria is feasible and would increase the burden of evidence.

Quality of Care

For various malignancies centralizing care in expert centers has led to a significant increase in quality of care, mainly illustrated by fewer surgical complications and improved surgical outcome. In the Netherlands this has been demonstrated for ovarian, esophageal and pancreatic cancer. Centralization of care resulted in an increase of complete cytoreduction of ovarian cancers (42% to 52%, $p<0.001$).[44] For esophageal cancer, an increase of hospital volume from 20 to 60 esophagectomies per year was associated with a decrease of 6 months mortality (HR 0.67, 95% CI: 0.58-0.77).[45] Thirdly, overall survival for patients with pancreatic cancer in the Netherlands is longer in high-volume centers.[46]

In this thesis, patient were studied who were referred to an expert center for a NEN. It was demonstrated that a high percentage of patients had significant changes in diagnosis or treatment. This could potentially reflect a patient benefit, but survival rates, or patient satisfaction were not studied. One study demonstrated an increase in survival for patients treated in an Australian expert center: median overall survival was 112 months in the expert center in Australia versus only 32 months for patients treated elsewhere.[47] This might be partly caused by patients receiving more lines of therapy, but the large difference in median survival does however suggest a certain bias.

Whether centralization also benefits the patient with a NET has not been proven yet, since only few papers report on patient satisfaction or survival in NET expert centers. However, the rarity of NETs and the lack of guidelines, make the benefit hardly undisputed. The benefit for a patient with a NET lies mainly in the cooperation of multiple dedicated specialties, centralized in the expert center. For the diagnosis, staging and grading of a NET experience in the

pathology diagnosis is required and correct selection and interpretation of nuclear imaging is essential. Thereafter, the treatment of the NET patient needs to be approached through a combination of endocrine and oncological principles. All these specialties are needed for treatment of the NET patient.

Additionally. ENETS has certain requirements to be registered as a so-called "ENETS Center of Excellence" (CoE). These requirements include representation of different medical specialties (as mentioned above), a fixed structure for patient care and a minimum number of NET patients. The fixed structure with a multidisciplinary team (MDT) has been proven effective in various cancers and this benefit can be extrapolated to the NET MDT. However, for NET the volume standard has never been studied, but it is advised by ENETS (80 patients) and through the Dutch SONCOS standardization reports (50 patients). These numbers suggest that volume equals quality, but rather than focusing on a number the emphasis should lie on various fixed quality of care criteria.

In conclusion, centralization has not been proven beneficial for NET patients, but benefit may be assumed. To obtain and maintain the benefit, expert centers should be obligated to continuously analyze their endpoints to prove their benefit and identify points for improvements.

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