Chapter 8

Summary, discussion and future perspectives
Medullary thyroid carcinoma (MTC) arises from the parafollicular C-cells and accounts for approximately 5% of all thyroid cancers.\(^1\) MTC occurs sporadically (75%) or in a familial form as part of one of the inherited syndromes known as familial MTC (FMTC) and Multiple Endocrine Neoplasia type 2 (MEN 2). A MEN2A and MEN2B variant of the syndrome are discerned and mutations in the ‘REarranged during Transfection’ (RET) gene are responsible for these syndromes. Other manifestations of the MEN2 syndromes include pheochromocytoma (MEN 2A and 2B) and hyperparathyroidism (MEN2A) or neurofibromatosis (MEN2B).\(^2\)

At presentation most MTC patients have an asymptomatic palpable solitary thyroid nodule or palpable lymph node(s). Fifty percent of the patients have lymph node metastasis and 10% will have distant metastasis at the time of diagnosis.\(^3,4\) Usually, the first diagnostic procedure for thyroid nodules is fine needle aspiration cytology (FNAC) with ultrasound guidance. However, without the use of specific immunohistochemical analyses, the sensitivity of this procedure for detecting MTC is limited.\(^5\) As MTC originates from the calcitonin producing C-cells, this hormone may also be used as a screening tool in patients with thyroid nodules to detect MTC in an early stage of the disease. This application is a matter of discussion as a proportionate number of patients has an elevated calcitonin caused by other conditions than MTC.\(^6,7\)

The initial treatment of patients with sporadic MTC without identified metastases consists of a total thyroidectomy with an elective lymphadenectomy of regional lymph nodes in the central compartment (level VI) (central compartment dissection (CCD)), according to the current American Thyroid Association (ATA) guidelines. If the disease has already spread to the lateral lymph nodes of the neck, a modified radical uni- or bilateral dissection of the lateral lymph nodes (levels II-V) (lateral node dissection (LND)) is also indicated.\(^8\) As surgery offers the only chance on cure, some surgeons not only perform an elective CCD, but also an elective LND because of a relative risk of nodal (micro) metastases. A considerable number of MTC patients (>50%) are beyond cure, because the disease has been so broadly metastasized, even when MTC presented as a locoregional disease.\(^9,10\) Unlike other types of thyroid cancer, MTC patients will not benefit from adjuvant radioactive iodine treatment.\(^11\) Therefore, adequate surgery in MTC is of crucial importance for optimal locoregional control and potential cure.
Although more than 50% of MTC patients cannot be cured despite extensive surgery, many patients with minimal residual disease have a good life expectancy.\textsuperscript{12,13} To monitor disease progression, careful follow-up, consisting of regular determinations of the tumour markers calcitonin and carcinoembryonic antigen (CEA) is important.\textsuperscript{14} If these tumour markers are increasing, diagnostic work-up with morphological and functional imaging (including Positron Emission Tomography (PET)) is advocated. Calcitonin and CEA doubling times are currently the most reliable markers for progression, but accurate determination requires serial measurements which are time consuming. Early detection of progression may be important, because appropriate therapeutic interventions may delay symptomatic deterioration. Depending on the extent of the disease, based on imaging and the rate of elevation of tumour markers, several therapeutic strategies can be considered; a ‘wait and see’ approach with close monitoring, surgical intervention (for locoregional control) or treatment with systemic (targeted) therapy.

Chemotherapy has not yet been proven to be effective in the palliative treatment of MTC. Recent preclinical and clinical research involving tyrosine kinase (TK) inhibitors, have shown an improvement of the progression free survival in MTC patients.\textsuperscript{15,16} However, it is unknown whether the effectiveness of these inhibitors is dependent on the somatic or germ line RET mutation of the tumour or patient. Different RET mutations give rise to different configurationally changes of the RET protein.\textsuperscript{17} For instance, the mostly found mutation found in sporadic MTC, the ‘MEN2B mutation’, changes the ATP pocket of the RET protein, to which these inhibitors bind. We therefore speculate that selecting patients based on specific RET mutations may increase the effectiveness of targeted therapy. Furthermore, most TK inhibitors have several targets activating multiple pathways, which can cause, next to tumour regression, also side-effects such as cardiac toxicity or hand-foot syndrome.\textsuperscript{18,19} Therefore, careful selection of patients is important in which mutation specific therapy can be of value.

This thesis covers several important clinical issues in the diagnosis and treatment of primary and recurrent (inherited) MTC. The aim of this thesis was to evaluate and to improve both diagnostic and therapeutic modalities in the treatment of patients with MTC by: (1) Addressing the value of calcitonin testing for detection of MTC in patients with thyroid nodules; (2) Evaluating the recommendations regarding surgical treatment by the current ATA guidelines for MTC patients; (3) Investigating the value of \textsuperscript{18}F-FDG PET and \textsuperscript{18}F-DOPA PET for detection progressive recurrent MTC and (4) Evaluating different tyrosine kinase inhibitors for treatment of MTC.
In **Chapter 1** a general introduction and the aims and outlines of this thesis are presented.

In **Chapter 2** three patients with different stages of disease were presented to illustrate the variety in clinical presentation and behaviour of MTC. Based on these patients a short overview of the presentation, diagnosis, treatment and follow-up of MTC was provided.

In **Chapter 3** the diagnostic accuracy of the calcitonin test for detection of MTC in patients with thyroid nodules was evaluated. MTC patients detected in an early stage of the disease have a better prognosis, so early detection of MTC in patients with thyroid nodules can be beneficial. As almost all MTC’s secrete calcitonin, standard determination of calcitonin may detect these tumours in patients with thyroid nodules. However, no consensus exists whether or not to perform routine calcitonin testing in patients with thyroid nodules. A meta-analysis was performed to evaluate the diagnostic accuracy of the calcitonin test. Sixteen studies were eventually included. Summary estimates for different cut-off values were determined. Sensitivity was high for lower basal cut-off values and combined basal and stimulated calcitonin testing. Specificity did slightly increase with higher cut-off values and stimulated testing. Overall, the diagnostic accuracy of calcitonin testing was high. However, for the interpretation of this accuracy, the low prevalence of MTC has also to be taken into account. The rarity of MTC decreases the positive predictive value and thereby the clinical applicability of routine calcitonin testing for the detection of MTC. The low positive predictive value carries the risk of patients being operated unnecessarily. Moreover, the cost-effectiveness of calcitonin screening for the early detection of MTC is still a matter of discussion following the overall low prevalence of MTC.

In **Chapter 4** we investigated, whether the current American Thyroid Association (ATA) recommendations are of clinical benefit for MTC patients with respect to the surgical treatment. No evidence exists for the optimal surgical treatment, especially with regard to the extent of the lateral lymph node dissection (LND). Therefore, different surgical strategies are advocated by experts and guidelines. The ATA guidelines recommend total thyroidectomy and LND of the central compartment (level VI) as the initial treatment. In case of regional node involvement, systematic LND of the lateral compartments (level II – V) should be performed. Retrospectively, we reviewed the surgical and pathology reports of 86 patients and compared the clinical outcome (reoperations, biochemical cure, survival and complications) of the patients treated by ATA-compliant surgery versus the patients treated by ATA-non-
compliant surgery. Furthermore we examined to which extent clinical outcome of patients was influenced by (1) one-step versus a two-step intended curative, (2) the location of the initial curative surgery (experienced referral centre versus non-centre hospital), and (3) patient and tumour characteristics. Our results indicated that patients treated adequately according to ATA guidelines had significantly fewer reoperations compared to the inadequately operated patients. Moreover, these patients remained significantly more often biochemically cured. We could not demonstrate a significant effect on the clinical outcome of the patients treated in an experienced referral centre hospital, but these patients received significantly more often adequate surgery. Tumour size and lymph node involvement showed to be the most important predictors for clinical disease free (DFS) and overall survival (OS).

In Chapter 5 we discussed the role of Positron Emission Tomography (PET) imaging using different radiotracers in the staging and follow-up of papillary thyroid cancer (PTC) and MTC. PET imaging is based on the use of positron emitting isotopes. For the detection of thyroid cancers, several radiotracers are available. $^{18}$Fluorine-Fluorodeoxyglucose ($^{18}$F-FDG), a glucose analogue, is frequently used. While its use for discriminating between benign and malignant thyroid nodules is contradictory, $^{18}$F-FDG can be of value in the follow-up of differentiated thyroid cancer (DTC). Especially in patients with a negative radiiodine scan but a detectable tumour marker thyroglobulin $^{18}$F-FDG can localize disease activity. Other available tracers for the use during follow-up of DTC are $^{11}$C-Methionine PET ($^{11}$C-MET-PET) and $^{124}$Iodine-PET ($^{124}$I-PET). While the value of $^{11}$C-MET-PET seems limited, $^{124}$I-PET may be a superior diagnostic tool in comparison to the $^{131}$Iodine whole body scintigraphy (WBS). In MTC patients, $^{18}$F-FDG also has been used, but $^{18}$Flurorine-dihydroxyphenylalanine ($^{18}$F-FDOPA) seems to be more sensitive. This radiotracer makes use of a strongly upregulated transmembrane transport of amino acids via the large amino acid transporters. In conclusion, PET imaging is a useful diagnostic tool in thyroid cancer although the optimal radiotracer depends on the type of cancer and the intent of imaging (staging/follow-up).

In Chapter 6 we evaluated the outcome of both $^{18}$F-FDG PET and $^{18}$F-DOPA PET with calcitonin and carcinoembryonic (CEA) doubling times in 47 MTC patients. Early identification of MTC patients with progressive residual disease is relevant because appropriate therapeutic interventions may delay symptomatic deterioration. Calcitonin doubling time is the most reliable marker for progression, but for accurate calculation, serial
measurements over a considerable period are needed. Most morphological imaging techniques like CT or MRI have moderate sensitivities for detecting recurrent MTC, but in the last decade $^{18}$F-FDG PET and $^{18}$F-DOPA PET have become available for staging and follow-up of MTC. We assessed the ability of $^{18}$F-FDG PET and $^{18}$F-DOPA PET to discriminate patients with progressive disease and patients with stable disease. PET positivity was compared with biochemical parameters (calcitonin and CEA serum levels and doubling times) and survival. In a subgroup of patients whole body metabolic burden (WBMTB) was assessed with standardized uptake value and the number of lesions. The WBMTB was compared with biochemical parameters.

We observed that $^{18}$F-FDG-PET positivity was significantly correlated with both calcitonin and CEA levels and their doubling times. Although $^{18}$F-DOPA PET positivity was significantly correlated with the calcitonin and CEA levels, no significant correlation existed with doubling times. $^{18}$F-DOPA PET detected significantly more lesions compared to $^{18}$F-FDG PET in the 21 patients included in WBMTB analysis. However, $^{18}$F-FDG PET positive was a more important indicator for poor survival. Both scans are therefore important in the follow-up of patients; while $^{18}$F-DOPA PET is better in assessing the extent of the disease, $^{18}$F-FDG PET can more accurately identify patients with progressive disease.

In Chapter 7 we described the effects of four different tyrosine kinase inhibitors on MTC and PTC cell lines. MTC and PTC can be caused by activating or rearrangements in the RE-arranged during Transfection (RET) gene. This gene encodes for the RET tyrosine kinase (TK) receptor which is involved in cellular growth and proliferation. Several TK inhibitors have been tested in clinical trials, but it is unknown if there is specificity for particular RET mutations and which inhibitor is the most effective. We cultured three cell-lines expressing a MEN2A (MTC-TT), a MEN2B (MZ-CRC-1) mutation, and a RET/PTC (TPC-1) rearrangement. We treated these cell lines with four different tyrosine kinase inhibitors (axitinib, sunitinib, vandetanib and cabozantinib (XL184)) and compared the effect on cell proliferation, RET expression and autophosphorylation, and RET downstream pathways (Mitogen-Activated Protein Kinase (MAPK) pathway; involved in cell differentiation, proliferation and survival).

A dose-dependent decrease in cell proliferation was found in all four tested tyrosine kinase inhibitors. Cabozantinib was the most effective inhibitor of the MEN2A and RET/PTC cell line, whereas vandetanib was the most effective inhibitor for the MEN2B cell line. Both cabozantinib and vandetanib were able to decrease RET autophosphorylation and RET
expression levels in MEN2A and MEN2B cells. However, only vandetanib exerted this effect by inhibiting RET transcription. A marked decrease in RET phosphorylation was detected for RET/PTC cells, but RET/PTC expression was increased after exposure to cabozantinib. With regard to downstream targets of RET, the MAPK pathway, and more specifically Extracellular Signal-regulated Kinase (ERK) phosphorylation and expression, was markedly decreased in MEN2A and MEN2B cells after exposure to cabozantinib and vandetanib respectively. In RET/PTC cells no change in ERK phosphorylation and expression was observed. Our results show that both vandetanib and cabozantinib are potent inhibitors for tumour progression in MTC. We also found a specificity of the TK inhibitors for different RET-mutations, suggesting mutation-specific therapies might be of benefit for MTC and PTC patients.

Discussion and future perspectives

Although several new diagnostic and therapeutic technologies have been developed, the prognosis of patients with medullary thyroid cancer has not improved in the last decades. The current diagnostic and therapeutic approaches will be discussed and future perspectives are provided.

Calcitonin as a routine test in patients with thyroid nodules

Whether or not to perform routine calcitonin testing is still a matter of debate. Although a clearly elevated calcitonin level is highly suggestive for MTC, moderately elevated levels are seen in a large number of non-MTC patients. While the European Thyroid Association (ETA) guidelines recommend routine determination of calcitonin, the ATA guidelines do not recommend either for or against routine measurement. Based on current literature, routine testing is performed in several centres. In our systematic meta-analysis we showed that the calcitonin test had a high diagnostic accuracy in terms of sensitivity and specificity. However, the high diagnostic accuracy itself does not advocate routine calcitonin testing. Due to the low prevalence of MTC in patients with thyroid nodules (range 0.11%-0.85%), the positive predictive value of the calcitonin test is low. This is especially true if a low basal calcitonin cut-off value is used and no additional stimulation tests are performed. With a cut-off value of 10 pg/ml, only 7.5% of patients with an elevated calcitonin level will have a histological proven MTC. Another important problem for evaluation of the routine use of the
calcitonin test is the variation between assays, which make comparison between different study groups difficult.\textsuperscript{25} Although not all patients with elevated calcitonin levels will be operated on, a considerable proportion of these patients will be operated unnecessary, if the supposed diagnosis MTC was not confirmed with immunohistochemical (IHC) based cytological examination.

Cheung et al. performed a formal cost-effectiveness model on the routine calcitonin test in thyroid nodules and concluded that calcitonin testing had a comparable cost-effectiveness to mammography.\textsuperscript{26} However, if we apply our findings in this model, routine calcitonin testing is not cost-effective, especially due to the lower prevalence we established in the evaluated studies. Cost-effectiveness can be improved in several ways, such as applying the calcitonin test only in subgroups of patients with a higher prevalence of MTC. Further studies should focus on identification of such subgroups (e.g. young male patients, or patients with large thyroid nodules). Based on current literature there seems no role for routine calcitonin testing in all patients with thyroid nodules.

Instead of using the calcitonin test as a triage test next to or before FNA, calcitonin testing can also be used as an add-on test after FNA. Determining calcitonin levels in patients with inconclusive FNAC (Bethesda 3) results might be a more cost-effective approach in comparison to routine calcitonin testing in all thyroid nodule patients. Measuring calcitonin in FNA aspirates can increase the sensitivity of FNAC for diagnosis of MTC. Recently the use of FNA-calcitonin measurement has been advocated in addition to routine calcitonin testing as a possible alternative to stimulated calcitonin testing.\textsuperscript{27} The (cost-)effectiveness of this approach is unclear as it still requires routine calcitonin testing and needs further evaluation.

Another application of the calcitonin test may be as a routine pre-operative test. The reported sensitivity of routine pre-operative testing is lower compared to routine calcitonin testing in all patients. This lower sensitivity is likely the result from a verification bias; studies including only pre-operative patients have histological verification in all calcitonin-negative patients while studies including all patients have only histological confirmation in a limited number of calcitonin-negative patients (e.g. operated for other causes).\textsuperscript{28,29} The sensitivity of pre-operative calcitonin testing is for detection of MTC is higher than FNAC and can increase the rate of correct pre-operative diagnoses. This can result in more adequate initial surgical procedures which is of crucial importance in MTC patients. Few studies have reported on the value of pre-operative calcitonin testing and more research is needed whether this approach can be cost-effective.
Summary, discussion and future perspectives

Optimal surgical treatment for MTC patients

Although the current ATA guidelines provide specific recommendations for the surgical approach, the effect of adherence to these recommendations on the outcome of MTC patients is unclear. We demonstrated that patients who were not treated adequately according to guidelines had more locoregional reoperations and less biochemical cure at follow-up. We also observed that patients treated in a non-centre institute had less often adequate surgery compared to patients treated in a centre. Our findings underscore the importance to organize the treatment of MTC patients in specialized tertiary referral centres, with maximal surgical experience but also a multidisciplinary approach including endocrinologists, surgeons, radiologists, nuclear physicians and pathologists. One of the main problems in the treatment of MTC is to determine accurately the extent of the disease. Even with state of the art diagnostic methods it is difficult to determine locally advanced disease and in particular optimal nodal staging. On the other hand, although accurate imaging is crucial for optimal locoregional surgery, it remains unclear when the point of cure is passed due to undetectable distant metastases even with extended (elective) surgical dissections.\(^{30}\)

Although total thyroidectomy and central compartment lymph node dissection are accepted as standard procedure in common practice, it still remains unclear whether or not to perform a (bi)lateral lymph node dissection of level II-V in MTC patients, especially in the elective treatment of patients without apparent lymph node metastases in the lateral compartments. A number of authors recommend an elective lateral lymph node dissection whenever lymph node metastases are present in the central lymph node compartment (level VI).\(^{31}\) Other authors suggest a (uni)lateral lymph node dissection when calcitonin levels are elevated >20 pg/ml and a bilateral lymph node dissection with calcitonin >200 pg/ml. Using such a cut-off level for calcitonin minimizes the risk that MTC patients receive inadequate surgery. However, as a consequence there is a risk of over treating patients as nearly 90% of MTC patients with calcitonin between 20-200 pg/ml have no lateral lymph node metastases.\(^{32}\) Long-term results on cure and survival of the different proposed strategies are lacking in patients without apparent lateral lymph node metastasis, and the current ATA guidelines are indeterminate. In absence of such data, careful follow-up of treated patients is crucial. Either way, comprehensive pre-operative evaluation is important and functional imaging techniques can play an important role pre-operative staging. These techniques can also identify patients who may not benefit from surgical treatment. The optimal surgical treatment for MTC patients thus remains difficult; another reason to advocate treatment in an experienced centre.
Functional imaging in the follow-up of MTC

We showed that $^{18}$F-FDG PET was superior in detecting patients with progressive disease, while $^{18}$F-DOPA PET was better in assessing the extent of residual disease. We therefore proposed a flow diagram with a combined approach of $^{18}$F-FDG PET and $^{18}$F-FDOPA PET in patients with increasing tumour markers. Using this flow diagram, progressive MTC patients who do not benefit from (local) surgical treatment but are candidates for systemic treatment can be identified. A proportion of patients with progressive disease - indicated by rapidly increasing tumour markers - still have false negative imaging results. Other imaging modalities in these patients are necessary for detection of recurrent tumour lesions to assess the optimal (therapeutic) approach. However, first results for new tracers such as $^{68}$Ga-somatostatin analogues or $^{11}$C-methionine are not convincing. Compared with $^{18}$F-FDG PET and $^{18}$F-FDOPA PET, $^{68}$Ga-somatostatin analogue did not identify additional lesions or led to a change in TNM status. Although $^{11}$C-MET PET had a higher sensitivity for detecting cervical metastasis compared to $^{18}$F-FDG PET, it was not superior compared to ultrasound.

Further applications of nuclear imaging may be valuable in the early evaluation of therapeutic response of targeted therapy, thereby identifying patients who may benefit from this treatment. A preclinical study with vandetanib showed for instance that $^{18}$F-FDG PET was able to assess metabolic changes after three days of treatment. A down-regulation of key genes in the glycolysis pathway was observed resulting in a reduction of uptake of deoxyglucose both in vitro and in vivo. Other improvements in the future may focus on developing radiotracers which are able to selectively image mutated receptors, as was shown for a mutant form of EGFR in lung carcinoma. As somatic mutations exist in the RET receptor in a fair proportion of sporadic MTC patients, using selective radiotracers may better identify these lesions and also serve as a new treatment modality.

New treatment modalities

In the last years, new systemic therapeutic options have become available for the treatment of MTC patients. We showed in a comparison of four different TK inhibitors targeting RET that vandetanib and cabozantinib were the most effective TK inhibitors. This was also confirmed in two phase III clinical trials evaluating these TK inhibitors with a significant effect on progression free survival (PFS). Although these TK inhibitors show promising results, only effects on PFS have been reported, while no benefits on overall survival (OS) have been demonstrated yet. With vandetanib an estimated PFS of 30.5 months was reported versus 19.3
months in the placebo group. For cabozantinib a PFS of 11.2 months was observed versus 4 months in the control group, but this study included only progressive patients (i.e. showing progression compared to imaging obtained within the prior 14 months). Targeted therapies are generally better tolerated in comparison to cytotoxic chemotherapeutic regimens, although a large proportion of patients develop serious side-effects which may have a great impact on the quality of life. Furthermore, timing for initiation of these therapies has to be made with consideration of the natural course of disease progression, as a large proportion of patients have stable or slowly progressive disease. However, in patients presenting with progressive disease there may be a role for (neo) adjuvant systemic therapy.

Besides the RET activated pathway, also other receptors and pathways can be potential therapeutic targets in MTC. Indeed, over-expression of VEGF, VEGFR and MET have been described in MTC.\textsuperscript{41-43} It also has been shown that inhibition of RET can lead to over activation of signalling through EGFR.\textsuperscript{44} Inhibiting multiple kinases can therefore be beneficial to suppress such escape mechanisms. Most inhibitors currently in use are already multikinase inhibitors, thus exerting their effects on multiple kinases. However, the optimal combination of inhibition with regard to tumour regression and side effects may advocate the use of multiple inhibitors. Combinations of TK inhibitors with classic chemotherapeutic agents may also be beneficial as was reported in an in vivo study showing an additional effect of cisplatin to sunitinib.\textsuperscript{45}

In the meanwhile new TK inhibitors are being developed and tested. Ponatinib was shown to be a potent inhibitor of RET kinases in a pre-clinical study.\textsuperscript{46} A phase II study with this new drug is currently recruiting for patients with advanced MTC (clinicaltrials.gov). Other TK inhibitors evaluated for MTC are lenvatinib and AZD1480.\textsuperscript{47,48} Not only tyrosine kinases can be therapeutic targets for medullary thyroid carcinoma. A recent in vitro study showed promising results with an agent targeting mitochondria in MTC tumour cells.\textsuperscript{49}

In conclusion, the treatment options for patients with advanced metastatic MTC are being extended. However, although results of clinical trials look promising, no definite improvement of survival has been established. Further studies are needed to identify more effective TK inhibitors, possible in combination with other TK inhibitors or with agents blocking other potential targets. Careful consideration must be given to outweigh the benefits of possible disease control versus side effects of these new therapies. Therefore treatment of this rare neuroendocrine tumour should be performed in an experienced centre to further enhance diagnostic and therapeutic strategies to ultimately improve the outcome of MTC patients.
References


