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The management of patients with RAI-R thyroid cancer and the evolution towards personalized treatment

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INTRODUCTION

Thyroid cancer is the most frequent endocrine malignancy and represents 5% of all thyroid nodules. About 95% of thyroid cancers are differentiated cancers (DTCs), with an excellent prognosis (survival rates >95% at 25 years), above all for the papillary histotype. Their incidence is increasing, because of the widespread of diagnostic ultrasound and fine-needle aspiration biopsies that bring to the incidental diagnosis of small and indolent tumors.

The treatment of thyroid cancer is based on surgery (lobectomy or thyroidectomy), radioiodine therapy (in selected cases) and levo-tiroxine therapy (suppressive, semi-suppressive or replacement, as appropriate). DTC is usually indolent, but in less than 10% of patients distant metastases (above all, lung and bone metastases) occur, representing the most frequent cause of thyroid cancer-related death. Radioactive-iodine (RAI) remains the treatment modality of choice in patients with RAI avidity metastases and may be associated with local treatments such as radiotherapy or surgery. However, one-third of metastatic patients develops RAI refractoriness. Thus, the therapeutic approach for these patients should be very prudent with a careful analysis of risk and benefits. According to guidelines, if disease is asymptomatic, stable or minimally progressive, active surveillance is recommended. Novel systemic agents are considered first-line therapy for progressive, symptomatic and/or life-threatening cancers. Before tyrosine kinase inhibitors (TKIs) administration, both clinician and patient must agree that benefits are expected to outweigh the TKI-related risks, particularly regarding quality of life worsening. To date, TKIs approved for the treatment of RAI-R DTCs are: cabozantinib, sorafenib, levatinib, selpercatinib, larotrectinib and entrectinib. In our Institute, lenvatinib

has been administered to 18 patients, affected by RAI-refractory metastatic cancer. In this study, we analysed the progression-free survival, the treatment response and the adverse events occurred in these patients. Moreover, we described our approach towards the oligoprogressive disease, namely, the progression of one or few metastatic sites among an otherwise TKI-responsive cancer, an aspect not yet addressed in the guidelines. Finally, a focus on the need to move towards a personalized therapy based on the study of genetic mutations.

CHAPTER I
METASTATIC RADIOIODINE - REFRACTORY THYROID CANCER

1.1 Differentiated thyroid cancer: epidemiology, diagnosis and treatment

Thyroid cancer is the most frequent endocrine malignancy and represents 5% of thyroid nodules. The 95% of these cancers are differentiated thyroid cancers (DTCs), which comprise papillary, follicular and Hürthle cell histotypes. DTCs derive from follicular cells and maintain the ability to produce thyroglobulin, to be stimulated from TSH, to capture iodine.

The papillary thyroid cancer (PTC) represents the 80-85% of DTCs and tends to be biologically indolent with an excellent prognosis (survival rates >95% at 25 years). PTC can occur at any age, even if most tumours are diagnosed in patients within the third to fifth decade of life. Women are more frequently affected than men, with a 2-4:1 ratio. Microscopically, PTC shares common features. The neoplastic papillae contain a central core of fibrovascular (occasionally just fibrous) tissue lined by one or occasionally several layers of cells with crowded oval nuclei. The tumour invades lymphatic vessels leading to multifocal lesions and to regional node metastases. Venous invasion rarely occurs and metastases outside the neck are unusual (5–7% of cases). Certain subtypes of PTC, such as tall cell variant and diffuse sclerosis variant, are associated with more aggressive clinical behaviour, while classic, solid and follicular variant have a better prognosis [1]. The follicular thyroid cancer (FTC) represents the 5-15% of all DTCs. Histologically, FTCs display variable morphology ranging from small/medium-sized

follicles containing colloid to trabecular or solid growth pattern. According to local aggressiveness, it is classified as 'minimally invasive without angioinvasion', 'minimally invasive with angioinvasion' and 'widely invasive.' FTC develops distant metastases through blood more frequently than PTC [2]. A recent diagnostic category, defined by *Nikiforov et al*. as NIFTP (noninvasive follicular thyroid neoplasm with papillary-like nuclear features), has overcome the previous definition of 'noninvasive encapsulated follicular variant of papillary thyroid carcinoma' and comprises a very indolent form of cancer, that doesn't require further treatment after surgery [3]. Hürthle cell carcinoma (HCC) or oncocytichistotype represents the 3% of all DTC. Oncocytic cells are large, polygonal cells with marked eosinophilic, granular cytoplasm due to abundant mitochondria. HCC is subdivided into minimally invasive or widely invasive subgroups. Minimally invasive carcinomas are fully encapsulated tumours with microscopically identifiable foci of capsular or vascular invasion (< 4 foci), in contradistinction to widely invasive tumours, which have extensive vascular invasion (> 4 foci) and extrathyroidal invasion [4]. Anaplastic thyroid cancer (ATC) is a very aggressive tumour, with a mean survival time of 6 months from the diagnosis. It may represent the transformation of a well-differentiated thyroid cancer, which has lost the ability to uptake iodine or synthesize thyroglobulin. The incidence is low and the patients are generally elderly and female. ATC is characterized by spindled or giant cells, bizarre neoplastic cells that may be multinucleated, or atypical cells with high mitotic activity and a syncytial pattern [5]. Poorly differentiated thyroid cancer (PDTC) resembles ATC due to its aggressive nature but has partial overlap with FTC/PTC, retaining follicular elements and thyroglobulin production, so its behaviour is more aggressive than DTCs but less aggressive than ATCs. The Turin Proposal algorithm defines poorly differentiated carcinoma on the basis of the presence of

solid/trabecular/insular growth pattern, absence of conventional nuclear features of papillary carcinoma, and the presence of at least one of the following features: convoluted nuclei, mitotic activity ≥ 3/10 HPF, or tumor necrosis [6].

The fifth edition of the World Health Organization (WHO) histologic classification of thyroid neoplasms released in 2022 includes newly recognized tumor types, subtypes, and a grading system. The term "variant" to describe a subclass of tumor has been replaced with the term "subtype" in order to avoid confusion with genetic variants. Follicular cell-derived neoplasms are categorized into three classes: benign tumors, low-risk neoplasms, and malignant neoplasms. The terms "follicular nodular disease" and "differentiated high-grade thyroid carcinoma" are introduced to account for multifocal hyperplastic/neoplastic lesions and differentiated thyroid carcinomas with high-grade features, respectively. The term "Hürthle cells" is replaced with "oncocytic cells", Hürthle cell adenoma and Hürthle cell carcinoma are now called oncocytic adenoma and oncocytic carcinoma (OCA), respectively.

The new tumor type "high-grade follicular cell-derived carcinomas" has two histologic subtypes, traditional poorly differentiated thyroid carcinoma (PDTC) and a new subtype "differentiated high-grade thyroid carcinoma (DHGTC)" that arises from PTC, FTC, or OCA.

Invasive encapsulated follicular variant of papillary thyroid carcinoma (IEFVPTC) is now considered a separate entity and no longer a subtype of PTC because has a RAS-like mutational and transcriptomic profile similar to that of FTC. Like FTC, IEFVPTC can invade vessels in the capsule and develop distant metastasis.

Invasive encapsulated follicular and cribriform morular variants of papillary thyroid carcinoma (PTC) are now redefined as distinct tumor types, given their different genetic alterations and clinicopathologic characteristics from other PTC subtypes. Finally, a histologic grading

system based on the mitotic count, necrosis, and/or the Ki67 index is used to identify high-grade follicular-cell derived carcinomas and medullary thyroid carcinomas [7].

According to the data estimated from the Surveillance, Epidemiology, and End Results (SEER) Program, thyroid cancer represents 3% of all new cancer cases in the U.S.A. The number of new cases of thyroid cancer was 15.8 per 100.000 men and women per year and the number of deaths was 0.5 per 100.000 men and women per year, based on 2012-2016 data. Using statistical models for analysis, rates for new thyroid cancer cases have been rising on average 1.9% each year over the last 10 years, while death rates have been rising on average 0.7% each year over 2007-2016 [8]. The increase in PTC incidence is related to overdiagnosis, resulting from the introduction and increasing widespread use of diagnostic ultrasound and other imaging modalities and fine-needle aspiration biopsies that have allowed for incidental detection and diagnosis of mostly indolent localized cancers. However, the increasing thyroid cancer mortality rates among patients diagnosed with advanced-stage PTC is not consistent with the notion that overdiagnosis is the only responsible for the changing trends in PTC incidence, but possibly it is due also to changes in exposure to environmental risk factors [9]. Exposure to ionizing radiation is a well documented risk factor for cancer, especially in childhood, and exposure has increased in the general population in recent decades primarily because of more widespread use of diagnostic medical examinations. A potential endogenous carcinogenic factor may be the increased TSH (for iodine deficiency, autoimmune thyroiditis), because it is the major growth factor for thyroid follicular cells. However, a role of TSH in inducing thyroid cancer, documented in rodents, is not confirmed in humans. Obesity and smoking have been associated to several type of cancers, but the relationship with thyroid cancer is not demonstrated. Endocrine-

disrupting chemicals (e.g. pesticides, bisphenol A) also have been suspected to contribute to thyroid cancer incidence trends through their effects on thyroid hormone metabolism [10].

A crucial role for the diagnosis of thyroid cancer is played by neck ultrasound (US), which is an easily accessible, noninvasive, and cost-effective exam, even if it is operator-dependent. US allows to distinguish benign nodules that can be managed conservatively from those with suspicious or malignant features requiring fine-needle aspiration (FNA). Certain features of thyroid nodules on ultrasound are consistently predictive of malignancy and are used as criteria for FNA. Indeed, an ETA Executive Committee set up a task force to create guidelines and a standardized risk stratification system, called EU-TIRADS, to assess the risk features of thyroid malignancy [11]. See Table 1

Tab.1 EU-TIRADS (European Thyroid Imaging Reporting and Data system) classification

CATEGORY	**US FEATURES**	**MALIGNANCY RISK, %**
EU-TIRADS 1: normal	No nodules	None
EU-TIRADS 2: benign	Pure cyst Entirely spongiform	~0
EU-TIRADS 3: low risk	Ovoid, smooth isoechoic/hyperechoic No features of high suspicion	2-4
EU-TIRADS 4: intermediate risk	Ovoid, smooth, mildly hypoechoic No features of high suspicion	6-17
EU-TIRADS 5: high risk	At least 1 of the following features of high suspicion: - Irregular shape - Irregular margins - Microcalcifications - Marked hypoechogenicity (and solid)	26-87

The cytological diagnosis of thyroid nodules is essential to decide the following step. There are several system to classify different

cytopathologic categories and relative cancer risk: “the Bethesda System for Reporting Thyroid Cytopathology system” [12], “the British Royal College of Pathologists classification” [13], and “the Italian Classification and Reporting of Thyroid Cytology”, a consensus statement established by AIT, AME, SIE and SIAPEC-IAP [14]. See Table 2

Tab.2 Italian Classification and Reporting of Thyroid Cytology

CODE	DIAGNOSTIC CATEGORY	EXPECTED RISK OF MALIGNANCY (%)	SUGGESTED ACTIONS
TIR 1	Non-diagnostic	Not defined	Repeat US-guided FNA after at least 1 month
TIR 1 C	Non-diagnostic-cystic	Low (variable on the basis of clinical findings)	Evaluate the clinical setting and/or repeat FNA
TIR 2	Non-malignant/benign	<3	Follow-up
TIR 3 A	Low-risk indeterminate lesion (LRIL)	<10	Repeat FNA/clinical follow-up
TIR 3 B	High-risk indeterminate lesion (HRIL)	15-30	Surgery
TIR 4	Suspicious of malignancy	60-80	Surgery (consider frozen section)
TIR 5	Malignant	>95	Surgery

In case of indeterminate cytology (25% of all cytological specimens), it can be useful to apply the molecular biology tests to decide for surgery or follow-up. The molecular pathogenesis of the majority of thyroid cancer involves dysregulation of the mitogen-activated protein kinase (MAPK) and phosphatidylinositol-3 kinase (PI3K)/AKT signaling pathways. The MAPK pathway is frequently activated in thyroid cancer through point mutations of the BRAF and RAS genes and RET/PTC and TRK rearrangements. Indeed, BRAF and RAS point mutations and RET/PTC

and PAX8/PPARγ rearrangements are the most common genetic alterations found in thyroid cancer and have been used for cancer detection in thyroid nodules with indeterminate FNA cytology. The presence of any of these mutations, with the exception of RAS, which has a low positive predictive value for cancer, is an indication for surgery [15]. Molecular tests can be divided into two groups: "rule-in" or "rule-out" tests. The ability of a clinical test to "rule in" or "rule out" malignancy depends on its positive predictive value (PPV) and negative predictive value (NPV), respectively. The rule-out test is the Afirma Gene Expression Classifier (GEC), regarding 167 genes using microarray technology, whilst the "rule-in" test is the Panel 7-gene, which analyzes: BRAF, KRAS, HRAS, NRAS, and chromosomal translocations resulting in RET/PTC1, RET/PTC3, and PAX8/PPARγ fusions. Other newly tests are: ThyroSeq, a next-generation sequencing (NGS)-based platform that expands the list of oncogenic mutations and gene fusions; and a hybrid approach combining ThyraMIR, a microRNA gene expression classifier, with the existing 7-gene mutation/fusion panel [16].

According to guidelines established by the American Thyroid Association (ATA) in 2015, the gold standard for the treatment of DTC, is surgery. For patients with thyroid cancer >4cm, or with gross extrathyroidal extension (clinical T4), or clinically apparent metastatic disease to nodes (clinical N1) or distant sites (clinical M1), the initial surgical procedure should include a total thyroidectomy. For patients with thyroid cancer >1 cm and <4 cm without extrathyroidal extension, and without clinical evidence of any lymph node metastases (cN0), the initial surgical procedure can be either a bilateral procedure (near total or total thyroidectomy) or a unilateral procedure (lobectomy). For thyroid cancer <1 cm (microcarcinoma) without extrathyroidal extension and cN0, the initial surgical procedure should be a lobectomy unless there are clear indications to remove the contralateral lobe [17]. For

microcarcinomas, *Ito et al.* suggest an active surveillance (observation without immediate surgery), due to a very low risk of progression and metastases, avoiding the possibility of surgical complications such as permanent vocal cord paralysis and permanent hypoparathyroidism [18]. Accurate histopathological examination of the specimen after surgery is essential for the further diagnostic and therapeutic approach. The DTC risk classification system is based on a combination of primary tumor diameters, histology, extrathyroidal extension and the patient's age at diagnosis, aiming to predict the risk of local recurrence and developing metastases, and the cancer-related mortality. The TNM classification depends on the size of primary tumor, the number and localization of metastatic lymph nodes and number of distant metastases. The American Joint Committee on Cancer (AJCC) uses the combination of TNM Classification and an age of more than 55 years at diagnosis as risk factor.

Since the AJCC/TNM risk of mortality staging system does not adequately predict the risk of recurrence in DTC, the 2009 version of the ATA thyroid cancer guidelines proposed a three-tiered clinico-pathologic risk stratification system that classified patients as having low, intermediate, or high risk of recurrence:

- low-risk patients were defined as having intrathyroidal DTC with no evidence of extrathyroidal extension, vascular invasion, or metastases;
- intermediate-risk patients demonstrated either microscopic extrathyroidal extension, cervical lymph node metastases, RAI-avid disease in the neck outside the thyroid bed, vascular invasion, or aggressive tumor histology;
- high risk patients had gross extrathyroidal extension, incomplete tumor resection, distant metastases, or inappropriate postoperative serum Tg values.

The initial recurrence risk should be modified over time during follow-up, depending on the clinical course of the disease and the response to therapy. These categories below are used to describe the clinical status at any point during follow-up:

- excellent response: no clinical, biochemical, or structural evidence of disease.
- biochemical incomplete response: abnormal Tg or rising anti-Tg antibody levels in the absence of localizable disease.
- structural incomplete response: persistent or newly identified loco-regional or distant metastases.
- indeterminate response: nonspecific biochemical or structural findings that cannot be confidently classified as either benign or malignant. This includes patients with stable or declining anti-Tg antibody levels without definitive structural evidence of disease.

After surgery, the decision about the next therapeutic step is decided on the base of the risk classification: the ATA guidelines don't recommend the use of radio-active iodine (RAI) ablation after lobectomy and after thyroidectomy for ATA low-risk DTC patients, it should be considered after total thyroidectomy in ATA intermediate-risk level DTC patients and it is routinely recommended after total thyroidectomy for ATA high-risk DTC patients. Postoperative administration of RAI after total thyroidectomy is aimed to: destroy any thyroid remnant in the thyroid bed (remnant ablation) to facilitate detection of recurrent disease and initial staging by tests such as Tg measurements or whole-body RAI scans, improve disease-free survival by cleaning persistent microscopic foci of cancer (adjuvant therapy), improve disease-specific and disease-free survival by treating persistent disease in higher risk patients (RAI therapy). To ensure a high uptake of radioiodine, an elevated serum level of TSH is required (>30 mU/L). This level is believed to increase the expression of the sodium iodine symporter (NIS) in benign and

malignant follicular cells of the thyroid. This TSH level can be reached by waiting not less than 3 weeks after thyroidectomy or after a withdrawal (4–5 weeks) of levothyroxine (L-T4). The subsequent period of hypothyroidism decreases the quality of life significantly in many patients. Alternatively, to avoid the risk of hypothyroid-related complications in patients with significant medical or psychiatric comorbidity, recombinant TSH (rhTSH) can be administered intramuscularly (an injection of 0.9 mg rhTSH in 2 consecutive days) [19]. The dose of RAI given to DTC patients depending on the aim of iodine administration. The low dose of 1100 MBq radioiodine activity is sufficient for thyroid remnant ablation as compared to 3700 MBq radioiodine activity with similar quality of life, less common adverse effects, and a shorter hospital stay in a radiation protection unit [20]. Among adjuvant therapy, the range of potentially recommended doses is wide and the data less strong to help choice. Clinicians should choose increasing dose with increasing perceived risk based on clinical and pathologic features (e.g., presence of gross extrathyroidal extension or lateral cervical nodal involvement). In most cases, treatments are limited to the range of 1.1–5.6 GBq. The desire to use the minimum effective dose for adjuvant therapy is similar to that for remnant ablation. But, adjuvant therapy lays on a biologic question. β particles have a range in tissue on the order of millimetres, and thousands of β particles must traverse a cell to provide a high likelihood of lethal DNA damage. For this reason, to be statistically likely, there must be a relatively high number of β-emitter molecules in and around the tumor cell. When there is a macroscopic focus of disease, β particles from various areas overlap and lethal doses are realized. This is why relatively low doses have been shown to be effective in remnant ablation. In microscopic foci of disease, on the other hand, the radiation dose may be insufficient to kill tumor cells because a significant fraction will be deposited outside them.

Therefore, the relatively low but effective remnant ablation doses may be less successful in the adjuvant goal of killing residual microscopic disease [21]. Finally, in case of radioiodine therapy, the goal should be to give the maximum permissible dose to maximize the probability of controlling the tumor, avoiding radiation toxicity and complication (above all, on lung and bone marrow). There are usually three ways to choose the therapeutic dose of RAI in locoregional or metastatic thyroid cancer disease: a) to administer an empirically fixed dose, b) to administer a dose determined by the upper permissible limit of radiation to be absorbed in blood and the whole body and c) to administer a dose calculated according to tumor and/or lesions dosimetry. Lesional dosimetry is based on the absorbed dose of the target tissue, in order to prevent both suboptimal and excessive administration of radioactivity. It is safer than empirical approach, that remains the most widespread. Empirical doses are generally safe for the typical patient, but clinicians must be careful in case of cardiac and renal diseases and pay attention to the age of patients [22].

The third mainstay of the treatment for DTCs, after surgery and radioiodine treatment, is the thyroid hormone suppression. The goal of TSH suppression is to reduce endogenous stimulation of remnant DTC cells, given that TSH stimulates the number, size and activity of thyrocyte cells from which DTC arises. On the other hand, TSH suppression is a state of iatrogenic subclinical thyrotoxicosis obtained by supra-physiological doses of levothyroxine, and has been associated with increased resting heart rate, tachyarrhythmias, and predisposition towards a reduction in cardiac function, especially in elderly patients. Moreover, thyrotoxicosis is an established cause of osteoporosis. For this reason, it is important to consider the balance between risks and benefits of TSH suppression. Retrospective and prospective studies have demonstrated that TSH suppression to below 0.1 mU/L may improve outcomes in

high-risk thyroid cancer patients, while no such evidence of benefit has been documented in low-risk patients. Indeed, the ATA guidelines recommend an initial TSH suppression below 0.1 mU/L for high-risk thyroid cancer patients, an initial TSH suppression to 0.1–0.5 mU/L for intermediate-risk thyroid cancer patients, and for low-risk patients TSH may be maintained at the lower end of the reference range (0.5–2 mU/L). The following dynamic risk stratification guides the clinicians through the decision about TSH suppression/replacement [17].

1.2 Definition and pathogenesis of radioiodine-refractoriness

DTC is usually indolent. However, distant metastases, mostly in lung and bone, occur in less than 10% of patients and represent the most frequent cause of thyroid cancer-related death. RAI remains the main treatment modality in patients that show RAI uptake and may be associated with local treatments such as radiotherapy or surgery. In particular, as ATA guidelines suggest [17], if locoregional lymph node metastases are detectable, surgery should be performed and RAI used after surgery or as an alternative therapy if surgery is not possible/planned. In the case of micrometastases of the lung (< 1 cm in maximum diameter), RAI ablative therapy is carried out with curative intent. On the contrary, macronodular pulmonary metastases are unlikely to achieve complete remission after RAI ablation. The complete surgical resection of isolated bone metastases leads to an improved outcome. A combination of different therapeutic approaches such as RAI, percutaneous ablation, and other local therapies could be helpful for unresectable symptomatic metastases. For the treatment of metastases, RAI standard activities of 100–200 mCi are usually empirically given. As *Durante et al.* demonstrated, RAI is highly effective in patients younger than 40 years, with small metastases and with RAI uptake after ablation. Patients should be treated until the disappearance of any uptake or until a cumulative activity of 600 mCi has been administered. In contrast, in patients with no tumor response after several RAI treatment or with no RAI uptake, the treatment should be avoided and other treatment modalities should be used when tumor progression has been documented [23]. In conclusion, RAI-avidity is a favourable prognostic factor, while older age at diagnosis (≥55 years), RAI non-avidity, preoperative or late diagnosis of metastases and macro-nodular lung metastases are predictive of reduced progression-free survival and cancer-specific survival [24].

One-third of metastatic patients develops radioactive iodine refractoriness (RAI-R), which can be defined by at least one of the following criteria:

- the malignant/metastatic tissue is unable to take up iodine and the post-RAI whole body scan is negative;
- the maximum activity of RAI has been reached;
- RAI up-take is present only in some lesions but not in others;
- the disease progresses despite its ability to take up RAI [25].

When thyroid gland has not been removed, RAI treatment is not usually administered and these patients are usually managed as radio-iodine refractory. The third category is the only that may yet benefit from a combination of RAI and other therapies, for all the others, RAI therapy is not recommended [17].

For thyroid cancer the possibility to use radioiodine as therapy is due to the residual ability of tumor cells to accumulate iodine, through the sodium iodide symporter (NIS) [26]. The NIS is a membrane glycoprotein that transports two sodium ions and one iodide ion into the cytosol of benign and tumoral thyroid cells from extracellular fluid. Since radioiodine also can be taken up by the NIS, radioiodine can be used to visualize or selectively kill DTC cells. Although DTC retains most of the biochemical properties that are typical of normal thyroid follicular cells, and the ability to concentrate radioiodine is generally considered to indicate a more differentiated phenotype, a variety of abnormalities have been demonstrated. Malignant tumors show up as hypofunctioning areas on thyroid scintigraphy, indicating that the loss of iodide uptake ability is hallmark of thyroid carcinogenesis. Several previous studies reported a lower expression of NIS mRNA in samples of thyroid carcinomas compared to normal tissues, but above all, a predominant intracellular localization of NIS, which could be responsible for the thyroid's inability to concentrate iodine. The AMPK and PI3K pathways lead to NIS

lysosomal degradation, such as mTOR, hyperactivated in thyroid cancer, and MAPK pathway [27]. The $BRAF^{V600E}$ mutation is frequent in RAI refractory and fluorodeoxyglucose (FDG)–positron emission tomography (PET) positive metastatic tumors, and this mutation is associated with a lower NIS expression and lower radioactive iodine uptake, both *in vitro* and *in vivo* [28].

Recent studies using drugs that selectively inhibit the MAPK pathway showed promising results for restoring radioiodine uptake in RR-DTC: selumetinib, a selective MEK inhibitor and dabrafenib, a selective BRAF inhibitor. However, thyroid tumor cells bearing the $BRAF^{V600E}$ overexpress neuregulin 1 and the human epidermal growth factor receptor 3 (HER3) signaling pathway when treated with RAF or MEK inhibitors, a phenomenon that leads to drug resistance. Thus, the association of MEK or RAF inhibitors with the HER3 inhibitor lapatinib or the anti-HER3 MAB results in MAPK pathway suppression without a rebound phenomenon and increases NIS expression. To overcome the rebound of MAPK signaling, an allosteric MEK inhibitor (CH5126766, known as CKI) could be a new candidate. CKI binds selectively to the non-phosphorylated form of MEK, and makes RAF/MEK complex stable and inactive. Thus, the CKI suppresses the feedback induction of MEK phosphorylation that occurs after ERK pathway inhibition in tumors exposed to other MEK inhibitors. Morever, inhibiting mTOR induces radioiodine uptake through increased TTF1 expression. Recently, several clinical trials have been performed to evaluate the effect of an mTOR inhibitor (everolimus) and the combined used of a TKI (sorafenib) and an mTOR inhibitor (temsirolimus). These clinical trials appear to be effective, but they did not evaluate the changes of radioiodine uptake and effectiveness of combined radioiodine therapy [29].

If no radioiodine-accumulating tumor tissue is detectable, clinical diagnostics should include the search for non-radioiodine-avid tumor

tissue using F^{18}-FDG-PET combined with computed tomography.

In effect, Glucose transporters 1 (GLUT1), which facilitate glucose uptake in cancer, are upregulated and overexpressed during dedifferentiation of cancer cells. For this reason, an inverse relationship between I^{-131} and FDG uptake ("flip-flop phenomenon") has been described for thyroid cancers during dedifferentiation [30].

FDG-PET and PET-CT are highly accurate diagnostics tools for DTC recurrence in patients who present a negative whole-body scintigraphy, with a primary impact on therapeutic management of metastatic patients [31].

1.3 Therapeutic options: local and systemic therapies

The therapeutic approach for patients who developed RAI refractoriness must be very prudent with a careful analysis of risk and benefits, considering that a complete remission is unlikely. The progression of metastatic lesions is evaluated through Response Evaluation Criteria in Solid Tumours (RECIST) version 1.1. According to RECIST, only measurable lesions (target) are taken into account for evaluation, namely, metastases of at least 10 mm in maximum diameter for solid lesions and ≥ 15 mm in short axis for lymph nodes, measured by CT scan. Progressive disease (PD) is defined as increase in at least 20% of the sum of diameters of target lesions or an absolute increase of 5 mm or the appearance of one or more new metastases, whilst partial response (PR) is a reduction from baseline ≥ 30%, complete response (CR) is the disappearance of all lesions and stable disease (SD) includes all other measurement group [32].

In case of progression of one single lesion or multiple lesions in one single organ, local treatment should be applied. If the progression regards multiple lesions or single lesions in multiple organs, clinicians should consider the progression timing: slow growth (<20% in 12-14 months), could benefit of a "wait and see" approach with biochemical and instrumental controls every 4-6 months, in case of rapid growth, systemic therapy is required [33].

Several local treatment modalities other than surgery may be used to treat brain, lung, liver, and bone lesions from thyroid carcinoma. These techniques can be a less aggressive alternative to surgery and may be indicated in cases not suitable to surgery, such as poor clinical status, multiple previous surgical resections, local recurrence at the site of previous surgery, or refusal of additional surgery. External beam radiotherapy (EBRT) has the primary objective to stop the growth or at

least to reduce the rate of growth in order to control or prevent symptoms. EBRT is associated with improved local disease control and a longer progression free survival time. With the same medical objective, but also to reduce the pain, EBRT can be used in bone metastases. Brain metastases may be also treated with EBRT, with a stereotactic radiosurgery and/or whole brain radiotherapy, and a rapid and reliable response may be obtained. The recent introduction of stereotactic EBRT has opened up the possibility of treating small metastatic lesions in any organ including lungs, for which a diffuse radiation exposure is contraindicated due to the high risk of radiation fibrosis and respiratory dysfunction. For osteolytic bone metastases, the cementoplasty is generally in combination with other local treatments, such as radiofrequency ablation or EBRT. Its aim is to obtain the reinforcement of bone defect through percutaneous injection of polymethylmethacrylate cement. If metastases are not amenable to local control, or if the disease is rapidly progressive, systemic therapy has to be considered and TKIs are considered the first line of systemic therapy. In fact, thyroid cancer poorly responds to traditional cytotoxin chemotherapy, which is considered only after failure of TKIs [17]. Doxorubicin was approved for use in thyroid cancer by the FDA in 1974 and has some utility in anaplastic thyroid cancer, but it is associated with a response rate of up to 40% for progressive differentiated cancers that do not respond to radioactive iodine. Doxorubicin, epirubicin, taxol, and cisplatin have all been used in various combinations; responses do not seem to be any better than single agent with increased toxicities [34]. Combination therapy of gemcitabine plus oxaliplatin (GEMOX regimen) is well tolerated and seems to be effective in advanced differentiated thyroid cancer, but data are limited because of the small number of patients involved [35]. The mechanisms responsible for the widespread refractoriness to chemotherapeutic drugs observed in thyroid cancers

may be explained by different reasons. One of the theories is based on autocrine production of IL-4 and IL-10 by tumor cells that up-regulated antiapoptotic proteins, Bcl-2 and Bcl-xL [36].

A new therapeutic strategy is based on immunotherapy (PD1-PDL1 pathway), already approved for different types of cancer. No information from clinical trials with anti-PD-1/PD-L1 directed therapies enrolling patients affected by aggressive thyroid cancers is available. However, different preclinical studies demonstrated the efficacy of PD-1/PD-L1 axis blockade in restraining growth of ATC-derived cell lines injected in mice [37]. Differently from chemotherapy, target therapy based on tyrosine kinase inhibitors is citotostatic, not citotoxic, but it results in higher efficacy with less side effects. The decision to start TKIs is a crucial point, that should include patient-related medical factors (age, health status, comorbidities, and contraindications) and patient preferences and acceptance, all discussed by a multidisciplinary board [33].

CHAPTER II

SYSTEMIC THERAPY WITH TKIs AND THE MANAGEMENT OF OLIGOPROGRESSION

2.1 What are TKIs and how do they act?

Protein kinases act by protein phosphorylation, a pivotal mechanism in signal transduction pathways that regulates proliferation, differentiation, migration, metabolism and anti-apoptotic signalling. The serine/threonine and tyrosine kinases are the most important protein kinases, which are characterized by their ability to catalyse the phosphorylation of serine/threonine or tyrosine aminoacid residues in proteins, respectively. Two classes of tyrosine kinases are distinguished: tyrosine kinase receptors and cellular tyrosine kinases. Tyrosine kinase receptors consist of an extracellular ligand binding domain, a transmembrane domain and an intracellular catalytic domain. Dimerization of two receptors upon ligand binding results in autophosphorylation of the tyrosine residues of the intracellular catalytic domains, which leads to an active conformation and subsequent activation of the signal transduction cascade within the cell, through cellular tyrosine kinases, located in the cytoplasm or in the nucleus. Because of their important effects on cells, tyrosine kinases inhibitors have been developed as anticancer agents. They represent a multi-target therapy because they stop several signaling pathways simultaneously. In human cells, 56 receptor tyrosine kinases and 32 cellular tyrosine kinases are expressed; among these, the ABL, SCR, EGFR, PDGFR and VEGFR families have been the primary targets for development of tyrosine kinase

inhibitors [36]. Several TKIs have been developed and approved for different type of cancer and the success but at same time the appearance of resistance mechanisms bring to the need to develop new molecular therapies [37]. The TKIs bind to different receptors with different affinities but share the same mechanism of action, namely, competitive ATP inhibition at the catalytic binding site of tyrosine kinase [38]. See figure 1

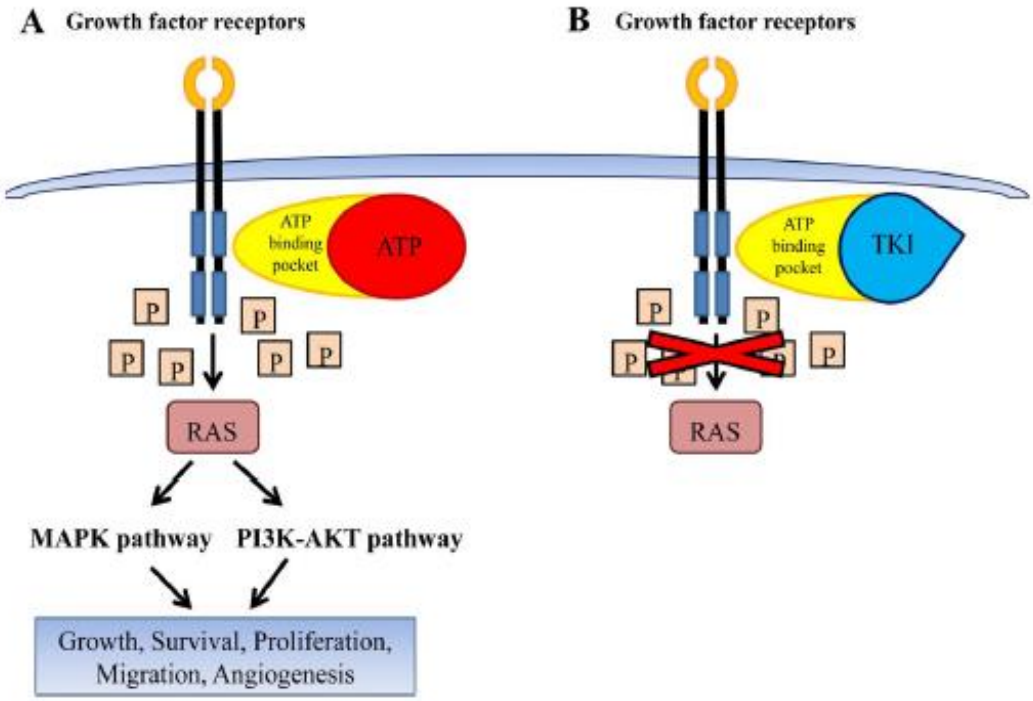

Fig.1 Competitive ATP inhibition by TKIs at the catalytic binding site of RTKs.

From Viola D. (2016). *Treatment of advanced thyroid cancer with targeted therapies: ten years of experience*. Endocrine-Related Cancer, 23, R185-R205

VEGF receptors are members of a family of transmembrane tyrosine kinase receptors that mediate signal transduction from extracellular signaling ligands, to intracellular signaling cascades. VEGFR-2 is thought to be the

most important mediator of angiogenesis. When activated, VEGFR-2 activates both MAPK and mTOR pathways. The result of activation of these intracellular signaling cascades is the promotion of endothelial cell survival, proliferation, and migration-key steps in tumor angiogenesis. The importance of VEGFR-2 and angiogenesis in thyroid cancer tumor progression is supported by the observation that thyroid cancers are highly vascular and that VEGF expression correlates with risk for the development of metastatic disease in PTC. For these reasons, VEGFR-2 inhibition is the most effective new therapeutic strategy developed to date in the treatment of these tumors [39].

A phase III study of vandetanib, already approved for medullary metastatic cancer, is currently ongoing in advanced RAI-R DTCs. The clinical trial (VERIFY study) started in 2013 and enrolled 243 patients with differentiated locally advanced or metastatic thyroid cancer who are refractory or unsuitable for radioiodine therapy. The aim is to determine the efficacy and safety of vandetanib 300 mg when compared to placebo in participants who have never received target therapies. The estimated study completion date is December 2022 [40].

To date (September 2022), TKIs approved for the treatment of RAI-R DTCs are: cabozantinib, sorafenib, levatinib, selpercatinib, larotrectinib and entrectinib.

Cabozantinib, already approved for medullary metastatic cancer, has been recently approved by the FDA and the EMA for treating adults with radioactive iodine (RAI)-refractory DTC who progressed during previous treatment with TKIs targeting the VEGFR (i.e. sorafenib or lenvatinib). The recommended dose is 60 mg orally once daily. The approval decision was

based on data from the COSMIC-311 trial (NCT03690388), a global, randomised, double-blind, placebo-controlled, phase III trial conducted in patients aged >16 years with RAI-refractory DTC (papillary or follicular and their variants). Patients were randomised 2:1 to treatment with cabozantinib tablet or placebo. Interim analysis of progression-free survival (PFS) revealed significant improvement in the cabozantinib arm versus placebo (5.7 versus 1.9 months). Adverse effects were manageable (palmareplantar erythrodysesthesia, hypertension and fatigue). Five percent of patients experienced AEs leading to drug discontinuation. There were no treatment-related deaths [41].

Sorafenib, an oral multitarget tyrosine kinase inhibitor that inhibits C-RAF, B-RAF, RET, c-KIT, PDGF-R and VEGFR 1-3, already used for the treatment of advanced hepatocellular carcinomas and advanced renal cell carcinomas, was approved also for the treatment of refractory advanced DTC by the FDA in 2013 and by the EMA in 2014. The DECISION study, a multicenter, randomized, double-blind, placebo-controlled, Phase III trial, has led to the approval of sorafenib (400 mg orally twice-daily) [42]. In this study, 417 patients with RAI-refractory DTC and progression within the past 14 months according to the RECIST v1.0 criteria, were randomized to sorafenib (n=207) or placebo (n=210). Patients who had received prior targeted therapy, thalidomide, or chemotherapy for thyroid cancer were excluded. The primary endpoint of the study was PFS, assessed every 8 weeks by central independent blinded review during a median follow-up of 16.2 months and the median PFS was significantly longer in sorefenib group than in placebo group (10.8 vs 5.8 months respectively). Regarding the safety, the most common adverse events (AEs) in the sorafenib group, monitored using Common Terminology Criteria for Adverse Events

(CTCAE v3.0), were: hand–foot skin reaction (HFSR), diarrhoea, alopecia, rash/desquamation, fatigue, weight loss, and hypertension. The number of deaths in the double-blind part of the study was low in both sorafenib and placebo groups (12 and 6, respectively), with the majority of causes being related to underlying disease and only one death in each arm attributed to study drug. Interesting information have been obtained via the exploratory analysis of outcomes of patients receiving open-label sorafenib postprogression in the phase 3 DECISION trial [43]. This analysis demonstrated that sorafenib may continue to suppress tumor growth rates because the median PFS of patients receiving this drug after progression was still lower than that of patients treated with the placebo and comparable to those who started receiving the drug from the beginning of the trial (9.6 months).

Lenvatinib is an oral multitarget tyrosine kinase inhibitor. It inhibits VEGFR 1-3, FGFR 1-4, PDGFR α, RET, c-KIT. The SELECT study, a phase III multicenter, randomized, double-blind, placebo-controlled study, was developed to evaluate the PFS of patients with RAI-R thyroid cancer [44]. It started in 2011 and involved 392 patients (261 received lenvatinib 24 mg die and 131 received placebo) with disease progression within the prior 13 months, including whose who had received prior TKI therapy. If patients receiving placebo went on progression according RECIST 1.1, a crossover to open-label lenvatinib was available, as well as a dose reduction for adverse events until drug interruption. The primary endpoint was PFS and it was significantly longer in lenvatinib group than in placebo group (18.3 vs 3.6 months), with no significant difference in patients already treated with a TKI (15.1 months), nor according to the BRAF and RAS mutational status. The most frequent AEs were: hypertension, diarrhoea, decreased appetite,

weight loss, nausea and proteinuria. Interruption or dose reduction due to AEs were frequent, resulting in a mean drug dose of 17,2 mg die. The most common side effects that required a dose adjustment were diarrhoea, hypertension, proteinuria and decreased appetite. 6 of 20 deaths that occurred in lenvatinib group, not due to disease progression but related to study treatment, were one case of pulmonary embolism, one of hemorrhagic stroke, one of general physical deterioration, and three cases of sudden deaths [44].

Head-to-head comparisons between the two pairs of drugs (i.e. lenvatinib versus sorafenib, and cabozantinib versus vandetanib) have never been undertaken. For these reason, the optimal sequence cannot be established on currently available evidence. Lenvatinib efficacy has been tested in cabozantinib-naive patients as well as in patients who had received one prior treatment regimen with an TKI (including sorafenib). So, it can be used after sorafenib-progression. But the decision is always complex and should be individualised for each patient considering the regulatory aspect in that country [45].

Selpercatinib is a novel, ATP-competitive, highly selective, small-molecule RET kinase inhibitor. The safety and efficacy of selpercatinib was evaluated in LIBRETTO-001, a phase 1–2 clinical trial involving adolescent and adult patients with any solid tumor type harboring an activating RET alteration. The percentage of patients with an objective response was 79% for RET fusion–positive thyroid cancer. As for safety, the most common grade 3 or 4 adverse events were hypertension, increased alanine and aspartate aminotransferase levels, hyponatremia, and diarrhea [46]. It was approved by AIFA on September 2021 and reimbursed from 2022 by the national health system for RET fusion–positive thyroid cancer.

Larotrectinib and entrectinib are tropomyosin receptor kinase (TRK) inhibitors approved by FDA and EMA for solid tumours harbouring functional neurotrophic tyrosine receptor kinase (NTRK) fusions after several trials included adults and paediatric patients with metastatic and progressive solid tumours harbouring an NTRK gene fusion (including thyroid cancers) [47,48].

Considering that the cytostatic effect of the TKIs is not durable, due to the development of drug resistances and that the TKI-related adverse events can severely affect the patients quality of life, the choice of when the patients have to start the therapy is crucial [49]. In particular both the site and speed of growth of metastatic lesions have to be considered. For example, small lesions but with exceptionally rapid growth and/or related to severe symptoms not amenable to local control, such as pain, also have to be considered to be treated with TKIs. To evaluate the progression of metastases, it is useful to consider both tyroglobulin (Tg)-doubling time and the SUV on PET-CT scan. There are some major contraindications to TKI therapy. These drugs can induce haemorrhage from metastatic lesions, thus, they should be avoided in the presence of substantial risk of bleeding. Untreated brain lesions should be managed first with surgery or stereotactic radiotherapy in order to prevent haemorrhage during TKI administration. Metastases adjacent to main blood vessels such as carotid artery, represent a relative contraindication, as well as the risk of tissue necrosis, fistula formation and poor wound healing [50]. The general conditions of the patient should always be assessed through the Eastern Cooperative Oncology Group (ECOG) performance status scale before TKI start. The cardiological evaluation should focus on blood pressure, since TKIs, in particular lenvatinib, can cause early hypertension, and on QTc interval. Moreover, it

is necessary to evaluate electrolytes (sodium, potassium, magnesium, calcium) and renal and hepatic function. Lastly, TSH and FT4 should be monitored because a high percentage of patients become hypothyroid and require an increase of L-T4 daily dose. Clinical and biochemical control must be performed regularly in order to promptly treat the AEs, the severity of which is evaluated according to the Common Terminology Criteria for Adverse Events (CTCAE) version 4.0.

In case of AEs of :

- Grade 1 (mild, asymptomatic or mild symptomatic), clinical or diagnostic observation is sufficient;
- Grade 2 (moderate severity), minimal, local or noninvasive intervention (such as specific treatment) can be the right choice;
- Grade 3 (severe or medically significant but not immediately life-threatening), hospitalization should be considered and the drug must be reduced or temporarily discontinued until the resolution of the toxicity;
- Grade 4 (life-threatening consequences), urgent intervention is indicated, with the definitive interruption of the drug;
- Grade 5 event refers to death related to AE [51].

2.2 The definition of oligoprogressive disease

TKIs do not result in long-term cancer control. Indeed, in many cases, within 1–2 years the cancer develops genetic alterations that overcome the inhibition of the targeted kinases. The resistance to TKIs can develop in several ways, such as: secondary mutation of the tyrosine kinase, gene amplification and subsequent overexpression of the protein kinase, activation of other signalling pathways, lower intracellular drug concentrations because of extracellular sequestration of the inhibitor by binding to α acid glycoprotein, decreased expression or activity of drug influx pumps, increased expression or activity of drug efflux pumps [52]. Acquired resistance to EGFR tyrosine kinase inhibitors was first studied in patients with non–small-cell lung cancer (NSCLC), in order to help creating standard entry criteria for the studies of such patients in clinical trials [53]. Oncologists proposed to subtype the setting of progressive disease in patients with acquired resistance to EGFR- or ALK-directed TKI therapy in NSCLC into: 1) CNS sanctuary PD, 2) oligo-progressive disease (OPD) and 3) systemic PD [54]. Actually, TKI resistance can result in a wide variety of progression patterns, because not all patients develop the same pattern of progression in terms of the extent and/or sites. In oncological practice, disease progression according to RECIST is interpreted as a marker of treatment failure and, consequently, as a need to change therapy. However, every scenario should be managed according to a tailored approach. The definition of oligoprogressive disease was first introduced in 2012 to describe widespread systemic tumour at diagnosis, which shows, after a prolonged response or, at least, a stability of disease on systemic treatment, a limited progression in few metastatic sites and organs. In our scenario, OPD refers to the progression in one or few sites of disease within a

multimetastatic TKI-treated cancer. Since the other lesions are well controlled by the TKI-therapy, it is clear that RECIST progression is not the single determining factor for terminating TKI, especially because of the paucity of systemic therapeutic options other than TKIs for thyroid cancer. In contrast to widespread systemic progression, oligoprogression may be managed by not only changing systemic therapy, but also by maintaining the same systemic treatment beyond progression and adding metastasis-directed therapy to primary systemic treatment. Although most of the evidence derives from studies conducted in patients with EGFR mutated NSCLC, the same approach can be extended to other molecular driver alterations, such as ALK, ROS1, BRAF and other less common genetic aberrations. Thus, in the presence of OPD, the use of local ablative therapies (LATs), such as radiotherapy or surgical resection, against the progressive sites allows to continue an ongoing TKI therapy [55]. The necessity of reviewing RECIST in the era of target therapy has been addressed in a recent paper by *Morgan et al.*, in which several controversies regarding the application of these criteria are examined [56]. For example, while RECIST consider central nervous system (CNS) and extra-CNS lesions equally for the assessment of treatment response, they propose to consider a bicompartmental model because of the different pharmacokinetics of drugs. So, if there is progression in only one compartment, the systemic therapy should not be changed or interrupted because it can continue to act in the other, but local therapy must be combined. Another open question is how to consider a lesion after local therapy, because it should be considered as a new baseline for the following reassessment of treatment response. Finally, if a patient has a partial response but then develop a slow progression, it should be considered as a real progression only when it meets the criteria of PD from the nadir and not from baseline.

2.3 The role of local therapies

According to the ATA differentiated thyroid cancer guidelines, in selected cases of focal disease progression amenable to directed therapy, it is reasonable to administer local ablative therapy (LAT) while maintaining the current TKI therapy. Concerning the available techniques, in addition to surgery, most indications derive from studies on solid tumours other than thyroid tumors, since data from TKI-treated patients are limited [55].

The LATs include different techniques: conventional radiotherapy, stereotactic body radiation therapy (SBRT) and percutaneous thermal ablative therapies, such as radiofrequency (RFA) and cryotherapy ablation. Randomized prospective studies comparing the efficacy and tolerability of these different techniques are lacking, and their choice in clinical practice is based on clinicians' experience, lesion location, patient status and preference. The main principle of these techniques is to selectively treat the lesion, be minimally invasive, and be well tolerated with relatively few side effects [17].

Radiation is a physical agent, which is used to destroy cancer cells, through electrically charged particles, which deposit energy, killing cells directly or causing genetic changes resulting in cell death. Radiation can be used with a curative or palliative intent. Moreover, it can be combined with other treatment modalities, including surgery, chemotherapy or immunotherapy. Radiation therapy delivered in a fractionated regime is the most widespread. It is based on the concept that normal cells proliferate relatively more slowly compared to the rapidly proliferating cancer cells and therefore have time to repair damage before replication. Different techniques are possible: 3D conformal radiotherapy (3DCRT) based on CT imaging allows accurate

localization of the tumour and critical normal organ structures for optimal beam placement and shielding. Intensity modulated radiation therapy (IMRT) allows the radiotherapist to create irregular-shaped radiation doses that conform to the tumour whilst simultaneously avoiding critical organs. IMRT is made possible through inverse planning software and computer-controlled intensity-modulation of multiple radiation beams during treatment. When critical structures are close to the tumour, a slight positional error may also lead to inadvertent radiation of the normal organs. Image-guided radiotherapy (IGRT) avoids such errors by information acquired through pre-radiotherapy imaging, (e.g., a daily cone-beam CT scans acquired before each treatment). Stereotactic body radiation therapy (SBRT), also called stereotactic ablative radiotherapy (SABR), enables to deliver high radiation doses in few fractions to the target tumoral lesion with a high degree of precision, minimizing the radiation of normal surrounding tissue. It has been used in several trials to treat brain (stereotactic radiosurgery, SRS), liver, lung, and bone metastases [57,58]. Several randomized studies aim to evaluate the efficacy of SBRT, comparing to chemotherapy alone, are ongoing [59,60,61].

Concerning bone lesions, radiotherapy plays an important role because it can complement surgery in case of incomplete resection. Moreover, it is extensively used for pain control. The major limitation of radiotherapy in spine lesions is the cumulative dose to the spinal cord. SBRT compared to standard radiotherapy demonstrated a higher efficacy on tumor control and a reduced radiation damage to the spinal cord, especially in patients who need to be re-irradiated [17]. A local tumor control rate of bone lesions for SBRT ranging from 88% to 100% was reported especially for lesions that were previously surgically resected, with a pain relief rate of 30%–83%. SBRT

protocols differed among studies, with a maximum of 30 Gy administrated in one to five fractions but a single dose of 12.5–15 Gy seems to achieve similar results, even if a unique dose of 8 Gy is the most commonly administered dose for palliation of bone metastases. Spinal myelopathy or vertebral fractures are the most important side effects, especially in case of large-volume lesions.

Percutaneous thermal ablation aims to destroy tumor cells by heat (radiofrequency ablation) or cold (cryoablation). RFA is performed in liver, lung, and bone lesions. In primary and secondary lung tumors, RFA has been mostly used, for patients not amenable to surgery. It preserves normal parenchyma, can be used in patients with limited pulmonary reserve or with multifocal or bilateral metastatic disease, can be repeated, and requires a minimum hospital stay. RFA may be considered for lung lesions <3 cm of diameter, without soft tissue or mediastinum invasion and without contact with large vessels. The most frequent complications are: pain, fever, haemorrhage, pneumothorax [62]. RFA is mostly used for liver metastases and studies have shown that prolongs survival time, especially for lesion of diameter ≤3 cm [63]. Cryoablation seems to cause more complications than RFA, but there are no randomized controlled trial comparing the two techniques [64]. RFA or cryoablation of bone lesions showed promising results with rapid (1–7 days) and long-lasting pain control. Cryoablation is frequently associated with cementoplasty to consolidate the bone and avoid subsequent complications, and can treat larger lesions than RFA. Local pain or transient neurological deficit or vertebral fracture can occur in 5%–6% of bone lesions treated [17].

Two novel treatments for liver lesions are transarterial chemoembolization (TACE) and transarterial radioembolization with Yttrium-90 (TARE), a

modern technique that seems to ensure better tumor control in patients with hepatocellular carcinoma [65]. The complications of local treatment of liver lesions are: intestinal perforation, abdominal pain, or intraperitoneal bleeding. Published experience using local therapy in thyroid cancer patients is limited, and recommendations are currently based on more robust evidence in other solid tumors, above all NSCLC. *Weickhardt et al.* firstly investigated the benefits of LATs in patients with oligoprogressive metastatic ALK-positive or EGFR-mutant NSCLC treated with crizotinib and erlotinib, respectively [66]. In this study, the median PFS was 9 months with crizotinib and 13.8 months with erlotinib. The 49% of patients showed oligoprogression (defined as progression in four extra-CNS sites or fewer) and were subjected to LATs (radiotherapy or surgery), while continuing the TKI. The extension of PFS after-LAT (PFS2) was 6.2 months. Since then, several retrospective studies have demonstrated the benefit of LATs in patients who developed oligoprogression [67,68,69]. However, only few are the ongoing prospective and/or randomized trials designed to evaluate LAT for oligoprogressive cancer patients, in particular, 4 studies for NSCL and 1 for clear cell renal cancer [55].

CHAPTER III

THE EXPERIENCE IN OUR INSTITUTE

3.1 The use of lenvatinib in clinical practice

In 2015, our Centre of Endocrinology in "Policlinico Federico II" of Naples participated to the multi-centre Expanded Access Program (EAP) for compassionate use of lenvatinib in the treatment of Radioiodine-Refractory Differentiated Thyroid Cancer, with University of Pisa as coordinator and other 14 Italian centres. This program was valid until lenvatinib become commercially available in Italy. The primary objectives of the study were: to make lenvatinib available to patients with RR-DTC, to confirm the safety data of lenvatinib in TKI naïve patients, to evaluate the quality of life (QoL), assessed using EORTC QLQ-C30 model, to confirm the activity of the drug.

The EAP consisted of 2 phases: a pretreatment phase and a treatment phase. The pretreatment phase was up to 28 days in duration and consisted of a screening period. The treatment phase started with the first dose of therapy. Subjects received lenvatinib at 24 mg daily. Dose reductions due to AEs were performed based on the previous dose level (24 to 20, 14, and 10 mg daily). Treatment with lenvatinib terminated when one of the following occurred: evidence of global disease progression, unacceptable toxicity, death or patient withdrawal of consent. From April 2015 to May 2016, we enrolled 6 patients, over a total of 94 patients within the EAP, with a mean follow-up of 36 months [70]. The study showed that the treatment responses were: partial response in 34 patients (36%), stable disease in 39 patients (41%) and progressive disease in 13 patients (14%). Response was not

evaluable in 8 patients. No complete remission was observed. Median progression-free survival (PFS) was 10.8 months (vs 18.3 in SELECT study) [44]. The lower PFS compared to SELECT study could be explained with a worst ECOG performance status in our population compared to SELECT (15% of patients with ECOG 2 vs 5%, respectively). 82 patients presented at least one AE. The most frequent were asthenia, hypertension and diarrhea. No AEs of grade 5 were reported. Although not statistically significant, a trend to a better overall survival was observed in patients treated in Institutions with 5 or more enrolled subjects. The EAP ended in June 2016, after lenvatinib became commercially available. Then, lenvatinib was prescribed according to the Italian Drug Authority (AIFA) indications. From September 2016 to September 2022, we prescribed lenvatinib to 18 patients, each of them met the criteria of radio-iodine refractoriness. The characteristics of patients are described in Table 3. They included 12 females and 6 males (F:M ratio of 2:1), with a median age of 67.2 years (46-90 ys). The most common histological type of thyroid cancer was follicular cell cancer. All patients received RAI therapy after total thyroidectomy, from a minimum of 265 to a maximum of 1200 mCi (median dose of 580 mCi), except for one patient with histological diagnosis of poor differentiated carcinoma. 7 patients received radiotherapy before starting lenvatinib, and 1 patient was treated with a previous-line TKI (sorafenib), within a clinical trial. The sites of metastasis were: lung (12), bones (10), neck (6), cavernous sinus (1), hypopharynx and larynx (1), liver (1), cerebral (1), skin (1).

Tab. 3 Baseline characteristics of patients receiving lenvatinib.

BASELINE CHARACTERISTICS		
Median age, years	67.2	
Sex	Male	6/18
	Female	12/18
Histology	Follicular	9/18
	Papillar	8/18
	Poor differentiated	1/18
ECOG PS	0	10/18
	1	4/18
	2	4/18
Metastatic lesions	Lung	12/18
	Bones	10/18
	Neck	6/18
	Cavernous sinous	1/18
	Hypopharynx and larynx	1/18
	Liver	1/18
	Cerebral	1/18
	Skin	1/18
Prior TKI therapy	RAI therapy	17/18
	Radiotherapy	7/18
	Sorafenib	1/18

ECOG PS: Eastern Cooperative Oncology Group Performance Status
RAI: radioactive iodine

14 patients started lenvatinib at a dose of 24 mg, 4 patients started with a lower dose because of their worst general health conditions (ECOG 2). All patients received levo-tiroxine as suppressive therapy, in order to maintain the value of TSH <0,1 mU/L. All patient with bone metastasis received denosumab 120 mg 1 injection a month. The median follow-up was of 37.1 months, with a range from 2 to 72 months and there are no real life studies in the literature with such a long follow-up [71,72,73,74,75].

4 patients died during follow-up: 1 patient died from progressive brain metastases 2 months after therapy start, 1 patient died from larynx stenosis after 14 months of therapy and the other two died because the global progressive disease after 12 and 16 months after therapy start, respectively. So the overall survival was 78%. The progression-free survival was 64% at 36 months and 48% at 60 months. See figure 2

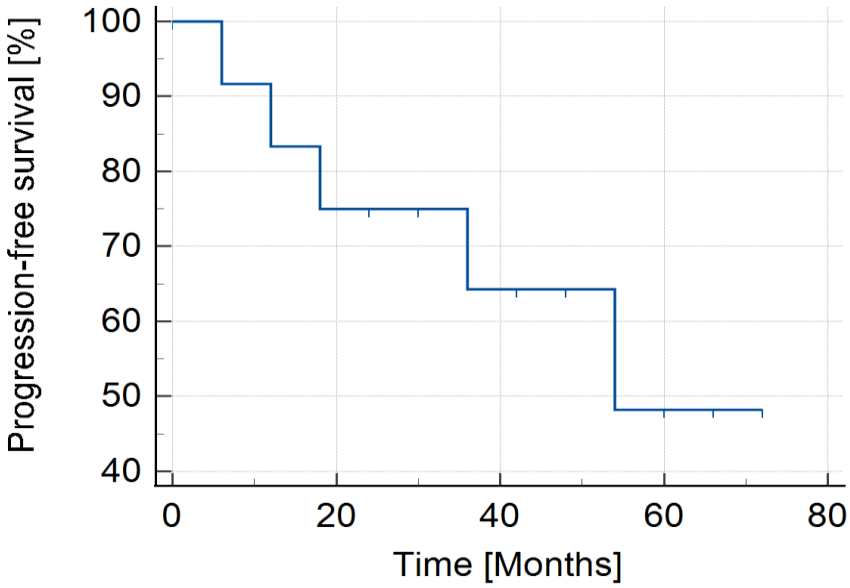

Fig. 2 Kaplan-Meier of progression-free survival in patients treated with lenvatinib in our Institute.

6 patients permanently discontinued lenvatinib: 1 patient due to gastrointestinal perforation after 3 months of therapy, 1 patient for the appearance of proteinuria (grade 3) after 6 months of therapy, 1 patient for global disease progression in lung, 1 patient after the appearance of a gastrointestinal stromal cancer, 2 patients for personal decision. The response to the therapy was: stable disease in 8 patients (44%), partial

response in 4 patients (22%), global progressive disease in 3 patients (17%), oligoprogressive disease in 3 patients (17%). No complete remission was observed. See figure 3

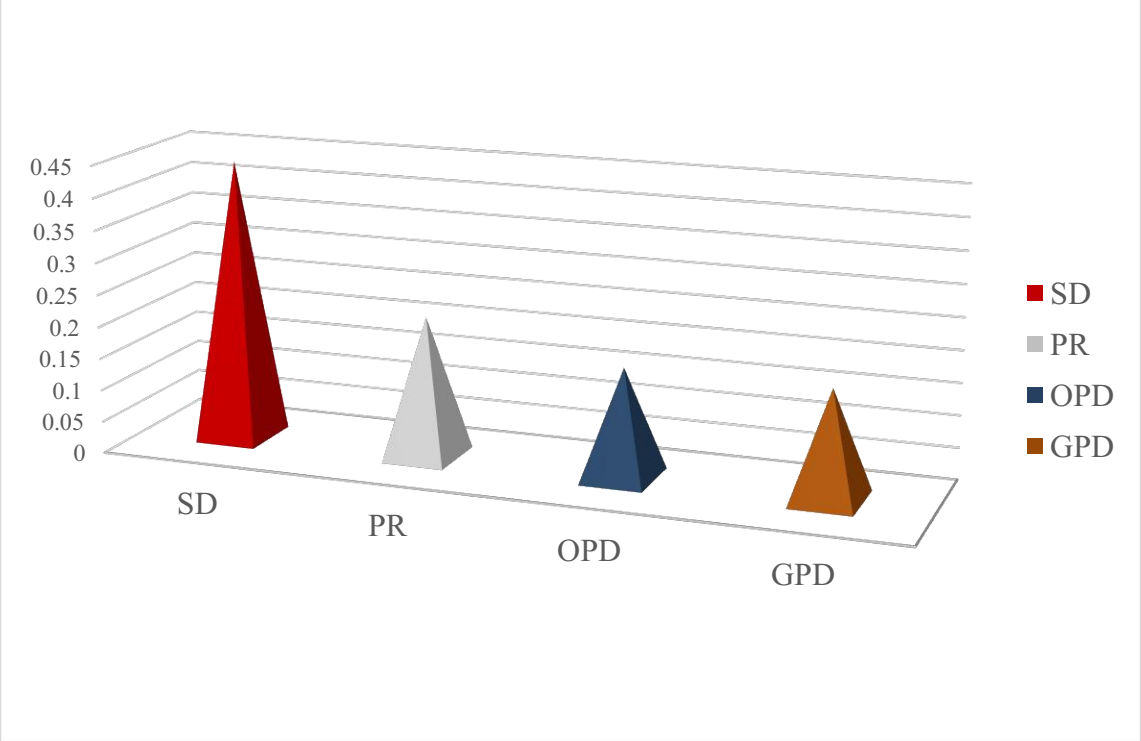

Fig. 3 Response to therapy with lenvatinib

GPD: global progressive disease; OPD: oligoprogressive disease; SD: stable disease; PR: progressive disease.

Concerning the treatment-related toxicity, the most common AEs (Fig. 4) were:

- hypertension (13),
- diarrhoea (13),
- asthenia (12), nausea (12),
- weight loss (11),

- anorexia (11),
- disphonia (9), oral mucositis (9),
- hypothyroidism (5).

Less common AEs were:

- vomiting (4), acute abdominal pain (4),
- rectal mucositis (3),
- eritema (2), proteinuria (2),
- folliculitis (1).

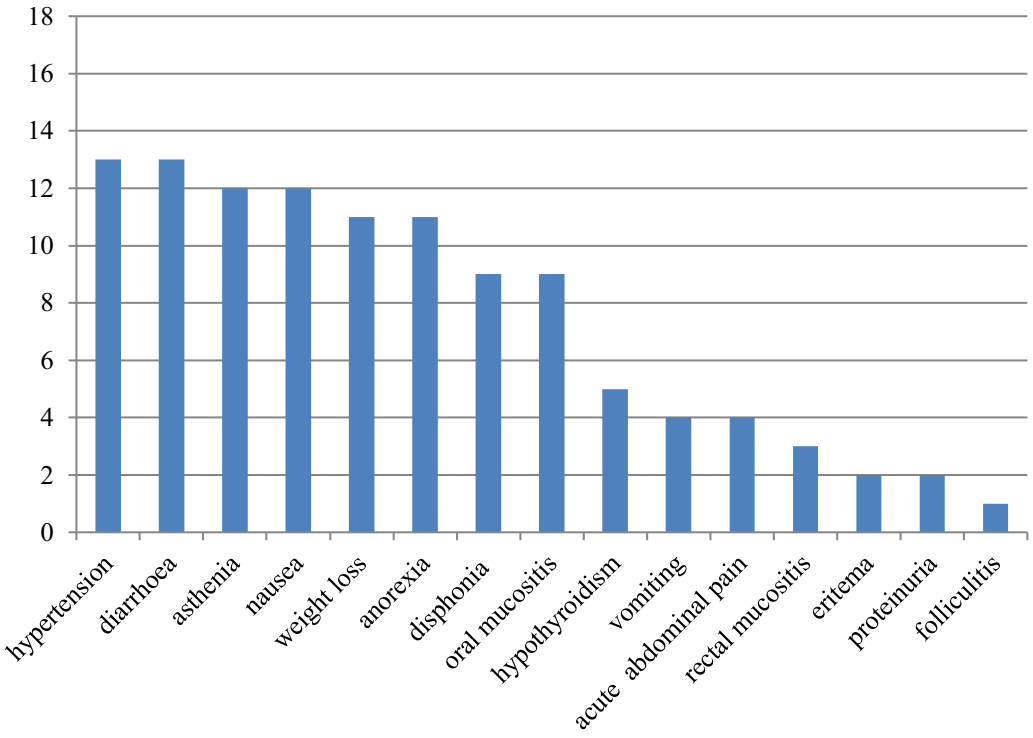

Fig. 4 The AEs during treatment with lenvatinib

As regards the severity of these effects, except for gastrointestinal perforation and 1 case of cholecystitis (grade 4), all the adverse events were of grade 1-3 (Fig. 5):

- hypertension: 8 cases of grade 1 and 5 cases of grade 2;
- diarrhoea: 8 cases of grade 2 and 4 cases of grade 3;
- asthenia: 8 cases of grade 2 and 4 cases of grade 3;
- weight loss: 1 case of grade 1, 7 cases of grade 2 and 3 cases of grade 3;
- nausea: 4 cases of grade 1, 5 cases of grade 2 and 3 cases of grade 3;
- anorexia: 3 case of grade 1, 5 cases of grade 2 and 3 cases of grade 3;
- disphonia: 2 cases of grade 1, 5 cases of grade 2 and 1 cases of grade 3;
- oral mucositis:1 case of grade 1, 5 cases of grade 2 and 3 cases of grade 3;
- hypothyroidism: 5 cases of grade 1;
- vomiting: 1 case of grade 1, 2 cases of grade 2 and 1 case of grade 3;
- acute abdominal pain: 1 case of grade 2, 2 cases of grade 3 and 1 case of grade 4;
- rectal mucositis: 3 cases of grade 2;
- eritema: 2 cases of grade 2;
- proteinuria: 1 case of grade 2 and 1 case of grade 3.
- folliculitis: 1case of grade 3.

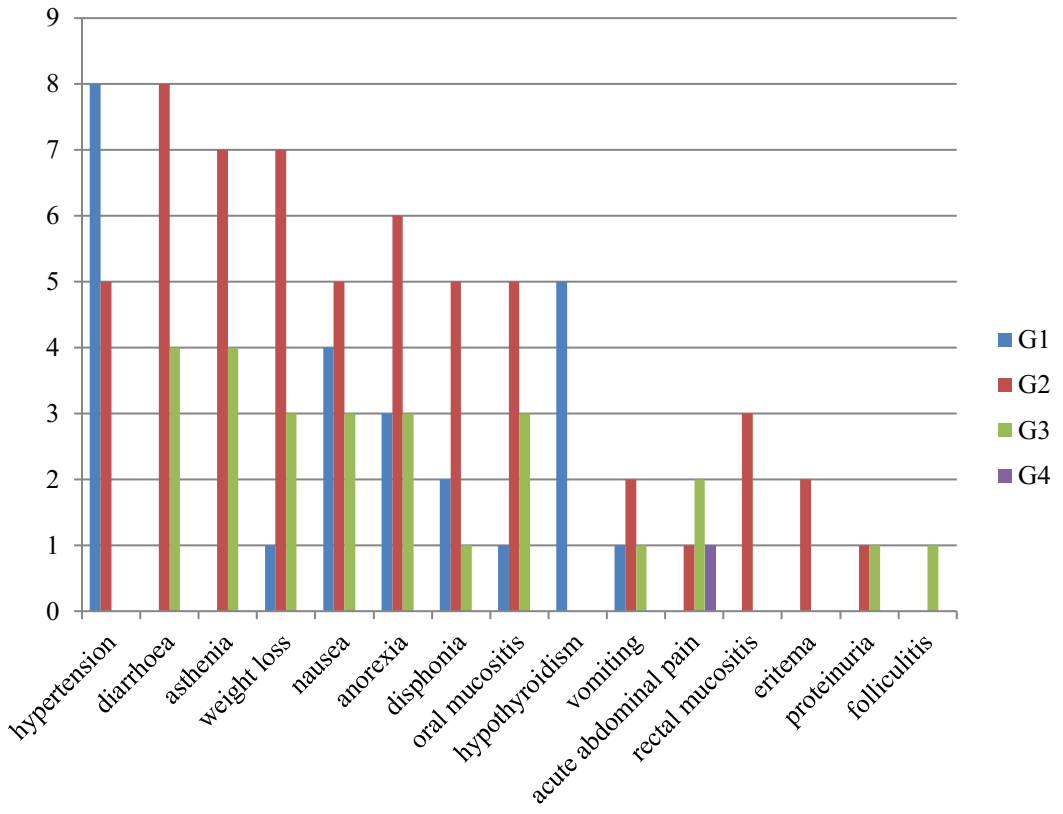

Fig. 5 Severity of AEs

Some of these AEs could severely affect patients' quality of life or endanger their health. For this reason, management of each of them was accurate and our approach varied on a CTCAE grading basis. The earliest AE occurring after lenvatinib start was hypertension. The approach consisted of increasing the anti-hypertensive therapy the patient already took or adding drugs, preferably ACE-inhibitors or angiotensin II blockers or calcium antagonists. Also diarrhoea tended to develop early (within 1 month). So patients were encouraged to avoid caffeine, fiber, dairy and greasy food. When symptomatic treatment was need, most patients were able to obtain good control with loperamide at the onset of diarrhoea, until a maximum of 16 mg daily, associated to probiotics. Most patients complained asthenia, in some

cases associated to hypothyroidism and the following need to increase L-T4 daily dose, in the others it could be linked to depression and emotional distress or weight and muscle mass loss. If needed, patients were encouraged to start a specific programme with a psychologist and/or a physiotherapist. It has been reported that primary adrenal insufficiency (PAI) occurs frequently in patients treated with lenvatinib, and that replacement treatment with cortisone acetate consequently reduces the sense of fatigue, even if none of our patients had demonstrated adrenal insufficiency [76].

A nutritional path was also important to counteract weight loss, which could be related to nausea, vomiting, anorexia, oral mucositis. Mucositis, oral and/or rectal could manifest as mouth ulcers and sensitivities, and rectal lesions. An accurate personal hygiene resulted to be essential, associated to products containing lidocaine and/or sucralfate to provide relief. Eritema, appearing above all on hands and feet, required patients education: wearing cotton gloves and socks, avoiding hot and cold water, applying regularly emollients and moisturing cream. Finally, the 4 cases of acute abdomen pain were caused by cholecystis and biliary tract inflammation, surgically or medically treated.

3.2 Our approach to the oligoprogressive disease

Among the 18 patients treated with lenvatinib in our centre, 3 of them met the criteria of oligoprogression. This is a topic not yet covered in the guidelines or in other scientific publications, but the management of other types of tumor has convinced us of the possibility of improving the prognosis of our patients appling local ablative therapies on the site of progression, while maintaining systemic therapy.

The first case is a 61-year-old man who underwent total thyroidectomy in 2010 for a follicular thyroid carcinoma (oncocytic subtype). Over a period of 7 years, he received a total dose of 405 mCi of RAI. When bone metastases appeared (vertebrae D6), it was treated with local techniques: a first course of EBRT with 20 Gy on D6 in 2017. After a year, a new metastases appeared on L4, so in June 2018, he underwent laminectomy on L4. In September 2021, PET/TC showed a progression of disease: D6 lesion progressed and three new lung metastasis appeared. So, he started lenvatinib at dose of 24 mg. The disease remained stable until February 2022, when the D6 lesion progressed and a new metastasis appeared on D7. Since all other target lesions were stable, we decided to treat the oligoprogression through RFA on D6 and D7, following by vertebroplasty, maintaining the systemic therapy. See figure 6.

Till now, the patient continues to assume lenvatinib (at a lower dose of 10 mg) and the last control shows stable disease (July 2022).

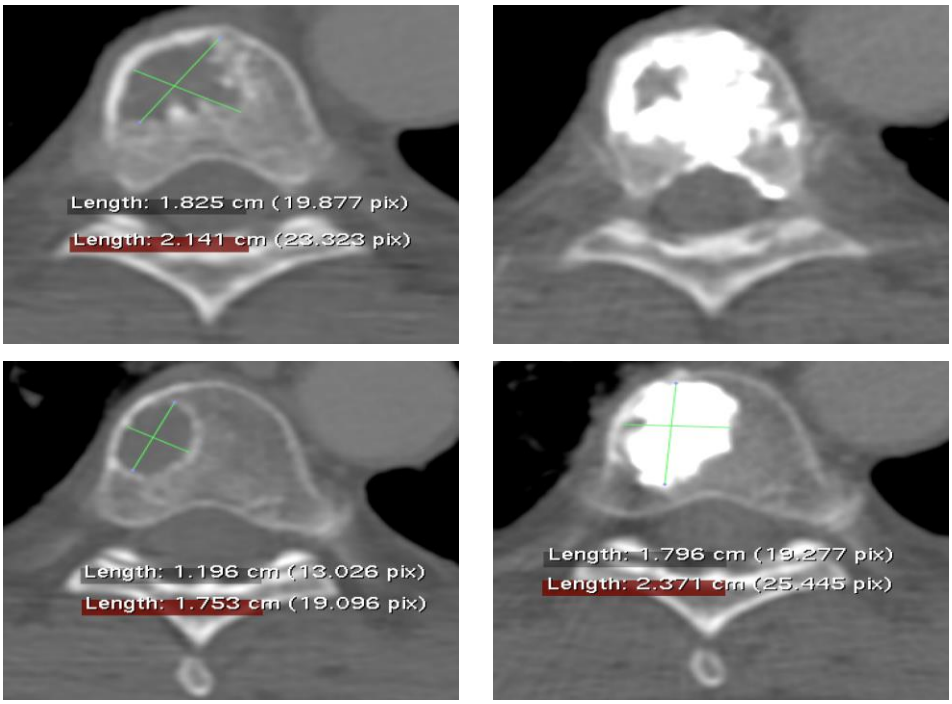

Fig. 6 Lesions D6-D7 pre and post-LAT

The second case is a 59-year-old woman who underwent total thyroidectomy in 2012 for a follicular thyroid carcinoma (oncocytic subtype), followed by RAI therapy (cumulative dose of 318 mCi). In August 2015, a symptomatic lesion on D8 was treated through EBRT (total dose of 20 Gy in 5 fractions). The following controls (CT-PET and MRI scan), showed the presence of several metastases (D1, D5, L1, right femoral neck), so on September 2018 she started taking lenvatinib at the dose of 24 mg, progressively reduced when important adverse effects appeared. The disease remained stable until January 2022, when lesion on D5 progressed, while the systemic treatment was controlling all the other sites of metastases. So, she underwent a course of EBRT at the cumulative dose of 30 Gy in 10 sessions on D5, while maintaining the assumption of lenvatinib. The last control shows stable disease (July 2022). See figure 7.

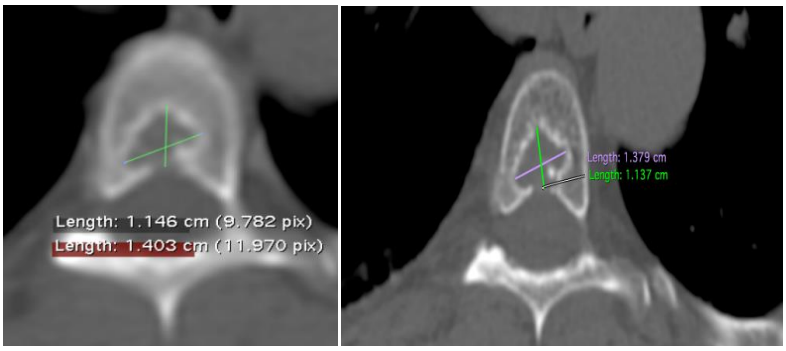

Fig. 7 Lesion D5 pre and post-LAT

The third case is a 69-year-old women who underwent total thyroidectomy in 2015 for a follicular thyroid carcinoma (oncocytic subtype), followed by RAI therapy (cumulative dose of 357 mCi). In November 2016, a CT/PET showed recurrence in the right neck and pulmonary metastases, while a following MRI showed a metastasis of the cavernous sinus, which incorporated a piece of the right internal carotid artery. For this reason, she started lenvatinib at the dose of 10 mg in June 2017. It was able to control the disease, and in July 2018, the patient convinced herself to underwent to SBRT for the metastasis of the cavernous sinus. The disease was stable until December 2020, when neck recurrence progressed. So we decided to associate local therapy (EBRT 30 Gy in 10 sessions), to lenvatinib. See figure 8

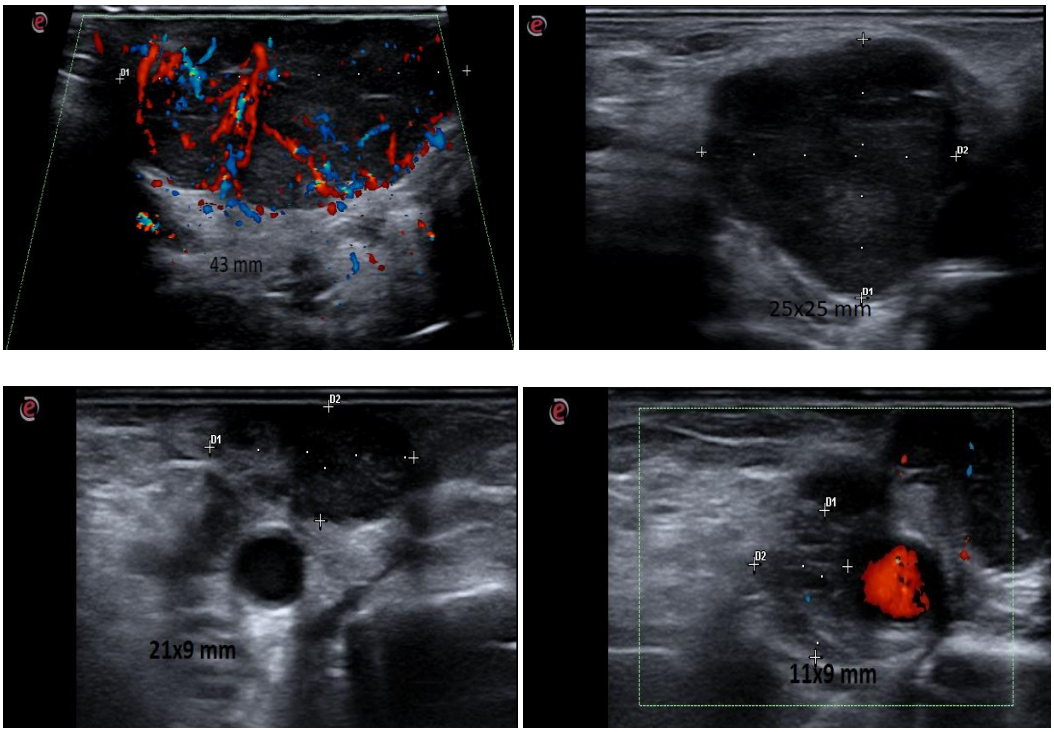

Fig. 8 Neck lesions pre e post-LAT

3.3 Towards a personalized treatment

The onset of progression prompted us to reflect on the need to find other therapeutic options. The treatment with targeted therapies has changed the therapeutic strategies and the disease prognosis, however drug resistance remains the main reason for treatment failure. Thus, the understanding of both molecular pathways implicated in tumorigenesis, and tumoral escape mechanisms, are of paramount significance for the development of new therapies for DTC. For those patients not responding to the approved TKIs or those not tolerating treatment-related adverse events, novel targeted therapies could be used based on the tumor's molecular signature. For this reason, a genetic test targeting actionable cancer mutations should be considered to individualise therapy. That's why the ESMO guidelines recommend the use of Next-Generation Sequencing (NGS) in clinical practise, so that the study of gene panels can increase the probability of finding a druggable target [45].

BRAF V600E mutation is the most common somatic mutation in thyroid cancer of follicular cell origin. BRAFV600E is located in the 15th exon of the BRAF gene. Its mutation is the T1799A point mutation in this exon, which changes its coded product, resulting in the substitution of valine (V) by glutamate (E). In patients with $BRAF^{v600e}$ mutation, the lost of gene expression is correlated to the activation of the MAPKinase pathway which has been shown to result in reduced expression of sodium/iodide symporter (NIS), which, in turn, leads to reduced iodide uptake, and, hence, resistance to the effects of RAI therapy. So, these patients develop RAI-refractoriness very early. Trials conducted with BRAF inhibitors showed significant drug resistance, due to reactivation of the MAPK pathway. The combination of BRAF and MEK inhibitors can significantly inhibit the growth of TC cells,

reduce the survival rate of clone formation, reducing the activity of the MARK signalling pathway [77]. A treatment combining of trametinib (MEK inhibitor) plus dabrafenib (BRAF inhibitor), already approved for melanoma and anaplastic thyroid cancer, can be an important therapeutic option for patients with BRAF mutated advanced TC. It should be emphasized that, with regard to efficacy, the first studies demonstrate the non-superiority of the combined therapy compared to dabrafenib monotherapy [78].

A 66-year-old man was diagnosed with poor differentiated thyroid carcinoma after total thyroidectomy in July 2021. The NGS study on tumour tissue showed a BRAF V600E mutation. The aggressive cancer had caused tracheal invasion and needed a tracheal stent. Beyond this, a CT scan showed metastases in mediastinal lymph nodes and lung micrometastases. Despite RAI therapy (120 mCi), the local and distant lesions progressed. The risk of tracheal fistula formation contraindicated the use of lenvatinib, so treatment with dabrafenib–trametinib was requested as a compassionate use. In June 2022, patient was started on the combination of dabrafenib 150 mg twice a day and trametinib 2 mg once a day. After 3 months of therapy, the CT scan showed a partial response. See figure 9

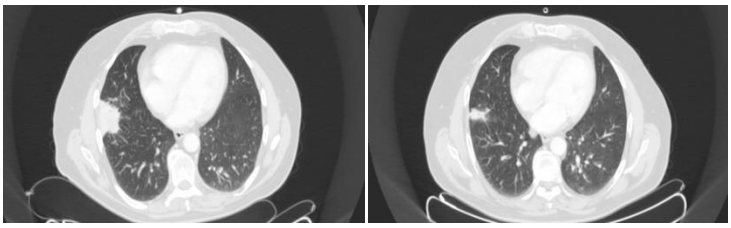

Fig. 9 Partial response in pulmonary metastases

Neither dabrafenib nor trametinib is currently approved for papillary thyroid cancer, however responses have been observed to those drugs in thyroid tumors carrying the BRAFV600 mutation.

This approach demonstrates the need for personalized treatment for each patient. Further studies are needed to help clinicians make the best choice among available treatment options.

CONCLUSION

During the last decades, the knowledge on thyroid cancer molecular biology has led to the evolution of a number of novel therapies for these tumors, mainly tyrosine kinase inhibitors. This study analysed the results of the treatment with lenvatinib in 18 patients during a median follow-up of 37.1 months. 14 patients received lenvatinib at a starting dose of 24 mg, while 4 patients started with a lower dose, due to worst general condition. Moreover, the dose has been reduced progressively according to the severity of adverse events. The most common adverse events (grade 1-3) have been: hypertension, diarrhoea, asthenia, weight loss, nausea, anorexia. All the AEs have been treated with specific drugs, as indicated in guidelines. The progression free-survival (PFS) has been 64% at 36 months and 48% at 60 months. The overall survival (OS) has been 78%. The response to the therapy has been: stable disease (44%), partial response (22%), global progressive disease (17%), oligoprogressive disease (17%). In case of oligoprogression, we decided to treat the progressive sites of disease with local ablative therapy and to continue the systemic therapy with lenvatinib. There are scarce published data for this approach in thyroid cancer, because of the relatively short time since the approval of TKIs in this scenario and the heterogeneity of the clinical presentations.

Finally, the appearance of drug resistance makes the development of more effective further targeted therapies necessary, towards a personalized approach, based on tumour' mutational pattern.

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