PHARMACOTHERAPY IN ADVANCED THYROID CANCER

Voichiţa Mogoş
Clinic of Endocrinology, „St. Spiridon" Hospital Iaşi
University of Medicine and Pharmacy „Gr.T.Popa" Iaşi

PHARMACOTHERAPY IN ADVANCED THYROID CANCER (Abstract): Thyroid cancers are the most common carcinomas of the endocrine system. Their behavior depends of histology, extension of the disease and patients-related factors. Differentiated thyroid cancers arising from follicular epithelium may be cured with combined surgery and radioiodine therapy. In 10-15 % of cases patients may develop metastases which are cause of death. In advanced differentiated thyroid cancers of follicular origin combined therapy with radioiodine and TSH suppression may result in a long survival if metastases are still iodine avid are made iodine avid by redifferentiation therapy. Classical chemotherapy has no significant effect in differentiated advanced thyroid cancers. The knowledge regarding genes and gene products involved in cancer development, dedifferentiation, angiogenesis, tumor progression, and apoptosis allowed the development of a new arsenal of therapeutic agents designed to target these elements. Antibodies, small molecules, antisense nucleotides, and other agents directed against RET-RAF-MAPK, the main pathway of tumor initiation and growth or against other growth factors and their receptors. Most of these therapeutic agents proven to be efficient in preclinical trials and some enter into clinical trials.

KEY WORDS: DIFFERENTIATED THYROID CANCER, RET–PROTOONCOGENE, ONCOGENE INHIBITORS, ANGIOGENESIS INHIBITORS, MODULATORS OF GROWTH AND APOPTOSIS.

BACKGROUND

Differentiated thyroid cancers are the most common cancers of endocrine system.

Most of differentiated thyroid cancers may be effectively cured in patients with localized disease with combined surgery and ablative radioiodine therapy. 10-15 % of patients may develop distant metastases which are the cause of the disease [1].

Advanced thyroid cancer is defined as: presence of distant metastases not including cervical lymph node metastases; recurrent locally invasive disease of the neck.

STAGING OF THYROID CANCER

According to American Association Joint Committee on Cancer Classification, advanced thyroid cancer includes [2]: stage IV disease; stage II disease (distant metastases) in patients less than 45 years old; any occurrence of anaplastic thyroid cancer is also considered a advanced disease because of its extremely poor prognosis even after aggressive surgical resection.

Distant metastases occur in 5-23 % of patients with DCT, most of them having less differentiated cancers [3]. Most important sites of metastases are [4,5]: lung - 45-49%; bone only- 24-39%; other single sites 4-8%; multiple sites – 12-19%.

Metastases may arise from papillary cancer in 51 % of case and from follicular cancers in 49 % of cases. Lung metastases from DTC are associated with thoracic pain and dyspnea, and bone metastases with swelling, pain, fractures [3].

Distant metastases may be identified through [1,6,7]:
- thyroglobulin measurement basal and under TSH stimulation (endogenous or exogenous) if there are functional metastases;
- X-ray: enlarged mediastinal lymph nodes may be seen in children with metastatic papillary thyroid cancer;
- bone metastases are usually osteolytic and may be detected in advanced stages; bone CT, skeletal and whole body MRI, combined SPECT/CT;
- ^{18}F-FDG –PET that is positive more frequently in thyroglobulin and iodine-negative DTC metastases. High FDG uptake in a large tumor has a negative prognostic impact;
- ^{124}I PET could be used in metastases with iodine uptake as well as simultaneous dual isotope acquisition with ^{131}I scan and ^{99m}Tc dysphosphonates.

Risk factors for developing metastatic disease are [1]:
- age: children with papillary thyroid cancer and patients over 45 years have a high risk;
- sex: male gender;
- tumor characteristics: aggressive histology (poor differentiated follicular cancer, Hurthle cell carcinoma, insular carcinoma), multifocallity, locally invasive tumor and extracapsular invasion).

Prognostic factors in patients with distant metastasis of differentiated thyroid cancer are [5]: age under 45 years, and particularly iodine avidity and histology.

TREATMENT OF ADVANCED DTC
Patients with advanced thyroid cancer have a few therapeutic options for effective disease control [7]. Primary treatment in patients with distant metastases of DTC reported by Sampson E [5] was: thyroidectomy – 82%, radioiodine – 88%, excision of metastases – 20%, external beam irradiation – 47%, chemotherapy – 6%. ^{131}I therapy is the most important therapy for advanced differentiated thyroid cancer

Local and regional recurrences of differentiated thyroid cancer
The recommended treatment is based on combination of surgery and radioiodine therapy in cases with radioiodine uptake [7]. The use of an intraoperative probe may be useful to improve success rate of surgery.

DTC – lung metastases
In the case of radioiodine uptake treatment consists of ^{131}I administration after prolonged suppressive T4 therapy withdrawal.
Administration of ^{131}I activity ranges from 1.7 to 7.4 MBq or higher every 4-8 month in the first 2 years and thereafter at longer intervals. 2-5 days after iodine administration WBS will assess the response to treatment. Thyroglobulin monitoring will guide further treatment.
WBS is not required before treatment because it does not modify indication for treatment and may induce stunning effect (reduction of uptake of the subsequent therapeutic iodine dose). There is no maximum cumulative dose of radioiodine that can be given in patients with persistent disease, but most remissions are obtained with doses equal or lower that 22 GBq

(600 mCi) [7]. Lithium may increase iodine uptake and induce a longer half-life of ^{131}I in the tumor [1,7]. Early diagnosis of metastatic disease is crucial. Small lung metastases may be cured with radioiodine, but its efficiency in large lung metastases is controversial (Dinneen SF quoted by Zanotti-Fregonara P [1]). If metastatic disease is unresponsive either primary or secondarily to radioiodine therapy and complet surgery is not possible external beam irradiation therapy may be indicated [1]. Gamma knife radiosurgery may be useful in selected patients for palliation [2].

DTC – bone metastases

Bone metastases, if iodine avid, may be treated with a combination of surgery and ^{131}I. Usually 100-150 mCi of ^{131}I are given every 4-6 month. In non iodine-avid metastases other could be used: external beam irradiation, bisphosphonates, embolisation and cementoplasty [1,7]. Only a few cases reveal complete response after external irradiation [3]. In selected cases CT-guided percutaneous radiofrequency ablation or percutaneous ethanol injection for bone metastases and solid lung metastases could be used [8]. Palliative surgery for bone metastases when there are neurological or orthopedic complications is useful to debulking large tumors and may be curative in patients with a single of a few bone metastases.

DTC - brain metastases

Brain metastases are rare and carry a worse prognosis [7]. When possible they will be operated and if non iodine avid or non resectable external beam irradiation may provide palliation and a better quality of life [9].

Thyrotropin suppressive therapy

Rationale and recommendations for THS suppressive therapy [7] are: to provide appropriate thyroid hormone in order to achieve normal blood levels; to inhibit THS – dependent growth of thyroid cancer cells by decreasing TSH levels to less than 0.1 mIU/L.

In patients considered to be in complete remission at any time of the follow up there is no need for TSH suppression and therapy may be shifted from suppressive doses to replacement doses. LT4 is the drug of choice. LT3 use is limited to short time therapy before withdrawal for preparation for WBS and ^{131}I therapy. The dose of T4 should be sufficient to reduce TSH levels under 0.1 mIU/L. TSH will be assessed every 3 months and the dose of T4 will be adjusted with 25 μg in order to achieve suppressive levels.

TSH suppressive therapy is mandatory in patients with persistent disease including those with detectable levels of thyroglobulin and no signs or symptoms and in high risk patients for 3-5 years.

Long term THS suppressive therapy may result in: weight loss, atrial fibrillation, cardiac dysfunction and bone loss. In elderly patients and in patients known with cardiac disease suppressive therapy should be avoided due to the risk of arrhythmias and tromboembolism [7].

Accelerated bone turn-over and subsequent bone loss may be seen during THS suppressive therapy. TSH suppressive therapy is associated with minimal bone loss at distal radius in men, minor bone loss in lumbar spine and femoral neck in a small number of patients [10]. Other studies report no risk for osteoporosis in men and women treated with suppressive doses of thyroid hormones, no bone turn-over markers modifications except for increased PTH. When adjusted to maintain TSH below the detection limits for a second generation TSH assay thyroxine therapy does not induce significant bone loss and does not increase the risk for osteoporosis in patients with DTC [11].

Human Recombinant TSH (hrTSH) for diagnostic and therapeutic purposes in DTC; hrTSH is produced by Chinese hamster ovary cell line transfected with TSH human subunit gene.

The protocol for hrTSH administration prior diagnostic or therapeutic [131]*I administration* [12] is: 0.9 mg hrTSH day 1 and 2; ^{131}I is given day 3; thyroglobulin measurements in days 1-3-5; WBS in day 5.

Adverse effects of hrTSH: nausea, fatique, headache. Painful swelling of bone metastases was reported [13]. No toxicity and antibodies directed against hrTSH have been seen. hrTSH provides a stimulation of tumor cells as good as T4 withdrawal. Some authors reported better stimulation of metastases of DTC with thyroid hormone withdrawal than by hrTSH and other consider it of value in metastatic disease. Remnant uptake may be lower in hrTSH stimulated patients because renal iodine clearence is preserved in euthyroid patients and reduced in hypothyroid patients.

Patients with increased thyroglobulin and negative for iodine scan may be investigated by FDG-PET that is more accurate after hrTSH stimulation than under T4 suppressive therapy.

hrTSH is particularly suitable because:
-   preserves quality of live of patients avoiding prologed hypothyroidism,
-   it decreases morbidity associated with prolonged thyroid hormones withdrawal,
-   it is indicated in patients unable to rise endogenous TSH and in those in whom prolonged withdrawal is contraindicated,
-   in patients with spine metastases and spinal cord compression a short course of high dose corticoisteroids will avoid worsening of neurological symptoms,
-   hrTSH is particularly suitable in advanced reccurent or metastatic DTC, in patients who are intolerant to TSH stimulation by L4 withdrawal.

Differentiated thyroid cancer generally has a good prognosis. 50% of patients with distant metastases of DTC die in 10 years after diagnosis. The role of radioiodine in the management of DTC is beyond dispute but the therapy is impaired by decreased expression of NIS during dedifferentiation process. Therefore strategies to improve iodide uptake in metastases are mandatory.

Stategies to enhance ^{131}I uptake and retainment into DTC neoplastic cells

Lithium therapy **-** 300mg 3 times per day or 10mg/kg.b.w. per day is a potential adjuvant of ^{131}I therapy by increasing 2 fold the dose of radioiodine retained by metastatic tumor in DTC [7,12].

Redifferentiation of non iodide–avid DTC - The ability of thyroid cancer cell to uptake ^{131}I allows the use of ^{131}I for diagnosis and treatment purposes including the treatment of metastatic disease.

Retionoids **-** are derivatives of vitamin A(retinol) that increase NIS mRNA expression and iodide uptake in thyroid cancer line cell. They bind to RXR retinoid nuclear receptor that regulates cell growth and differentiation. In clinical trial cis-retionoic acids increase iodide transport in 26-40% of cases. 1-1.5 mg/kg/day of *isotretinoin* or *bexarothene* 300mg per day for 6 week prior to ^{131}I uptake stimulated by rhTSH may increase radioiodine uptake in some cases but not in all detectable metastases [14].

Histone deacetylase inhibitors - induce through a unclear mechanism cell cycle arrest and dedifferentiation. They re-establish NIS expression, Tg mRNA and iodide accumulation [15].

Superoylanilide hydroxamic acid inhibits papillary and anaplatic thyroid carcinoma cell growth and dedifferentiation in vitro [16].

Valproic acid - inhibit histone dyacetylase and promote re differentiation, NIS expression, iodide uptake and is a growth suppression agent in poorly differentiated thyroid carcinoma cells [17]. Retinoids may be associated with histone deacetylase inhibitors (Cras A 2007, quoted by Zanoti-Fregonara P [1]).

Gene therapy - adenovirus – mediated in vivo NIS transfer is directed to introduce NIS code into less differentiated thyroid carcinoma in order to induce or enhance iodine avidity [15].

Classical chemotherapy has a little or no role in the management of advanced differentiated thyroid cancer. It is restricted to patients with progressive disease, uncontrolled by surgery and 131 iodine therapies [7].

Studies with chemotherapy in radioresistant DTC are limited.

Single agent *doxorubicin* or a combination of *doxorubicin and cysplatin* provide a 10-20% partial and transient response without impact on prolonged survival in patients with metastatic DTC [7]. Combined therapy with bleomicin (30mg/day for 3 days), adriamicin ($60mg/m^2$ day 5, cisplatinum 60 mg/m^2 day 5 produced a transient response in some cases [18]. Doxorubicin in association with interferon beta was tried in advanced non-medullary thyroid cancer with modest antitumor activity but increased toxicity [19].

Combined chemotherapy using *carboplatin* and *epirubicin* under THS endogenous or exogenous stimulation demonstrated a rate of complete and partial response of 37% of cases and 81% of patients remain with stable disease [20].

Novel therapies in (clinical) trials for DTC

New insights on the genetic and molecular pathogensis of cancers, including thyroid cancer allowed the development of new therapies targeting the mechanisms involved in thyroid cancer development. These therapies are in different phases of in vitro and in vivo trials with promising results. Target therapy, a new generation of anticancer therapy, is designed to interfere with specific molecular targets which are genes or their protein products believed to play a crucial role in cancer biology: aggressive behaviour, metastatic spread, loss of iodine uptake ability and resistance to conventional therapies.

These therapies are grouped in the following categories [21]: oncogene inhibitors, angiogenesis inhibitors, modulators of growth or apoptosis, other targeting therapies. Most agents presented later are being testes in vitro, on xenografts models and some of them are now in clinical trials [22].

1. Oncogene inhibitors

Tyrosine kinase inhibitors target the activated RET/PTC oncogene, responsible for a proportion of PTCs. PTC development is associated with RET/PTC rearrangements that occur in 2.5 to 40% of cases, most of them in radiation-induced PTCs. RET/PTC was also noticed in Hurthle cell carcinoma. Constitutive activation of RET is responsible for sporadic and hereditary MTC (FMTC, MEN 2A and MEN 2B). Thus, there is rationale to try RET inhibitors in different forms of thyroid cancers [23].

Inhibitors of RAS, RAF, and MEK kinase target various members of the signaling pathway for RET action.

RET tyrosine kinase inhibitors in DTC and MTC:
- Imatinib was proven to be well tolerated but induces only a transient stable disease when used in advanced MTC [24];

- ZD6474 – Zactima a kinase inhibitor acts as antiangiogenic, RET blocking agent and inhibits EGFR (tested in PTC cell lines) [24];
- Pyrazolopyridines PP1 and PP2 [23,24];
- Indolocarbazole derivatives inhibit RET in MTC cell lines;
- Sorafenib (BAY 43-9006) a multikinase inhibitor inhibits RET/PTC autophosphorilation is useful in all thyroid malignancies associated with RET autoactivation due to germline mutations or RET/PTC rearrangements [1,25,26];
- SU11248 (Sunitinib), is an indolinone derivative that target specifically RET/PTC oncogenes acting as tyrosine kinase inhibitor and by the same mechanism inhibit PDGFR and VEGFR being particularly useful in PTC [27];
- An antibody able to induce RET internalisation was produced by Yano (2000, quoted by de Groot 2006 [23]) but its efficacy was not yet proven in thyroid cancers.

RAS is a GTP-binding protein involved in cell proliferation and survival. Mutations of RAS have been reported in thyroid cancer. *RAS inhibitors* from preclinical and clinical trials there are:
- Antisense drugs are a single strand DNA sequences of 13-25 nucleotides complementary to targeted mRNA, that interfere with ribosomal assambly and bloks gene expression. ISIS 2503 a first generation antisense nucleotides that selectively inhibits RAS [25];
- Farnesyl transferase inhibitors inactivate RAS [25];
- Tipifarnib, Lonafarnib, Manumicin (a farnesyl transferase inhibitor) and paclitaxel [22,25];
- Phenyacetate an aromatic fatty acid that increases radioiodine uptake and thyroglobulin production and inhibits VEGF in DTC cancer cells [22].

BRAF inhibitors:
- BRAF kinase belongs to the RAF family of serine/threonine kinases, a downstream effector for RAS activation, acting through BRAF – MAPK – ERK pathway [22]. 44% of PTC and 24% of ATC carry a point BRAF mutation [28], this mutation being the most prevalent mutation in thyroid malignancies [24], RAF inhibition may be achieved [22];
- Antisense nucleotides designed to inhibit C-RAF ISIS 5132 (I phase study);
- BAY-43-9006 - Sorafenib was tested in advanced PTC and anaplastic thyroid cancer.

MEK inhibitors are kinase inhitors, CI – 1040, PD 03225901, ARRY – 142886, which proven to be efficient and well tolerated in other cancer in clinical trials and will be probably used in thyroid cancer in which MEK activity is involved [25].

NTRK1 incodes for a high afinity receptor for NGF and its interraction with NGF is blocked by CEP – 701 an indolocarbazole derivative [24].

Akt/mTOR (Akt/ mammalian target of rapamicin) acts through IP3 pathway and may be overexpressed in some sporadic follicular thyroid cancers. KP-372 an Akt inhibitor induces inhition of cell prolipheration and apoptosis in thyroid cancer cell lines [25].

Rapamicin (Sirolimus) is an macrolide antibiotic with immunosuppressive and antutimor properties. Its analog CCI-779 is on phase I trial in thyroid cancer therapy [22].

LY 294002 inhibitor of IP3 pathway was used in MTC cell lines [25].

2. Angiogenesis inhibitors

The most important factors associated with angiogenesis and tumour progression are: FGFR, EGFR, cMET (Hepatocyte Growth Factor), VEGF and IGF-1 receptor [22].

VEGF-R is the most prominent factor involved in tumor angiogenesis and is overexpressed in papillary, poorly differentiated and metastatic DTC

Inhibitors of VEGFR

- Recombinant human monoclonal antibodies against VEGF reduce angiogenesis. Bevacizumab inhibits angiogenesis in xenografts models and in association with chemotherapy and radiotherapy is now in phase II clinical trial for head and neck solid tumors [22];
- Vatalanib is small molecule VEGFR inhibitor and tyrosine kinase inhibitors and reduces follicular tumor cells xenograft in animal models and AC013736 an oral inhibitor of tyrosine kinase fraction of VEGF and PDGF receptors is now in phase II trial in non iodine-avid thyroid cancer [25];
- ZD6474 inhitis VEGF, RET oncogene an MEN 2a and 2b [22];
- Combrestatin, a tubulin–binding protein inhibitor with vascular targeting properties may be useful in poorly differentiated thyroid cancer, but its cardiac side effects need careful avaluation [22].

EGF and *EGFR* in PTC and associated with poor prognosis. It acts through RAS-RAF MAPK cascade and PI3 kinase pathway. Bloking of EGF and EGFR results in cell arrest in G1 phase, apoptosis, antiagiogenetic activity and down regulation of metaloproteases [22].

Inhibitors of EGFR

- recombinant human monoclonal antibodies agains EGFR - were tested in thyroid cancer cell lines [22]. Mab 4253 an anti AGFR antibody was tried in papillary cancer cell lines [25];
- AG1478 is a tyrosine kinase inhibitor small molecule that antagonises EGF – mediated angiogenesis and local invasion;
- Gefitinib (Irresa, ZD1839) a tyrosine kinase inhibitor that blocks EGFR is in trials for iodine refractory DTC, advanced DTC and ATC xenografts [29]. A phase II clinical trial with gefitinib 250 mg.per day in 27 patients with different forms of thyroid cancers (PTC, FTC, ATC, MTC) failed to prove any significant result in terms of partial response rate [30].

FGFR 4 is over-expressed in advanced and metastatic thyroid cancer. Its inhibitor is PD 17307 - NVP-AEE 788 a member of pyrazolopirimidine class inhibits tyrosine kinases coupled with both EGFR and VGFR with antiangiogenetic effects in experimental bone metastases of FTC [31].

Thalidomide is an antiangiogenic factor was used in a phase II clinical trial including follicular, insular carcinoma refractory to radioiodine and MTC with distant metastases. 800 mg tolerated dose induced partial response in 18 % and stable disease in 32 % of cases with a median survival of 23.8 months for responders and 11 months for non-responders [32].

3. Modulators of growth or apoptosis

Apoptosis is the programmed cell death induced by internal and external stimuli**.**

HSP 90 is a multichaperone protein complex that mediates maturation and stability of different proteins involved in tumorigenesis: RAF, Akt. Inhibition of HSP90 lead to degradation of these proteins and interruption of signal transduction that is essential for tumor progression.

HSP 90 inhibitors [2,22]: Geldamicin and its derivatives 17 AAG (Tanespimycin) and 17 DMAG (phase I/II clinical trials).

Tumor necrosis factor-related apoptosis inducing ligand TRIAL activates apoptosis by acting on its receptors TRIAL R1 and TRIAL R2 and caspase pathway. TRIAL-induced apoptosis is inhanced by cycloheximide and paclitaxel. Recombinant TRIAL induces apoptosis in xenografts models. TRM-1, a monoclonal antibody against TRIAL R1 with stimulating properties was not studied in preclinical trials [22].

Proteazome inhibitors - Bortezomib acts on ubiquity – proteasome system involved in cell survival and proliferation as proteasome inhibitor inducing apoptosis through activating intrinsic apoptotic pathway. It produces a light cytotoxic effect on anaplastic thyroid carcinoma cells, which is enhanced by TRIAL that activates the extrinsic apoptotic pathway [33].

Histone deacetylase inhibitors. Valproic acid usually used as an anticonvulsivant drug is a class 1 diacetylase inhibitor, activates in poorly differentiated thyroid cancer cell lines the intrinsec apoptotic pathway involving caspase 3 and 9 and also activates cell growth arrest in G1 phase [17].

Tiazolidindione and derivatives -PPARγ is considered a tumor suppressor factor. PAX8/PPARγ fusion oncogene was seen in follicular carcinoma and supresses wild type PPARγ activity. Troglitazone and rosiglitazone, ligands for PPARγ induce apoptosis by increasing expression of c-myc in PTC cell lines [34] inhibit tumor cell growth and upragulate NIS mRNA in papillary and follicular cell lines [25].

High afinity PPARγ agonist RS5444 produces growth inhibition in anaplastic thyroid cancer cell lines. PPARγ agonists induce reversion of epidermal/mezenchymal transition which is critical for anaplastic transformation of differentiated thyroid cancer. They also increase cyclin-dependent apoptosis, increase cyclin-dependent kinase inhibitors P21 and P27, decrese BcL-x expression and decrease caspase 3 and caspase 7 activities.

4. Cyclooxigenase 2 inhibitors

Activation of COX 2 is over expressed in different malignancies including thyroid carcinoma. A phase II clinical trial with celocoxib failed to prove any results in advanced differentiated thyroid cancer [35]. Cyclooxygenase 2 inhibitors seem to be active in reversing chemoresistance of MTC cells [36].

5. Other therapies

Demetilating agents. Reducing aberrant metilation of gene promoters that inhibit gene expression and is associated with loss of NIS, failure of 131 I treatment and aggressive behavior of DTC may be achieved by 5 azo 21 deoxycytidine and sodium butirate, epigenetic modifying agents that act through to induce NIS expression (NIS mRNA) and iodine uptake in NIS – deficient thyroid cancer cells culture [37].

Gene therapy : viral vectors were used to introduce: NIS gene in order to restore iodine uptake in poorly differentiated or anaplastic thyroid carcinoma cells [15]; Wt (wild type) p53 gene that is critical regulator of cell cycle progression by activation the cascade of events that lead to apoptosis [38].

Prodrug suicide gene therapy – cytoreduction gene therapy

Thymidine kinase gene is introduced within the cells and placed under the control of thyroglobulin gene promoter that is expressed only in thyroid cells. Once activated thymidine kinase is able to react with Ganclyclovir resulting in DNA strand breaks and subsequent cell death [22,38,39].

Treatment of advanced MTC

MTC represent 5-10 % of thyroid cancers and occurs sporadic form in 70% of cases or is familial in 30 % of cases. Independently of the form the main event leading to its development is a germ line or somatic mutation or RET proto-oncogene that leads to auto-activation of RET tyrosine kinase receptor [23]. Until now a successful surgical treatment is possible only in the first stage of the disease, when tumor size is less than 1cm [40].

Successful treatment may be achieved only in the firs stage of the disease, with tumor size of less than 1cm. with surgical cure of 100%. Distant metastases may be present at diagnosis in 7-23% of patients. In most patients metastases occur later during evolution and impair quality of life [41].

Systemic chemotherapy reduces tumor size in 10% of cases and has no impact on survival. Chemotherapy in MTC is limited to patients with advanced or rapid progressive metastatic disease.

Drugs investigated in clinical trials for MTC

Doxorubicin alone or in association with cysplatin resulted in les than 20% tumor response. Same results have been reportd with fluorouracil, dacarbazine, streptozotocine, cyclophosphamide and vincristine. Somatostatin analoques andd interpheron have not proven efficacy [41].

Transarterial chemoembolisation of the hepatic artery with doxorubicin 50-70mg. per treatment for liver metastases is associated with regression of metastases in 42 % and stabilization in 42% of patients for 5-28 month [42]. Bone metastases may be treated by surgery, external radiation therapy, embolization or cementoplasty [41].

Immunotherapy - The aim of immunotherapy is to enhance the body immune response to specific antigens of thyroid cancer by vaccination with dendritic cells able to present these antigens or by trasfering immunostimulatory substances [40].

Vaccination with mature dendritic cells

Dendritic cells are highly potent antigen presenting cells used for anticancer vaccination. They may be used in MTC patients not cured by surgery. Two studies quoted by Vezossi [40] showed radiological response (tumor reduction by 50% assessed by Xray) in 1/7 and 4/10 patients, and biological response (reduction of CEA and calcitonin) in 3/7 and 7/10 patients. Hormonal and radiological stabilization of the disease was noticed in 4/7 and 3/10 patients. The treatment has a good tolerability. In another study granulocyte macrophage colony-stimulating factor and alpha interferon combating tumor- specific calcitonin were used [43].

Transfer of immunostimulatory substances

Studies on murine models were performed by introducing IL2 or IL12 into MTC cells by adenoviral vectors [38].

Radioimmunotherapy

Intense expression by MTC cells of CEA allowed the use of anti CEA antibodies labelled with ^{131}I to target these cells. 9-30 treated patients were followed 12-121 months. Moderate regression (less than 50%) was noticed in 7-29 %, and stable tumor in 35-73%. Combined treatment with myelodepressant labeled anti-CEA antibodies coupled with autologous graft of hematopietic cells rezulted in reduction of tumor size in 10 of 12 treated patients (Chatal J, Juweid M, Kraeber-Bodere F, quoted by Vesozzi [40]).

Targeted radiotherapy

Medullary thyroid carcinoma cells may express somatostatin receptors. Yttrium labelled octreotide [(90) Y DOTA]-TOC was used in 39 patients with metastatic medullary thyroid

carcinoma in whom an activity of 12,6GBq was delivered, with a 29% response and prolonged survival [44].

Treatments targeting RET gene or protein

Germ line RET mutation are found in 95% of hereditary MTC and 40-70 of sporadic MTC. Oncogenic activity of RET may be inhibited by: dominant negative RET mutants that will create a RET protein without oncogenic activity or tyrosine kinase inhibitors, inhibitors of RET protein-kinase or inhibition of tumorigenic patways upstream of tyrosine kinase [40].

Dominant negative RET mutants - Adenoviral vectors expressing dominant negative RET mutants have being used in some studies as gene therapy. Their use results in reduced quantity of RET protein at cell surface and the oncogenic capacity of RET [40].

Thyrosine kinase inhibitors - Imatinib (Glivec) reduces tumor size in animal model but the concentration needed to induce clinical efects in humans seems to be too high. Recently transient stable disease have been reported with Imatinib 600 mg per day in 9 patients [45]. A phase II study combining tyrosine kinase inhibitor imatinib with chemothrapy (dacarbazine and capecitabine) is in way. Other tyrosine kinase inhibitors in preclinical and clinical trials are: vandatanib, sorafenib, motesamib and sunitinib. They act not only on RET protein kinase but also on other tyrosine kinases associated with VEGFR, EGFR, FGFR being a multitarget inhibitors [2,41]. Irinotecan a topoisomerase poison in association with tyrosine kinase inhibitor CEP-751 block in S phase MTC cells [46].

Use of suicide genes - The suicide gene system involves a combination of herpex simplex virus type thymidine kinase and ganciclovir. Physiologically, intracellular kinases will convert ganciclovir monophosphate in ganciclovir triphosphate wich in toxic for cells [38].

COX 1 and COX 2 inhibitors – indometacin could induce inhibition of tumor growth and calcitonin secretion in MTC cell lines [47].

Radioiodine therapy following iodine symporter (NIS) gene expression - NIS may be transfected into MTC cells which will express the symporter and may be targeted by radioiodine [38,48].

CONCLUSIONS

Differentiated thyroid cancers arising from follicular epithelium have an excellent prognosis as long as they are limited and may be treated with surgery and radioiodine. During evolution some initially differentiated thyroid cancer loses their ability to respond to radioiodine and this is the cases in which there are a few or none therapeutic options. Conventional chemotherapeutic agents have shown not significant activity in differentiated thyroid cancer and MTC. In the last years the new acquisitions regarding the genetics of thyroid tumors development allowed the use of new therapeutic agents targeting advanced thyroid carcinomas. Some of them are in preclinical trials and others inter into clinical trials.

REFERENCES

1. Zanotti-Fregonara P, Hindie E, Faugeron I, Moretti JL, Rovasi L, Rubello D, Taubert ME. Update on the diagnosis and therapy of distant metastases of differentiated thyroid carcinoma. *Minerva Endocrinol.* 2008 [Epub. ahead to print].
2. Morris JC, Bible KC, Smallridge RC. Clinical trials for advanced thyroid cancer. *Mayo Clinic Endocrinology Update 2007*; available online at www .mayoclinic. org/ endocrinology.
3. Schlumberger M, Challeton C, De Vataire F, Travagli JP, Gardet P, Lumbroso JD. Radioactive iodine treatment end external radiotherapy for lung and bone metastases from thyroid carcinoma. *J Nucl Med.* 1996; 37(4): 598-605.
4. Hag M, Harmer C. Differentiated thyroid carcinoma with distant metastases at presentation: prognostic factors and outcome. *Clin Endocrinol.* 2005; 63(1): 87-93.

5. Sampson E, Brieley J, Le LW, Rotstein L, Thang RW. Clinical management and outcome of papillary and follicular (differentiated) thyroid cancer presenting with distant metastasis at diagnosis. *Cancer.* 2007; 110(7): 1451-1456.

6. Muresan MM, Olivier P, Leclere J, Sirveaux F, Brunaund L, Klein M, Zarnegar R, Werhya G. Bone metastases from differentiated thyroid carcinoma. *Endocrie Relat Cancer.* 2008; 15(1): 37-49.

7. Pacini F, Schlumberger M, Dralle H, Elisei R, Smit WA, Wiersinga W. and the European Thyroid Cancer Task Force: European Consensus for the management of patients with differentiated thyroid cancer of the follicular epithelium. *Eur J Endocrinol.* 2006; 2: 154-203.

8. Monchik JM, Donatini G, Jannucilli J, Dupuy DE. Radiofrequency ablation and percutaneous injection for recurrent local and distant well differentiated thyroid carcinoma. *Ann Surg.* 2006; 244(2): 296-303.

9. Yousuf K, Archibald SD. Brain metastases from papillary adenocarcinoma of the thyroid. *J Otolaryngol.* 2006; 35(6): 366-372.

10. Karner I, Hrgavic Z, Sijanovic S, Bucovic D, Klobucar A, Usadel KH, Fassbender WJ. Bone mineral density changes and bone turnover in thyroid carcinoma patients treated with supraphysiological doses of thyroxine. *Eur J Med Res.* 2005; 10(11): 480-488.

11. Heijckmann AC, Huijberts MSP, Gensens P, de Vries J, Monheere PPCA, Wolfenbuttel BHR. Hip bone mineral density, bone turn-over and risk of fracture in patients on long-term suppressive L-thyroxine therapy for differentiated thyroid carcinoma. *Eur J Endocrinol.* 2005; 153(1): 23-29.

12. Mazzaferri EL, Kloos RT. Current approaches to primary therapy for papillary and follicular thyroid cancer. *J Clin Endocrinol Metab.* 2001; 86(4): 1147-1463.

13. Lippi F, Capezzone M, Angelini F, Taddei D, Molinaro E, Pinchera A, Pacini F. Radioiodine treatment of metastatic differentiated thyroid cancer in patients on L-thyroxine using recombinant human TSH. *Eur J Endocrinol.* 2001; 144(1): 5-11.

14. Liu YY, Stokel MP, Pereira AM, Corssmit EP, Morreau HA, Romijn JA, Smit JWS. Bexarotene increases uptake of radioiodine in metastases of differentiated thyroid carcinoma. *Eur J Endocrinol.* 2006; 154 (4): 525-531.

15. Carvahlo DP, Ferreira AC. The importance of sodium/iodide symporter (NIS) for thyroid cancer management. *Arq Bras Endocrinol Metab.* 2007; 51(5): 672-682.

16. Luong QT, O'Kelly J, Braunstein GD, Herschman JM, Koeffer HP. Antitumor activity of suberoylanilide hydroxamic acid against thyroid cancer cell lines in vitro and in vivo. *Clin Cancer Res.* 2006; 12(18): 5570-5577.

17. Catalano MG, Fortunati N, Pugliasi M, Constantino L, Pali R, Bosco O, Baccuzzi G. Valproic acid induces apoptosis and cell cycle arrest in poorly differentiated thyroid cancer cells. *J Clin Endocrinol Metab.* 2005; 90(9): 1383-1389.

18. De Besi P, Busnardo B, Taso S, Girelli ME, Nacamulli S, Simioni N, Casara D, Zorat P, Fiorentino MV.:Combined chemotherapy with bleomicin, adriamicin and platinum in advanced thyroid cancer. *J Endocrinol Invest.* 1991; 14(6): 475-480.

19. Argiris A, Agarvallo SS, Karamonzis MV, Burmeister LA, Corly SE. A phase II trial of dixirubicin and interferon 2b in advanced non-medullary thyroid cancer. *Invest New Drugs.* 2008; 26(2): 183-188.

20. Santini E, Battici V, Elisei R, Montanelli A., Mazzeo S, Basolo F, Pinchera A, Pacini F. Cytotoxic effects of carboplatinum and epirubicin in the setting of an elevated serum thyrotropin for advanced poorly differentiated thyroid cancer. *J Clin Endocrinol Metab.* 2002; 87(9): 4160-4165.

21. Cooper DS, Doherty GM, Haugen BR, Kloos RT, Lee SL, Mandel SJ, Mazzaferri EL, Melver B, Sherman SI. and the American Thyroid Association Guidelines Taskforce: Management guidelines for patients with thyroid nodules and differentiated thyroid cancer. *Thyroid.* 2006; 16(2): 4-25.

22. Braga Basaria M, Ringel MD. Clinical review 158: Beyond radioiodine: a review of potential new therapeutic approach for thyroid cancer. *J Clin Endocriol Metab.* 2003; 88(5): 1947-1960.

23. de Groot JWB, Links TP, Plukker JTM, Lips CJM, Hofstra RMW. RET as a diagnostic and therapeutic target in sporadic and hereditary endocrine tumors. *Endocrine Reviews* 2006; 27(5): 535-560.

24. Fagin JA. How thyroid tumors start and why it maters: kinasemutants as targets for solid cancer pharmacotherapy. *J Endocrinol.* 2004; 83(2): 249-256.

25. Coelho SM, Pines Carvalho D, Waisman M. New perspective in the treatment of differentiated thyroid cancer. *Arq Bras Endocrinol Metab.* 2007; 51(4): 612-624.

26. Carlomagno F, Anaganti S, Guida T, Salvatore G, Troncone G, Wilhelm SM, Santoro M. BAY 43-9006 inhibition of oncogenic RET mutants. *J Nat Cancer Institute.* 2006; 98(5): 326-334.

27. Kim DW, Jo YS, Jung HS, Chung HK, Song JH., Park KC, Park SH, Hwang JH et al. An orally administered multitarget tyrosine kinase inhibitor, SU11248, is a novel potent inhibitor of thyroid oncogenic RET/papillary thyroid cancer kinases. *J Clin Endcorinol Metab.* 2006; 91(1): 4070-4076.

28. Salvatore G, de Falco V, Salerno P, Nappi TC, Pepe S, Troncone G, Carlomagne F, Melillo RM, Wilhelm SM, Santoro M. BRAF as a therapeutic target in aggressive thyroid carcinoma. *Clin Cancer Res.* 2006; 12(5): 1623-1629.
29. Schiff BD., Mc Murphy AB., Jassen SD., Younes MN., Doan D., Yigitbasi OG., et all.: Epidermal growth factor receptor (EGFR) is overexpressed in anaplastic thyroid cancer and Gefitinib inhibits the growth of anaplastic thyroid cancer. Clin.Cancer. Res. 2004, 10: 8594-8562.
30. Pennell NA, Daniels GH, Haddad RI, Ross DS, Evans T. A phase II study of gefitinib in patients with advanced thyroid cancer. *Thyroid.* 2008; 8(3): 317-323.
31. Younes MN, Yigitbasi OG, Park YW, Kim SJ, Jansen SA, Hawtorne VS. Antivascular therapy of human follicular thyroid cancer experimental bone metastases by blockade of epidermal growth factor receptor and vascular growth factor receptor phosphorilation. *Cancer Res.* 2005; 65(11): 4716-4727.
32. Ain KB, Lee C, Williams KD. Phase II trial with thalidomide for therapy of radioiodine unresponsive and rapidly progressive thyroid carcinomas. *Thyroid.* 2007; 17(7): 663-670.
33. Conticello C, Adamo L, Giufrida R, Vicari L, Zauner A, Eramo A, Anastasi G, Memeo L. Protesome inhibitors sinergize with tumor necrosis factor-related apoptosis-induced ligand to induce anaplastic thyroid carcinoma cell death. *J Clin Endocrinol Metab.* 2007; 92(5): 1938-1942.
34. Ohta K, Endo T, Haraguchi K, Hershman JM, Onay AT. Ligands for peroxisome proliferator-activated receptor γ inhibit growth and induce apoptosis of human papillary thyroid carcinoma. *J Clin Endocrinol Metab.* 2001; 86(5): 2170-2177.
35. Mrozek F, Kloos RT, Rinigel MD, Krestyl L, Snider P, Arbogast D, Kies M, et al. Phase II study of celecoxib in metastatic differentiated thyroid carcinoma. *J Clin Endocrinol Metab.* 2006; 91(6): 2201-2204.
36. Zatelli MC, Luchin A, Piccin P, Tagliati F, Bottoni A, Vignali C, Bondanelli M., et al. Cyclooxigenase 2 inhibitors reverse chemoresistance phenotype in medullary thryoid cercinoma by a permeability glycoprotein-mediated mechanism. *J Clin Endocrinol Metab.* 2005; 90(10): 5754-5760.
37. Provenzano MJ, Fitzgerald MP, Krager K, Domman FF. Increased iodine uptake in thyroid carcinoma after treatment with sodium butyrate and dicitabine (5-Aza-dC). *Otolaryngol Head Neck Surg.* 2007; 137(5): 722-728.
38. Spitzweg C, Morris C. Gene therapy for thyroid cancer: current status and future prospectives. *Thyroid.* 2004; 14(6): 424-434.
39. Kin SB, Ahn IM, Park JS, Cho HJ, Gang G, Suh C, Lee JS, Kim WK, Kim SH. Growth inhibition and chemosensitivy of poorly differentiated human thyroid cancer line (NPA) transfected with p53 gene. *Head Neck.* 2001; 23(3): 223-229.
40. Vezzosi D, Bennet A, Caron P. Recent advances in treatment of medullary carcinoma. *Ann Endocrinol.* 2007; 68: 147-153.
41. Schlumberger M, Carlomagno F, Baudin E, Bidart JM, Santoro M. New therapeutic approaches to treat medullary thyroid carcinoma. *Nat Clin Endocrinol Metab.* 2008; 4(1): 22-32.
42. Fromigue J, De Boere T, Baudin E, Dromain C, Leboulleux S, Schlumberger M. Chemoembolisation for liver metastases for medullary thyroid carcinoma. *J Clin Endocrinol Metab.* 2006; 91(7): 2496-2499.
43. Papewalis C, Wuttko M, Jacobs B, Domberg J, Willemberg H, Boehring T, et al. Dendritic cell vaccination induces tumor epitope-specific immune response in medullary thyroid carcinoma. *Horm Metab Res.* 2008; 40(2): 108-116.
44. Iten F, Müller B, Schindler C, Rochlitz C, Oertli D, Mäcke HR, Müller-Brand J, Walter MA. Response to [90Yttrium-DOTA]-TOC treatment is associated with long-term survival benefit in metastasized medullary thyroid cancer: a phase II clinical trial. *Clin Cancer Res.* 2007;13(22 Pt 1): 6696-6702.
45. Frank-Raue K, Fabel M, Delorme S, Haberkorn U, Raue F. Efficacy of imatinib mesylate in advanced medullary thyroid carcinoma. *Eur J Endocrinol.* 2007; 157(2): 215-220.
46. Strock GJ, Park JI, Rosen M, Rugerri B, Denmeade SR, Ball DW, Nelkin BD. Activity of irinotecan and thyrosine kinase inhibitor CEP-751 in medullary thyroid carcinoma. *J Clin Endocrinol Metab.* 2006; 91(1): 79-84.
47. Zatelli MC, Luchin A, Piccin D, Tagliati F, Bottoni A, Vignali C. et al. Cyclooxygenase 2 inhibition reverse chemoresistance phenotype in medullary thyroid carcinoma bu a permeability glycoprotein-mediated mechanism. *J Clin Endocrinol Metab.* 2005; 90(10): 5754-5760.
48. Cengic N, Baker CH, Schutz M, Goke B, Morris JC, Spitzweg C. A novel therapeutic strategy for medullary thyroid cancer based on radioiodine therapy following tissue-specific sodium iodide symporter gene expression. *J Clin Endocrinol Metab.* 2005; 90(8): 4457-4464.