Treatment of advanced thyroid cancer with targeted therapies: ten years of experience

David Viola[1], Laura Valerio[1], Eleonora Molinaro[1], Laura Agate[1], Valeria Bottici[1], Agnese Biagini[1], Loredana Lorusso[1], Virginia Cappagli[1], Letizia Pieruzzi[1], Carlotta Giani[1], Elena Sabini[1], Paolo Passannati[1], Luciana Puleo[1], Antonio Matrone[1], Benedetta Pontillo-Contillo[2], Valentina Battaglia[2], Salvatore Mazzeo[2], Paolo Vitti[1] and Rossella Elisei[1]

[1]Department of Clinical and Experimental Medicine, Section of Endocrinology, University of Pisa, Pisa, Italy
[2]Diagnostic and Interventional Radiology, Department of Translational Research and New Technologies in Medicine and Surgery, University of Pisa, Pisa, Italy

Correspondence should be addressed to R Elisei
Email
rossella.elisei@med.unipi.it

Abstract

Thyroid cancer is rare, but it is the most frequent endocrine malignancy. Its prognosis is generally favorable, especially in cases of well-differentiated thyroid cancers (DTCs), such as papillary and follicular cancers, which have survival rates of approximately 95% at 40 years. However, 15–20% of cases became radioiodine refractory (RAI-R), and until now, no other treatments have been effective. The same problems are found in cases of poorly differentiated (PDTC) and anaplastic (ATC) thyroid cancers and in at least 30% of medullary thyroid cancer (MTC) cases, which are very aggressive and not sensitive to radioiodine. Tyrosine kinase inhibitors (TKIs) represent a new approach to the treatment of advanced cases of RAI-R DTC, MTC, PDTC, and, possibly, ATC. In the past 10 years, several TKIs have been tested for the treatment of advanced, progressive, and RAI-R thyroid tumors, and some of them have been recently approved for use in clinical practice: sorafenib and lenvatinib for DTC and PDTC and vandetanib and cabozantinib for MTC. The objective of this review is to present the current status of the treatment of advanced thyroid cancer with the use of innovative targeted therapies by describing both the benefits and the limits of their use based on the experiences reported so far.
A comprehensive analysis and description of the molecular basis of these therapies, as well as new therapeutic perspectives, are reported. Some practical suggestions are given for both the choice of patients to be treated and their management, with particular regard to the potential side effects.

Key Words
- advanced thyroid cancer
- targeted therapy
- tyrosine kinase inhibitors
- *RET*
- *BRAF*

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Introduction

Thyroid cancer is the most common endocrine malignancy, accounting for approximately 4% of all human malignancies (Hayat *et al.* 2007). The majority of thyroid tumors (85–90%), namely, differentiated thyroid cancer (DTCs), arise from follicular cells and are further classified as either papillary (PTCs, 75–80%) or follicular thyroid cancers (FTCs, 5–10%). Both anaplastic thyroid cancers (ATCs) and poorly DTCs (PDTCs) are also derived from follicular cells, and they represent 2–3% and 3–5% of all thyroid tumors, respectively. The remaining 1–2% of thyroid tumors originate from parafollicular C-cells and are classified as medullary thyroid cancers (MTCs) (Veiga *et al.* 2013).

The survival rates of patients affected by thyroid cancer are highly variable and depend on the histotype

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and the degree of differentiation. Rates are 95 and 80% after 35–40 years for PTC and FTC, respectively; 65% for MTC after 10 years; less than 20% for PDTC at 5 years; and less than 10% for ATC at 6 months after the initial diagnosis (Elisei & Pinchera 2012).

The cellular origin of the tumor has important implications for planning the therapeutic and follow-up strategies. In fact, tumor cells of both PTC and FTC are able to take up and organify iodine and to secrete thyroglobulin (Tg) under stimulus from thyrotropin-stimulating hormone (TSH). Because of the preservation of these properties, the majority of DTCs are curable via surgery and radioactive iodine (^{131}I) therapy. Recurrences can be identified early and then cured by measuring the basal and/or TSH-stimulated serum Tg levels and via neck ultrasound (Pacini *et al*. 2001, Torlontano *et al*. 2006). However, in approximately 10% of cases, the patients have an advanced stage of the cancer at the time of diagnosis, with local invasion and/or distant metastases in the lungs (50%), bone (25%), lungs and bone (20%), and other sites (5%), and curing these cases with conventional therapeutic procedures is unlikely (Durante *et al*. 2006). In about one-third of advanced DTCs, the metastatic lesions have a very low avidity for iodine at the time of diagnosis, and ^{131}I therapy has no effects. This is also what normally happens in cases of ATC and PDTC whose tumoral cells are so dedifferentiated compared to the follicular cells from which they originate that they are no longer able to take up iodine, secrete Tg, or respond to TSH stimulus. For these cases of ATC and PDTC, there is no rationale for the use of ^{131}I, and, as an alternative, other conventional therapies, such as external beam radiotherapy (EBRT) and chemotherapy, have been unsuccessfully employed so far.

A similar approach has been used with advanced MTC tumors, which are not able to concentrate ^{131}I because they are derived from parafollicular C-cells, which have a totally different embryological origin from follicular cells and are not involved in iodine metabolism, are not TSH dependent, and do not produce Tg. However, they do produce several other peptides, among which the most important and specific is calcitonin (Ct) (Pacini *et al*. 1991). Despite the different cellular origin that suggests that MTC should be unresponsive to RAI, a beneficial effect of RAI treatment has been described *in vitro* and *in vivo* in rat studies (Ott *et al*. 1987). The efficacy of this therapy for MTC was investigated several years ago also in humans, but the multiple studies performed showed contradictory results (Deftos & Stein 1980, Ott *et al*. 1987, Bayraktar *et al*. 1990). A recent controlled multicenter study concluded that RAI is not appropriate for the treatment of MTC and that the beneficial effect of RAI, if present, was due to the so-called bystander effect (Meijer *et al*. 2013).

Until recently, no effective therapeutic options have been available for patients with any type of advanced thyroid cancer. In fact, EBRT has significant toxicity and mainly plays a palliative role, and classical cytotoxic chemotherapies have shown disappointing efficacy. In fact, despite the relevant toxic effects of chemotherapy, studies have shown that they have only a transient and poor response rate (10–20%), with no prolongation of survival in response to the use of either a single therapeutic agent or in combination (De Besi *et al*. 1991, Orlandi *et al*. 1994).

Fortunately, in the last decade, an increase in our understanding of the molecular mechanisms underlying thyroid carcinogenesis and the first description of compounds able to inhibit the catalytic activity of a tyrosine kinase receptor involved in the process, resulting in antiproliferative effects, has opened up an era of targeted cancer therapies that represent new and important therapeutic options (Yaish *et al*. 1988).

The objective of this review is to present the current status of the treatment of advanced thyroid cancers using these innovative targeted therapies by describing both the benefits and the limits of their use based on the experiences reported so far.

Molecular alterations in thyroid cancer and the rationale for targeted therapies

In the past three decades, several molecular alterations have been described in tumors originating from follicular and parafollicular cells. In 1986, the first activated oncogene was found in the DNA extracted from an irradiated PTC tumor and transfected into a cell line (Fusco *et al*. 1995). After this report, several studies demonstrated the presence of the same activated oncogene in other instances of PTC and particularly in those related to radiation exposure (Nikiforov *et al*. 1997, Elisei *et al*. 2001). The oncogene of interest is *RET*, which lies on chromosome 10 and codes for a tyrosine kinase membrane receptor that is normally involved in cell proliferation and tumoral transformation via the mitogen-activated protein kinase (MAPK) pathway, also known as the Ras–Raf–MEK–ERK pathway (Fig. 1) (Arighi *et al*. 2005). The activation of the *RET* oncogene in PTC is due to a rearrangement (i.e., *RET/PTC*) of its intracellular tyrosine kinase region with a ubiquitous gene partner characterized by the presence of a coiled–coiled domain that determines the

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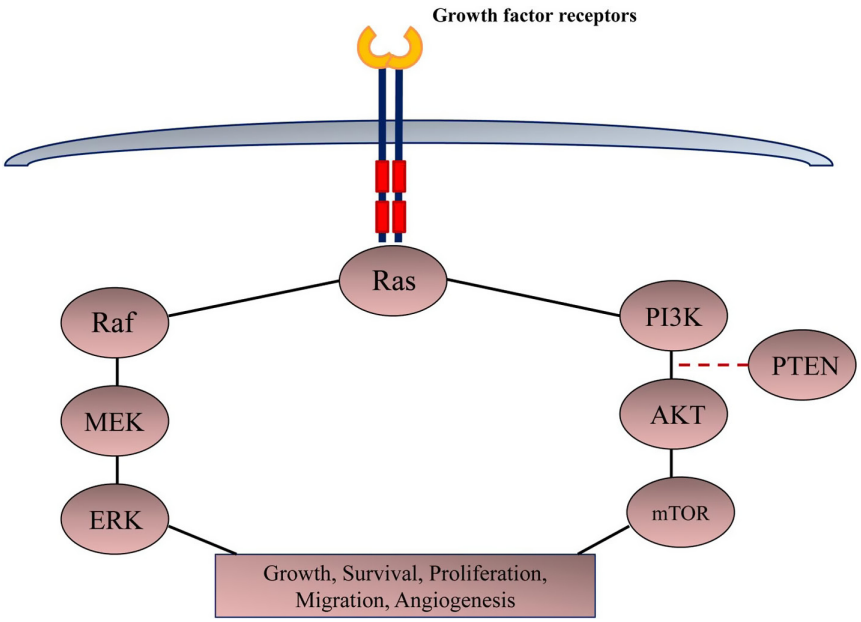

Figure 1
Schematic representation of the two most important intracellular pathways, MAPK and PI3K/AKT, in thyroid cells.

activation of *RET* in the follicular cells in which it is not usually expressed or is only expressed at a very low level (Tallini & Asa 2001). Several *RET/PTC* rearrangements (Table 1) have been described, almost exclusively in PTC cells. The exceptions are a few cases of leukemia and lung adenocarcinomas that show peculiar and exclusive *RET/PTC* rearrangements that have not yet been reported in PTC (Ballerini *et al*. 2012, Kohno *et al*. 2012, Lira *et al*. 2014, Nakaoku *et al*. 2014). The prevalence of *RET/PTC*-positive cases is approximately 20%, and it has apparently been decreasing in recent decades (Romei *et al*. 2012).

Currently, the most frequent mutation found in PTC is $BRAF^{V600E}$ mutation, which is present in approximately 40% of cases and is being found at an increasing rate worldwide (Smyth *et al*. 2005, Mathur *et al*. 2011, Romei *et al*. 2012). The $BRAF^{V600E}$ mutation, which causes the constitutive activation of a serine/threonine kinase, was shown to be an initiating event for the disease and also to promote proliferation, tumorigenicity, and dedifferentiation processes through the activation of the MAPK pathway (Knauf *et al*. 2005, Liu *et al*. 2007). Moreover, $BRAF^{V600E}$ mutation seems to increase the expression of vascular endothelial growth factor (VEGF) and hypoxia-inducible factor-1 alpha (HIF1α), and thus, targeting the product of the $BRAF^{V600E}$ mutation may have the dual effects of blocking the tumor's progression and reducing tumor angiogenesis (Jo *et al*. 2006, Zerilli *et al*. 2010). Although still controversial, there is a general agreement that the $BRAF^{V600E}$ mutation is associated with more aggressive clinical–pathological features, loss of ^{131}I avidity, and increased recurrence and mortality rates (Nikiforova *et al*. 2003, Elisei *et al*. 2008, Riesco-Eizaguirre *et al*. 2006, Xing *et al*. 2013, 2015).

Table 1 Different types of *RET/PTC* rearrangements in human tumors.

Oncogene	Donor gene	Chromosomal location	Tumor type
RET/PTC1	*CCDC6*	10q21	PTC, NSCLC, CRC
RET/PTC2	*PRKAR1A*	17q23	PTC
RET/PTC3RET/PTC4	*NCOA4*	10q11.2	PTC, NSCLC, CRC
RET/PTC5	*GOLGA5*	14q	PTC
RET/PTC6	*TRIM24*	7q32-34	PTC
RET/PTC7	*TRIM33*	1p13	PTC, NSCLC
ELKS-RET	*ELKS*	12p13.3	PTC
RET/PTC8	*KTN1*	14q22.1	PTC
RET/PTC9	*RFG9*	18q21-22	PTC
PCM1-RET	*PCM1*	8p21-22	PTC
ΔRFP-RET	*TRIM27*	6p21	PTC
HOOK3-RET	*HOOK3*	8p11.21	PTC
ERC1-RET	*ERC1*	12p	PTC
AKAP13-RET	*AKAP13*	15q24-25	PTC
TBL1XR1-RET	*TBL1XR1*	3q26.32	PTC
FKBP-RET	*FKBP*	20p13	PTC
SPECC1L-RET	*SPECC1L*	22q11.23	PTC
RET-ANK3	*ANK3*	10q21	PTC
ACBD5/RET	*ACBD5*	10p12.1	PTC
MYH13-RET	*MYH13*	17p13.1	MTC
KIF5B-RET	*KIF5B*	10p11.22	NSCLC
CUX1-RET	*CUX1*	7q22.1	NSCLC
KIAA1468-RET	*KIAA1468*	18q21.33	NSCLC
BCR-RET	*BCR*	22q11.23	CMML
FGFR1OP-RET	*FGFR1OP*	6q27	CMML

PTC, papillary thyroid cancer; MTC, medullary thyroid cancer; CRC, colorectal cancer; NSCLC, nonsmall cell lung cancer; CMML, chronic myelomonocytic leukemia.

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H-, *N-*, and *K-RAS*-activating point mutations in specific hot spots at codons 12, 13, and 61 are also present in PTC cells, particularly in the follicular variant of PTC (Bhaijee & Nikiforov 2011). These mutations cause the loss of GTPase activity so that the kinase becomes constitutively activated. *RAS* mutations can activate two different pathways, namely, the MAPK and the phosphoinositide-3-(PI3K/AKT) pathways (Fig. 1), the latter being the preferential way for proliferation as demonstrated by a higher prevalence of AKT phosphorylation in *RAS*-mutated tumors (Xing 2013). Several other oncogenes have been found to be activated in PTC but with a much lower prevalence than those mentioned previously (Giordano *et al*. 2014). It is worth noting that these oncogene mutations are usually mutually exclusive, and only rarely are two or three present in the same tumoral tissue (Nikiforov 2011, Giordano *et al*. 2014).

Although *H-*, *N-*, and *K-RAS* mutations are found in a relatively small percentage of PTCs, they represent the most frequent genetic alterations in FTCs, and they are also significantly present in PDTCs and ATCs (Xing 2013). Other common genetic alterations in FTCs are *PTEN* deletion/mutation, paired box 8-peroxisome proliferator-activated receptor-gamma (*PAX8/PPARg*) rearrangement, and *PIK3CA* and *IDH1* mutations. In PDTCs, beta-catenin (*CTNNB1*), *p53*, and $BRAF^{V600E}$ mutations can be also present. Although the most common oncogene alteration in ATCs are *p53* point mutations, $BRAF^{V600E}$, *PIK3CA*, *PTEN*, *IDH1*, and *ALK* mutations have all been reported in these aggressive thyroid tumors, in which, unlike in other hystotypes, it is not uncommon to have multiple genetic alterations in the same tumoral tissue (Eng *et al*. 1996, Smallridge *et al*. 2009, Soares *et al*. 2011).

The most common genetic alterations found in MTC cells are *RET*-activating point mutations. Unlike in follicular cells, parafollicular C-cells normally express the *RET* oncogene and a simple heterozygous nucleotide substitution can determine its constitutive activation in this cell lineage. Germline *RET* mutations are present in approximately 95% of hereditary forms of MTC, which represent approximately 25% of all MTCs, whereas somatic *RET* mutations (mainly M918T) are present in approximately 45% of sporadic cases, which represent the other 75% of the cases. Recently, *RAS* mutations, mainly *H-* and *K-*mutations, have been reported in approximately 17% of *RET*-negative sporadic MTCs (Ciampi *et al*. 2013). In addition, *RAS* and *RET* are mutually exclusive in MTC cases. A few anecdotal MTC cases harboring a *RET* or *ALK* rearrangement have been very recently described (Grubbs *et al*. 2015, Ji *et al*. 2015).

In addition to the above-mentioned molecular alterations, *HGF*, *MET*, and *VEGF*, as well as their receptors, are overexpressed in both MTCs and DTCs, and seem to play an important role in the pathogenesis, progression, and recurrence of these diseases (Papotti *et al*. 2000, Capp *et al*. 2010, Karaca *et al*. 2011, Koo *et al*. 2014).

Additional factors promoting thyroid cancer tumorigenesis are gene amplifications and copy number gains. These genetic abnormalities involve genes encoding tyrosine kinase receptors (TKRs), such as *VEGFR*, *MET*, *EGFR*, *PDGFR*, *KIT*, and *PI3K/AKT* pathway kinases, including *PIK3CA*, *PIK3CB*, 3-phosphoinositide-dependent protein kinase 1 (*PDPK1*), and *AKT* (Abubaker *et al*. 2008, Liu *et al*. 2008, Santarpia *et al*. 2008). The higher prevalence of these alterations in ATCs than in FTCs or PTCs and the consequent overexpression of the corresponding proteins are likely responsible of a more aggressive phenotype.

In addition to these genetic alterations, epigenetic abnormalities have been described to play an important role in human cancer and in thyroid cancer tumorigenesis, cell differentiation, and proliferation (Xing 2007). Oncogenes and the abnormal activation of the pathways involved in thyroid cancer tumorigenesis cause the aberrant methylation of thyroid-specific genes involved in iodine metabolism and/or of tumor suppressor genes, leading to the loss of radioiodine avidity and increased tumor growth, invasion, and metastasization. The activation of PI3K/AKT signaling through the aberrant methylation of *PTEN* leads to a self-amplifying loop that maintains the constitutive activation of PI3K/AKT signaling (Xing 2013).

Another important pathway that is linked to the tumorigenesis of thyroid cancer is the nuclear factor-kappa B (NF-κB) pathway. This pathway, which is physiologically involved in the inflammatory response, has recently been shown to control proliferative and antiapoptotic signaling in thyroid cancer cells (Xing 2013). Interestingly, the activation of this pathway seems to upregulate the same proteins that are overexpressed by the MAPK pathway, and the oncogenic mutations that act through the MAPK pathway (i.e., $BRAF^{V600E}$, *RET/PTC* rearrangements, and *RAS*) cause the activation of NF-κB (Xing 2013). Targeting both pathways could have a synergistic effect on the proliferation of $BRAF^{V600E}$-mutant cells (Xing 2013).

Despite the many genetic alterations that have been described for thyroid cancer and the most recent efforts to find other activated oncogenes, approximately 5–10% of PTCs, 50–60% of MTCs, and 10% of ATCs and PDTCs are still negative for all known genetic abnormalities (Soares *et al*. 2011, Giordano *et al*. 2014, Ji *et al*. 2015).

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Targeted therapies: what are they and how do they act?

The increasing knowledge about the molecular alterations underlying thyroid cancer that has been obtained in the last decade has greatly increased the interest in developing new drugs for targeted treatments. The families of drugs that have primarily been investigated for the treatment of thyroid cancer are small molecules, namely, tyrosine kinase inhibitors (TKIs) and monoclonal antibodies (mAbs). Both families of drugs are able to bind to one or multiple RTKs, thus inhibiting their tyrosine kinase activity (Yaish *et al*. 1988). One of the main differences between TKIs and mAbs is that the latter are unable to penetrate the tumoral cell plasma membrane. For this reason, it is likely that mAbs would be more effective against circulating cancer cells than against solid tumors.

Of these two families, TKIs are the most extensively studied. Tyrosine kinases are enzymes responsible for the control of mitogenic signals via the phosphorylation/dephosphorylation of many intracellular proteins involved in the signal transduction cascade. The enhanced catalytic activity that is responsible for uncontrolled cell growth is the molecular rationale for the use of TKIs in thyroid cancer treatment. The first description of a drug able to inhibit the catalytic activity of RTKs and its potential use as an antiproliferative agent was demonstrated in 1988 (Yaish *et al*. 1988).

Since 2001, when the first TKI (imatinib) was approved for the treatment of chronic myelogenous leukemia, many of these drugs have been studied for the treatment of thyroid cancer (Druker *et al*. 2001). These drugs 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 (Fig. 2). Almost all TKIs investigated in relation to thyroid cancer are multitarget drugs, with the exception of selumetinib, which binds to only one receptor (MEK) and, unlike the others, works by blocking tumor growth and acts via the reinduction of ^{131}I uptake in dedifferentiated DTC cells (Table 2).

Due to the high prevalence of the *BRAF*^{V600E} mutation in PTCs, drugs targeting the MAPK pathway, one component of which is a product of this gene, have been the most extensively studied. However, considering that the PI3K/AKT/mTOR pathway is another important pathway for the development of thyroid tumors, other drugs have also been investigated. The most studied group of drugs in relation to this pathway in thyroid cancer has been mammalian target of rapamycin (mTOR) inhibitors. More recently, due to the relative inefficacy of this group of compounds in treating solid tumors, a new drug (BEZ235) targeting both PI3K and mTOR has been developed (Lin *et al*. 2012). The possible synergistic effects of gene amplifications, copy number gains, *PIK3CA* alterations, and *BRAF* mutations suggest that a more effective result could be obtained by simultaneously targeting both the PI3K/AKT and MAPK pathways (Xing 2013).

Another important target that is often overactivated in DTC, MTC, and ATC is the NF-κB pathway. The inactivation of this pathway is now possible with a proteasome inhibitor, bortezomib, through a complex mechanism that prevents the degradation of a factor (i.e., inhibitory-kappa B) that normally inhibits this pathway. This drug is also able to induce programmed cell death by increasing the expression of tumor necrosis factor-related

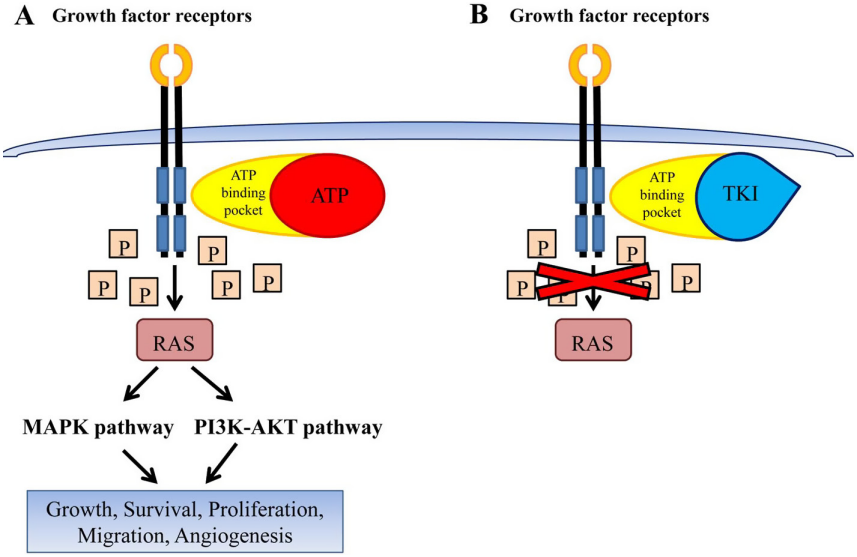

Figure 2
Competitive ATP inhibition by TKIs at the catalytic binding site of RTKs.

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Table 2 TKIs and their molecular targets.

Drug	VEGFR1	VEGFR2	VEGFR3	c-KIT	RET	PDGFR	FGFR1-3	EGFR	Others
Axitinib	+	–	+	+	–	+	–	–	
BEZ235	–	–	–	–	–	–	–	–	Dual PI3K/mTOR
Cabozantinib	–	+	–	+	+	–	–	–	MET, *KIF5B-RET* rearrangement
Imatinib	–	–	–	+	–	+	–	–	*Bcr-Abl*
Lenvatinib	+	–	+	+	+	+	+	–	*KIF5B-RET, CCDC6-RET, NcoA4-RET* rearrangement
Motesanib	+	–	+	+	+	+	–	–	
Nintedanib	+	–	+	–	–	+	+	–	
Pazopanib	+	–	+	+	–	+	–	–	
Ponatinib	–	–	–	–	+	+	+	–	*Bcr-Abl*, *FLT3*, *KIT*
Selumetinib	–	–	–	–	–	–	–	–	MEK
Sorafenib	–	+	+	+	+	+	–	–	*Raf*, *FLT3*
Sunitinib	+	–	+	+	+	+	–	–	*FLT3*
Vandetanib	–	+	–	+	+	–	–	+	*KIF5B-RET* rearrangement
Vemurafenib	–	–	–	–	–	–	–	–	$BRAF^{V600E}$, *CRAF*
Crizotinib	–	–	–	–	–	–	–	–	*MET*, *ALK*, *ROS1*

Bcr-Abl, Abelson and breakpoint cluster region fusion gene; EGFR, epidermal growth factor receptor; FGFR, fibroblast growth factor receptor; *KIT*, v-kit Hardy-Zuckerman 4 feline sarcoma viral oncogene; *Raf*, v-raf murine sarcoma viral oncogene homolog; $BRAF^{V600E}$, valine-to-glutamic acid substitution of *BRAF* gene; *CRAF*, v-raf murine sarcoma viral oncogene homolog 1; *FLT3*, Fms-like tyrosine kinase 3; MEK, mitogen-activated protein kinase; MET, hepatocyte growth factor receptor; PDGFR, platelet-derived growth factor receptor; RET, rearranged during transfection receptor; *RET* gene fusions, *KIF5B-RET*, *CCDC6-RET*, and *NcoA4-RET*; VEGFR1,2,3, vascular endothelial growth factor receptors 1, 2, and 3; ALK, anaplastic lymphoma kinase; *ROS1*, c-ros oncogene 1.

apoptosis-induced ligand that induces apoptosis through the activation of caspases (Gild *et al*. 2011, Xing 2013).

Angiogenesis is also a very important process in tumor progression and an attractive target for therapy (Xing 2013). Angiogenesis is promoted by VEGF, which is overexpressed in response to intratumoral hypoxia via the overactivation of HIF1α. This transcriptional factor is upregulated not only by hypoxia but also via growth factor signaling pathways, such as PI3K/AKT and MAPK, and is expressed in thyroid cancer cells, especially in ATC cells, but not in normal thyroid tissue (Burrows *et al*. 2010, Zerilli *et al*. 2010). An important target of HIF1α is the *MET* oncogene that is upregulated in many thyroid cancers and promotes angiogenesis as well as cellular motility, invasion, and metastasis (Rong *et al*. 1994, Ramirez *et al*. 2000, Scarpino *et al*. 2004). Almost all TKIs target VEGF receptor (VEGFR) with different affinities, but only one of them (i.e., cabozantinib) also blocks HIF1α signaling (Table 2).

Another promising drug that targets angiogenesis is combretastatin A-4 phosphate (CA4P), a microtubule-depolymerizing agent (Sosa *et al*. 2014). This drug is strictly related to colchicine, the classic tubulin-binding agent that was discovered to have a damaging effect on tumor vasculature in the 1930s. This vascular-targeting agent, also known as fosbretabulin, impairs tumoral vasculature function, reduces tumoral blood flow, and induces tumoral ischemic necrosis. CA4P exerts its activity via two mechanisms. First, it binds to tubulin dimers, interferes with microtubule polymerization, and induces mitotic arrest and apoptosis in endothelial cells. Second, it inhibits endothelial cell migration and capillary tube formation by disrupting the function of vascular endothelial-cadherin (VE-cadherin) that is an important determinant of microvascular integrity.

The role of mAbs in treating thyroid cancer is still uncertain. There are at least three ways of using this large, new, and varied family of drugs. The first is by using a mAb that is directed against tyrosine kinase receptors or their ligands, mainly VEGF (VEGF mAb) to block their function, thus exerting an antivascular effect (Bauer *et al*. 2002). The second is by using a mAb that is directed against an antigen expressed by the tumor (e.g. carcino embryonic antigen (CEA)) and is conjugated with a radioisotope (e.g. ^{131}I, ^{90}Y) as a way to reach the tumor cells and kill them with the radioactivity (Juweid *et al*. 2000). The third is by targeting and inhibiting specific receptors (e.g. CTLA-4, PD-1) with mAb (e.g. ipilimumab, pembrolizumab, etc.) to negatively regulate the immune response by promoting the immune system to attack tumor cells (Koguchi *et al*. 2015).

Clinical trials of targeted therapies in advanced, progressive thyroid cancer: the experiences of the last decade

After the discovery of *in vitro* evidence that some TKIs could interfere with thyroid cancer cell growth

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(Carlomagno *et al*. 2002, 2006, Salvatore *et al*. 2006), several clinical trials were designed (Table 3). The first international clinical trial started in 2005 and explored the efficacy of motesanib diphosphate (AMG706) on progressive, locally advanced or metastatic, radioiodine refractory (RAI-R) DTC (ClinicalTrials.gov identifier NCT00121628). Ninety-three patients with evidence of disease progression as assessed by the investigator, based on the Response Evaluation Criteria in Solid Tumors (RECIST), within the 6 months prior to the start of the drug study were treated with 125 mg of AMG706, administered orally once daily (Sherman *et al*. 2008). At the end of the study, an objective response (OR) was achieved in 14% of the cases, a stable disease (SD) in 67% of the cases, and 8% of the cases had a progressive disease (PD) (Sherman *et al*. 2008). In 35% of the patients, a SD was obtained for 24 weeks or longer. The median progression-free survival (PFS) was estimated to be 40 weeks (Sherman *et al*. 2008). The same drug was investigated for the treatment of locally advanced or metastatic, progressive, or symptomatic MTC in a single-arm phase 2 study (ClinicalTrials.gov identifier, NCT00121628). A total of 91 patients were enrolled and treated with AMG706. Of these patients, only 2% achieved an OR, 81% had an SD (48% for 24 weeks or longer), and 8% had a PD; 9% of the patients were not able to be evaluated for a response. The median PFS was 48 weeks (Schlumberger *et al*. 2009). Although the rate of OR was rather low for both DTC and MTC, a significant proportion of patients achieved SD, and this can be considered a clinically beneficial outcome. Despite these promising results, the drug never reached the market. The main reasons for this are attributable to the relative inefficacy of the drug in treating other solid tumors and to the design of the studies. Both studies used a single-arm design were performed in a relatively small population of patients, and, particularly in case of the MTC trial, many patients were enrolled because they were symptomatic but did not have radiological evidence of disease progression. Moreover, the lack of a placebo arm makes the interpretations of the data from these studies regarding the drug's efficacy and toxicity quite difficult.

Soon after the AMG706 study, an international, multicentric, phase 2 study examining the effect of axitinib (Table 3) on MTC and DTC was started (ClinicalTrials.gov identifier NCT00389441). Fifty-two cases of locally advanced, unresectable, or metastatic MTC or RAI-R DTC with disease progression demonstrated in the previous 12 months were treated with 5 mg axitinib, orally administered twice daily. At the end of the study, 35% had a PR and 35% had an SD for 16 weeks or longer. The median PFS was 16.1 months, and the median overall survival (OS) was 27.2 months (Locati *et al*. 2014). In this study, as in the previous studies, the single-arm design makes the interpretation of the results rather difficult and, although the data appeared encouraging, no further studies have been planned for this drug.

More recently, two new compounds, vandetanib and cabozantinib, have been investigated for the treatment of patients with advanced, unresectable, locally advanced or metastatic MTC in two phase 3 trials (ZETA and EXAM trials) (Table 3). In the first trial (ClinicalTrials.gov identifier NCT00410761), 331 patients were enrolled and randomly assigned to receive 300 mg of oral vandetanib once daily or a placebo (2:1). At the data cutoff point, after a median follow-up of 24 months, a significant PFS prolongation with vandetanib relative to the effect of the placebo (30.5 vs 19.3 months, respectively) was observed (hazard ratio (HR), 0.46; 95% CI, 0.31–0.69; $P<0.001$). Moreover, a statistically significant difference in the effect of vandetanib relative to that of the placebo was observed in the OR rates ($P<0.001$) and disease control rates ($P=0.001$), as well as for the biochemical response ($P<0.001$). Although there was apparently a better response to vandetanib in MTC patients with the somatic *M918T RET* mutation, MTC cases with no somatic *RET* mutation also showed a positive response (Wells *et al*. 2012). An OS analysis was not performed because at the end of the study, the data were still too preliminary to make any conclusions regarding the relative long-term survival of patients affected by MTC. These data allowed for the approval of vandetanib (Caprelsa, AstraZeneca) from the FDA (2011) and the EMA (2013) for use in the treatment of symptomatic or progressive, unresectable, locally advanced or metastatic MTC.

Currently, an international, multicentric phase 3 clinical trial (VERIFY trial) (ClinicalTrials.gov identifier NCT01876784) exploring the efficacy of vandetanib in treating RAI-R DTC is being conducted. The study was motivated by the positive results obtained in a phase 2 study performed in France (ClinicalTrials.gov identifier NCT00537095) in locally advanced or metastatic cases of RAI-R DTC (PTC, FTC, or PDTC). In this latter study, 72 patients were randomized to receive vandetanib (300 mg per day) or a placebo (1:1). At the end of the study, the patients in the vandetanib arm had a statistically significant increase in PFS compared to that of the placebo arm (11.1 vs 5.9 months for patients in the vandetanib and placebo arm, respectively; HR 0.63; 60% CI 0.54–0.74; one-sided $P=0.008$) (Leboulleux *et al*. 2012). As mentioned previously, vandetanib has been already approved for the treatment of advanced MTC, and this study might allow

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Table 3 Results of clinical trials with tyrosine kinase inhibitors in thyroid cancer patients.

Drug	Tumor	Phase	Patients (n)	PR (%)	SD > 6 months (%)	Median PFS (months)	Median OS (months)	Most frequent AEs (%) (any grade)	References
Axitinib	MTC DTC	2	52	35	NE	16.1	27.2	Diarrhea (60) Hypertension (54) Fatigue (48) Anorexia (37)	Locati *et al.* (2014)
Motesanib	MTC	2	91	2	48	12	NE	Diarrhea (41) Fatigue (41) Hypothyroidism (29) Hypertension (27) Anorexia (27)	Schlumberger *et al.* (2009)
	DTC	2	93	14	35	10	NE	Diarrhea (59) Hypertension (56) Fatigue (46) Weight loss (40)	Sherman *et al.* (2008)
Vandetanib	MTC	3	331	45	87	NE	NE	Diarrhea (56) Skin rash (45) Nausea (33) Hypertension (32)	Wells *et al.* (2012)
	DTC	2	145	8	57	11.1	NE	Diarrhea (74) Hypertension (34) Acneiform rash (27) Asthenia (26) Anorexia (26)	Leboulleux *et al.* (2012)
Cabozantinib	MTC	3	330	28	NE	11.2	NE	Diarrhea (63) Hand-and-foot syndrome (50) Weight loss (47) Anorexia (45) Nausea (43) Fatigue (40)	Elisei *et al.* (2013)
Sorafenib	DTC	3	417	12.2	42	10.8	NE	Hand-and-foot syndrome (76) Diarrhea (68) Alopecia (67) Skin rash (50) Fatigue (49) Weight loss (46) Hypertension (40) Anorexia (31)	Brose *et al.* (2013)
	ATC	2	20	10	25	1.9	3.9	Skin rash (65) Fatigue (60) Weight loss (60) Diarrhea (35) Hypertension (20)	Savvides *et al.* (2013)
Lenvatinib	DTC	3	392	64.8	29.8	18.3	NE	Hypertension (67) Diarrhea (59) Fatigue or asthenia (59) Anorexia (50) Wight loss (46) Nausea (41) Stomatitis (35)	Schlumberger *et al.* (2015)
Fosbretabulin with Paclitaxel/ Carboplatin	ATC	2/3	80	20	NE	3.3	5.2	Neutropenia (56) Anemia (34) Hypertension (33) Leukopenia (31) Fatigue (31) Alopecia (31) Diarrhea (23)	Sosa *et al.* (2014)

DTC, differentiated thyroid cancer; MTC, medullary thyroid cancer; ATC, anaplastic thyroid cancer; PR, partial response; SD, stable disease; PFS, progression-free survival; OS, overall survival; NE, not estimated.

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its use in treating RAI-R DTC as well if the data of the phase 2 study are confirmed. A limit of the VERIFY study is that to be enrolled, RAI-R DTC patients had to be 'naïve' of any other treatment; thus, the efficacy of vandetanib as second-line treatment cannot be evaluated.

The EXAM trial (ClinicalTrials.gov identifier NCT01908426) was also a double-blind, phase 3 trial comparing the safety and efficacy of cabozantinib to that of a placebo in treating advanced and progressive MTC patients (Table 3). Due to the positive results observed in the phase 1 study, the EXAM trial allowed for the inclusion of patients who had previously been treated with other TKIs (i.e., vandetanib, motesanib, sorafenib, etc.) (Kurzrock *et al*. 2011). A total of 330 MTC patients were randomly assigned to receive cabozantinib (140 mg orally) once daily or a placebo (2:1). In contrast with the ZETA trial, an important inclusion criterion was the documented, central radiographic progression of MTC per the RECIST. The OR rate was 28 and 0% for the cabozantinib and placebo treatments, respectively. The estimated median PFS was 11.2 and 4.0 months for the cabozantinib and placebo groups, respectively (HR, 0.28; 95% CI, 0.19–0.40; $P<0.001$). The longer median PFS durations observed in the ZETA trial with respect to that observed in the EXAM trial may be explained by the different levels of the severity of diseases in the two groups of patients, as demonstrated by the fact that the PFS durations of the two placebo groups were also significantly different (19.3 months in the ZETA trial and 4.0 months in the EXAM trial). In fact, although symptomatic patients without evidence of progressive disease were enrolled in the ZETA trial, the presence of disease progression was a fundamental inclusion criterion in the EXAM trial. In addition, in the EXAM trial, tumor response and PFS were both independent of *RET* mutation status, and more importantly, prolonged PFS with cabozantinib treatment was also observed in the subgroup of patients who had prior TKI treatment (Elisei *et al*. 2013). As for ZETA trial, OS was not improved in the patients treated with cabozantinib compared to those treated with the placebo. However, newly collected data show that OS was significantly increased in patients treated with cabozantinib when the analysis was restricted to the subgroup of *M918T-RET*-mutated MTC patients (Schlumberger *et al*. 2015*b*). The positive results in terms of the safety and efficacy of cabozantinib allowed for the approval of this drug (Cometriq, Exelixis, San Francisco, CA, USA) by the FDA in 2012 and the EMA in 2014 for the treatment of patients with progressive, metastatic MTC. At the time of this review, no other drugs are under clinical evaluation in phase 3 studies for the treatment of advanced MTC.

The inhibitory effect of sorafenib in the treatment of thyroid tumors has been recently explored in an international, multicentric, phase 3 study (DECISION trial) (ClinicalTrials.gov identifier NCT00984282) (Table 3), which included 417 patients affected by locally advanced/metastatic RAI-R DTC who were randomly assigned to receive sorafenib (400 mg orally) twice daily or a placebo (1:1) (Brose *et al*. 2014). Patients were allowed to enter the study only if they demonstrated disease progression per the RECIST within the previous 14 months and only if they had not been previously treated with other TKIs or chemotherapy. At the data cutoff point, patients treated with sorafenib had a longer and statistically significant difference in PFS relative to that of patients receiving the placebo (10.8 vs 5.8 months, respectively; HR, 0.587; 95% CI (0.45–0.76); $P<0.0001$). The median OS has not been reached yet and additional analyses of the OS are planned. However, the results will be affected by the large proportion of patients in the placebo arm (71%) who have crossed over to treatment. No complete response (CR) was observed, and PR was documented in 12.2% of the patients in the sorafenib arm and 0.5% in the placebo arm. The median duration of PR was 10.2 months. Valuable information has been obtained via the exploratory analysis of outcomes of patients receiving open-label sorafenib postprogression in the phase 3 DECISION trial (Schlumberger *et al*. 2014*b*). This analysis demonstrates that sorafenib may continue to suppress tumor growth rates following tumor progression because the median PFS of patients receiving this drug was still lower than that of patients treated with the placebo (6.7 vs 5.3 months). This suggests that, despite the evidence of tumor progression, in the absence of an alternative drug, it may be better to continue to treat the patients with sorafenib, especially if it is well tolerated. Moreover, this exploratory analysis also demonstrated that those patients in the placebo arm who started receiving sorafenib following tumor progression showed a comparable PFS to those who started receiving the drug from the beginning of the trial (9.6 vs 10.8 months). This indicates, although does not prove, that delaying the initiation of sorafenib treatment should not greatly affect the response to the drug (Schlumberger *et al*. 2014*b*). The positive results in terms of the safety and efficacy of sorafenib allowed for the approval of this drug (Nexavar, Bayer) by the FDA in 2013 and by the EMA in 2014 for the treatment of patients with RAI-R DTC. However, it is worth noting that sorafenib had already been approved by both the FDA and the EMA

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for the treatment of advanced hepatocellular carcinomas (Llovet *et al*. 2008) and advanced renal cell carcinomas (Escudier *et al*. 2007). For this reason, and based on the evidence of its clinical benefits obtained in previous phase 2 studies (Gupta-Abramson *et al*. 2008, Kloos *et al*. 2009), sorafenib can be used 'off label' in many countries, and several studies confirming the efficacy of the drug in treating RAI-R DTC have been already reported (Marotta *et al*. 2013, Pitoia 2014).

A phase 2 study exploring the efficacy of lenvatinib in treating RAI-R DTC shown to be progressing in the 12 months prior to the trial has been recently published (Cabanillas *et al*. 2015). Fifty-eight patients were enrolled and received 24 mg lenvatinib orally, once daily. A partial response (PR) was observed in 50% of the patients, and the median PFS was 12.6 months. It is worth noting that patients previously treated with a VEGFR-directed treatment had a higher rate of response in terms of PR than did naïve patients (59 vs 46%, respectively). The results were so promising that a phase 3, multicenter, randomized, placebo-controlled study with lenvatinib (SELECT trial) (ClinicalTrials.gov identifier NCT01321554) (Table 3) was immediately started. This trial was designed with the same therapeutic scheme, and 392 patients were randomized to receive either lenvatinib or the placebo (2:1). Patients were enrolled in the study based on cases of centrally assessed RAI-R disease and radiological evidence of disease progression within the 13 months prior to the study. In contrast to the DECISION trial, prior TKI treatment was not part of the exclusion criteria. At the end of the study, patients treated with lenvatinib had a longer and statistically significant difference in PFS relative to that of patients treated with a placebo (18.3 vs 3.6 months, respectively; HR 0.21; 99% CI (0.14–0.31); $P<0.001$) (Schlumberger *et al*. 2015*c*). A benefit in terms of PFS was present regardless of the *BRAF* or *RAS* mutation status of the patients or prior TKI treatment, and it was also observed in patients with PDTC. The overall response rates were more than 50% in all metastatic sites (brain, bone, liver, lungs, and lymph nodes), and a PFS benefit associated with lenvatinib was present in all cases independent of the sites of metastasis, with the exception of patients with brain metastases in which PFS fell to 8.8 months in patients treated with lenvatinib and to 3.7 months in those receiving the placebo (Habra *et al*. 2015). At the first data cutoff period, OS was not different in patients treated with lenvatinib than in those treated with the placebo, even if a higher rate of response was present in patients treated with lenvatinib, when the potential bias introduced by patient crossover was considered (Schlumberger *et al*. 2015*c*). More recently, a higher and statistically significant difference in the OS rate using a rank-preserving structural failure time model of patients treated with lenvatinib than in those treated with placebo in the SELECT study was reported at the European Cancer Congress (HR=0.53; 95% CI: 0.34–0.82, $P=0.0051$) (Guo *et al*. 2015). Moreover, when the OS analysis was performed on subgroups of patients, a statistically significant increase of OS was observed in patients >65 years (Brose *et al*. 2015) with respect to younger and in follicular with respect to papillary histotype (Elisei *et al*. 2015). Lenvatinib has also been tested in MTC patients in a phase 2 study from which very promising results have been recently published (Schlumberger *et al*. 2015*a*).

Another interesting drug that was investigated for the treatment of RAI-R, progressive, metastatic or unresectable PTC patients positive for the *BRAF*V600 mutation is vemurafenib (ClinicalTrials.gov identifier NCT01286753) (Table 3). In this open-label, exploratory, phase 2 study, 51 patients were assigned to two cohorts: patients naïve for TKI treatment ($n=26$) and patients previously treated with TKIs ($n=25$). Both cohorts were treated with oral vemurafenib (960 mg, twice daily). At the end of the study, no cases of CR were observed and PR was present in 35% of the TKI treatment-naïve cohort and in 26% of the TKI-treated cohort. The clinical benefit, namely, CR+PR+SD≥6 months, was present in 58% of the TKI treatment-naïve cohort and in 36% of the TKI-treated cohort. The median PFS was 15.6 months in the TKI treatment-naïve cohort and 6.8 months in the TKI-treated cohort (Brose *et al*. 2013). More recently, these results have been confirmed in an off-label study (Dadu *et al*. 2015).

A very interesting drug, selumetinib, has been recently evaluated for use in treating RAI-R thyroid cancer. This drug, in contrast to the other TKIs, acts as a highly selective uni-target therapy (Table 2) that does not work directly on tumor growth but has been demonstrated to induce or enhance ^{131}I uptake and retention in a mouse model and in a subgroup of patients with thyroid cancer that was RAI-R (Chakravarty *et al*. 2011, Ho *et al*. 2013). The patients whose tumors were positive for *RAS* mutations showed a very good response, thus suggesting a major role for this drug in treating RAS-positive RAI-R DTCs. This study led to the design of a multicentric, international, phase 2 study (ASTRA trial) (ClinicalTrials.gov identifier NCT01843062) of selumetinib versus a placebo to improve the rate of thyroid remnant ablation in intermediate- to high-risk DTC patients. The ASTRA study is still enrolling DTC patients who have been

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recently submitted for total thyroidectomy and who have no gross residual disease but who have had a T3 or T4 thyroid tumor with at least five micro-lymph node metastases or one metastatic lymph node bigger than 1 cm. The enrolment is expected to be completed in spring 2016. The use of selumetinib for the treatment of RAI-R metastatic disease is now being explored in two multicenter, phase 2 studies in North America. The first, which is investigating the possibility of treating patients with RAI-R PTC in terms of OR rate (CR and PR), is ongoing but no longer recruiting participants (ClinicalTrials.gov identifier NCT00559949). The second is a double-blind, phase 2 study of RAI comparing the use of selumetinib or a placebo for the treatment of RAI-avid recurrent or metastatic advanced (stage IV) DTC and PDTC (ClinicalTrials.gov identifier NCT02393690).

Another promising drug for the treatment of patients with advanced thyroid cancer is pazopanib. This drug, which targets VEGFR, PDGF, c-KIT, and other kinases, was investigated in 39 patients with RAI-R and rapidly progressing (in the 6-month period before enrolment) metastatic DTC in a phase 2 study (ClinicalTrials.gov identifier NCT00625846). Among the 37 patients who were able to be evaluated for a response, a PR was observed in 18 patients (49%), with a calculated response duration longer than 1 year in 66% (Bible *et al*. 2010).

The same authors investigated the efficacy of pazopanib in a cohort of patients with advanced and rapidly progressing (within 6 months) MTC. Among the 35 patients, a PR was obtained in 5 patients (14.3%) with a median PFS of 9.4 months and a median OS of 19.9 months (Bible *et al*. 2014). Despite the positive results of these phase 2 studies, no phase 3 trials are ongoing.

A phase 2 study of pazopanib as a monotherapy for ATC demonstrated only minimal clinical activity (Bible *et al*. 2012). More recently, an *in vitro* and *in vivo* study has reported a synergistic antitumor effect of combining pazopanib with a microtubule inhibitor, such as paclitaxel (Isham *et al*. 2013). Based on these findings, some efficacy may be demonstrated in a phase 2 study sponsored by the US National Cancer Institute (Bethesda, MD, USA) in which an intensity-modulated radiation therapy and the use of paclitaxel with or without pazopanib in ATC patients are being explored (ClinicalTrials.gov identifier NCT01236547).

Other than pazopanib, several other TKIs have been studied in the treatment of ATC but almost exclusively in phase 2 studies (Savvides *et al*. 2013). The major limit of these studies is that the rarity of ATC that does not allow trials to enroll large numbers of patients in a short period of time. A phase 2 trial in patients affected by ATC and treated with sorafenib was performed using the same scheme as the DECISION trial (Savvides *et al*. 2013). Among the 20 ATC patients enrolled, 10% experienced a PR and 25% SD according to RECIST. The duration of the response in the two patients with a PR was 10 and 27 months, whereas in the patients experiencing SD, the median duration of the response was 4 months, with a range of 3–11 months. The overall PFS was 1.9 months, with a median of 3.9 months and a 1-year survival rate of 20%. Interestingly, one of the patients who had a PR had previously shown disease progression while being treated with other antivascular agents (Savvides *et al*. 2013).

The biggest study on ATC was performed a few years ago using combretastatin (CA4P). In this open-label, multicentric, international study, 80 patients were randomly assigned in a 2:1 ratio to receive carboplatin/paclitaxel (CP) chemotherapy with or without CA4P. At the end of the study, the median OS was 5.2 months in the CP/CA4P arm (55) and 4.0 months in the CP arm (25). It was of interest that the 1-year survival rate was 26% for the CP/CA4P arm and 9% for the CP arm. Unfortunately, no statistically significant difference was observed in PFS time between the two arms. It is likely that if the population enrolled was larger, the primary objective of the study would have been reached, but unfortunately, ATC is a really rare disease and the number of patients enrolled was not enough to achieve the required statistical power (Sosa *et al*. 2014).

Interestingly, a remarkable response to crizotinib has been recently reported in a patient affected by ATC that presented an *ALK* rearrangement (Godbert *et al*. 2015). Despite this very interesting case, no clinical trials have yet been started.

The possibility of using mAbs to block ATC cells growth was investigated in a nude-mouse xenograft model using VEGF-mAbs. Despite the significant reduction of ATC growth, after more than 10 years of investigation, this treatment has not reached the clinical stage (Bauer *et al*. 2002). Since 1995, many studies have investigated the possibility of treating thyroid cancer patients with radioimmunotherapy (RIT). In particular, MTC patients were treated with mAbs directed against CEA and labeled with radioisotopes (Juweid *et al*. 1995, 1999). Moreover, the combination of RIT and doxorubicin has been shown to have a potential synergistic therapeutic effect, which may be due to a radiosensitizing effect of the chemotherapeutic agent (Behr *et al*. 1997). Nevertheless, there are no studies showing any actual clinical benefit of these types of therapies.

More recently, a phase 1 study has investigated the toxicity and therapeutic potential of an anti-CEA-mAb

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Table 4 Clinical trials still ongoing and/or actively recruiting participants.

Drug	Tumor	Study type	Recruitment status	ClinicalTrials.gov identifier
Selumetinib	PTC	Phase 2	Active, not recruiting	NCT00559949
	DTC, PDTC	Phase 2	Recruiting	NCT02393690
	DTC	Phase 3	Recruiting	NCT01843062
Everolimus	DTC	Phase 2	Active, not recruiting	NCT00936858
Everolimus and Vatalanib	MTC	Phase 1	Active, not recruiting	NCT00655655
Lenvatinib	DTC	Phase 1/2	Recruiting	NCT02432274
	DTC, PDTC, ATC, MTC	Observational	Recruiting	NCT02430714
	DTC	Phase 4	Recruiting	NCT02211222
Cabozantinib	MTC	Phase 4	Recruiting	NCT01896479
Vandetanib	MTC	Phase 4	Active, not recruiting	NCT01496313

PTC, papillary thyroid cancer; DTC, differentiated thyroid cancer; PDTC, poorly differentiated thyroid cancer; MTC, medullary thyroid cancer; ATC, anaplastic thyroid cancer.

combined with autologous hematopoietic stem cell rescue in patients with rapidly progressing metastatic MTC and showed promising results (Juweid *et al.* 2000). A phase 1/2 study with a high-dose ^{90}Y-labeled, humanized mAbs alone or in combination with doxorubicin and peripheral blood stem cell rescue has been recently completed, but the results are not yet available (ClinicalTrials.gov identifier NCT00004048). Despite these efforts, in the absence of a large, randomized, phase-3 study to compare the efficacy and toxicity of RIT with a placebo or TKI treatment, the benefits of this type treatment remain unclear.

The results of all these clinical trials are summarized in Table 3. Numerous additional clinical trials on the same and on different drugs that are still ongoing and/or actively recruiting participants are summarized in Table 4.

Pitfalls of targeted therapies

The escape phenomenon

To date, the main limitation of targeted therapy is the fact that after a variable period of time from the beginning of the treatment, cancer cells start to grow again, likely due to the development of an escape mechanism. This phenomenon is almost always present, independent of the type of TKI used and the type of human tumor treated. The most likely explanation is that the tumoral cells develop a mechanism of resistance to the treatment (Wang *et al.* 2009, Arao *et al.* 2011, Finke *et al.* 2011).

There are two main types of resistance: a 'primary' resistance that is intrinsic to the tumor cells and is present before the treatment has been started and a 'secondary' resistance that develops after a certain period of exposure to the TKI. An example of primary resistance in MTCs is represented by the *V804M-RET* mutation that confers resistance to vandetanib treatment *in vitro* by preventing the binding of the drug to the receptor (Carlomagno *et al.* 2004). An example of secondary resistance in thyroid tumors treated with TKIs is still unknown, but it is possible that secondary site mutations could develop during therapy. These new mutations have been demonstrated in lung adenocarcinoma cells treated with TKIs and can be represented by *MET* amplification or by *RAS*, *BRAF*, and *PIK3CA* gene mutations (Ohashi *et al.* 2012). Usually, they are located downstream from the TKI target or in parallel pathways and result in a mechanism to bypass the action of the drug.

The challenge is to find the mutations or other alterations that determine the resistance to the drug because patients should not be further treated with the same drug once the efficacy of the TKI is lost and should be treated with other drugs that specifically target the new alterations. To accomplish this, tissue samples should be collected in clinical trials both before starting the TKI treatment and during the treatment when the escape phenomenon arises (Bible *et al.* 2015). Supporting this concept are the results obtained by studying the mechanisms of resistance to imatinib in patients affected by chronic myeloid leukemia. After the discovery of imatinib resistance mutations, new drugs, such as dasatinib, nilotinib, and bosutinib, have been developed for use as second- and third-line therapies for use in treating CML patients after the development of escape from imatinib (Stein & Smith 2010, Amsberg & Koschmieder 2013). This type of research and its findings are also important for RAI-R DTC patients who become resistant to sorafenib, vandetanib, or lenvatinib (Isham *et al.* 2014).

The cytostatic action

All TKIs act as cytostatic and not as cytotoxic agents, implying that tumoral cells are not killed but made quiescent and nonproliferative. This is also the reason why CR, with very few exceptions (Schlumberger *et al.* 2015*c*),

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has never been reported in clinical trials employing TKIs. It is also why the shrinkages of the tumoral masses observed in many cases and the findings of a significant PR are mainly due to the antiangiogenic actions and the partial ischemia of the tumoral core. The cytostatic action represents a limit because, although clinically satisfactory, once started, TKIs should be continued indefinitely until evidence of tumoral progression appears or until the appearance of severe side effects. Moreover, there is also some evidence that once the TKI treatment is stopped, the progression of the disease can become even more rapid. This is of particular relevance in patients, either women or men, who intend to have children. These patients should stop taking the drug for at least 12–14 and 3–6 months, respectively. There is not yet sufficient evidence to predict how much an interruption of TKI treatment of this length affects the disease progression or if it can make the progression even worse because of recovery by the tumor from the cytostatic action. For this specific problem (i.e., having children), the preservation of gametes before starting the TKI treatment should be taken into consideration. However, this would still not be enough to avoid drug interruption in women.

Drug toxicities and side effects

Despite the different receptors targeted, the vast majority of TKI-related adverse events (AEs) are common to different drugs. The most frequent AEs are diarrhea, anorexia, weight loss, fatigue, hypertension, hypothyroidism, hand-and-foot syndrome, and skin rash. The most frequent drug-related AEs for the different drugs investigated in the largest clinical trials are summarized in Table 5. The majority of these AEs are generally mild or moderate (G1–G2), and only in less than 5–10% of cases are they severe or life threatening (G3–G4), based on the common terminology criteria for adverse events (Shah *et al*. 2013). Death related to AEs (G5) is, fortunately, a very rare event.

The majority of AEs are easily prevented or managed with drug treatment, but in a nonnegligible percentage of cases, dose reduction (up to 79% for cabozantinib), interruption (up to 66.2% for sorafenib), or withdrawal (up to 26% for lenvatinib) was needed in clinical trials (Brose 2013, Elisei *et al*. 2013, Cabanillas *et al*. 2015).

Because of this, after drug approval, a number of phase 4 studies were designed to evaluate the efficacy/tolerability of the drugs at lower doses, which can be better tolerated, and to determine if the same results on the disease progression are observed (vandetanib: NCT01496313; cabozantinib: NCT01896479; lenvatinib NCT02211222)

Table 5 Prevalence of TKI-related adverse events in different studies.

Drug	Tumor	Phase	Adverse events														References
			Hypertension (%)		Diarrhea (%)		Skinrash (%)		Anorexia (%)		Nausea (%)		Weight loss (%)		Fatigue (%)		QTcprolangation (%)		
			G_{1-2}	G_{3-4}	G_{1-2}	G_{3-4}	G_{1-2}	G_{3-4}	G_{1-2}	G_{3-4}	G_{1-2}	G_{3-4}	G_{1-2}	G_{3-4}	G_{1-2}	G_{3-4}	G_{1-2}	G_{3-4}	
Axitinib	MTC	II	48	6	50	10	NE	NE	31	6	27	0	25	10	36	12	NE	NE	Locati *et al*. (2014)
	DTC																		
Motesanib	DTC	II	31	25	46	13	NE	NE	23	4	26	2	35	5	42	4	NE	NE	Sherman *et al*. (2008)
	MTC	II	17	10	28	13	NE	NE	24	3	24	2	20	2	33	8	NE	NE	Schlumberger *et al*. (2009)
Vandetanib	MTC	III	23	9	45	11	41	4	17	4	33	0	10	0	18	6	6	8	Wells *et al*. (2012)
	DTC	II	34	0	64	10	25	0	25	1	25	0	NE	NE	18	5	9	14	Leboulleux *et al*. (2012)
Cabozantinib	MTC	III	24	8	48	15	18	1	41	4	42	1	43	4	31	9	NE	NE	Elisei *et al*. (2013)
Sorafenib	ATC	II	20	0	35	0	50	15	5	5	25	0	55	5	55	5	NE	NE	Savvides *et al*. (2013)
	DTC	III	31	9	63	5	45	5	30	2	20	0	41	6	44	6	NE	NE	Brose *et al*. (2015)
Lenvatinib	DTC	III	26	41	51	8	15	< 1	44	5	39	2	36	9	50	9	7	1	Schlumberger *et al*. (2015c)
Combretastatin	ATC	II/III	25	4	19	2	NE	NE	11	0	23	0	NE	NE	27	2	7	4	Sosa *et al*. (2014)

MTC, medullary thyroid cancer; DTC, differentiated thyroid cancer; ATC, anaplastic thyroid cancer; NE, not estimated.

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(Table 4). From a practical point of view, side effects should be known and prevented by both patients and doctors to avoid the need for and the risk of unnecessarily stopping the drug treatment. Some practical suggestions are given in the 'Management of thyroid cancer patients undergoing TKI treatment: practical suggestions' section. In particular, patients should be instructed to report any types of side effects as soon as they appear to allow doctors to immediately start a therapeutic strategy.

According to some observations, TKI toxicities could also be used as a surrogate marker of the efficacy of the drug. TKI toxicities have recently been classified as being an 'on-target' toxicity (On-TT) and an 'off-target' toxicity (Off-TT) (Shah *et al.* 2013). An On-TT is a primary effect of the drug that occurs because of a common pathway/target among neoplastic and normal cells, whereas an Off-TT is a secondary effect of the drug that is due to the inhibition of the kinases that are not the intended target of the drug. A paradigmatic example is the association between TKI-induced hypertension and drug efficacy in terms of OR, PFS, and OS outcomes in patients treated with sunitinib and sorafenib for renal cell carcinoma and hepatocellular carcinoma (Rini *et al.* 2011, Estfan *et al.* 2013). In cases of thyroid cancer, this phenomenon has been recently reported for hypertension in patients treated with lenvatinib (Choi *et al.* 2015).

Future steps: targeting different pathways and combined therapies

A genotype-directed therapy is the goal of targeted therapy; however, due to the cross talk between signaling pathways, it is unlikely that treatment with a single TKI or targeted drug will be effective. In fact, blocking a single pathway has been shown to lead to the activation of other pathways that then overcome the drug's effects (Bernards 2012). Moreover, it is likely that concurrent and/or subsequent genetic molecular alterations drive tumor growth, invasion, and metastases simultaneously or at different times through different pathways in aggressive tumors. This hypothesis is consistent with several studies, showing that more aggressive thyroid cancers such as PDTCs and ATCs often show more than one gene alteration (Sobrinho-Simoes *et al.* 2008, Smallridge *et al.* 2009). Nevertheless, dual inhibition of the MAPK and mTOR pathways or the MEK and mTOR pathways has shown a strong inhibitory synergism in thyroid cancer cell lines, including those from ATC (Jin *et al.* 2009, Liu *et al.* 2010). Similarly, a synergistic effect was demonstrated through the combination of a dual inhibitor of PI3K/mTOR (BEZ235) and an RAF inhibitor (RAF265) in MTC and DTC cell lines both *in vitro* and in a mouse xenograft model (Jin *et al.* 2011). Another way of overcoming drug resistance mechanism and/or enhancing drug efficacy is the combination of TKIs or other target agents with chemotherapy or radiotherapy. In fact, it has been demonstrated that treatment with BEZ235 and paclitaxel, imatinib and docetaxel, and efatutazone, a PPARγ agonist, and paclitaxel have synergistic effects *in vitro* compared to the effect of treatment with a single agent (Copland *et al.* 2006, Kim *et al.* 2012, Lin *et al.* 2012). These combinations were demonstrated to be safe and tolerable in ATC patients in a phase 1 trial (Smallridge *et al.* 2013). Similarly, it was demonstrated that an mTOR inhibitor (everolimus) enhances the response to cytotoxic chemotherapy (doxorubicin) and EBRT in PTC (Lin *et al.* 2010).

Which thyroid tumors are good candidates for targeted therapies?

In general, good candidates for targeted therapies are those thyroid tumors that are defined as RAI-R according to well-defined criteria (Schlumberger *et al.* 2014*a*). This group generally includes approximately 66% of DTCs that have distant metastases at the time of diagnosis, which cannot be cured with ^{131}I because they were initially or became RAI-R over time. RAI-R diseases are more frequent in older patients because of the presence of macroscopic metastatic diseases and/or a PDTC. It is worth noting that RAI-R thyroid tumors generally show an 18FDG-PET-positive scan and that, independent of the ability of metastatic lesions to take up ^{131}I, 18FDG-PET-positive metastatic patients have a very lower survival rate (Robbins *et al.* 2006) and they are very good candidates for targeted therapies.

The group of good candidates for targeted therapies also includes the vast majority of ATC and PDTC cases that have locally advanced disease and distant metastases at the time of presentation because, despite the fact that these tumors originate from follicular cells, the complete lack of cell differentiation makes the disease unresponsive or scarcely responsive to ^{131}I treatment and also to other conventional treatments such as ERBT and chemotherapy.

Approximately 30% of patients with MTC, particularly those with a somatic *RET* mutation and those with distant metastases at diagnosis, have a survival time that is not longer than 5–10 years (Gharib *et al.* 1992). These MTC patients are good candidates for targeted therapies

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because no other therapies are available at the present time (Orlandi *et al.* 1994).

Although necessary, the RAI-R feature is not sufficient per se to determine if a thyroid tumor is a good candidate for targeted therapy. It is well known that, with the exception of ATC, thyroid cancer grows slowly, especially DTC and MTC. Patients with these cancers often survive for a long time, and in many cases, the quality of their life is very good. For this reason, only patients with an advanced and progressive disease should undergo a treatment with targeted therapies. According to expert opinion (Schlumberger *et al.* 2014*a*), good candidates are patients with a RAI-R metastatic thyroid tumor with lesions that are radiologically measurable and have been in progression over the previous 12–14 months, as defined by the RECIST (Eisenhauer *et al.* 2009). Due to the presence of two specific serum markers in thyroid cancer that could correlate with the tumor burden, namely, calcitonin (Ct) for MTC and Tg for DTC, many researchers have investigated their doubling time to assess the progression of the disease (Laure Giraudet *et al.* 2008, Miyauchi *et al.* 2011). Despite the reliability of these markers, progressive diseases must be assessed or confirmed with standardized imaging, which should be repeated every 6–12 months, and the rate of progression should be calculated using the RECIST (Eisenhauer *et al.* 2009). Only a few exceptions to this general rule are accepted and include cases with a large tumor burden, the presence of the disease in sites where its progression can be harmful (e.g. trachea, spinal cord, brain), or a high level of 18-FDG uptake (Deandreis *et al.* 2011, Cabanillas & Sherman 2012). Other exceptions can be made for symptomatic patients and patients with paraneoplastic diseases, such as Cushing's syndrome in those with MTC. In fact, several reports have demonstrated the utility of TKIs for hypercortisolism control. The fact that several different TKIs can induce this positive effect demonstrates that this is a class effect (Baudry *et al.* 2013, Fox *et al.* 2013, Barroso-Sousa *et al.* 2014, Marques *et al.* 2015, Pitoia *et al.* 2015).

Another very important consideration is that, in any patient, before starting a targeted therapy, the possibility of controlling the disease via local treatments (e.g. surgical treatment, chemoembolization, radiofrequency) should be considered, especially if the number of metastatic lesions is relatively small (Schlumberger *et al.* 2012, 2014*a*).

Management of thyroid cancer patients undergoing TKI treatment: practical suggestions

Once the decision to start the TKI treatment has been made, the patient should be evaluated for any concomitant diseases and treated for these if necessary. Frequent clinical and biochemical control tests should be performed, especially during the first weeks of treatment. When using TKIs that can result in hand-and-foot syndrome, the use of urea-based cream, as well as comfortable shoes and gloves, should be suggested during the winter for the care of the patient's hands and feet. Patients treated with vandetanib or cabozantinib should be advised to avoid exposing their skin to aggressive soaps and direct sunlight. Diarrhea should be controlled with the use of loperamid, and attention should be paid to identifying and avoiding those aliments that worsen the symptoms. A supportive multivitamin drug can also be suggested. Many patients become anorexic and lose weight, sometimes very significantly. The use of Megace and/or steroids can be suggested to improve the appetite and regain weight. However, in some cases, side effects such as fatigue and severe anorexia cannot be treated with any drug, and only the reduction of the daily dose of the TKI can control the symptoms. The reduction of the daily dose often allows for a good compromise between the possibility of continuing the treatment and the tolerability of the side effects. As a matter of fact, in almost all phase 3 studies, the median dose used by patients was lower than the starting dose, and phase 4 studies aimed at determining if lower doses are still effective are ongoing for several TKIs. From a practical point of view, it is important that patients are aware of the risk they run if they stop the drug (i.e., a rapid increase in the rate of cell growth) because sometimes they spontaneously decide to stop the drug to better manage the side effects. Patients should be instructed to report any side effects as earlier as possible to allow the doctors to immediately take care of them. Providing good information and good communication with doctors and/or dedicated nurses is the best way to help patients during this therapeutic program (Worden *et al.* 2015).

Conclusions and future perspectives

TKIs represent a new and promising approach to the treatment of advanced RAI-R DTC, MTC, and likely also PDTC and ATC. At the present time, sorafenib and lenvatinib have been approved by the FDA and the EMA for the treatment of progressive RAI-DTC, and vandetanib and cabozantinib have been approved for treating advanced and progressive MTC. After 10 years, patients with these diseases, who have been without any therapeutic options until now, finally have options for slowing tumor growth and better controlling some symptoms.

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However, many areas of uncertainty in the treatment of thyroid cancer patients with targeted therapies remain to be elucidated. Despite the efficacy of TKIs demonstrated in many phase 3 studies in terms of PFS, only a limited amount of data regarding the actual prolongation of patient survival are available at the moment. In addition, these drugs have a nonnegligible toxicity that has an impact on the patients' quality of life; for this reason, determining the right time to start these treatments represents a goal for future clinical research. We still do not know if these diseases should be treated at the very early stages or when the tumor burden becomes significant. While waiting for a better clarification of this issue, only advanced and progressive diseases should be treated with TKIs. Comparative and sequential clinical studies will tell us which treatment strategies are the best to begin with and which are the best for a sequential treatment regimen or if it is better to combine different targeted therapies, possibly at lower doses to decrease toxicities and at the same time increase efficacy. The use of new drugs when the escape phenomenon occurs is necessary, and molecular studies of metastatic and growing tumoral tissues during the treatment will be fundamental. We still do not know if the molecular signature of the primary tumor could help determine the choice of the most appropriate drug; indeed, the results of the phase 3 studies do not strongly support the hypothesis that patients with molecular alterations in genes considered as the main targets of these TKIs (e.g. *M918T-RET* for vandetanib or cabozantinib, and $BRAF^{V600E}$ for sorafenib) respond to treatment better than those without the mutations. Cross-resistance between drugs should be discovered to avoid unnecessary, useless, and harmful treatments. The associations between different TKIs, between these drugs and innovative compounds, and their association with classical cytotoxic chemotherapy and EBRT should be carefully evaluated in preclinical models as well as in clinical trials. Further understanding of the molecular basis of cancer growth, invasion, and metastasization, and the mechanisms through which resistance develops will hopefully lead to the development of new targeted drugs that could selectively and definitely kill cancer cells and spare normal cells or at least lower drug toxicity to ameliorate the patient's quality of life.

Declaration of interest

Rossella Elisei is a consultant and/or keynote speaker for Exelixis/Sobi, AstraZeneca, Genzyme, Bayer, and Eisai. D Viola is a consultant and/or keynote speaker for Sobi and Genzyme. E Molinaro and V Bottici have been keynote speakers for Genzyme. The other coauthors have nothing to disclose. Nevertheless, this review is an unconditioned and independent work reporting published data and reflecting personal experience.

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