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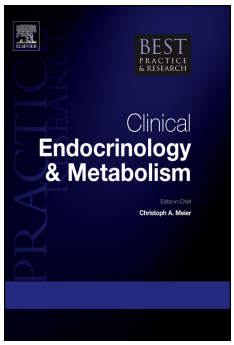

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PROTEINE KINASE INHIBITORS FOR THE TREATMENT OF ADVANCED AND PROGRESSIVE RADIOREFRACTORY THYROID TUMORS: FROM THE CLINICAL TRIALS TO THE REAL LIFE.

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ABSTRACT

The last ten years have been characterized by the introduction in the clinical practice of new drugs named tyrosine kinase inhibitors for the treatment of several human tumors. After the positive conclusion of two international multicentric, randomized phase III clinical trials, two of these drugs, sorafenib and lenvatinib, have been recently approved and they are now available for the treatment of advanced and progressive radioiodine refractory thyroid tumors. We have been involved in most clinical trials performed with different tyrosine kinase inhibitors in different hystotypes of thyroid cancer thus acquiring a lot of experience in the management of both drugs and their adverse events. Aim of this review is to give an overview of both the rationale for the use of these inhibitors in thyroid cancer and the major results of the clinical trials. Some suggestions for the management of treated patients in the real life are also provided.

KEY WORDS

Protein kinases, thyroid carcinoma, vascular endothelial growth factor, oncogenes, RECIST.

INTRODUCTION

Differentiated thyroid cancer (DTC) is one of the human cancer with the lowest mortality and represents 0.3% of all cancer deaths [1]. However, about 10 % of thyroid cancer can have a poor prognosis with a local advanced or diffuse metastatic disease that can severely affect the quality of life of patients until death [2]. The most important prognostic factors for poor prognosis are, among others, the advanced age and the advanced stage at diagnosis [2].

The conventional therapy for DTC is represented by surgical treatment (i.e total thyroidectomy +/- cervical lymphadenectomy) and then radioiodine (131-I) therapy. Among patients with metastatic disease at diagnosis, which represent 5% of all DTC [2], about 25% can be definitively cured with 131-I treatments, 45% have metastases that are initially able to take up iodine but they will never reach the definitive cure and 30% have distant metastasis unable to take up iodine since the first 131-I treatment [3]. These two latter groups represent those cases defined as radioiodine refractory (RAI-R) DTC and, together with the poorly differentiated thyroid cancer (PDTC), that account for about 5% of all thyroid cancer, represent the real clinical challenge since no therapies to cure these patients are available. Fortunately, these tumors are slowly growing in the majority of cases and an active surveillance represents the most widely recognized medical attitude, at least until the evidence of progression [4].

Aim of the present review is to answer the question of how to manage patients with advanced RAI-DTC who enter into progression, particularly considering that the majority of these patients are middle age subjects still in the working period of their life.

THE NEW THERAPIES

Until few years ago, the therapeutic options for the treatment of progressive RAI-R DTC were confined to external beam radiotherapy (EBRT) and conventional chemotherapy which had a significant toxicity with a low efficacy (i.e. 10-20% of partial response), usually not durable and with no evidence of an increase of the overall survival [5].

In the last 20 years, a bulk of evidences have been accumulated regarding the several molecular aberrations located in the cell signaling pathways of malignant cells. In particular it has been discovered that several tyrosine kinases (TK), which are physiologically involved in the cell growth and angiogenesis (Fig 1A), are mutated or overexpressed in tumor cells and represent important targets for drugs that are able to inhibit their activity and, for this reason, named tyrosine kinase inhibitors (TKIs) [6]. TKIs are typically used as anticancer drugs and the first product approved in

clinical practice was imatinib (Gleevec®) in 2001, that substantially improves the outcome of patients with chronic myelogenous leukemia [7]. Over the last decades several TKI aiming to inhibit different TK have been generated and proven to be effective anti-solid tumor and anti-leukemic agents. They are small molecules whose inhibitory activity can be operated by different mechanisms: they can compete with the adenosine triphosphate (ATP) at the binding site of a TK receptor or with the substrate or both or, can also act in an allosteric modality, namely by binding to a site located outside the active site, thus affecting its activity by determining a conformational change of the kinase [8,9] (Fig 1B). While some TKIs act on tyrosine, others act on serine or threonine or even histidine residues and for this reason nowadays the drugs able to inhibit the action of one or more kinases are better called multitarget tyrosine kinase inhibitors (TKIs). These drugs can simultaneously act against several kinases although with different affinities as demonstrated by different values of the dissociation constant (Kd) and of minimal inhibitory concentration (IC_{50}) (Table 1).

Several kinases belonging to the two most important signaling pathways involved in cell growth and proliferation, the mitogen-activated protein (MAP) kinase/extracellular signal-regulated (ERK) pathway and the phosphatidylinositol-3 kinases (PI3K)/AKT/mTOR pathway, have been found to be altered in thyroid cancer, as better explained in the subsequent paragraph. From 2005 up to date several TKIs and mammalian target of rapamycin (mTOR) inhibitors [10] have been studied in advanced and progressive RAI-R DTC with promising results but only two of them, sorafenib (Nexavar®) and lenvatinib (Lenvima®), have been approved by Food and Drug Administration (FDA) and European Medical Agency (EMA).

Rationale of targeted therapies in thyroid cancer.

a) The molecular alterations involved in cell proliferation.

The pathogenesis of thyroid cancer is a multistep process involving from one side, genetic mutations both in oncogenes and tumor suppressor genes, causing anomalous cells proliferations and from the other, alterations of genes involved in angiogenesis, essential for local invasion and metastatic tumor spread [11].

Papillary (PTC) and follicular (FTC) thyroid cancer are the two histotypes into which DTC can be distinguished and they represent about 85% and 15% of DTC, respectively [12]. The most prevalent oncogenic alteration in PTC is the activating point mutation of BRAF oncogene V600E that is present in about 60% of cases [13]. BRAF proto-oncogene encodes for a serine/threonine protein kinase that plays a role in regulating the MAP kinase/ERKs signaling pathway, which in turn

regulates cell division, differentiation, and secretion [14]. The second most common genetic alteration in PTC is represented by a series of rearrangements of RET oncogene, named RET/PTC rearrangements that make the tyrosine kinase portion of the RET oncogene constitutively activated in follicular cells [15]. They are present in about 15% of PTC [13] and are substantially related to ionizing radiation exposure [16], although they have been reported also in sporadic cases [17]. The RET proto-oncogene encodes for a tyrosine kinase receptor for members of the glial cell line-derived neurotrophic factor (GDNF) family. It also plays an important role in regulating the MAP kinase/ERKs signaling pathway and when inappropriately activated it induces cell growth and proliferation [18]. The third most prevalent oncogene alterations in PTC are represented by activating point mutations of RAS oncogene, mainly N-RAS (8.5%) and, to a lesser extent H-RAS (3.5%) [13]. RAS proto-oncogene activates not only the MAP kinase cascade but also the PI3K/AKT/mTOR pathway which stimulates protein synthesis and cellular growth, and inhibits apoptosis. Other molecular alterations have been found in DTC but at a rather low prevalence and their pathogenic role is not yet fully demonstrated [13].

The molecular alterations of FTC are different from those of PTC and RAS point mutations, mainly N-RAS and H-RAS are the most common with a prevalence of about 40% [19]. BRAF mutations and RET/PTC rearrangements have never been reported in FTC while in FTC a paired box 8 (PAX 8)/peroxisome proliferator activated receptor γ (PPAR γ) fusion gene is the second most common alteration [20]; also tumor suppressor gene PTEN deletions or mutations, PIK3CA and isocitrate dehydrogenase 1 (IDH1) mutations are present in FTC although to a lesser frequency [19].

b) ***Angiogenesis and expression of angiogenic factors***

Both angiogenesis, the development of new blood vessels from an existing vasculature, and vasculogenesis, the *de novo* formation of blood vessels from primitive precursor cells, are dependent on several growth factors and their associated TK. The vascular endothelial growth factor (VEGF) plays a fundamental role in the regulation of both these processes [21]. The molecular interactions between VEGF, which consists of a family of five members (VEGF-A, B, C, D and placental growth factor - PIGF), and their receptors VEGF-R 1, 2 and 3 play a pivotal role in new blood vessel formations, lymphangiogenesis [22] and in physiological and pathological angiogenesis [23]. VEGF-R are also TK receptors since they are characterized by seven IgG-like extracellular domains, a transmembrane domain and an intracytoplasmatic TK domain [24]. Two of the 3 isoforms of VEGF-R (VEGFR 1 and 3) are also present in soluble forms (sVEGF-R) and are powerful anti-angiogenic and anti-lymphangiogenic factors by sequestering VEGFs [25]. Tumors cannot grow more

than 2 mm without angiogenesis. By stopping the growth of blood vessels, the means by which tumors can nourish themselves, both the growth of the primary and the metastatic process are cut. Both VEGFs and VEGF-R1 are known to be related to thyroid tumorigenesis and there are studies showing that some isoforms are more expressed in lymphnode metastases of PTC [26,27]. The presence of VEGFs and VEGF-R in tumoral thyroid cells and the cross talk between these cells and the neighboring endothelial cells (Fig 2) is the rationale to use antiangiogenic drugs in advanced and progressive RAI-R DTC and PDTC.

Several TKIs are VEGF-R inhibitors although with different IC_{50} with respect to the inhibited receptor (Table 1). It is clear, that the inhibition of VEGF-R signaling, slow down angiogenesis and tumor vascularization; more than 80% of tumor vessels are destroyed during TKI therapies [28]. Unfortunately, it has been clearly demonstrated that when these drugs are stopped, there is a rapid revascularization [29,30]. In vivo studies demonstrated that the regression of tumor vessels in mice is very fast after the beginning of TKI therapy, but at the stop of the therapy also the re-growth of the vessels is equally rapid and in about 7 days from the withdrawal, tumor is fully revascularizated and vessels appear completely formed, functional and the ability to be stimulated by VEGF is fully restored [31]. Taking into consideration these findings, the therapy with antiangiogenic drugs should be as continuous as possible and daily dosage reduction is preferred over treatment discontinuation, which should be as short as possible, of occurrence of severe adverse drug reactions (ADRs).

One concern is that angiogenesis suppression may drive tumors towards an enhanced local invasiveness and metastatic behaviour. One explanation of this conundrum is that tumors treated with antiangiogenic drugs become less vascularized and hypoxic but the hypoxia can in turn stimulate neoangiogenesis to restore the delivery of oxygen. The intra-tumor hypoxia is indeed a fundamental process to stimulate the production and release of VEGF-A, PIGF and VEGFR-1[32]. These molecules are overexpressed under the stimulation of the transcriptional factor called hypoxia inducible factor-1 alpha (HIF1α) which is also upregulated by other important pathways like PI3K/AKT and MAPK. Several studies showed that HIF1α is expressed in thyroid cancer but not in normal thyroid cells [33,34]. On this regard, it is of interest that MET oncogene, which is upregulated in many thyroid cancers [35], is a target of HIF1α and is able to promote angiogenesis, cellular motility, invasion and metastasis [36-38].

NEW THERAPIES FOR ADAVNCED AND PROGRESSIVE RAI-R DTC

Current systemic therapeutic options for advanced and progressive RAI-R DTC are represented by target TKI therapies specifically directed against signal transduction pathways and or genetic

alteration of DTC (Table 1). Although several TKIs have been tested on advanced and progressive RAI-DTC and PDTC only two drugs have been approved by both FDA and EMA for the therapy of these patients. A description of the two drugs and the phase III clinical trials that allowed them to reach the clinical practice is provided in the following paragraphs.

a) Sorafenib

Sorafenib is an oral, small multi-kinase inhibitor molecule. This drug is an inhibitor of the VEGF-R 1, 2 and 3, RET and RET/PTC, RAS and BRAF/MEK/ERK signaling pathways and platelet-derived growth factor receptor β (PDGFRβ) [39]. The drug was approved for the treatment of advanced kidney cancer [40] in 2005 and advanced hepatocellular cancer [41] in 2007. Several studies of phase 2 showed interesting results in patients affected with advanced RAI-R DTC [42,43] and the drug, on the basis of those results, has been used "off label" until recently.

In 2013 sorafenib was approved by the FDA for the treatment of RAI-R DTC when a clinical and radiological progression of the disease is observed. The approval was obtained after the results of the DECISION trial (ClinicalTrials.gov identifier NCT00984282) [44], a multi-center, randomized, double-blind, placebo-controlled, phase 3 study performed to evaluate safety and efficacy of the drug in RAI-R DTC. Randomization of the study population between sorafenib (400 mg BID) and placebo arm was 1:1. Locally advanced and or metastatic progressive RAI-R DTC, evaluated by single investigators review, was the main inclusion criteria of the 419 patients enrolled in the study. All patients previously treated with other systemic therapy (chemotherapy and or targeted therapies) were excluded and patients in clinical progression in the placebo arm during the study could switch to the sorafenib arm (i.e cross over from placebo to therapy arm).

The primary endpoint of the DECISION trial was to assess the progression-free survival (PFS); patients in the sorafenib arm demonstrated a statistically significant longer PFS with respect to the those randomized in the placebo arm (10.8 vs 5.8 months, respectively) [HR 0.59; 95% CI, 0.45-0.76; P<0.0001]. Also when considering specific subgroups based on age, sex, tumor histology, type (bone or lung), number and size of metastases and uptake of ^{18}FDG the improvement of PFS was confirmed in each subgroup.

Some post-hoc analysis showed information rather relevant for the clinical practice. In a subgroup of patients in which maximum diameter of metastatic lesion was < 1.5 cm, clinical response to therapy was less significant respect to those with larger lesions [45]; for these patients, an active surveillance should be preferable until the evidence of a clear tumor progression. In another subgroup of patients, the presence of mutations of BRAF and RAS gene, showed no impact on the clinical and radiological response to the drug [44].

Regarding the two most important secondary objectives of the DECISION study, the overall survival (OS) was not improved in patients treated with the drug [HR 0.80; 95% CI 0.54-1.19; P=0.14] and the most plausible reason is that a significant proportion of patients crossed over from placebo to treatment arm (71.4%). At variance, the objective response rate (ORR) was significantly improved in the sorafenib arm with respect to placebo (12.2% vs 0.5%; p<0.0001) and this result was favorably surprising since the drug, as all TKIs, is cytostatic rather than clearly cytotoxic and we would expect the block of the growth and the stabilization of the disease, as it happened in 41.8% of cases, but not necessarily the reduction of the lesions.

Interestingly, in the DECISION study, a subgroup of patients who continued open-label treatment with sorafenib after the first evidence of disease progression, showed a further PFS of 6.7 months that was lower than that observed during the first analysis (i.e. 10.8 months) but anyway longer than that observed in the placebo arm (i.e. 5.3 months) [46]. In clinical practice, this evidence suggests that, despite the evidence of tumor progression, in the absence of alternative local and or systemic therapies, it could be better to continue treatment with sorafenib, especially if it is well tolerated. Furthermore, this post-hoc analysis also demonstrated that when sorafenib was started in patients who enter into progression and were in the placebo arm (crossover), this subgroup showed a PFS of 9.6 months that was rather similar to that observed in those who received the drug from the beginning (i.e. 10.8 months). This observation implies that in case of delaying the beginning of the therapy, this should not significantly influence the response to the drug [46].

In the DECISION trial a series of ADRs were reported. Hand-foot skin syndrome (76.3%) was the most frequent followed by diarrhea (68.6%), alopecia (67.1%), cutaneous rash or desquamation (50.2%), fatigue (49.8%), weight loss (46.9%) and hypertension (40.6%). Most of the ADRs were of grade 1 or 2, but serious ADRs have been reported too and one death was related to the use of the drug.

On the basis of the results of the phase III trial, the use sorafenib was expanded to the treatment of patients with advanced and progressive RAI-R DTC and PDTC with the approval of both FDA and EMA.

b) Lenvatinib

Lenvatinib is an oral, multitargeted inhibitor of the VEGF-R 1, 2 and 3, fibroblast growth factor receptor 1-4 (FGF-R 1-4), platelet-derived growth factor receptor α (PDGFR α), RET and KIT signaling pathways [47].

In a phase II study, lenvatinib was able to produce a partial response (PR) in 50% of 58 patients enrolled and followed for about 14 months; median PFS was 12.6 months. In the majority of patients ADRs of any grade were observed; in particular from 10 to 12% of grade 3-4 (hypertension, weight loss, diarrhea and proteinuria) [48].

The efficacy and safety of lenvatinib was assessed in a double-blind, randomized, multicenter, phase 3 study (SELECT trial - ClinicalTrials.gov identifier NCT01321554) [49]. The randomization of the study population between lenvatinib (24 mg/daily) and placebo arm was 2:1. At variance with DECISION study, the procedure to define the progression of DTC following Response Evaluation Criteria in Solid Tumors (RECIST) were supervised by a central reviewer. In this trial 329 patients affected by locally advanced and or metastatic progressive RAI-R DTC were enrolled. Previous systemic treatment with one VEGF-targeted drug was allowed, and was reported in 25% of the entire study group. Patients in the placebo arm with evidence of progressive disease were allowed to cross over to the drug. The primary endpoint of the study was the evaluation of PFS. Patients treated with lenvatinib showed a statistically significant longer PFS with respect to those entered in the placebo arm (18.3 vs 3.6 months) [HR for progression or death 0.21; 99% CI 0.14-0.31; P<0.001]. The presence of a BRAF or RAS mutation did not change the response to the therapy. In the subgroup of patients previously treated with VEGF-targeted drugs (25%), median PFS was significantly improved compared with that of patients in the placebo arm (15.1 vs 3.6 months) and similar, although a little lower, to that of patients treated with lenvatinib as first systemic therapy (18.7 vs 3.6 months). These results demonstrated the efficacy of lenvatinib as second line therapy which is an important finding from a clinical point of view.

The ORR in the lenvatinib arm was 64.8% (vs 1.5% in the placebo arm, p<0.001) and was observed in all metastatic sites (brain, bone, liver, lungs and lymph nodes). In case of brain metastases, PFS was reduced to 8.8 months in the lenvatinib arm and 3.7 months in the placebo arm [50]. It is wirth to note that among those patients who obtained an objective response according to RECIST, 165 showed a PR and 4 a complete response (CR). Beside these outstanding results, it is relevant to say that the death of 6 patients was related to the drug.

After the first data-lock period, also in the SELECT study the OS did not significantly differ between the two arms. However, a post-hoc analysis of two subgroups, the group of the oldest patients (> 65 yrs) [51] and those affected by FTC [52], showed a statistically significant increase of the OS with respect to the youngest and to PTC, respectively.

Treatment related ADRs of any grade are reported in more of 40% of the lenvatinib arm and were characterized by hypertension (67.8%), diarrhea (59.4%), fatigue (59%), decrease appetite (50.2%)

and weight (46.4%) and nausea (41%). In 37 patients (14.2%) treatment was discontinued due to the severity of ADRs.

Of note, about 82% of patients in the lenvatinib arm (starting dose 24 mg/daily) required a dose reduction during the treatment consequently, the mean dose during the follow up was 17.2 mg/daily. The optimal dose of lenvatinib that balanced clinical benefit and ADRs remains unclear and for this reason a new clinical trial comparing 24 mg vs 18 mg per day of lenvatinib will be planned.

On the basis of the results of the phase III trial, lenvatinib was approved by the FDA and EMA in 2015 for the treatment of patients with advanced and progressive RAI-R DTC.

OPTIMIZING THE INITIAL CHOICE AND TIMING OF SYSTEMIC THERAPY WITH TKIs IN RAI-R DTC

RAI-R DTC can remain asymptomatic and stable for a long period of time also in case of metastatic disease, therefore the most difficult decision is when to start systemic therapy. Patients with small (<1.5 cm) metastatic lymph nodes, multiple (sub)centimetric lung lesions or asymptomatic stable bone metastases, should be safely considered for an active surveillance with imaging evaluation every 6 months, especially if the serum thyroglobulin (Tg) values are stable [45]. The experts recommend to take into consideration the TKI treatment in patients with multiple metastatic lesions assessable at imaging studies, larger than 1-1.5 centimeter, with a progressive disease (PD) according to RECIST over the previous 12-14 months [53].

Serum Tg doubling time (DT) has been demonstrated to be a reliable marker to evaluate the progression of the disease [54]. However, despite this information is very useful for monitoring disease activity and making an appropriate time table for the imaging controls (Fig 3), the progression of the disease must be evaluated with standardized imaging after choosing one or more target lesion(s) to be monitored over the time. To this purpose, the best imaging technique is the computerized tomography (CT) scan with iodine contrast medium (icm). This is particularly true for neck and chest, while for abdomen and brain a nuclear magnetic resonance (NMR) would be better [55]. 18FDG positron emission tomography (PET)/CT scan can be useful for prognostic purposes [56] but not to monitor the disease progression. Rate of progression should be assessed by performing a total body CT scan with icm every 6 to 12 months depending from case to case and calculated using RECIST [57]. Only a progression of at least 20% of the target lesion(s) or the appearance of new lesion(s) in the last 12-14 months can be considered as a clinical relevant progression and the opportunity to start with a TKI should be considered. The exceptions to this general rule include cases with larger tumor burden, the presence of the disease in sites where its progression can be

dangerous (e.g., trachea, spinal cord, brain) or diffuse metastatic lesions with high level of 18-FDG uptake [58,59].

Before starting a TKI treatment in a patient with a PD according to RECIST, the site(s) and the number of the metastatic lesions should also be considered [53,60] and whenever possible local treatments should be considered first [61]. Local treatments such as surgery, chemoembolization, ethanol ablation, radiofrequency, cryotherapy and others are useful particularly in those cases with a limited number or even better single progressive metastatic lesion(s) [62-64] (Fig 4).

Other aspects to be considered before starting a TKI therapy include the general health conditions of the patient evaluated according to the Eastern Cooperative Oncology Group **(**ECOG) scale of performance status [65] since the ADRs of the TKI can be relevant and it is very important that the patient is in good health before starting the therapy. A cardiological evaluation with particular regard to blood pressure level is strongly suggested before starting A TKI and in particular lenvatinib whose most frequent ADR is the increase of blood pressure. A particular care of hands and foot should be suggested in particular when sorafenib is planned and a dermatological supervision can be useful if some lesions are already present. Advanced age per se is not a contraindication and patients > 65 years treated with lenvatinib can even obtain an improvement of the OS [49]. Obviously, all the comorbidities must be taken into account, stabilized and an accurate check of the concomitant drugs must be done to avoid drug-drug interactions [66].

It is evident that the decision to start a systemic therapy requires a discussion in a multidisciplinary care team, including endocrinologists, oncologists, surgeons, radiotherapists, radiologists and others and patients should be preferentially treated in centers with experience in treating advanced thyroid cancer.

The choice of the first line drug

Once the decision to start a TKI treatment is taken, the drug must be chosen among those that are available that, to our knowledge, at the present are only sorafenib and lenvatinib. In many cases the choice is dictated by the local availability of the drug. As an example, in many European countries sorafenib can be prescribed but it is not refundable and the choice of lenvatinib is forced, or viceversa. Nevertheless, we will compare the two drugs according to the results of the two phase 3 clinical trials: SELECT for lenvatinib and DECISION for sorafenib.

Several are the differences between the 2 phase III studies starting from the inclusion criteria up to the results and side effects (Table 2). Differences in PFS and ORR have been observed in the two studies but it is important to underline that the aggressiveness of the thyroid tumors in the two

cohorts of enrolled patients was also different as demonstrated by the shorter PFS of the patients enrolled in the placebo arms of the two studies. However, the OS was not improved in both studies likely because both study designs included the cross-over from placebo to drug arm in case of progression. Only two subgroups of patients, those affected by FTC respect to those affected by PTC and those older respect to those younger than 65 yrs at the diagnosis, showed a significant increase of the OS when treated with lenvatinib [49].

As far as the ADRs are considered, the two drugs seem to induce very similar side effects although with a different prevalence being hypertension the most common for lenvatinib and hand and foot syndrome the most common for sorafenib (Table 2).

The choice to start systemic therapy with one rather than the other drug, if both available, should be personalized to the features of the patient and its disease. There are some observations to take into consideration before choosing sorafenib or lenvatinib as first line therapy. The shrinkage of metastatic lesions is more rapid with lenvatinib than sorafenib, may be related to its high anti-angiogenic activity due to the high affinity of lenvatinib for VEGF-R 2. Therefore, lenvatinib should be considered as first line therapy when there is an urgent need to reduce the size of a metastatic lesion, as in case of a vertebral lesion that is compressing the spinal cord, or in case of very rapidly growing disease. On the contrary, in case of infiltration of a critical structure as the trachea or the esophagus, a rapid shrinkage could be dangerous for the risk of fistula and sorafenib would be preferred for its slower antitumoral activity [67].

The choice of the drug should be also personalized taking into account the general health conditions of the patient, the presence of other relevant diseases, the use of other drugs that can interfere with that specific TKI treatment and the presence of symptoms that can be worsened by the therapy.

An accurate discussion with the patient regarding his/her health conditions, lifestyle, working commitments and the impact that the systemic therapy can have on all these aspects should always precede the choice of the drug.

The management of treated patients

Before starting a TKI treatment the hands and feet of the patients must be accurately observed and treated if any callosity or skin lesion are found. An abundant use of urea-based cream must be suggested to these patients. It is also required a cardiologist assessment with ECG and Qt measurement and a blood pressure evaluation with accurate pharmacological correction if elevated. Patients with local aggressive disease must be studied with tracheoscopy and esophagoscopy to ascertain the presence of tumoral infiltration which may represent a limit to the use of a very

aggressive therapy for the risk of fistula. The evaluation of several electrolytes (i.e sodium, potassium, magnesium) and in particular of calcium must be included in the general blood parameters evaluation [68].

After the beginning of TKI therapy, the patient must be under clinical and biochemical control every 15 days in the first 2 months and, if everything is doing well, less frequently thereafter. Particular attention must be done to the levels of thyrotropine stimulating hormone (TSH) and free T4 (FT4) because a high percentage of patients become hypothyroid and an increase of the daily dose of L-T4 is necessary [69]. The schedule of the periodical re-evaluation should be related to the clinical and biochemical conditions of the patient and the results of the CT imaging, thus varying from 3 to 6 months.

The management of the ADRs is depending on their severity evaluated according to the Common Terminology Criteria for Adverse Events (CTCAE) [70]. First of all, patients must be instructed to refer immediately to medical staff about the onset of every ADRs and not waiting as they usually do for the fear of the suspension of the drug. In case of ADRs of grade 1-2, careful surveillance with specific treatment can be the right attitude. At variance, patients who experience ADRs of grade 3-4, should reduce or temporarily discontinued the drug until the resolution of the toxicity. When the toxicity has recovered to grade 1, treatment could be restarted. In consideration of the dissimilar biological half-life of the TKIs, the suspension of the drug must be different to obtain the resolution of the AE.

As general rule, since TKIs are cytostatic and not cytotoxic, once they have been started they should be continued until the evidence of tumor progression and or appearance of life-threatening ADRs. In case of progression, if there is no a valid alternative, the drug should be continued since it has been observed, at least for sorafenib, that the PFS of patients who continue to be treated with the drug after the first evidence of progression, is still longer than that of patients in the placebo arm [46].

OUR EXPERIENCE WITH TKIs IN DIFFERENTIATED THYROID CANCER

We actively participated from 2005 in almost all phase II and phase III clinical trials for the treatment of advanced and progressive DTC with TKIs. Inside these clinical trials we tested motesanib [71], axitinib [72] and vemurafenib [73] in 1, 5 and 3 patients, respectively. Although all these studies obtained very promising results, unfortunately, none of these drugs was tested in a phase III study.

In the DECISION study, the phase III trial for sorafenib, and in the SELECT study, the phase III study for lenvatinib we enrolled 24 and 15 patients, respectively. From those patients enrolled in the

DECISION study, one patient is still taking sorafenib and after 6 years of therapy he has a very slow growing disease and a good quality of life. Unfortunately, 18/24 (75%) have been died for PD either during the study or during a second line therapy. Five patients are still alive with SD either without any therapy or under a second line TKI. The most important and frequent ADRs that our patients experienced was the hand and foot syndrome that often required the reduction of the daily dose of the drug.

We acquired the largest experience with sorafenib by using it as "off label" drug. We treated 66 progressive metastatic thyroid cancer patients with different hystotypes: 16/66 (24.2%) PTC, 13/66 (19.7%) FTC, 21/66 (31.9%) ATC, 5/66 (7.5%) MTC and 11/66 (16.7%) PDTC. After 3 months from the beginning of the treatment, in 19/66 (29%) we discontinued the drug for several reasons, 9/19 died for PD. At the end of follow up, until now (mean 240.88±354.6 days), 9/47 (19.1%) patients showed a PD, 13/47 (27.7%) a SD and 2/47 (4.3%) a PR; 11/47 (23.4%) patients withdrawn for ADRs and 9/47 (19.1%) died. In 3/47 (6.4%) patients there was a small progression and are waiting for a new assessment. In many of these cases we needed to reduce the daily dose to obtain a good compromise between the cytostatic effect of the drug and the ADRs: the median dose used was 600 mg/die [74].

From those patients enrolled in the SELECT study, 3/15 patients are still taking the drug, all at a lower dosage respect to the initial one (14 mg/day vs 24 mg/day) and have a SD with a good quality of life. Six patients (40%) have been died after a period of PR/SD while taking the drug. One withdrew the consent for the severe ADRs. The remaining 5 patients showed PD after a period of SD and 4/15 (26.6%) patients died for PD while one started another TKI.

From December 2014 we started lenvatinib treatment in an "expanded" and compassionate access in a total of 36 patients affected with thyroid tumors among which 21/36 (58.3%) PTC, 9/36 (25%) FTC, 6/36 (16.7%) PDTC. After the first evaluation (2 months from the beginning), 15/36 (42%) showed a PR, 13/36 (36 %) a SD, 5/36 (14%) a PD and 3/36 (8%) died before the first evaluation. At the last evaluation in February 2017, 15/36 (41.7%) patients died and among them 6/15 die for PD, 3/15 for serious ADRs (2 GI perforation, 1 esophageal fistula) and 6/15 for worsening of clinical conditions. Discontinuation of the drug occurred for the withdrawal of informed consent in 1/36 (2.8%), for the onset of serious AE in 1/36 (2.8%) and for PD in 2/36 (5.6%). Seventeen (47.2%) patients are still continuing the treatment and among them 3/36 (8.3%) showed a PR at the last evaluation, 9/36 (25%) a SD and 5/36 (13.9%) had a slight but not significant PD.

Finally, in phase III study with vandetanib for advanced and progressive DTC, we enrolled 5 patients. After a follow up of 41 months, none of the patients died during the trial; 3/5 (60%)

dropped out from the study for PD and two of these are now in treatment with a second line TKI; 2/5 (40%) patients are still under vandetanib treatment with a consistent SD. The ADRs of vandetanib are rather well manageable and no major ADRs have been observed during this study.

Thank to this experience we can affirm that these drugs are able to change the natural history of progressive RAI-R DTC and PDTC. In our experience, we observed very significant reductions of metastatic lesions and, especially with lenvatinib, the size reduction can be rapid and able to avoid compression of adjacent structures. However, all TKIs have severe ADRs that we learned to manage and control with a rather rapid learning curve. Nevertheless there are some ADRs such as fatigue and anorexia that are very difficult to manage and requires the reduction of the daily dose, if not the complete interruption of the drug. Over the years we also learned that it is better to reduce the daily dosage as soon as the symptoms begun to appear than to expect too much until the necessity to discontinue the drug treatment. Moreover, we understood that any ADRs may be prevented with an adequate physical examination and friendly discussion with the patient before starting the medication. A stringent program of controls must be performed especially in the first 2-3 months after the initiation of the therapy and a dedicated number of telephone through which the patients can reach the medical staff is mandatory. Finally, the most important issue that we realized as unavoidable is the escape phenomenon: sooner or later, after a period of good response, the disease starts again to grow (either with the evidence of new lesions or with the increase in size of known lesions) but, if no other TKI would be available, patients should better continue the drug since the discontinuation may be followed by a rapid increase of the tumoral burden.

CONCLUSIONS

Although several TKI have been tested in clinical trials, so far only sorafenib and lenvatinib have been approved for the treatment of advanced and progressive RAI-R DTC and PDTC. However, since these tumors are commonly slow growing tumors, it is convenient to start the systemic therapy with TKI only if a real PD is demonstrated. Moreover, if the PD is limited to a single or few metastases it can also be convenient to consider the possibility to use local treatments before starting the TKI. Once it has been decided to start the TKI treatment, the choice of the drug is strictly conditioned by the availability of the different types of TKIs. Also the characteristics of both the patient and disease must be considered. During the treatment the patients must be followed with rather frequent controls especially in the beginning and then according to the response to therapy. Patients should be treated in experienced centers because of the risk to have important ADRs that

are manageable but a specific experience is required. The major limit of TKIs is the unavoidable escape phenomenon that, sooner or later, can arise and determine a real challenge for clinicians. For this reason the TKI cannot be considered an arrival point but just a transition towards the development of other drugs that could block not only the tumor progression but mainly the tumor initiation.

LEGEND OF FIGURES

Fig. 1: **Panel A:** schematic representation of physiological activation of TK receptors. **A_1**: Inactive conformation of TK receptor; [N-tl: N-terminal lobe; C-tl: C-terminal lobe; PBR: Phosphate binding region; AR: Adenine Region; SR: Sugar Region; HR: Hydrophobic Region]; **A_2**: Active conformation of TK receptor determined by the binding with its physiological ligand; [ATP: Adenosine TriPhosphate; ADP: Adenosine DiPhosphate; P: Phosphorilated tyrosine residues]; **Panel B:** TKIs mechanisms of action. **B_1**: ***Type I TKIs*** (i.e. Sunitinib), recognize active conformation of the kinase and compete with ATP to bind the ATP-binding site; **B_2**: ***Type II TKIs*** (i.e. Sorafenib), indirectly compete with ATP by occupying the hydrophobic pocket adjacent to ATP-binding site; **B_3**: ***Type III TKIs*** (i.e.Vandetanib), covalently bind to cysteines at specific sites of the kinase (variably located) and prevent the activation of the kinase.

Fig. 2: Schematic representation of the cross talking between thyroid tumor cells and endothelial cells: tumor cells are able to produce VEGF that interacts with VEGF-R located on the cell membrane of both endothelial cells and thyroid tumor cells. Moreover, several tyrosine kinase receptors (i.e. RET, MET, EGF-R) are anchored on the cell membrane of thyroid cells and their physiological and pathological activity is related to the activation or over activation, respectively, of the MAP kinase /ERK and PI3K/AKT/mTOR pathways.

Fig.3: Algorithm for the active surveillance of RAI-R DTC for the identification of the right time to start therapy with TKI.

Fig.4: Examples of local treatment by radiofrequency ablation; **Panel A**: Liver metastatic lesion (segment VII – 15 mm) before treatment; **Panel B**: Necrotic tissue (20 mm) after 2 months from radiofrequency ablation; **Panel C**: Right paratracheal neoplastic recurrence (15 mm); **Panel D**: Disappearance of the lesion after 12 months from radiofrequency ablation.

PRACTICE POINTS

- TKI should be started only when a progression of the metastatic lesions is documented or when the tumor burden is very advanced, provided their radioiodine refractoriness
- An accurate multidisciplinary evaluation of the clinical features of the patient should be performed before starting TKI
- Patients must be instructed to report adverse events when they are still low grade
- Clinical and biochemical controls must be performed every 15 days during the first two months of treatment
- The interruption of the TKI treatment for adverse events should be avoided or reduced to a very short period of time; dose reduction should be preferred

RESEARCH AGENDA

- Other drugs for progressive RAI-DTC and PDTC should be developed to be used as second, third or successive therapy
- The identification of biomarkers able to provide information about the response to therapy should be pursued with dedicated studies
- Trials combining two TKI or a TKI and another type of drug (i.e. chemotherapy or immunotherapy) are needed

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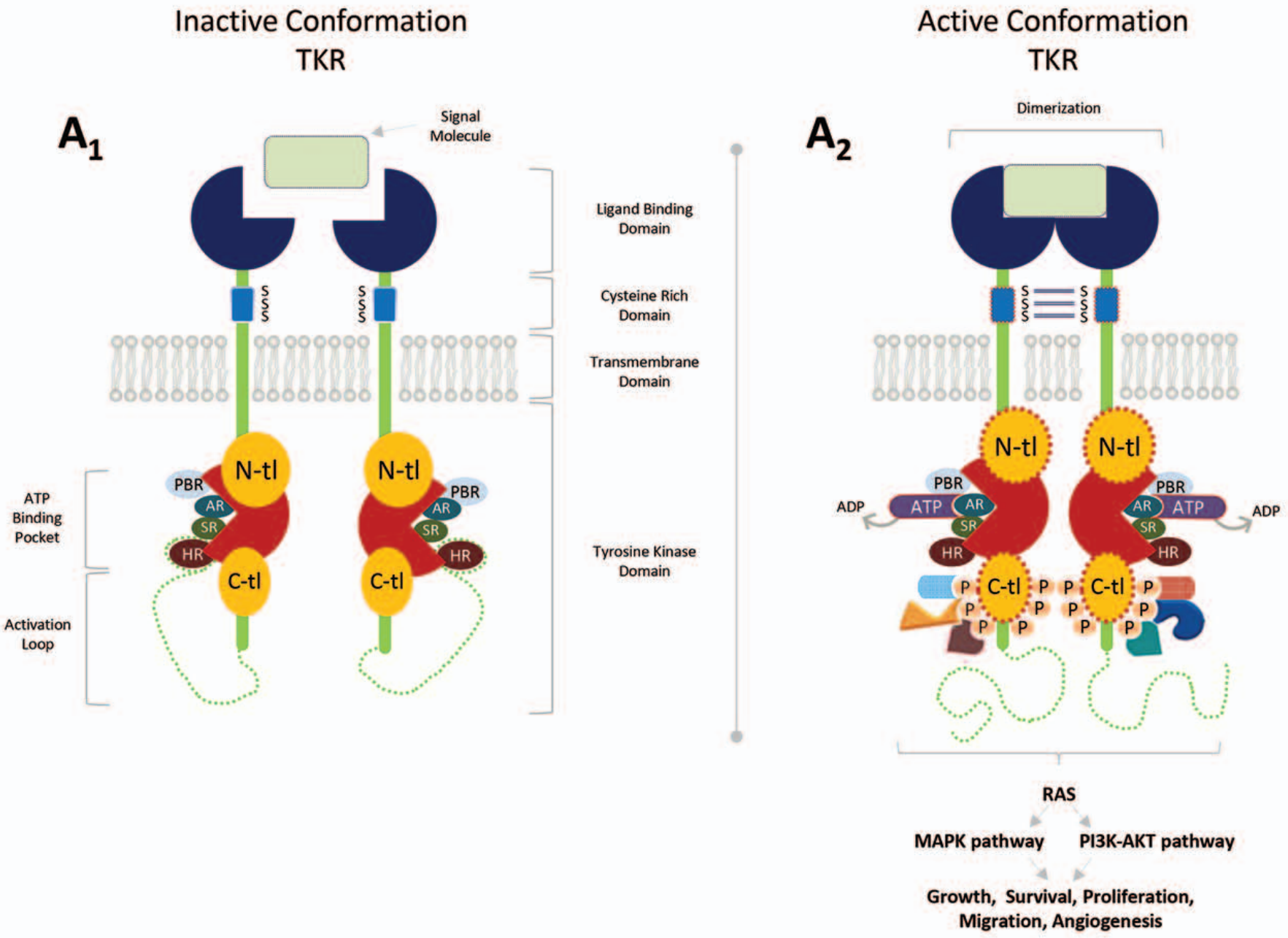
Tab. 1 – Molecular targets and other features of Tyrosine Kinase Inhibitors (TKIs) tested in phase II, III or IV clinical trials in thyroid cancer

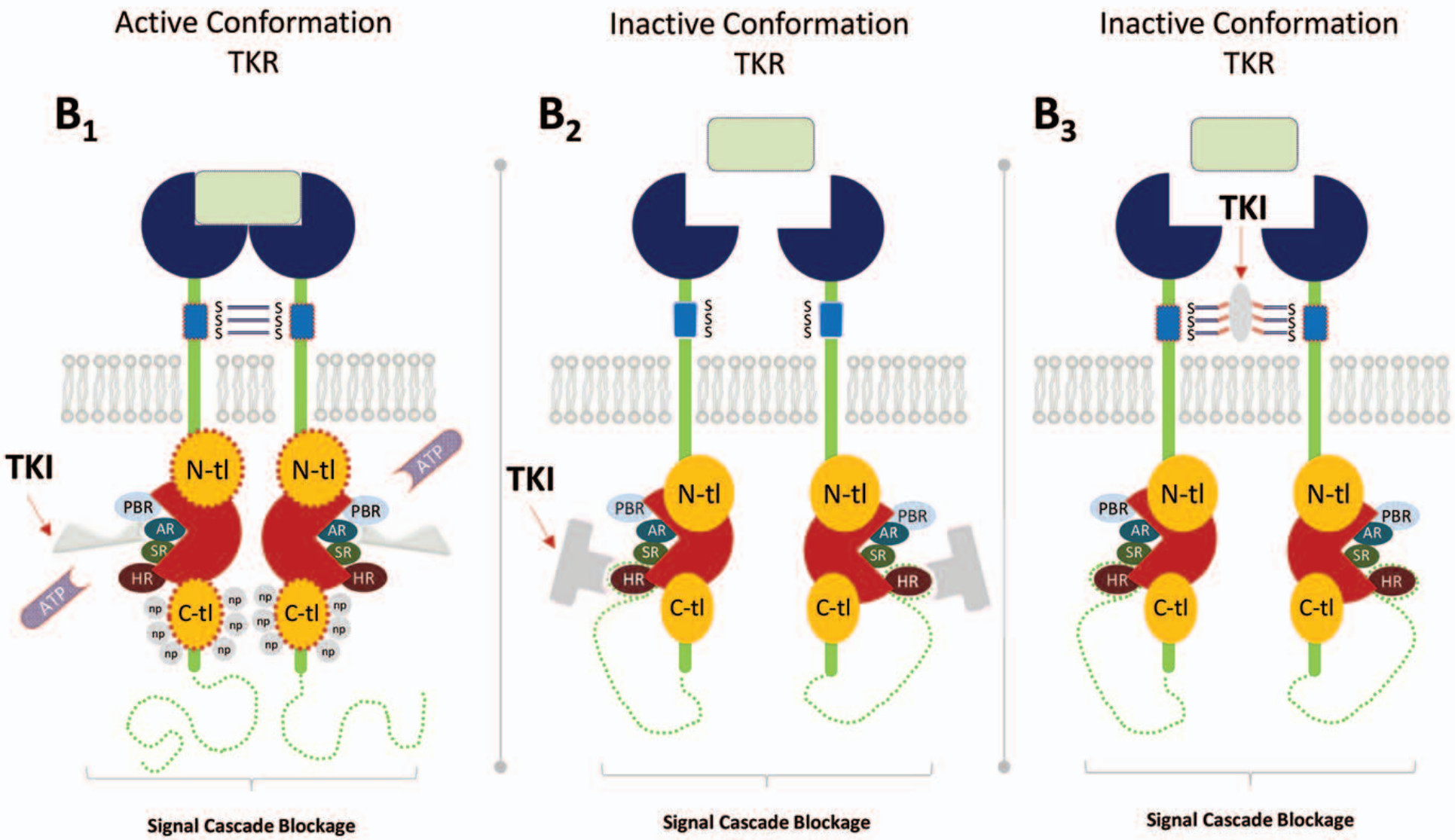
	VEGFR	*c-Kit*	*RET*	*PDGFR*	*FGFR*	*EGFR*	*Other Molecular Targets*	*Half-Life*	*Study phase*	*FDA and EMA approved for TC*
Sorafenib	+	+	+	+	-	-	Raf, FLT3	25-48 hrs	III	Yes for DTC and PDTC
Lenvatinib	+	+	+	+	+	-	RET-KIF5B, CCDC6-RET, NcoA4-RET rearrangement	28 hrs	III-IV	Yes for DTC and PDTC
Selumetinib	-	-	-	-	-	-	MEK	5-8 hrs	III	No
Vandetanib	+	+	-	-	-	+	RET-KIF5B rearrangement	19 days	III-IV	Yes for MTC
Cabozantinib	+	+	+	-	-	-	MET, RET- KIF5B rearrangement	55 hrs	III-IV	Yes for MTC
Sunitinib	+	+	+	+	-	-	FLT3	40-60 hrs	II	No
Axitinib	+	+	-	+	-	-	-	2.5-6 hrs	II	No
Motesanib	+	+	+	+	-	-	-	21.4-68.7	II	No
Vemurafenib	-	-	-	-	-	-	$BRAF^{V600E}$, CRAF	57 hrs	II	No
Nintedanib	+			+	+	-	-	10-15 hrs	II	No
Pazopanib	+	+	-	+	-	-	-	30.9 hrs	II	No
Ponatinib	-	-	+	+	+	-	Bcr-Abl, FLT3, KIT	12-66 hrs	II	No
Bevacizumab							dual PI3K/mTOR	11-50 days	II	No
Imatinib	-	+	-	+	-	-	Bcr-Abl	14-23 hrs	II	No
Everolimus	-	-	-	-	-	-	mTOR	30 hrs	II	No
Temsirolimus	+	-	-	-	-	-	mTOR	17.3 hrs	II	No

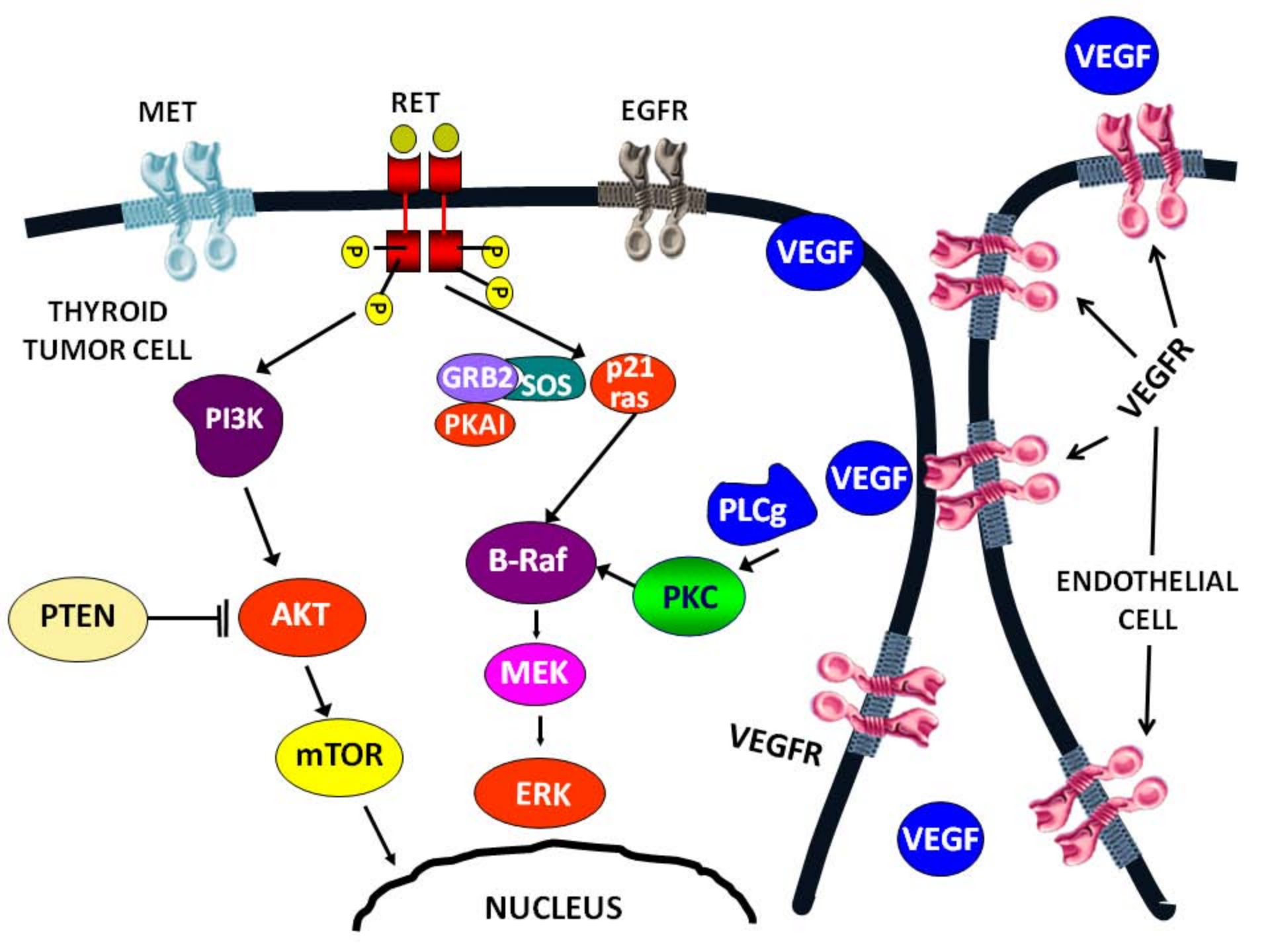
Bcr-Abl: Abelson and breakpoint cluster region fusion gene; **CSF-1R:** colony stimulating factor 1 receptor; **EGF-R:** epidermal growth factor receptor; **FGF-R:** fibroblast growth factor receptor; **KIT:** v-kit Hardy-Zuckerman 4 feline sarcoma viral oncogene; **Raf:** v-raf murine sarcoma viral 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 [HGF] receptor; **PDGFR:** platelet-derived growth factor receptor; **RET:** REarranged during Transfection receptor; **RET gene fusions: KIF5B-RET, CCDC6-RET** and **NcoA4-RET**; **VEGF-R:** vascular endothelial growth factor receptor.

Tab. 2 – Comparison of different features between DECISION and SELECT phase III clinical studies

	DECISION Trial (NCT00984282) (Progressive RAI-R DTC)	**SELECT Trial (NCT01321554) (Progressive RAI-R DTC)**
Progression Assessment	Local investigators by RECIST 1.0	Central reviewers by RECIST 1.1
Randomization ***(IP vs Placebo)***	1:1	2:1
Previous TKI	no	yes
PFS *vs* Placebo ***(months)***	10.8 *vs* 5.8	18.3 *vs* 3.6
ORR *vs* Placebo ***(%)***	12.2 *vs* 0.5	64.8 *vs* 1.5
Complete Response (CR - *%*)	0	1.5
Partial Response (PR - *%*)	12.2	63.2
Stable Disease ≥ 6 months (SD - *%*)	42	15.3
Progressive Disease (PD - *%*)	45.9	6.9
Death (%)	0.5	5.4
Crossover	71.4% of the placebo pts	83% of the placebo pts
Molecular Target	VEGFR, c-Kit, RET PDGFR, RAF, FLT3	VEGFR, c-Kit, RET, PDGFR, FGFR, RET-KIF5B, CCDC6-RET, NcoA4-RET rearrangement


Inactive Conformation
TKR
A1
Signal Molecule
Ligand Binding Domain
Cysteine Rich Domain
Transmembrane Domain
Tyrosine Kinase Domain
ATP Binding Pocket
Activation Loop
N-tl
C-tl
PBR
AR
SR
HR
Active Conformation
TKR
A2
Dimerization
ADP
ATP
P
RAS
MAPK pathway
PI3K-AKT pathway
Growth, Survival, Proliferation,
Migration, Angiogenesis


Active Conformation
TKR
B1
TKI
N-tl
N-tl
ATP
ATP
PBR
AR
SR
HR
PBR
AR
SR
HR
C-tl
C-tl
np
Signal Cascade Blockage
Inactive Conformation
TKR
B2
TKI
N-tl
N-tl
PBR
AR
SR
HR
PBR
AR
SR
HR
C-tl
C-tl
Signal Cascade Blockage
Inactive Conformation
TKR
B3
TKI
N-tl
N-tl
PBR
AR
SR
HR
PBR
AR
SR
HR
C-tl
C-tl
Signal Cascade Blockage


MET
RET
EGFR
VEGF
THYROID
TUMOR CELL
P
P
P
P
PI3K
GRB2
SOS
PKAI
p21
ras
VEGF
PLCg
VEGFR
VEGFR
ENDOTHELIAL
CELL
B-Raf
PKC
PTEN
AKT
MEK
mTOR
ERK
VEGF
NUCLEUS