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Current perspective

Multikinase inhibitors in thyroid cancer

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ABSTRACT

Biological agents are rapidly developing for the treatment of metastatic RAI resistant thyroid cancer. The most promising results were shown by agents that target BRAF and VEGFR rather than RET. BRAF V600E mutation seems to be positively associated with tumour response by using BRAF targeting agents.

With these agents impressive clinical responses and prolonged disease stabilisation were observed. This activity compares favourably with that of chemotherapy with less prominent toxicity, although typically associated drug side-effects should be promptly recognised and managed. To date no drug has proved to prolong survival, as such none of these agents has been approved.

1. Introduction

Thyroid cancer (TC) is uncommon, although it represents the most frequently occurring malignancy of the endocrine system. Over the last years, the incidence of TC has been increasing in some European countries,[1–3] in United States[4] and Canada.[5] In Europe 141,000 estimated cases and 35,300 deaths were recorded in 2002.[6] Survival was analysed for about 22,900 adults diagnosed with cancer of the thyroid in Europe during the period 1995–1999 and followed up to the end of 2003.[7]

Most thyroid cancers are differentiated carcinomas (DTCs) (95%) that derive from the follicular epithelial cells and are either papillary (PTC) (80%) or follicular (FC) (10%) and Hurthle cell (5%) thyroid carcinomas. Survival rates can be very different, accounting for their different phenotypes and radioiodine sensitivity. Another 5% are the neuroendocrine-derived medullary carcinomas (MTCs) and 1% are anaplastic carcinomas.

Standard therapies for patients with advanced DTC include surgery, radioactive iodine 131 (RAI) and thyroid-stimulating hormone (TSH) suppression. For MTC surgery represents the only recognised form of curative approach. RAI and TSH suppression play no role in MTC. Anaplastic carcinoma is primarily treated with surgery and radiotherapy or chemoradiation. In TC the cure probability depends on histotype, tumour stage, and RAI sensitivity. Even within the same histotype, mainly DTC and MTC, there are marked differences in relation to survival, that are explained by biological heterogeneity. Once the disease looses its ability to uptake iodine (25–50%), or in case it is intrinsically RAI refractory, treatment opportunities are very few, since active chemotherapy agents are lacking. Doxorubicin, cisplatin and dacarbazine-based chemotherapy obtained a low response rate, along with remarkable toxicities.[8–10]

RAI resistant thyroid carcinomas (RRTCs), metastatic medullary and anaplastic thyroid carcinomas represent one of the most appealing models for biological therapies.

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Our review focuses on the molecular basis of the disease and summarises the results of the studies that include targeted agents in this rapid evolving research field.

1.1. *Molecular basis*

Recent years have been characterised by significant advances in the understanding of the molecular basis of thyroid carcinogenesis. Many tumour-initiating genetic events have been already identified in TC. Interestingly, there is a typical morphological histopathological disease presentation that consistently correlates with specific molecular pathway deregulation and some of its features are associated with a more aggressive tumour behaviour.

Gene deregulation is responsible for unique PTC, FTC and MTC gene expression signatures (see below) that confer a distinct phenotype and biological properties. On the other hand, the activation of ubiquitous intracellular tumour growth pathways, such as MAPK signalling and PI3 K/Akt, is also involved. Other 'aspecific' alterations, such as loss of heterozygosity, PI3 K/Akt signalling pathway mutations and those involving CTNNB1, RAS and PTEN, are more frequently associated with poorly differentiated carcinomas or ATC, which can arise de novo or from pre-existing papillary or follicular carcinomas, and for which these alterations can be considered as a marker of tumour progression within a stepwise process rather than an initiating one. Supporting evidence to this is based on microarray studies that have identified genes and pathways preferentially deregulated in ATC, as compared with differentiated tumours.[11] Recently, FOXA1 gene has been described to be hyperexpressed in ATC cell lines, thus representing a possible therapeutic target.[12] Neither EGFR mutations nor gene amplifications were found in a little series of ATC, while high polysomy was detected by FISH in 61% of cases.[13]

RET/PTC oncogenes play a causative role in the pathogenesis of 5–30% of sporadic adult PTCs[14,15], while a higher percentage is recorded in tumours in children and young adults[16] and in individuals exposed to either accidental or therapeutic irradiation (50–80%).[17,18] RET/PTC oncogenes are produced by chromosomal rearrangements involving the RET proto-oncogene, encoding a kinase receptor binding the GDNF family of peptides. All types of *RET/PTC* oncogenes have in common the replacement of the extracellular ligand-binding domain of *RET* by *RET*-fused genes, which are expressed in the thyroid and encode proteins containing dimerisation domains; this property allows constitutive, ligand-independent activation of RET kinase leading to clonal expansion and neoplastic transformation of thyroid follicular cells. Thirteen different RET/PTC oncogenes have been so far described both in sporadic and in radiation-derived PTC, RET/PTC1 is the most frequent rearrangement in general TC population, while RET/PTC3 is most involved in case of radiation-derived PTCs and in tall-cell variant carcinomas.[19] RET/PTC oncogenes trigger the activation of multiple intracellular signal transduction pathways, including the MAPK pathway. It is still not clarified how the different types of RET rearrangements can affect the clinical behaviour of PTC. The distribution of the *RET/PTC* gene can be heterogenous among the tumour cells (clonal versus non-clonal). In this context tumour heterogeneity characterised by non-clonal RET distribution can lead to treatment resistance by employing drugs that specifically inhibit RET. In the hereditary and sporadic forms of medullary thyroid cancer (MTC), germ-line activating point mutations of the RET proto-oncogene are observed. These mutations cause constitutive, ligand-independent activation of RET TK activity and, in some case, they alter the RET substrate specificity. Mutations at different codons of RET are associated with the biological aggressiveness of the tumour[20] and they may display a different affinity for the TK inhibitors.

Chromosomal rearrangements involving the NTRK1, coding for the high affinity receptor for NGF, have been detected in a small fraction of PTC (up to 12%). The resulting TRK oncogenes, whose mechanisms of activation and action mirror those of RET/PTC oncogenes, exert a direct role and represent an early event in the process of thyroid carcinogenesis.

Another genetic alterations involved in the early pathogenesis of TC is the activation of BRAF. BRAF is one of three mammalian isoforms of serine-threonine Raf kinase family, which are intracellular effectors of the MAPK signal cascade. BRAF gene is frequently mutated in a wide range of human cancers. The prevalent mutation is the V600E which increases the BRAF basal kinase activity, resulting in constitutive activation and continuous phosphorylation of downstream effectors of the MAPK pathway.[21] The BRAFV600E mutation represents the most common genetic change in PTC, reported in up to 70% of cases.[22,23] Other and rare mechanisms, such as different point mutations, small insertions and deletions and gene rearrangements, contribute to BRAF oncogenic activation in PTC. Some of these alterations have been associated with radiation-induced tumours. BRAF V600E has a recognised prognostic role and it could represent also a key factor for PTC management. BRAFV600E mutation is also present in up to 24% of anaplastic thyroid carcinomas (ATCs) arisen in association with PTC.[24,25] BRAF mutation in fine-needle aspiration diagnostic biopsies could be useful to drive decisions on surgical extension.[26] Moreover, the detection of free circulating mutant BRAF in serum[27] could be employed as non-invasive diagnostic tool. RAI resistance in BRAF-mutated tumours has been associated with deregulation of genes involved in the movement and metabolisation of iodine in follicular cells. Moreover, since BRAF is downstream of *RET* and *Ras* pathway, its inhibition can be effective in tumours with upstream *RET* and *Ras* mutations.

Oncogenic *Ras* point mutations occur rarely in PTC (10%),[28] most frequently in poorly and undifferentiated carcinomas.[29] Some studies have reported a correlation between RAS mutations and dedifferentiation, probably due to chromosomal instability as well as less favourable prognosis in DTC. Ras genes (HRAS, KRAS, and NRAS) encode highly related small G proteins playing a central role in intracellular signalling. The mutant *Ras* becomes permanently switched in the active status, thus constitutively activating their downstream targets, leading to the activation of MAPK and PI3 K/AKT pathways.

Mutations on *BRAF* and *Ras*, and *RET*-rearrangements which involve about 70% of PTCs, trigger MAPK signalling. Interestingly, they are mutually exclusive and rarely expressed simultaneously within the same tumour,[28] indicating that a single oncogenic hit in this kinase-cascade is enough for PTC development.[30]

Another gene rearrangement that can result in TC tumourigenesis is the PAX8-PPAR gamma gene. This alteration is frequently seen in FTCs and tends to be present in tumours with a vascular invasion.

An inappropriate activation of *MET* has been described in many malignant tumours, including medullary thyroid cancer. *MET* gene amplification enhances the survival and the invasive advantage of neoplastic cells and its activation is generally the consequence rather than the cause of cancer features: for example in medullary thyroid cancer, *RET* can induce a transcriptional upregulation and activation of *MET*. A close cooperation between c-MET and vascular endothelial growth factor (VEGF) to promote neo-angiogenesis, cell motility and survival has been demonstrated[31] Moreover, the inhibition of c-MET seems to selectively modulate the activity of *Ras*-dependent signals, leaving the other pathways unaltered. Recent evidence on cell lines have demonstrated that silencing of MET results in arrest of tumour growth, regression of metastases and decreased generation of new metastases suggesting the importance of c-MET expression[32] in cancer development and therefore as a target for tailored drugs.

VEGF is the main stimulator of angiogenesis in the thyroid gland. Elevated levels of VEGF have been found in thyroid tumour tissue than in normal thyroid,[33] VEGF binds VEGFR-1 (fms-like tyrosine kinase-1) and VEGFR-2 (foetal liver kinase-1/kinase insert domain-containing receptor) which in turn activate MAPK signalling.[34] The intensity of VEGF expression is related in PTC with a higher risk of metastasis and recurrence, and a shorter disease-free survival.[35,36]

It seems that other pathways, such as VEGF receptor (VEGFR)- and epidermal growth factor receptor (EGFR)-dependent signalling, also participate in tumour growth and development.[37] In this context it has been shown that overexpression and activation of EGFR and VEGFR-2 were more pronounced in metastatic tissue from MTC rather than the primary tumour with a tendency to a differential EGFR expression in relation to more aggressive RET mutations.[38] Moreover, a dual inhibition of the EGFR and VEGFR is required to induce apoptosis and inhibit proliferation, independent of the activating mutation present, in thyroid cell lines.[39]

1.2. *Targeting oncogenic and signalling kinases for the treatment of thyroid cancer: clinical trials*

The concept of 'oncogene addiction' describes the phenomenon by which some cancers, containing multiple genetic abnormalities, appear to be dependent on a single over-active oncogene for their proliferation and survival. At present this is the keystone for effective target therapy and in thyroid cancer the concept holds.

Multiple oncogenes have been identified and many targeted agents (i.e. vandetanib, motesanib, and sorafenib) are currently available and many others are under investigation. MTC cells are, for example, addicted to oncogenic RET signalling but the RET tyrosine-kinase inhibitor (TKI) used in clinical trials that showed promising activity are not exclusively targeted at RET. This raises two questions: firstly, what are the real targets? And secondly, what is the best possible combination of drugs to improve efficacy, tackle primary and reduce acquired resistance?

A discrepancy is observed in TC between the amount of biological and preclinical studies and the relative paucity of published clinical studies and, surprisingly, there is still a poor correlation between the identified target and tumour response.

To date, no randomised phase III trials with any of these compounds have yet been published nor has any therapeutic biological agent yet been approved. Trials where new targeted agents are studied are often requiring a confirmed progressive disease according to RECIST criteria[40,41] as inclusion clinical criteria. Interestingly there is no univocal definition of how many months are required to define a progressive disease to enter a clinical trial for both DTC and MTC. Although the criteria is clearly adopted because of the TC natural history and because of the chosen trial's primary end-point which is often progression-free survival, a correlation between time frame of progression before entering the study and outcome has never been attempted which would at least validate its relevance.

In DTC studies, among the inclusion criteria a RAI resistant/refractory disease defined as the absence of ^{131}I uptake on any radioiodine scan; one or more lesions with progressive disease despite ^{131}I uptake or a cumulative activity delivered of ^{131}I more than 600 mCi are required. Correctly biochemical progression is not considered within the inclusion criteria. In our opinion RAI resistant/refractory tumours need to be better defined since this has been clearly associated with a specific tumour biological profile that could correlate with target drug's activity.

Table 1 reports the studies with molecules inhibiting thyroid specific oncogenic kinases. BRAF inhibitors are the only specific target agents that have been clinically tested. The other studied agents have no specific target since they inhibit VEGFR and other kinases. In general not only activity but also toxicity profiles are common among all these agents, including fatigue, cardiovascular (hypertension, asymptomatic QT interval prolongation and thromboembolism), gastrointestinal (diarrhoea, nausea and very rare cases of perforation) and mucocutaneous (hand foot syndrome, skin rash stomatitis, dysphonia, photosensitivity, keratoacanthomas and malignant squamous cell lesion[42]). Hypothyroidism is a remarkable side-effect, experienced with motesanib,[43,44] sorafenib,[45] imatinib[46] and sunitinib,[47] requiring adjustments in dosing of thyroid hormone replacement therapy. Acute cholecystitis is a very rare (5%) adverse event occurring during motesanib administration, for which the correlation with the

Table 1 – studies with molecules inhibiting thyroid specific oncogenic kinases.

Agent	Site of activity	No. of pts	Outcome
PLX 4032[60]	BRAF mutant V600E	3 Points with PTC with mut BRAF	1 RP, 2 prolonged SD
XL281[61]	BRAF mutant + wild type	5	4 Prolonged SD

Table 2 – Tyrosine kinase inhibitors and their targets.

Drug	VEGFR 1-2-3	VEGFR 1-2	VEGFR-2	VEGFR-3	RET	RET/PTC	BRAF	C-KIT	EGFR	C-MET	PDGFR	FGFR
Imatinib					+			+			+	
Axitinib	+											
Motesanib	+				+			+			+	
Sorafenib	+				+	+	+				+	
Sunitinib		+			+	+		+			+	
Vandetanib			+		+	+			+			
XL184			+		+			+		+		
E7080				+							+	+

Table 3 – Phase II clinical trials with multitarget agents in Radioiodine resistant Thyroid Cancer.

Author	Histotype	Drug	No. of pts evaluable for response	RR
Frank-Raue[62]	MTC = 8 hMTC = 1	Imatinib	9	0%
De Groot[46]	MTC = 15		15	0%
Ha[63]	ATC = 11		11	25%
Cohen[71]	PTC = 37	Sunitinib	43 (38)[a]	13% (18%)[a]
Cohen[a,70]	MTC = 6			
Ravaud[64]	PTC = 8 MTC = 4 ATC = 1 Other = 4		17	5%
Goulart[65]	DTC = 15 MTC = 3		18	44% (FDG response)
Carr[66]	DTC = 26 MTC = 7		29	38%
Sherman[43]	PTC = 54 FTC = 15 HCT = 17 Other = 7	Motesanib	93	14%
Schlumberger[32]	MTC = 91			
Kloos[72]	Arm A PTC = 41	Sorafenib	41	Arm A = 15%
	Arm B non-PTC = 11		11	Arm B = 0%
Gupta-Abramson[45]	PTC = 18 FTC = 9 MTC = 1 ATC = 1		30	23% RP
Brose[a59]	PTC = 25 FTC = 19 MTC = 4 ATC = 5		52 (55)[a]	PFS 84 weeks[a]
Nagaiah[67]	ATC = 15		15	13%
Lam[68]	MTC = 16 hMTV = 3		19	11%
Kober[69]	MTC = 5		5	40%
Wells[50]	hMTC	Vandetanib	30	20%
Haddad[49]	hMTC		19	16%
Fox[51]	hMTC (children, young adults)		7	28%
Cohen[53]	PTC = 29 FTC = 15 MTC = 12 ATC = 2 unk = 2	Axitinib	60	30% PFS 18.1 months

PTC = papillary thyroid cancer; FTC = follicular thyroid cancer; MTC = medullary thyroid cancer; hMTC = hereditary medullary thyroid cancer; HCT = Hürthle cell cancer; unk = unknown; ATC = anaplastic thyroid cancer.

a Updated studies.

drug assumption is still unknown.[43,44] As happens also for other target therapies, toxicities recover by the drug withheld while none of these toxicities can be considered as a surrogate marker of efficacy. On the other hand, toxicity intensity often correlates with treatment interruptions raising the question on the optimal long-term tumour exposure in order to maximise therapeutic effects. Interestingly, mostly in MTC patients, the assumption of TKI causes an immediate relief of disease-related symptoms as diarrhoea, regardless of response.

Over the last years, the employment of multikinases inhibitors (Table 2) has achieved promising results in the management of advanced TC. Phase II studies on biological agents targeting signalling kinases have been preliminarily reported. Table 3 contains the significant study features along with the results according to the histological type.

The most studied drugs are motesanib, sorafenib, sunitinib, axitinib, vandetanib and XL184. The latter compound has been investigated in a phase I study, where of 34 MTCs, 15 showed a PR irrespective of *RET* mutation,[48] the same happened with hereditary MTC (hMTC) with the employment of vandetanib,[49–51] thus suggesting the prominent role of angiogenesis, rather than that of *RET* related signalling.[52] This was also true in the subgroup analysis of patients with MTC in a phase II trial with axitinib,[53] indicating that *RET* inhibition might be less important than VEGFR inhibition, in general calling into question the role of specific thyroid cancer marker inhibitors. Interestingly, some of patients responding to XL184 were previously treated with other TKIs such as vandetanib, sorafenib and motesanib, suggesting the absence of cross-resistance among these drugs.

With these new agents biochemical response tends to occur irrespective of radiological response. Calcitonin reduction, carcinoembryonic antigen (CEA)[44] and a thyroglobulin (TG) reduction were evident in most patients as such not correlating with tumour response.

The best tool to evaluate tumour response for target therapy is still a matter of debate. RECIST criteria[41,42] seem not always to catch tumour modifications/response during biological therapies.[54,55] Functional imaging results are promising but unfortunately, in PTC the correlation between functional imaging (DCE-MRI and FDG-PET) and response has been studied without showing any significant correlation[56] while in MTC, FDG-PET has a recognised low sensitivity that prevents its use.[57]

Both sorafenib and sunitinib have already been approved by US FDA and European EMEA in other indications thus potentially available for off-label use in patients with metastatic TC, at least in some European countries. In this context, the experience with DTC at the MD Anderson (US) has been reported at the 2009 Annual ASCO Meeting. The majority of patients received sorafenib with a 20% of response rate. Notably one patient, refractory to sorafenib, responded to sunitinib. There was no difference in activity among the different histotypes, PTCs, FCs and Hurthle cell carcinomas.[58] Interestingly, there was a correlation between response and TG levels, which was not seen in a phase II trial with sorafenib,[56] as well as a correlation between response and lung disease, suggesting a possible tissue response specificity.

In the recent update of the phase II study with sorafenib there was a correlation between longer PFS and the presence of BRAF mutations.[59] This suggests, for the first time, the validity of specific targeting and the concept of 'oncogene addiction' in TC and underpins the importance of patient's selection in future studies, where molecular studies intended to better define predictive factors for response, molecular crosstalk among relevant pathways and resistance to biological therapies will be critical. In the meantime, biological profiling should be prospectively studied to guide standard treatment decision and possibly used to identify patients who might benefit from the integration of biological therapies.

Conflict of interest

None declared.

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