

Genomic alterations in thyroid cancer: biological and clinical insights

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Abstract

Tumours can arise from thyroid follicular cells if they acquire driver mutations that constitutively activate the MAPK signalling pathway. In addition, a limited set of additional mutations in key genes drive tumour progression towards more aggressive and less differentiated disease. Unprecedented insights into thyroid tumour biology have come from the breadth of thyroid tumour sequencing data from patients and the wide range of mutation-specific mechanisms identified in experimental models, in combination with the genomic simplicity of thyroid cancers. This knowledge is gradually being translated into refined strategies to stratify, manage and treat patients with thyroid cancer. This Review summarizes the biological underpinnings of the genetic alterations involved in thyroid cancer initiation and progression. We also provide a rationale for and discuss specific examples of how to implement genomic information to inform both recommended and investigational approaches to improve thyroid cancer prognosis, redifferentiation strategies and targeted therapies.

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Key points

- Most thyroid cancers are initiated by a single genomic alteration, typically *BRAF*^{V600E} or in RAS genes, which constitutively activate the MAPK signalling pathway.
- Driver mutations *BRAF*^{V600E} or in RAS genes determine multiple clinicopathological properties of thyroid tumours, including their histology, level of differentiation and routes for metastatic dissemination.
- Advanced thyroid cancers usually evolve from *BRAF*-driven or RAS-driven clones and become more aggressive and less differentiated via the acquisition of additional genomic alterations in specific genes.
- Preclinical models of thyroid cancers are valuable tools to understand the underlying mechanisms and biological consequences of pathogenic mutations, and to test novel therapeutic strategies.
- Genomic characterization is a useful tool to refine prognosis of patients with thyroid cancers and can predict their responses to strategies to induce redifferentiation for subsequent radioactive iodine treatment.
- *BRAF*^{V600E} mutation and *RET* or *NTRK* fusions are actionable alterations with matched drugs that are approved for the treatment of certain patients whose thyroid tumours have these genetic defects.

Introduction

Genomic and biological knowledge of cancer is changing the way we view, classify and manage tumours. Cancers arising from thyroid follicular cells (also called thyrocytes) are fairly simple entities that can be defined by their histology; however, they are now increasingly categorized by their mutational and transcriptomic properties (Table 1). The 2022 WHO classification of thyroid tumours¹ comprises the following three follicular-cell-derived well-differentiated subtypes: papillary thyroid cancer (PTC), follicular thyroid cancer (FTC) and oncocytic carcinoma of the thyroid (OCA, previously known as Hürthle cell cancer)¹. Collectively, these three subtypes can be referred to as differentiated thyroid cancer (DTC). PTC is by far the most common thyroid cancer type, followed by FTC. Both PTC and FTC typically have good prognosis^{2,3} and a 5-year survival rate close to 100% when localized in the thyroid gland (US cancer statistics can be found in [the SEER database](#)). PTC has multiple histological subtypes, including classic PTC, tall-cell PTC and the follicular variant of PTC, which can adopt an encapsulated or an infiltrative form. The follicular variant of PTC, particularly the encapsulated form, shares some properties with FTC^{1,4}.

At the other extreme of the spectrum, anaplastic thyroid cancers (ATCs) are rare but one of the most aggressive tumours overall and have a historical median survival of less than 6 months from diagnosis⁵. In terms of morphological features and prognosis, poorly differentiated thyroid cancer (PDTC) and differentiated high-grade thyroid carcinoma (DHGTC; a new category from the WHO 2022 classification) represent intermediate entities between PTC or FTC (hereafter in this Review, we use 'PTC–FTC' when referring to both tumour histological subtypes together) and ATC¹ (Table 1), with a 5-year survival of ~70% for PDTC^{6,7}.

The availability of ample next-generation sequencing (NGS) data from patients' tumours and the implementation of multiple in vivo models of the disease have considerably increased our knowledge of the genetic underpinnings and mechanistic consequences of thyroid cancer initiation and progression. NGS data have opened translational opportunities to refine the clinical management of patients with thyroid cancer, particularly of those with aggressive disease. In addition, preclinical models have functionally corroborated the causal role of most genetic alterations identified by NGS and have provided invaluable settings to test novel drugs and explore mechanisms of resistance to treatment.

In this Review, we discuss the genomic alterations that occur across the main types of follicular cell-derived thyroid cancer. We focus on their effects on thyroid cancer evolution, summarize their biological consequences and highlight their utility at improving the clinical outcomes of patients with thyroid cancer. Tumours that affect the parafollicular or C cell lineage within the thyroid gland are known as medullary thyroid cancers (MTCs) and are not covered in this Review.

Genomic and biological landscapes of thyroid tumours

Cross-cancer comparisons of genomic landscapes have shown that thyroid tumours rank low in genetic complexity. PTCs have a low mutation burden and their genomes are mostly diploid^{8,9}, facilitating genotype–phenotype correlation. The initiation of thyroid cancer requires the sustained activation of the mitogen-activated protein kinase (MAPK) pathway, which is typically achieved by a single genomic event. Progression of thyroid tumours from DTC to PDTC or ATC entails the acquisition of additional mutations in key effectors of pathways and functional groups other than MAPK. The main genetic alterations that determine thyroid cancer initiation and progression, along with their consequences, are summarized in the next sections.

Mutational events in thyroid cancer initiation

The Cancer Genome Atlas (TCGA) study showed that 90% of PTCs are initiated by the acquisition of a MAPK pathway-activating event¹⁰. In most tumours, this activation occurs via mutually exclusive mutations at either the *BRAF* or RAS oncogenes, whereas a subset of thyroid cancers are initiated by gene rearrangements involving receptor tyrosine kinase (RTK) genes, primarily *RET*.

***BRAF* and RAS mutations.** Oncogenic mutations at *BRAF*^{V600E} are the most common alterations in PTC. Alternatively, *BRAF* class II alterations (that is, non-V600E mutations such as *BRAF*^{K601E}) and *BRAF* fusions occur in a minority of PTCs. PTCs without mutations in *BRAF* usually have mutations in RAS oncogenes: *NRAS* is the predominant isoform over *HRAS* and *KRAS*¹⁰.

The presence of either *BRAF*^{V600E} or a RAS mutation determines multiple characteristics of the thyroid tumour. First, although these correlations are not absolute, *BRAF*^{V600E}-driven PTCs are typically enriched in the classic PTC and tall-cell PTC subtypes, whereas RAS-driven PTCs usually cluster in the follicular variant of PTC (Fig. 1a and Table 1). Second, these tumour-initiating events establish specific transcriptomic features in PTCs that are best captured by the TCGA's 71-gene expression signature known as the '*BRAF*^{V600E}–RAS score' (BRS), which represents a proxy for MAPK activation¹⁰. In PTCs, higher MAPK activation (defined by negative BRS values) inversely correlates with another transcriptomic signature, the 'thyroid differentiation score', which includes 16 genes related to thyroid function and iodine

Table 1 | Biological and clinical features of follicular cell-derived thyroid cancer types

Cancer type	Prevalence (%)	Differentiation	Distinctive histological features	Typical route of spread and frequency	Prognosis	Predominant mutational hallmarks	Predominant transcriptomic BRS	Predominant transcriptomic thyroid differentiation score
PTC	80–85	Good	Papillae, nuclear features	Local lymph node via lymphatic spread; rare	Good	<i>BRAF</i> ^{V600E} (~60%, enriched in classic and tall-cell subtypes); <i>RET</i> fusion (~7%, classic); <i>NRAS</i> > <i>HRAS</i> > <i>KRAS</i> (12–16%, follicular variant)	<i>BRAF</i> ^{V600E} -like (classic and tall-cell subtypes); RAS-like (follicular variant)	Lower in <i>BRAF</i> ^{V600E} -like; higher in RAS-like
FTC	5–10	Good	Follicles, absence of PTC nuclear features	Distant metastasis via haematogenous spread, usually to lungs and bone; rare	Good	<i>NRAS</i> > <i>HRAS</i> > <i>KRAS</i> (25–30%); <i>PAX8</i> – <i>PPARG</i> (~25%); <i>EIF1AX</i> (5–20%); <i>PTEN</i> (5–10%)	RAS-like	Higher in RAS-like
OCA ^a	~5	Good	Abundance of mitochondria, eosinophilic cytoplasm	Local lymph nodes and distant metastasis; intermediate	Good–intermediate	Mutations in mitochondrial respiratory complex I genes; near total haploidization of nuclear genome	Not defined	Not defined
DHGTC ^a	<5	Good	Papillary, follicular or solid growth; high mitotic rate and/or necrosis	Local lymph node via lymphatic spread; frequent	Intermediate	<i>BRAF</i> ^{V600E} ; <i>TERT</i> promoter; <i>PIK3CA</i> ; others	<i>BRAF</i> ^{V600E} -like	Low, lower in <i>BRAF</i> ^{V600E} -like
PDTC	<5	Poor	Solid or trabecular or insular growth; absence of PTC features	Invasive growth, lymph node and distant metastasis; frequent	Intermediate	<i>NRAS</i> > <i>HRAS</i> > <i>KRAS</i> (+ <i>EIF1AX</i>); <i>PTEN</i> ; <i>TERT</i> promoter; others	RAS-like	Low, higher in RAS-like
ATC	2	Absent	Undifferentiated growth, spindle or squamous cells, macrophage infiltration	Invasive growth, lymph node and distant metastasis; very frequent	Poor	<i>BRAF</i> ^{V600E} or <i>NRAS</i> > <i>HRAS</i> > <i>KRAS</i> ; <i>TP53</i> (40–70%); <i>TERT</i> promoter; <i>PIK3CA</i> ; <i>CDKN2A</i> ; <i>SWI</i> – <i>SNF</i> complex; others	<i>BRAF</i> ^{V600E} -like or ATC-like, regardless of driver mutation	Lowest, regardless of BRS or driver

ATC, anaplastic thyroid cancer; BRS, *BRAF*^{V600E}–RAS score; DHGTC, differentiated high-grade thyroid cancer; FTC, follicular thyroid cancer; OCA, oncocytic cancer of the thyroid; PDTC, poorly differentiated thyroid cancer; PTC, papillary thyroid cancer; > shows that mutations in each gene are more frequently found than in the next one listed. ^aThese are new terms that fulfil the 2022 WHO classification of thyroid neoplasms. OCAs were formerly known as Hürthle cell cancers, whereas DHGTC unifies entities that were previously referred to as either high-grade PTCs or PDTCs not fulfilling the Turin criteria¹.

metabolism. *BRAF*^{V600E}-like PTCs show higher MAPK outputs and lower thyroid differentiation scores, compared with RAS-like PTCs, which display lower MAPK outputs and higher thyroid differentiation scores (Fig. 1b). This difference portends important clinical ramifications for targeted and radioactive iodine (RAI) therapies, as discussed later in this Review^{2,10}.

Mechanistically, constitutive MAPK activation has been shown to impair the expression of genes (included in the thyroid differentiation score signature) that are required for normal thyroid function¹¹. These genes are activated by thyroid-stimulating hormone (TSH)–adenylyl cyclase–protein kinase A signalling and encompass those encoding the sodium–iodide symporter (NIS) and other effectors of thyroid metabolism and thyroid hormone synthesis. Crucially, the degree of MAPK pathway activation (and thus of thyroid differentiation) by *BRAF*^{V600E} or RAS mutations is different (Fig. 1b). This difference is a consequence of *BRAF* (V600E) being unresponsive to ERK-mediated negative feedback inhibition, resulting in higher MAPK output, compared with RAS-driven tumours¹². The higher MAPK activation in

BRAF^{V600E}-driven thyroid cancers is also the most plausible explanation for their higher cancer-transforming potential compared with RAS-driven thyroid cancer. Of note, tumours driven by *BRAF* class II mutations behave as RAS-like cancers.

Genetically engineered mouse models of thyroid cancer initiation have shown that thyroid-specific expression of the *BRAF* (V600E) oncoprotein almost invariably results in the induction of PTCs in mice^{13–15}. By contrast, induction of *Nras*, *Hras* or *Kras* oncogenic mutations in mice yields only thyroid hyperplasia and/or follicular-patterned thyroid tumours (that is, the follicular variant of PTC or FTC) after a long latency^{15–18}. The fact that RAS mutations are also found in follicular adenomas, which are sometimes considered to be precursor lesions of follicular-patterned carcinomas, supports the idea of RAS as a weaker oncogene in thyroid cells. A potential hypothesis to reconcile these data suggests that the clonality of RAS mutations in follicular adenomas is lower than in carcinomas, but data on this topic are still limited and contradictory^{19,20}. Whether RAS-mutant follicular adenomas represent an early stage of evolution to carcinomas remains unclear, as does

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whether they can remain as benign lesions without the potential to become invasive. Nevertheless, a 2023 publication showed that the engineering (via CRISPR–Cas9) of *BRAF*^{V600E} mutations into human

thyroid progenitor cells was sufficient to recapitulate PTC, whereas the *NRAS*^{Q61R} mutation recapitulated FTC, thus providing additional direct evidence of these genotype–histotype correlations²¹.

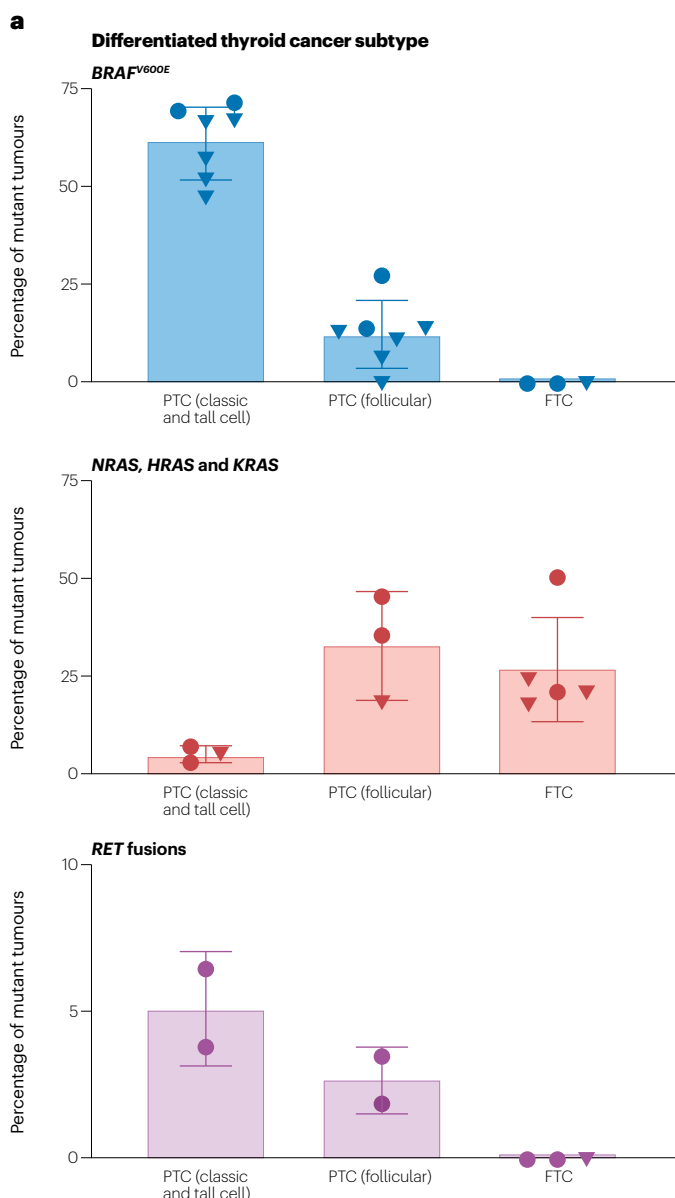
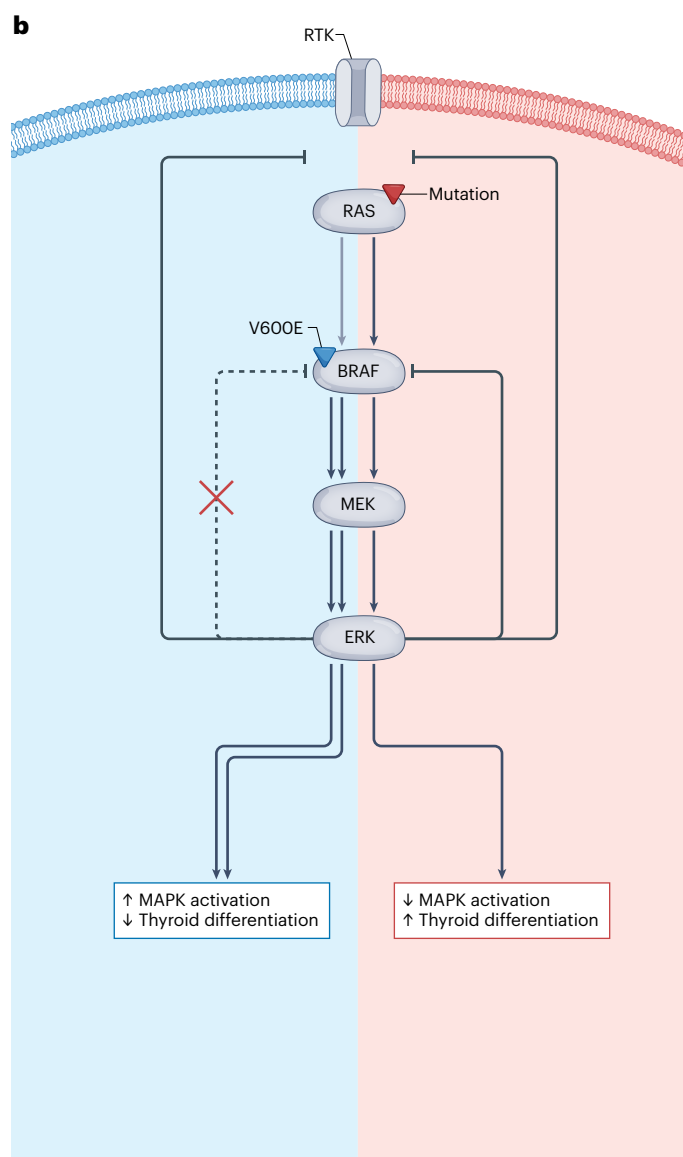


Fig. 1 | Genomic and biological characteristics of thyroid cancer initiation. **a**, Frequency of driver genetic alterations, including *BRAF*^{V600E} (top panel), *RAS* genes (middle) and *RET* fusions (bottom) in the main papillary thyroid cancer (PTC) subtypes and in follicular thyroid cancers (FTCs). *BRAF*^{V600E} is the initiating event in most PTCs of the classic and tall-cell subtypes. Mutations in *NRAS*, *HRAS* and *KRAS* oncogenes are enriched in tumours with follicular architecture, that is, the follicular variant of PTC (PTC, follicular) and FTC. *RET* fusions define PTC diagnosis and are enriched in classic PTC. Genomic data were compiled from selected next-generation sequencing (NGS) studies with information on specific PTC subtypes^{10,34,35} (circles in graphs) and from pre-NGS cohorts^{63,64,87,89,232–237} (triangles; lower frequencies might reveal technical limitations). Studies assessing ‘aggressive’ or ‘metastatic’ PTCs or FTCs were not considered here. The ‘PTC, follicular’ category probably includes entities now defined as infiltrative and encapsulated forms of the follicular subtype of PTC,



the invasive encapsulated follicular variant of PTC and possibly the non-invasive follicular thyroid neoplasm with papillary-like nuclear features (NIFTP).

b, Signalling properties of *BRAF*^{V600E}-driven versus *RAS*-driven thyroid cancers. Mutant *BRAF* and *RAS* activate the mitogen-activated protein kinase (MAPK) pathway at different intensities. Signalling via *BRAF*(V600E) (left half) does not respond to negative feedback signals (dotted line), resulting in higher MAPK output. MAPK signalling induced by oncogenic *RAS* (right) is partially attenuated by inhibitory feedback (solid black inhibitory arrows), leading to weaker MAPK output (compared with *BRAF*(V600E)). The degree of MAPK activation is inversely correlated with the expression of thyroid differentiation genes, which are involved in iodine uptake and metabolism. Owing to this factor, *RAS*-driven thyroid cancers tend to maintain higher levels of thyroid differentiation than their *BRAF*^{V600E}-driven counterparts.

Gene rearrangements. Approximately 5–10% of PTCs in adults are driven by gene rearrangements that result in the re-expression of the kinase domain of RET. These *RET* fusions are mutually exclusive with point mutations in *BRAF* or *RAS*, as well as with *BRAF* fusions, and represent an alternative mechanism by which thyroid cells can activate the MAPK pathway. *RET* fusion-driven PTCs show a *BRAF*^{V600E}-like BRS and are enriched in classic PTC histologies (Fig. 1a). The prevalence of fusion oncogenes, particularly those involving *RET*, is more frequent in paediatric PTCs^{22,23} and in thyroid tumours induced by ionizing radiation, such as those linked to the Chernobyl nuclear plant accident^{24,25}. The oncogenicity of *RET* fusion oncoproteins in thyroid cancer has been demonstrated in cell systems²⁶ and in genetically engineered mouse models^{27–29}.

A small subset of PTCs is driven by fusions implicating RTKs other than RET. These rearrangements usually involve *NTRK1*, *NTRK3* or *ALK* with various fusion partners^{10,30,31} and have been shown to induce thyroid tumours in vivo^{32,33}. Overall, gene rearrangements encompassing *RET*, *NTRK1*, *NTRK3*, *ALK* or other kinase genes distally activate MAPK signalling in tumours with wild-type *BRAF* and *RAS*. These gene fusions are particularly prevalent in paediatric PTC (60–70%)^{22,23} and patients with radiation-induced PTC (40–70%)^{24,25} compared with adult sporadic thyroid cancers (~15%)¹⁰.

FTC-associated driver alterations. FTCs are tumours predominantly driven by mutations in *RAS* genes (altered in 25–30% of FTCs), whereas *BRAF*^{V600E} and *RET* fusions are almost never seen^{34,35} (Fig. 1a). These associations further reinforce the observation of *RAS* mutations inducing follicular growth patterns in thyroid tumours and links *RAS*-driven FTCs and the follicular variant of PTC as entities with shared features. However, FTCs can be initiated by events that do not feed into MAPK signalling. Around 25% of FTCs (and some follicular adenomas) express the PAX8–PPAR γ fusion oncoprotein, in which thyroid transcription factor PAX8 drives the expression of PPAR γ , a nuclear receptor involved in adipocyte biology^{35–37}. Small subsets of FTCs have mutations in *TSHR*, *PTEN* and *EIF1AX* (5–10% of FTCs for each gene). *TSHR* mutations activate the downstream signalling from the TSH receptor³⁵, a key node for thyroid cell growth and function. *TSHR* mutations are also seen in hyperfunctioning thyroid nodules, which can result in hyperthyroidism^{38,39}. Loss-of-function mutations in *PTEN*, which encodes a negative regulator of the PI3K–AKT pathway, probably facilitate unrestricted signalling via this pathway^{34,35,40}. In vivo targeting of PAX8–PPAR γ plus *Pten* loss in mice induces thyroid tumours and increases tumour levels of phospho-AKT⁴¹. *EIF1AX*, which encodes a member of the protein translation initiation complex, is mutated in 5–20% of FTCs and in ~5% of follicular variant of PTCs and induces aberrant translation^{42–44}. Mutations in *PTEN* or *EIF1AX* can also occur in follicular adenoma, suggesting that their cancer initiation potency is lower than that conferred by other driver events (for example, *BRAF* mutations). However, *PTEN* or *EIF1AX* mutations can promote thyroid cancer progression when combined with other specific MAPK-activating events^{45,46}.

Mutations in *DICER1*, a gene encoding a ribonuclease involved in the processing of microRNA precursors, drive 1–2% of thyroid cancers, particularly those with a follicular pattern of growth. Somatic *DICER1* mutations cluster in the RNase IIIb protein domain⁴⁷ and are mutually exclusive with canonical *BRAF* and *RAS* drivers. This observation strongly suggests a functional effect that entails widespread microRNA deregulation and possibly MAPK activation^{47,48}. *DICER1* mutations associate with *RAS*-like thyroid cancers⁴⁸ and are thus particularly prevalent in FTC^{34,49,50}, but are also present in subsets of PTC^{10,51}, PDTC⁵²,

DHGTC and ATC⁵³. Mutations observed in *DGCR8*, another gene that encodes a component of microRNA biogenesis, in PTC–FTC^{44,54} further strengthen the role of this pathway in a subgroup of thyroid cancers. Of note, although isolated somatic mutations in *DICER1* exist, they often represent a second hit in the context of individuals who carry germline loss-of-function *DICER1* mutations. Germline defects in *DICER1* associate with DICER1 syndrome, which predisposes people to both benign and malignant thyroid lesions^{55,56} and probably explains why somatic *DICER1* mutations are more prevalent in paediatric patients with thyroid cancer^{49–52}.

The OCA subtype. Finally, although not discussed in this Review, the OCA subtype represents a separate entity with unique phenotypic features, such as the accumulation of abundant homoplasmic mitochondria, which correspond with distinctive genomic characteristics: near total haploidization of their nuclear genomes and mutations that disrupt genes encoding the mitochondrial respiratory complex I (refs. 57,58). The consequences of the disruption of mitochondrial respiration are beginning to be dissected and cause a reconfiguration of the metabolomic preferences of these cancers⁵⁹, which in turn convey specific therapeutic vulnerabilities in experimental OCA models⁶⁰. Mitochondrial mutations have also been found in tall-cell PTCs, suggesting that they might also affect the behaviour of this more aggressive PTC subtype^{59,61}.

Summary. Overall, the clonal nature and mutual exclusivity of alterations in *BRAF*, *RAS* genes, *RET* or others supports the idea that a single alteration is the crucial initiating event in most PTC–FTCs. Around 5–10% of PTC–FTCs lack unambiguous genetic drivers and might be initiated by focal or arm-level copy number alterations (gains and losses) or by epigenetic deregulation of key genetic programmes. Yet, the preponderance of constitutive MAPK pathway activation in the biology of thyroid cancer initiation is obvious, and its influence is maintained as PTC–FTCs progress to advanced forms (DHGTC, PDTC and ATC).

Mutational events driving disease progression

Most advanced thyroid cancers (that is, DHGTC, PDTC and ATC) probably either directly evolve from or share a common ancestor with DTC. Multiple layers of evidence are available to support this idea. First, some PDTCs and ATCs retain a co-existing well-differentiated component^{62,63}. Second, alterations in *BRAF*, *RAS* genes and *RET* were identified more than 20 years ago in PTC–FTC, and PDTC or ATC^{63–65}. In the past decade, the implementation of NGS technologies to characterize the mutational landscape across the spectrum of thyroid tumours^{10,34,40,45,46,66–78} clearly showed that most advanced cancers retain their driver truncal mutations (usually in *BRAF* or *RAS*). On top of those mutations, they have additional oncogenic alterations in key genes (Fig. 2), the prevalence of which correlates with the aggressiveness of the thyroid tumour subtypes (ATC is the most aggressive, PDTC or DHGTC are intermediate, PTC is the least aggressive). Whole-exome sequencing of multiple regions of ATCs and their concurrent well-differentiated areas suggests that ATCs diverge early from well-differentiated tumours and often share clonal mutations in *BRAF* or *RAS*^{62,79}. Finally, studies using single-cell RNA sequencing are beginning to delineate with unprecedented resolution the copy number and transcriptomic changes of premalignant-to-PTC-to-ATC evolution^{80–83}, but the effect of specific mutations in these gene expression changes is still not fully understood. Overall, ample evidence exists to support a model of tumorigenesis whereby the most advanced thyroid cancers arise from their

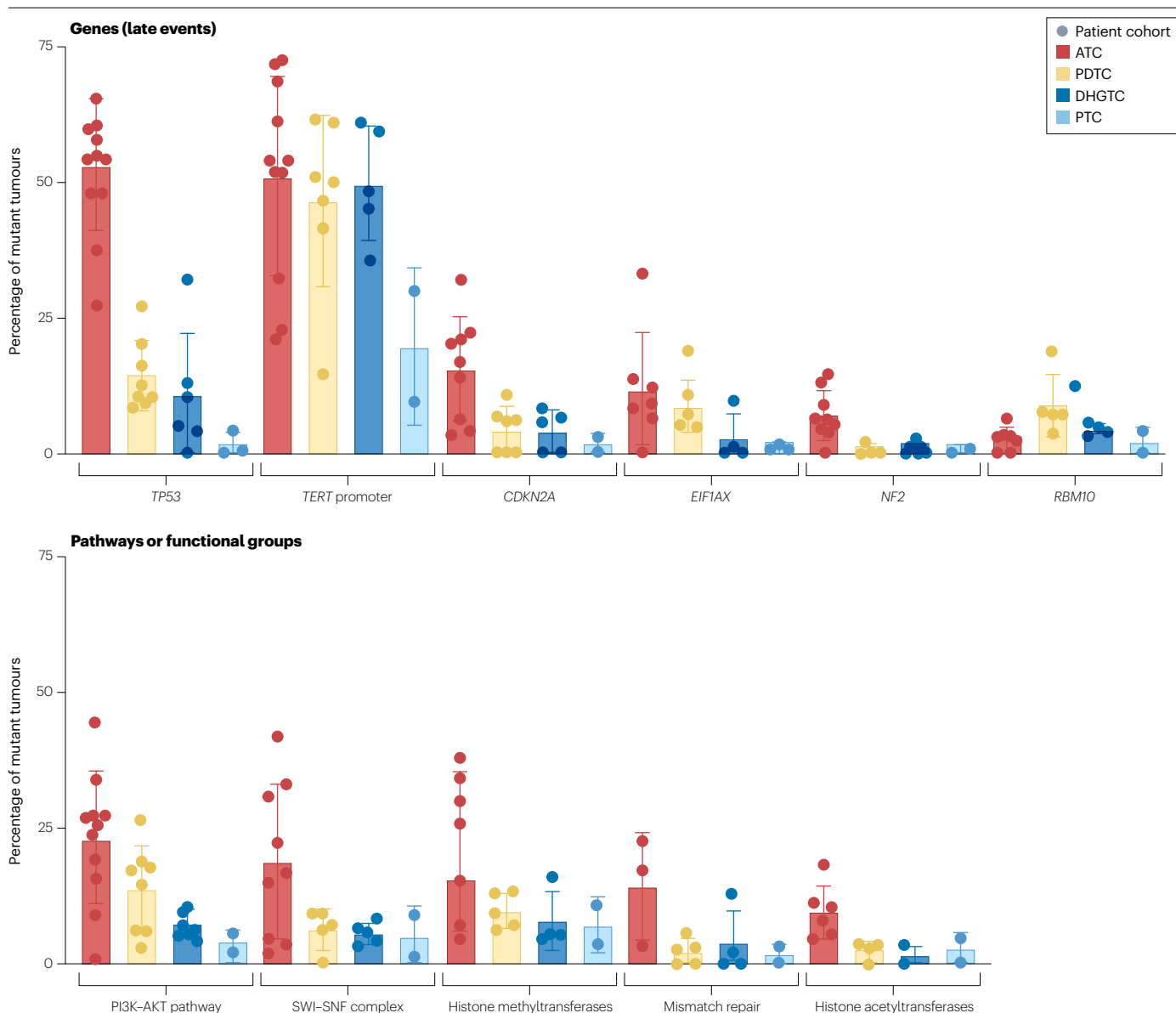


Fig. 2 | Mutational landscape of thyroid cancer progression. Frequency of late-event mutations in genes (top panel) and pathways (bottom) in anaplastic thyroid cancer (ATC), poorly differentiated thyroid cancer (PDTC), differentiated high-grade thyroid cancer (DHGTC) and papillary thyroid cancer (PTC). Each circle represents the average frequency of a given genetic alteration in an independent, non-overlapping patient group. Data were retrieved from studies published between 2014 and 2022, using next-generation sequencing platforms in thyroid cancer cohorts of at least 15 patients per subtype^{10,34,40,45,46,66–78}. The ‘long tail’ of genetic mutations in ATC (excluding early drivers, for example, *BRAF* or *RAS*) is shown in decreasing frequency, and their corresponding frequencies

in PDTC, DHGTC and PTC are displayed side by side. For the purpose of this figure, the DHGTC category (defined in 2022) was retrospectively applied to entities that would have previously been referred to as ‘high-grade’, ‘aggressive’ or ‘metastatic’ PTC. Pathway or functional group categories include the following genes: PI3K–AKT pathway (*AKT1*, *PIK3CA* and *PTEN*); SWI–SNF complex (*ARID1A*, *ARID1B*, *ARID2*, *SMARCB1* and *PBRM1*); histone methyltransferases (*KMT2A*, *KMT2C*, *KMT2D* and *SETD2*); mismatch repair (*MLH1*, *MSH2* and *MSH6*) and histone acetyltransferases (*CREBBP* and *EP300*). Alterations in the p16 (*CDKN2A*) gene include both mutations and deletions, when assessed.

well-differentiated counterparts via the sequential acquisition and selection of key genetic events, leading to a mutation-driven continuum in disease evolution.

Mutations that determine thyroid cancer progression affect cellular processes that are important for cancer biology. Figure 3 illustrates

the influence that these genetic alterations have in the framework of Hanahan and Weinberg’s hallmarks of cancer⁸⁴. Mutations that activate the PI3K–AKT pathway, disrupt p53 functions and cell cycle control, re-express telomerase, perturb the SWI–SNF chromatin remodelling complex and deregulate mRNA translation, among other processes,

are selected as thyroid tumours become less differentiated and more aggressive. Below, we summarize the mechanistic underpinnings provoked by the most common mutations involved in thyroid cancer progression, which were primarily unveiled by in vivo thyroid cancer models.

The PI3K–AKT pathway. The physiological functions of PI3K–AKT pathway activation facilitate cell metabolism, growth, proliferation and survival. By contrast, aberrant activation of this pathway can aid the uninhibited growth of cancer cells by increasing protein synthesis⁸⁵ and by interacting with unrestricted MAPK signalling in thyroid tumour progression. As discussed earlier, PI3K signalling has a role in the initiation of a subset of follicular-patterned thyroid tumours via mutations in *PTEN*, and PI3K activation can be achieved by RAS gene mutations⁸⁶. The key role of the PI3K pathway in thyroid cancer progression is best exemplified by the high prevalence of mutations in crucial genes of this pathway (*PIK3CA*, *AKT1* and *PTEN*) in PDTC and ATC^{87–90}. The PI3K pathway can also be activated by non-mutational mechanisms, for example, microRNA-mediated regulation⁹¹. In advanced disease, PI3K pathway mutations show specific patterns of co-occurrence: for example, *PIK3CA* and *AKT1* mutations associate with *BRAF*^{V600E}; by contrast, *PTEN* loss-of-function mutations cluster with mutations in RAS genes and *NF1* (ref. 45).

Work in mouse models has shown that thyroid-specific *Pten* inactivation with either *Kras*^{G12D} (ref. 18) or *Hras*^{G12V} (ref. 92) induces

metastatic FTCs. Mice with tumours driven by Ras genes and *Pten* displayed tumour overexpression of cyclin D1 and other genes, and an immunosuppressive tumour microenvironment^{18,92,93}. Co-targeting of *Pten* loss and *Braf*^{V600E} to the mouse thyroid gland resulted in tumour progression to PDTC and ATC^{94–96}; however, *PTEN* and *BRAF* are not co-mutated in tumours from patients with thyroid cancer. Further work in genetically engineered mouse models showed that PIK3CA(H1047R) cooperates with BRAF(V600E) in the pathogenesis of ATC by enhancing tumour cell proliferation, facilitating the epithelial–mesenchymal transition (EMT) and decreasing survival⁹⁴. Overall, the simultaneous activation of MAPK and PI3K signalling in preclinical mouse^{18,94,97,98} and human thyroid cancer models^{99–101} showed increased susceptibility to pharmacological blockade of these pathways. Although MAPK inhibitors are used as targeted therapies, the combined use of MAPK and PI3K pathway drugs has not yet been successfully translated to the clinic, primarily owing to toxicities¹⁰².

Disruption of p53 function. p53 is a tumour suppressor encoded by *TP53* that facilitates cell cycle control, DNA repair and apoptosis in response to various cellular stresses, thereby quenching the growth and proliferation of abnormal cells^{103,104}. Genetic inactivation of p53 is frequent in cancers, enabling mutant tumour cells to circumvent these checkpoints¹⁰⁵. *TP53* loss of function has long been identified as a key event in the progression to ATC^{106,107} and it is also found in subsets of DHGTC or PDTC, as well as in PTC–FTC with poor prognosis^{35,67,108} (Fig. 2).

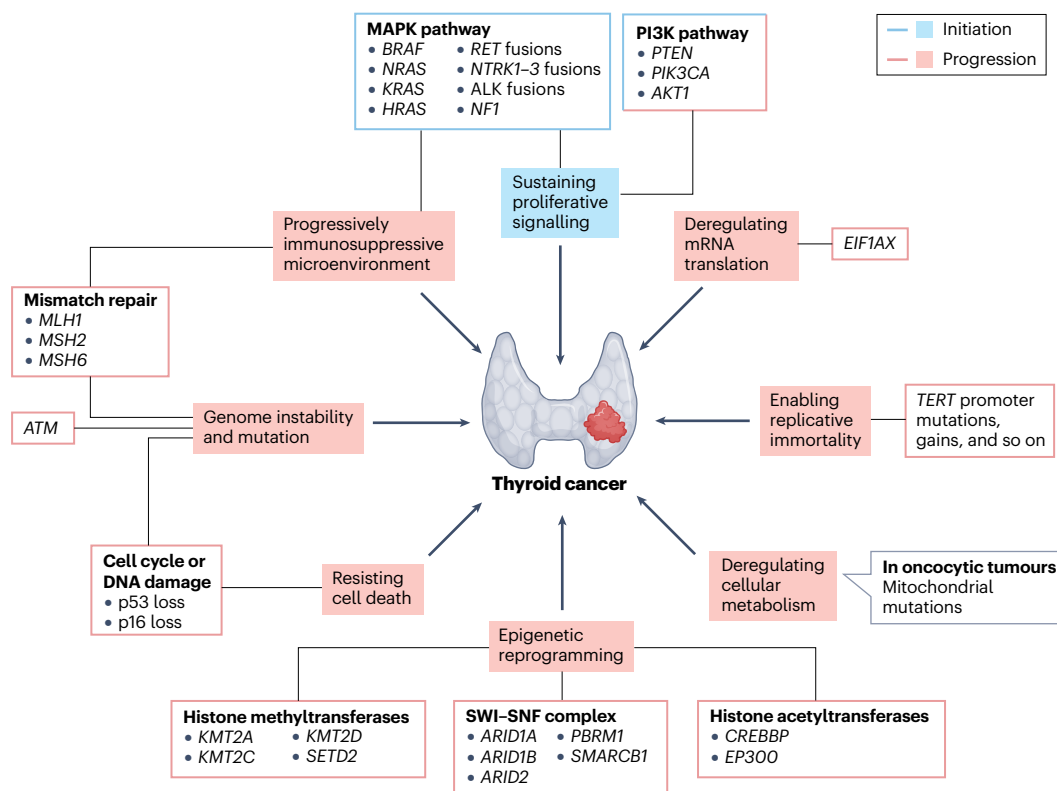


Fig. 3 | Genomic–biological hallmarks of thyroid cancer. This figure shows the main biological processes in thyroid cancer, colour-coded according to their roles in thyroid cancer initiation (blue) and/or progression (red), triggered by the genetic alterations found in thyroid tumours. Hanahan and Weinberg conceptualized ‘the hallmarks of cancer’⁸⁴, and, when applicable, the categories

defined by them are maintained here. The most frequent genomic mutations in thyroid cancers are shown, linked to their respective hallmarks by lines. Gene or protein names are shown in boxes, classified by the pathway or functional group to which they belong.

In vitro disruption of p53 induces thyroid dedifferentiation via loss of PAX8 expression in a rat thyroid cell¹⁰⁹ and via impaired DNA repair in a human thyroid cell line¹¹⁰. Furthermore, engineering loss of *Trp53* gene function in mouse models of thyroid cancer demonstrated the role of p53 in ATC transformation. The oncogenicity of p53 loss has been proved for thyroid tumours driven by *RET* fusion¹⁰⁹, *BRAF*^{V600E} (refs. 111–113), *HRAS*^{G12V} (ref. 114), *KRAS*^{G12D} (ref. 115), *Pten*¹¹⁶ and *STRN-ALK*¹¹⁷. ATCs from mice with these allelic combinations typically show thyroid dedifferentiation, unchecked tumour growth, EMT and metastases. Mouse models with *Braf*^{V600E} and p53 loss of function display the fastest and most striking features that mimic ATCs from patients, in which *BRAF*^{V600E} and p53 loss is the most common genetic combination. Further overactivation of the MAPK and PI3K signalling pathways, and tumour infiltration by immunosuppressive tumour-associated macrophages (a common feature in human ATC^{118,119}), were observed in *Braf*^{V600E}-driven mouse models of thyroid cancer upon p53 inactivation^{111–113}. Mouse ATCs induced by Ras mutations and p53 loss showed mixed PDTC–ATC histotypes, evaded apoptosis and/or acquired widespread copy number alterations^{114,115}.

Taken together, evidence indicates that loss of p53 function fast-tracks PTCs and FTCs to ATCs, probably by removing crucial barriers to oncogene-induced senescence and cell cycle checkpoints and by favouring genomic instability and DNA damage. Of note, loss-of-function mutations at *CDKN2A*, which encodes the cell cycle-related tumour suppressor p16, are enriched in a subset of DHGTC and in ATC^{46,66,120} (Fig. 2). Evaluation of p16 disruption in *BRAF*-driven and *HRAS*-driven preclinical thyroid cancer models showed cooperativity of these allelic combinations and potential therapeutic vulnerabilities via targeting CDK4 and CDK6 with CKD4/6 inhibitors^{121–123}.

Mutations in the *TERT* promoter and telomerase reactivation. The enzyme telomerase reverse transcriptase (encoded by *TERT*) maintains the protective telomeres found at chromosome ends, but it is not normally expressed in somatic cells. *TERT* also exerts non-telomeric roles^{124,125}. *TERT* promoter mutations are found across diverse types of cancer and are non-coding alterations that occur at c.–124C > T or c.–146C > T hot spots^{126–129}. These mutations are genetic markers of thyroid cancer progression, as shown by their increasing prevalence in aggressive disease and their co-occurrence with driver events at *BRAF* or *RAS*; *TERT* promoter mutations are most frequently found in ATC, followed by PDTC and DHGTC and least frequently in PTC^{130–132} (Fig. 2). *TERT* promoter mutations enable cancer cells to reactivate *TERT* transcription via the recruitment of epigenetic and transcription factors that are only partially elucidated^{132–137}. The upregulation of telomerase is a well-known trait in cancer¹³⁸, so *TERT* promoter mutations represent another mechanism, along with hypermethylation and/or copy number gains, by which cancer cells achieve *TERT* re-expression.

Mechanistically, both telomeric and non-telomeric effects mediated by *TERT* re-expression seem to operate in thyroid cancer progression. For example, a 2022 evaluation of human thyroid cancers showed that *TERT* upregulation tracks with highly proliferative tumours, shorter median telomere length and a more relaxed chromatin configuration at sub-telomeric regions compared with tumours without *TERT* overexpression¹³⁹. By contrast, a novel mouse model showed the ability of *Tert* to induce progression of *Braf*^{V600E}-driven thyroid tumours without crucial changes in telomere length¹⁴⁰. Instead, MAPK and PI3K pathways, as well as cytokine and chemokine signalling via the NF-κB pathway were overexpressed in these mice¹⁴⁰. The potential role of *TERT*-mediated cytokine and NF-κB signalling is indirectly supported

by RNA sequencing data that show overactivation of chemokine and cytokine signalling in human PTCs with *BRAF*^{V600E} mutation and *TERT* expression¹³⁵. Furthermore, *TERT* was demonstrated to regulate NF-κB transcriptional programmes in human breast and ovarian cancer cells¹⁴¹. Another mouse model published in 2023 confirmed that *TERT* reactivation can accelerate progression of *BRAF*-driven thyroid tumors via non-telomeric effects such as cytokine and PI3K signalling, and further dissected a role for ribosomal biogenesis¹⁴².

Deregulation of SWI–SNF-dependent chromatin accessibility. The biological function of SWI–SNF complexes is to reconfigure chromatin, thereby determining the accessibility and ultimately expression of certain genetic programmes. SWI–SNF activity associates with promotion of the terminal differentiation of cells¹⁴³, whereas its disruption induces stem cell-likeness¹⁴⁴. Truncating alterations that disrupt genes that encode members of the SWI–SNF chromatin remodelling complex (for example, *ARID1A*, *ARID1B*, *ARID2*, *SMARCB1* or *PBRM1*) occur in at least 20% of ATCs (Fig. 2). In *HRAS*^{G12V}-driven and *BRAF*^{V600E}-driven thyroid cancer mouse models, thyroid-specific inactivation of SWI–SNF subunits in vivo generates PDTC or ATC^{145,146}. Loss-of-function mutations of *Arid1a*, *Arid2* or *Smarchb1* were shown to elicit large-scale chromatin and transcriptional changes in mouse thyroid glands¹⁴⁶. Among those, thyroid tumours with combined *BRAF*(V600E) plus SWI–SNF impairment were locked in a dedifferentiated transcriptional state that could not be reversed by MAPK pathway inhibition¹⁴⁶, which would discourage the use of redifferentiation strategies for subsequent RAI therapy (discussed later). Instead, SWI–SNF-mutant tumours might present other therapeutic vulnerabilities¹⁴⁷, which remain to be tested in patients with thyroid cancer.

Mutant EIF1AX and RAS synergy and abnormal translation. EIF1AX is a component of the complex needed to initiate protein translation¹⁴⁸. Isolated mutations in *EIF1AX* are observed in follicular adenoma, the follicular variant of PTC and FTC^{10,35,42}. However, concomitant mutations in *EIF1AX* and *RAS* genes are clearly selected in advanced disease, particularly in those maintaining their follicular architecture. *EIF1AX* mutations at a C-terminal splice site (A113splice) are exclusive to thyroid cancers^{10,45,76}, whereas N-terminal domain mutations are also seen in tumours from other lineages, such as uveal melanomas¹⁴⁹. The study of *EIF1AX*(A113splice) mutants in several in vitro and in vivo models has proved their ability to cooperate with oncogenic *RAS* in the progression of thyroid cancer⁴³. In brief, *EIF1AX*^{A113splice} results in alternatively spliced transcripts that can induce oncogenic transformation in *RAS*-mutant human thyroid cancer cells. Thyroid-specific inducible expression of aberrant *Eif1ax*, when expressed in *Hras*^{G12V} mice, generates tumours that progress to PDTC; this finding is compatible with *RAS* and *EIF1AX* mutations being observed in advanced thyroid tumours from patients. Experiments in human thyroid cells show that *EIF1AX*(A113splice) mutants stabilize the translation complex, thereby increasing protein synthesis⁴³. In this preclinical model, mutant *RAS* and *EIF1AX* coordinately set in motion a series of cellular mechanisms that involve preferential translation of ATF4 (a sensor of amino acid deficiency), the stabilization of MYC and an increase in amino acid flux, which in turn activates mTOR⁴³. Taken together, the findings indicate that concurrent *EIF1AX* and *RAS* mutations induce thyroid cancer progression. These specific mutations might potentially confer therapeutic vulnerabilities (for example, to MEK, BRD4 and mTOR inhibition), but this hypothesis is yet to be assessed in patients whose tumours have this genetic combination.

Other genetic alterations. Mutations in other genes are clearly enriched in advanced thyroid cancer, but are detected in a smaller share of tumours than the mutations described in the previous sections (Fig. 2). Loss-of-function mutations in *NF2*, which encodes Merlin, are an example of this category. Merlin has a role in the Hippo pathway by preventing YAP-mediated activation of TEAD-dependent transcription of pro-growth genes^{150,151}. Mouse models using either thyroid-specific *Nf2* loss and *Hras*^{G12V} or constitutive activation of nuclear YAP (in *Hras*^{G12V} or *Braf*^{V600E} mice) demonstrated tumour progression to PDTC and a feedforward mechanism on YAP–TEAD-induced Ras transcription^{152,153}. Of note, loss-of-function mutations in the splicing regulator *RBM10* gene are particularly enriched in metastatic thyroid tumours from patients^{46,74,77} and probably facilitate alternative splicing events and changes in the extracellular matrix¹⁵⁴.

Finally, tumour sequencing studies have also unveiled mutations in genes within functional groups that are disproportionately altered in PDTC or ATC (Fig. 2), but for which thyroid-specific mechanistic assessment is either limited or remains to be performed. These include frequent loss-of-function alterations in two families of epigenetic regulators with roles in gene expression via histone modification; for example, histone methyltransferases (*KMT2A*, *KMT2C*, *KMT2D* and *SETD2*) and histone acetyltransferases (*CREBBP* and *EP300*). In addition, truncating mutations in the DNA mismatch repair pathway genes (for example, *MLH1*, *MSH2* and *MSH6*) and in *ATM* (involved in DNA damage) predominantly occur in ATCs^{45,76}. Functional studies in other tumour lineages and anecdotal evidence of primary thyroid tumours and cell lines with mismatch repair defects suggest that DNA repair deficiency might enhance genomic instability and hypermutation phenotypes in ATC^{45,76,155,156}. The presence of these neoantigens might elicit tumour infiltration by immune cells.

Summary. In summary, most thyroid tumours are initiated by a mutation that activates the MAPK pathway, but need additional mutations that alter other biological processes to progress towards less-differentiated and more-aggressive cancers. This evolution seems to be mostly linear and less convoluted than in other tumour types (Fig. 4), as distinct genotype–phenotype correlations can be drawn.

Genomics in action: clinical management

Genomic and biological understanding of thyroid tumours can be used to improve clinical care for patients with thyroid cancer, particularly for those with aggressive disease. Molecular testing is currently recommended for a subset of patients with aggressive thyroid cancers and for all patients with ATC¹⁵⁷. Below, we summarize these and other settings in which genomic knowledge can enhance thyroid cancer prognosis, refine RAI treatment and inform targeted therapies.

Gene mutations and thyroid cancer prognosis

As discussed, drivers of PTC–FTC initiation determine their histological and transcriptomic features^{10,34}. *BRAF*^{V600E}-like PTCs are more frequently invasive and show tropism to regional lymph nodes, whereas RAS-like PTCs tend to be encapsulated and hardly ever develop nodal metastases^{10,158}. In advanced disease, including metastatic PTC–FTC, DHGTC and PDTC, *BRAF*^{V600E}-driven tumours tend to spread locally via lymphatic routes, whereas RAS-mutant specimens more frequently show haematogenous spread and produce distant metastases that affect bone and lungs^{45,90,159} (Table 1). Despite the dependency on the MAPK activating driver mutation, reliance on a single mutation for prognostication has proved insufficient. This limitation is best exemplified

by the fact that *BRAF*^{V600E} mutations also occur in a large proportion of micro-PTCs (an often subclinical entity for which conservative strategies rooted in active surveillance approaches are increasingly used^{160,161}) or the fact that benign follicular adenoma can also have RAS mutations. In addition, a 2022 prospective study on patients with low-risk thyroid cancer showed that the presence of *BRAF*^{V600E} had no prognostic value in recurrence after RAI treatment¹⁶².

By contrast, the identification of additional mutations in PDTC or ATC (Fig. 2), together with the straightforward evolution of thyroid tumours (Fig. 4) eases the way for the use of genomics for prognostication in patients with PTC–FTC. Several studies have provided evidence of the prognostic value of *TERT* promoter mutations to stratify cohorts of patients with DTC with high-risk clinicopathological characteristics, such as increased rates of distant metastases, persistent disease or disease-specific mortality^{163–171}. The high-risk clinicopathological characteristics have been primarily studied for the *BRAF*^{V600E} and *TERT* promoter mutation combination, but it has also been shown in RAS-driven PTC–FTC with *TERT* promoter mutations¹⁶⁷. Overall, the presence of a *TERT* promoter mutation is a bona fide marker of poor prognosis (reviewed in^{172–174}) and has been incorporated into the clinical guidelines for the management of patients with thyroid cancer³. Furthermore, concurrent mutations in RAS and *EIF1AX*^{A113splice} are enriched in advanced thyroid tumours with follicular growth patterns^{10,40,45,76}. Unlike for *TERT*, *EIF1AX* mutations are fairly rare in PTC–FTC; consequently, no large studies have been conducted to comprehensively evaluate whether *EIF1AX*-mutated tumours behave notably differently from their counterparts with other genomic make-ups. However, evidence of RAS mutations and *EIF1AX*^{A113splice} enrichment in PDTC and ATC, as well as the data from mouse models⁴³, suggest that this genetic combination primes the follicular variant of PTC and FTCs towards progression. Thus, the combination of RAS mutations and *EIF1AX*^{A113splice} should probably be considered a poor prognostic factor. In addition, mutations in effectors of the PI3K–AKT pathway are rare in PTCs but portend clear prognostic importance, when they occur in the context of *BRAF*^{V600E}-driven or RAS-driven tumours. In this context, they have been associated with metastatic dissemination⁹⁰ and proposed as independent predictors of disease-specific mortality^{175–177}. They also predict worse overall survival in patients with ATC¹⁷⁸.

Indirect evidence exists to support other mutational biomarkers of PTC–FTC severity. This evidence comes from tumour sequencing studies in which specific cohorts of patients with ‘aggressive’, ‘metastatic’ or ‘high-grade’ PTC were evaluated^{46,66,67,77} (most of which would now probably be classified as DHGTC¹) as well as from a pan-cancer study of metastatic disease that included thyroid tumours⁷⁴. Mutations in loci such as *TP53*, *CDKN2A* (p16) and SWI–SNF complex genes are enriched not only in PDTC and ATC, but also in DHGTC (Fig. 2). In addition, *RBM10* mutations associate with metastatic PTC. Considering the genomic simplicity of thyroid cancers, we suggest that the finding of these rare events in PTC–FTC probably conveys poor prognosis.

Radioactive iodine and redifferentiation therapies

The ability of thyroid cells to take up and accumulate iodine primarily relies on NIS function and has been exploited for more than 80 years via the adjuvant use of RAI therapy in metastatic thyroid cancers, even though MAPK pathway activation suppresses expression of NIS (encoded by *SLC5A5*)^{11,26}. Full NIS functionality requires proper transcription from *SLC5A5* and appropriate trafficking of the protein to the basolateral membrane of thyroid cells^{179–181}. NIS regulation is complex and it is also influenced by non-MAPK inputs, such as

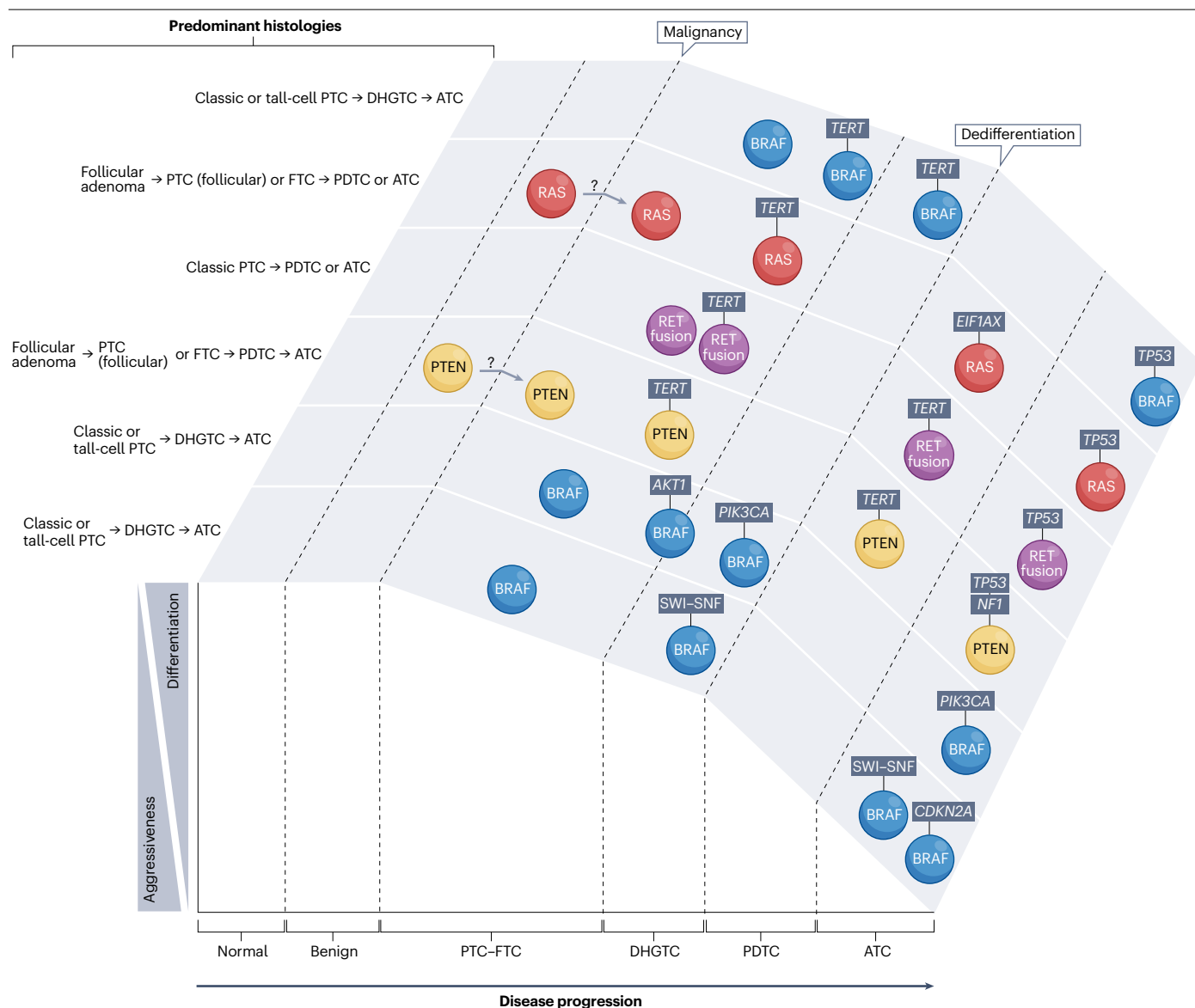


Fig. 4 | Schematic representation of the main genetic events implicated in thyroid cancer initiation and evolution. ‘Marble run’ depiction of thyroid cancer evolution. The most frequent patterns of progression, associated with specific mutations and thyroid cancer types, are shown in each lane. Predominant histologies are shown on the x-axis and the inverse correlation between thyroid differentiation and tumour aggressiveness is displayed on the y-axis. Marbles represent thyroid specimens and are coloured by their initiating genetic event, as tumours retain their driver dependency. *BRAF* (top lane) and *RAS* (second from top) mutations are frequent early events, but while *BRAF*^{V600E} is diagnostic (detected only in malignant tumours), *RAS* mutations are also found in benign specimens. Some thyroid cancers are driven by *RET* fusions (third lane)

or *PTEN* loss (fourth lane; also occurs in benign specimens). Mutated genes linked to tumour progression are shown as tags. They can either show no specific driver preference (such as *TP53*) or cooperate with defined drivers: the *RAS* and *EIF1AX* combination (second lane), or *AKT1* or *PIK3CA* mutations primarily co-occurring with *BRAF*^{V600E} (fifth lane). Advanced *BRAF*-driven tumours preferentially show differentiated high-grade thyroid cancer (DHGTC) histologies, whereas *RAS*-driven or *PTEN*-driven tumours tend to progress to poorly differentiated thyroid cancers (PDTCs). Mutations in *TP53* (top four lanes), loss of p16 (*CDKN2A*; sixth lane) or mutations in *SWI-SNF* complex genes (sixth lane) probably fast-track tumours towards anaplastic thyroid cancer (ATC). FTC, follicular thyroid cancer; PTC, papillary thyroid cancer.

transforming growth factor- β , reactive oxygen species signalling and other factors^{182–184}. The inverse relationship of MAPK signalling with NIS function depends on the specific MAPK-activating genetic event, which determines the intensity of MAPK pathway flux and in turn the expression of differentiated thyroid genes, including *SCLC5A5* (Fig. 1b).

Within *BRAF*-driven and *RAS*-driven tumours, variability can be seen in differentiation levels and iodine avidity, suggesting that additional events to truncal driver mutations have a role in tumour differentiation state¹⁸⁵. As tumours progress and dedifferentiate, they tend to further lose their ability to incorporate iodine (that is, they become RAI

refractory), as is invariably the case for ATCs. Work in *BRAF*^{V600E}-driven mouse models of thyroid cancer showed that genetic or pharmacological inactivation of MAPK signalling can partially restore NIS expression and RAI avidity in mouse PTCs^{186,187}. The clinical application of this idea entails conducting redifferentiation trials, in which treatment with MAPK inhibitors (typically targeting RAF and/or MEK) can potentially resensitize patients with metastatic and RAI-refractory thyroid tumours to RAI treatment (reviewed in refs. 188,189). Redifferentiation strategies for patients with RAI-refractory tumours are not yet recommended in thyroid cancer guidelines and should be considered investigational approaches. However, a growing body of evidence supports mutation-specific associations for both baseline RAI avidity and for restored RAI uptake upon redifferentiation strategies (Fig. 5), as well as an obvious need to systematically incorporate comprehensive genomic assessment of tumours from patients enrolled in these trials¹⁹⁰ (Fig. 6a).

So far, available data suggest that RAI-refractory tumours with RAS-like BRS, such as those driven by RAS^{191,192} or by *BRAF* class II mutations (for example, *BRAF*^{K601E})¹⁹³, might respond better to redifferentiation therapies. Whether responses in RAS-like tumours are explained by a slightly higher differentiation at baseline (compared with *BRAF*^{V600E}) and/or are affected by the specific MEK inhibitor tested remains to be fully elucidated (Fig. 5). Despite a lower baseline differentiation than RAS-like tumours, subsets of *BRAF*^{V600E}-driven RAI-refractory tumours responded to redifferentiation strategies that used targeted therapies against genetic drivers (Fig. 6a). In this regard, RAF inhibition (via small molecule vemurafenib or dabrafenib), alone or in combination with MEK or HER3 inhibitors, elicited some responses in tumour resensitization to RAI uptake^{194–200}. In addition, case reports show that targeted therapies specifically used in patients with *RET*-rearranged and *NTRK*-rearranged thyroid tumours can partially restore RAI uptake, suggesting that redifferentiation therapies are feasible in refractory tumours with alternative MAPK drivers^{201,202} (Fig. 6a).

Nevertheless, trusting exclusively in *BRAF*, *RAS* or *RTK* alterations to guide redifferentiation therapy is oversimplistic. In 2023, a retrospective study on exceptional responders to RAI showed that RAS-like lesions were enriched in those who responded to RAI therapy

versus a matched group of individuals who did not respond (who had more *BRAF*^{V600E} mutations)²⁰³. However, this study used an extended genomic panel, showing that those who did not respond to RAI also had *RBM10* and *SWI-SNF* gene mutations, as well as chromosome 1q gains. Thus, the study indicated that these additional genetic defects could be potential markers of RAI refractoriness at baseline and suggested that these patients would not benefit from redifferentiation trials²⁰³. The role of *SWI-SNF* mutations in determining lack of responsiveness to RAI treatment is further supported by the report of *ARID2* mutations in two patients from a 2022 redifferentiation trial with *BRAF*-mutated, RAI-resistant thyroid tumours¹⁹⁷, as well as by the previously mentioned *SWI-SNF*-disrupted animal models¹⁴⁶ (Fig. 5). Furthermore, the presence of *BRAF*^{V600E} with a *TERT* promoter mutation has been reported to correlate with low RAI avidity in patients with recurrent and metastatic PTC^{204–206}, but the predictive value of *TERT* promoter mutations in RAI refractoriness remains controversial.

In summary, although the optimal use of redifferentiation strategies in subsets of thyroid cancer cohorts is still a subject of debate, redifferentiation therapy should be considered in patients with slowly progressive disease. Moreover, molecular characterization should help to stratify individuals who would probably benefit from these therapies to ultimately optimize these approaches^{207,208} (Fig. 6a). Of note, in the context of redifferentiation trials, inhibitors are administered in the short term, thus circumventing most toxicities.

Actionable mutations: targeted therapies

Patients with advanced thyroid cancer have multiple systemic therapy options (Table 2), although none of these is curative at this time. Five of these drugs and drug combinations require identification of the targeted genetic alteration and are the focus of this section. The most exquisitely sensitive to genomically targeted therapy are those with *BRAF*(V600E)-mutant ATC, as well as patients with PTC or PDTC with fusion-driven thyroid cancer (Fig. 6).

Strategies that target RAF and/or MEK. The combination of dabrafenib (RAF inhibitor) plus trametinib (MEK inhibitor) has been approved by the US Food and Drug Administration (FDA) for *BRAF*(V600E)-mutant

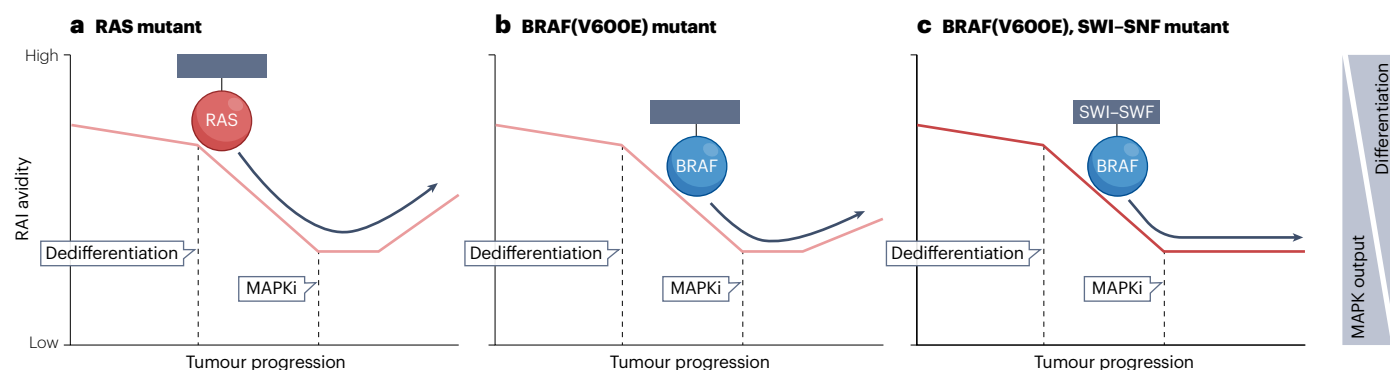
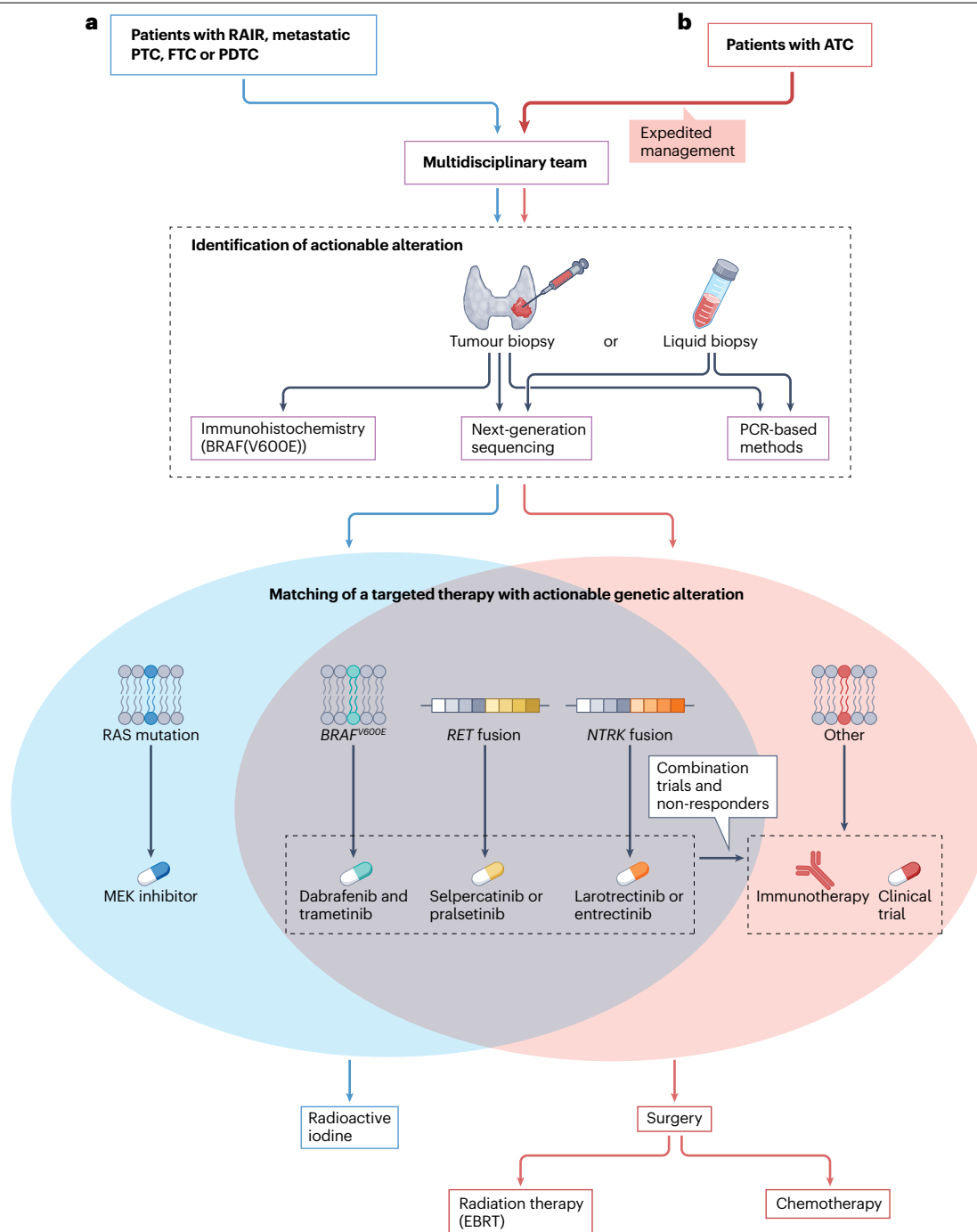


Fig. 5 | Role of tumour genotypes in response to radioactive iodine and redifferentiation therapies. Mitogen-activated protein kinase (MAPK) inhibition (MAPKi) strategies have been used to enhance radioactive iodine (RAI) avidity in RAI-refractory papillary thyroid cancer, follicular thyroid cancer and poorly differentiated thyroid cancer. Only a subset of redifferentiation trials include tumour sequencing. **a, b**, Limited data suggest that RAS-mutated tumours (panel **a**) might be more responsive to redifferentiation than their *BRAF*^{V600E} counterparts (panel **b**), because of either their higher baseline

differentiation or a better ability to restore iodine uptake. The observation that only certain subsets of RAS-driven and *BRAF*-driven tumours increase RAI avidity upon MAPKi treatment suggests that additional, not fully characterized, mutations (blank labels) might be driving sustained RAI refractoriness. **c**, Findings from mouse models and cohorts of patients, showing that loss-of-function mutations in members of the *SWI-SNF* complex lock *BRAF*-driven tumours in a dedifferentiated state. This finding suggests that these patients should be excluded from MAPKi-induced attempts to restore RAI uptake.



ATC and for BRAF(V600E)-mutant unresectable or metastatic solid tumours that have progressed following prior treatment and have no satisfactory alternative treatment options. In the most recent clinical trial of dabrafenib plus trametinib (published in 2022), which included 36 patients with ATC with 4-year follow-up, investigator-assessed overall response rate (ORR) was 61%, the proportion with stable or progressive disease was 24% or 12%, respectively, and the median progression-free survival (PFS) was 6.7 months, suggesting that resistance usually develops within the first year²⁰⁹. The median overall survival in this study

was 14.5 months. By contrast, a cross-cancer study that evaluated a single-agent BRAF inhibitor (vemurafenib) showed that only two of the seven patients with ATC evaluated achieved a response at 2 months²¹⁰. The low response rate compared with dabrafenib plus trametinib is striking and could be due to differences in the degree of MAPK shutdown via RAF inhibition versus combined RAF plus MEK blockade. Resistance to BRAF(V600E) inhibition has been described in thyroid cancer cell lines and patients via the acquisition of mutations in RAS^{211,212}, as well as in *NF1*, *NF2*, *SWI-SNF* or *PI3K-AKT* pathway effectors^{207,213}.

Review article

Fig. 6 | New mutation-informed treatment strategies for patients with aggressive thyroid cancers. Proposed approaches to manage patients with radioactive iodine refractory (RAIR), metastatic papillary (PTC), follicular (FTC) or poorly differentiated (PDTC) thyroid cancers (pathway **a**, blue tracks) and those with anaplastic thyroid cancers (ATC; pathway **b**, red tracks). **a**, Patients with RAIR PTC, FTC or PDTC, ideally with slowly progressive disease and who have received no more than 500 mCi of ^{131}I , can be considered for investigational redifferentiation regimes. Tumour (or liquid) biopsies should be used to identify tumour drivers via next-generation sequencing or PCR-based methods, and to match patients with appropriate targeted therapies. Patients with RAS-mutated tumours can be given off-label MEK inhibitors, whereas those with $\text{BRAF}^{\text{V600E}}$ or RET or NTRK fusions should be matched with their respective FDA-approved drugs, as indicated. Most protocols suggest treating patients for 4–6 weeks, after which restoration of iodine avidity can be determined by a diagnostic whole-body scan, followed by subsequent ^{131}I treatment. **b**, ATCs are rapidly growing

tumours that require an expedited assessment by a multidisciplinary team. Rapid evaluation of $\text{BRAF}^{\text{V600E}}$ mutation via immunohistochemistry and/or PCR is imperative, so that BRAF-directed therapy with FDA-approved dabrafenib (RAF inhibitor) plus trametinib (MEK inhibitor) can be initiated. NGS can identify other actionable targets, although single-agent therapies seem to result in rapid progression. Patients with mutations without matched FDA-approved inhibitors can benefit from enrolment in clinical trials and/or immunotherapy, particularly immune checkpoint blockade. Non-responders to approved targeted therapies can be considered for immunotherapy, alone or in combination (for example, dabrafenib plus trametinib plus PD1/PDL1 inhibitors). Responses to these therapies can facilitate surgical removal of the primary tumours and metastases. External beam radiation therapy (EBRT) to the pre-treatment field with or without radiosensitizing chemotherapy can be used after surgery or for palliative treatment.

Two strategies have been used to deter, delay or avoid resistance and subsequent death in patients with ATC: first, surgical resection after neoadjuvant dabrafenib plus trametinib; and second, triple therapy with the addition of an immune checkpoint inhibitor. Regarding the first approach, Fig. 6b shows how rapid determination of the genomic alteration and initiation of genomically driven targeted therapy can be used to achieve surgical complete resection in ATC. At this time, this approach is a well-established regimen for BRAF(V600E)-mutant ATC. The second strategy involves immunotherapy and is based on the progressively immunosuppressive microenvironment that ATCs show, which enable these cancers to avoid antitumour immune surveillance via the expression of immune checkpoints such as the PD1–PDL1 axis (reviewed in ref. 214). Mouse models of advanced PTC and ATC linked the presence of $\text{BRAF}^{\text{V600E}}$ mutation to the recruitment of immunosuppressive cells and provided the rationale for combinatorial therapies using RAF inhibition plus an immune checkpoint inhibitor^{215–217}. So far, trials in patients show that anti-PDL1 drugs improve survival by delaying progression but do not provide a cure. Indeed, a retrospective study showed that the addition of an immune checkpoint inhibitor to dabrafenib plus trametinib in patients with ATC (either before or after progression) does improve median overall survival²¹⁸. This strategy has also been tested in a prospective clinical trial with BRAF plus MEK inhibition plus an immune checkpoint inhibitor²¹⁹ in patients with ATC,

and with the addition of pembrolizumab (an anti-PDL1) to lenvatinib (a multi-RTK inhibitor) in patients with metastatic PDTC or ATC²²⁰. The addition of an immune checkpoint inhibitor results in added toxicity, particularly immune-mediated adverse events such as hepatitis, colitis and nephritis, in a minority of patients. However, the most impressive outcomes are achieved in patients with ATC who undergo surgical resection of the primary thyroid tumour after treatment with dabrafenib plus trametinib, with or without an immune checkpoint inhibitor²²¹. These data suggest that eliminating the largest source of possible emergence of resistance (the primary tumour) before progression ensues improves survival in this population. Of note, this study reported little long-term surgical morbidity²²¹.

Strikingly, patients with BRAF(V600E)-mutant PTCs do not achieve the strong responses to RAF-targeted therapy seen in ATC. They do, however, have more durable responses in terms of PFS. A 2022 study in patients with RAI-refractory PTC showed ORRs of 30% and 35% for dabrafenib plus trametinib versus single-agent dabrafenib, respectively²²². In addition, nonsignificant median PFSs of 15.1 and 10.7 months for dabrafenib plus trametinib and dabrafenib alone, respectively, were observed. Although response rates in patients with BRAF(V600E)-mutant PTCs are higher with FDA-approved lenvatinib than with RAF inhibitors, the benefit of using BRAF-directed therapy in PTC is the possibility of then attempting to give the patient RAI under

Table 2 | FDA-approved drugs for follicular cell-derived thyroid cancer

Year of approval	Drug name	Requires target identification	Target alteration	FDA indication
2013	Sorafenib	No	Multi-RTK	Metastatic RAIR PTC, FTC or PDTC
2015	Lenvatinib	No	Multi-RTK	Metastatic or recurrent RAIR PTC or FTC
2018, 2022	Dabrafenib+trametinib	Yes	$\text{BRAF}^{\text{V600E}}$ mutation	BRAF(V600E)-mutant ATC and PTC
2018	Larotrectinib	Yes	NTRK fusion	NTRK -rearranged thyroid cancer
2019	Entrectinib	Yes	NTRK fusion	NTRK -rearranged thyroid cancer
2020	Selpercatinib	Yes	RET fusion	RET -rearranged thyroid cancer
2020	Pralsetinib	Yes	RET fusion	RET -rearranged thyroid cancer
2021	Cabozantinib	No	Multi-RTK	Second-line metastatic RAIR PTC or FTC

ATC, anaplastic thyroid cancer; FDA, US Food and Drug Administration; FTC, follicular thyroid cancer; PDTC, poorly differentiated thyroid cancer; PTC, papillary thyroid cancer; RAIR, radioactive iodine refractory; RTK, receptor tyrosine kinase.

redifferentiation therapy. If RAI uptake is achieved, the BRAF-directed therapy could be discontinued and the patient can be observed to determine the response to RAI (Fig. 6a). Currently, no studies have investigated the sequential use of lenvatinib and RAF–MEK inhibition in thyroid cancer.

Strategies to target fusion-driven thyroid cancer. Patients with fusion-driven thyroid tumours (that is, those with gene rearrangements involving *RET*, *NTRK1* or *NTRK3*) can now benefit from the FDA-approved matched targeted drugs selpercatinib or pralsetinib (RET inhibitors), or larotrectinib or entrectinib (NTRK inhibitors) (Table 2). Patients with ATC treated with these drugs can achieve partial responses, but these are not as remarkable or as durable as they are in PTC–FTC.

In the larotrectinib study of *NTRK*-driven thyroid tumours, which enrolled 21 patients with PTC–FTC and seven patients with ATC, the ORR was 86% in PTC–FTC and 29% in ATC²²³. In PTC–FTC, the median PFS and overall survival were not reached, whereas in ATC, the median PFS was only 2.2 months and median overall survival was 8.8 months. This finding suggests that resistance occurs very early in ATC, possibly owing to blockade with a single agent. Preliminary thyroid-specific data with entrectinib were reported at a meeting in 2021 (ref. 224). The study enrolled 13 patients with *NTRK*-driven tumours, including ten with PTC and one with ATC. Although no conclusions can be made regarding the patient with ATC, in PTC the ORR was 54%, and the median PFS and overall survival were 19.9 months in the entire cohort.

Selective RET inhibitors are now used in adult and paediatric patients with advanced or metastatic *RET*-fusion-positive thyroid cancer that requires systemic therapy and is RAI refractory, as well as in *RET*-mutated MTC. The pralsetinib trial enrolled nine *RET*-fusion-positive patients with PTC and reported an ORR of 89%, but a median duration of response and median overall survival were not reached²²⁵. The selpercatinib trial enrolled 17 patients with *RET*-fusion PTC, OCA or PDTC and two with ATC, and the ORR was 79%²²⁶. One of the two patients with ATC achieved a durable response for more than 18 months. With so few patients with ATC enrolled, no conclusions can be made with regard to the efficacy of single-agent RET inhibitors in ATC. At this time, how resistance occurs in *RET*-rearranged thyroid cancer is unknown; however, data exist in lung cancer showing the emergence of *RET* solvent-front mutations^{227,228}. In two paediatric patients with advanced RAI-refractory PTC driven by *NTRK* and *RET* fusions, treatment with larotrectinib and selpercatinib, respectively, decreased tumour size and restored RAI uptake²²⁹.

Targeted therapies with future promise. Finally, clinical trials with pan-RAF or RAF dimer inhibitors that target the common alterations in thyroid cancer (such as those in NRAS, KRAS and HRAS oncoproteins) are on the horizon. Class II BRAF alterations could also be targeted with these drugs. MEK inhibitors have been used successfully to target *BRAF* fusions in other solid tumours²³⁰. Furthermore, drugs that target *ALK* fusions, which are approved for lung cancer and have shown some promise in *ALK*-rearranged ATC²³¹, should be studied in basket trials to evaluate their efficacy in other solid tumours.

Conclusions

The exponential increase in our knowledge of the genomic alterations and mechanisms that drive thyroid tumour initiation and progression is steadily making its way into improved clinical management of patients with thyroid cancer. However, many future challenges remain to be

addressed in this field. From the research standpoint, we still do not fully understand the genetic determinants and functional underpinnings of certain subsets of tumours, which might rely on copy number, transcriptomic or epigenetic modifications. We also need improved human-derived experimental models that retain the complexity and functionality of the original tumours and enable mechanistic studies and rapid testing of experimental treatments. On the clinical front, challenges related to optimal patient risk stratification, unresponsiveness and acquired resistance to approved therapies, and divergence in the access to ideal treatments, need to be addressed. Progress will hopefully be made towards the generation of more data and integration of the genomic and biological knowledge of thyroid tumours to inform traditional treatments and immunotherapy as more data become available. The acceleration of preclinical modelling and of drug discovery pipelines will lead to important advances towards clinical applications in the near future.

Published online: 04 December 2023

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Author contributions

The authors contributed to all aspects of the article.

Competing interests

M.E.C. has received grant support from Genetech, Exelixis, Eisai, Merck and Kura, and has received consultation fees from Lilly, Bayer and Exelixis. I.L. declares no competing interests.

Additional information

Peer review information *Nature Reviews Endocrinology* thanks Laura Boucai, Martin Schlumberger and the other, anonymous, reviewer(s) for their contribution to the peer review of this work.

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