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Preclinical Models of Follicular Cell-Derived Thyroid Cancer: An Overview from Cancer Cell Lines to Mouse Models

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The overall prognosis of thyroid cancer is excellent, but some patients have grossly invasive disease and distant metastases with limited responses to systemic therapies. Thus, relevant preclinical models are needed to investigate thyroid cancer biology and novel treatments. Different preclinical models have recently emerged with advances in thyroid cancer genetics, mouse modeling and new cell lines. Choosing the appropriate model according to the research question is crucial to studying thyroid cancer. This review will discuss the current preclinical models frequently used in thyroid cancer research, from cell lines to mouse models, and future perspectives on patient-derived and humanized preclinical models in this field.

Keywords: Thyroid neoplasm; Cell line; Models, animal; Animals, genetically modified

INTRODUCTION

Thyroid cancer is the most common endocrine malignancy and generally has an excellent prognosis. The overall 10-year survival rate is over 95%, but 5% to 10% of patients have distant metastatic disease, and one-third of these patients have radioactive iodine resistance with progressive disease [1,2]. Anaplastic thyroid cancer (ATC) is the most dedifferentiated and lethal of all cancers. Median survival of patients with ATC was reported as 5 to 6 months, even with multimodal treatment [3]. Over the past few years, targeted therapies have shown significant clinical benefit for patients with differentiated thyroid cancer (DTC) and ATC [4-7]. However, these therapies are not curative, and most patients eventually progress, underlying the critical need for more effective therapies [8]. Recent studies using immunotherapies also showed limited efficacy [9-12]. Preclinical experimental models are crucial for investigating thyroid cancer pathogenesis and developing novel effective therapies.

Recent progress in genetics and molecular biology has led to the development of many preclinical models with genetic characterization of these models [2,13,14]. Choosing the suitable model to ask specific questions is critical in understanding thyroid cancer pathogenesis and treatment. This review focuses on the preclinical models of follicular cell-derived thyroid cancer.

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We will provide the overview of available thyroid cancer cell lines and mouse models with a summary of their characteristics, advantages, and disadvantages.

CELL LINES

Cancer cell lines are the most widely used tool for *in vitro* and *in vivo* cancer research. Many thyroid cancer cell lines from different origins with various genetic characteristics are available for research, and the genomic landscape of the cell lines is well established (Table 1) [15]. It is critical to get cell lines form a trusted source that carefully validates the cell lines on a regular basis [16]. The University of Colorado Cancer Center has a repository of many of the authenticated thyroid cancer cell lines (https://medschool.cuanschutz.edu/colorado-cancer-center/research/shared-resources/cell-technologies/cell-culture-services). Thyroid cancer cells have driver mutations similar to those in primary human tumors: *BRAF* V600E is the most common driver mutation, and mutations in *RAS* genes are also frequently observed. However, thyroid cancer cell lines are profoundly dedifferentiated and have gene expression profiles similar to ATCs even though many originated from differentiated cancers. Furthermore, telomerase reverse transcriptase (*TERT*) promoter and tumor protein p53 (*TP53*) mutations appear to have been acquired or enriched under cell culture conditions [15].

Cancer cell lines have a significant limitation as they acquire genetic changes needed for immortalization and are not always representative of the original tumor. There are also issues of cross-contamination with other cell lines and infection with mycoplasma that can affect cell metabolism and other properties. Furthermore, the effect of the tumor microenvironment can't be assessed in these cancer cell lines [15,17]. However, cell lines are useful for large drug screens, and understanding the effects of cell signaling on proliferation, apoptosis, migration/invasion and anchorage-independent growth [15,17]. Cell lines are also generally easy to grow and provide a nearly limitless research tool.

Table 1. Commercially Available Thyroid Cancer Cell Lines according to the Origin of Tumor and Genetic Characteristics

Origin of tumor	Cell lines	BRAF	NRAS	HRAS	KRAS	PTEN	Other driver	TERT promoter	TP53
Anaplastic	8305C	V600E	●[a]					-146C>T	●[b]
	8505C	V600E						-146C>T; -149C>T	●[b]
	BHT101	V600E						-124C>T	●[b]
	HTC-C3	V600E						-124C>T	●[b]
	HTH104	V600E						-124C>T	
	IHH4	V600E						-124C>T	
	KHM-5M	V600E						-124C>T	●[b]
	KTC2	V600E						-124C>T	
	SW1736	V600E						-124C>T	●[a]
	TCO1	V600E						-124C>T	●[b]
	THJ21T	V600E						-124C>T	●[b]
	THJ560	V600E						-124C>T	●[b]
	CUTC60	V600E						-124C>T	●[b]
	THJ-16T	Fusion						-124C>T	●[b]
	ASH3		Q61R			●[a]		-146C>T	●[b]
	KMH2		Q61R					-124C>T	
	HTH7		Q61R					-146C>T	●[b]
	HTH83			Q61R				-124C>T	●[b]
	C643			G13R		●[b]		-124C>T	●[b]
	JEM493			Q61R				-124C>T	
	CAL62				G12R				●[b]
	THJ29T						FGFR2-OGDH fusion	-146C>T	●[a]

(*Continued to the next page*)

Table 1. Continued

Origin of tumor	Cell lines	BRAF	NRAS	HRAS	KRAS	PTEN	Other driver	TERT promoter	TP53
Papillary	BCPAP	V600E						-124C>T; -125C>T	●[b]
	K1	V600E						-124C>T	
	KTC1	V600E						-146C>T	
	LAM1	V600E						-124C>T	●[c]
	LAM136	V600E						-146C>T	●[b]
	MDA-T32	V600E						-124C>T	●[b]
	MDA-T41	V600E							●[a]
	MDA-T85	V600E						-124C>T	
	MDA-T120	V600E						-124C>T	●[b]
	CUTC5	V600E							●[b]
	CUTC48						CCDC6-RET fusion	-124C>T	
	TPC1						CCDC6-RET fusion	-124C>T	
Follicular	FTC133					R130*	NF1 p.C167*	-124C>T	●[b]
	FTC236					R130*		-124C>T	●[b]
	FTC238					R130*		-124C>T	●[b]
	SDAR1					V54fs		-146C>T	●[a]
	SDAR2					V54fs		-146C>T	●[a]
	TT2609-CO2		Q61R					-124C>T	●[b]
	CUTC61			Q61R				-124C>T	●[a]

PTEN, phosphatase and tensin homolog; TERT, telomerase reverse transcriptase; TP53, tumor protein p53.
[a]Truncated; [b]Missense; [c]In frame mutation.

To overcome some of the problems of cancer cell lines, researchers have established organoid models or cell lines from patient-derived xenografts (PDXs) that show gene expression profiles similar to the original tumor [18-20]. Papillary thyroid carcinoma (PTC) organoids also preserve most of the microenvironment of the originating tumors and better approximate patient drug responses [20].

MOUSE MODELS

Preclinical animal models are critical in cancer research, and the mouse model is considered most useful due to their short lifespan, small body size, and genetic similarities to humans [21]. In the past, thyroid cancer studies were mainly performed *in vitro* with thyroid cancer cell lines, then confirmed *in vivo* using immunosuppressed xenograft models. The traditional technique of the xenograft model has been subcutaneous human tumor implants on the flank of an immunocompromised mouse. More recently, investigators have used orthotopic implants of human thyroid cancer cells into the thyroid bed as well as intracardiac injections in immunocompromised mouse [22,23]. Currently, genetic manipulation using the Cre-loxP system developed various genetically engineered mouse models (GEMMs) for thyroid cancer, and the mouse models continue to evolve [2,24]. The summary of each mouse model's characteristics is shown in Table 2 and the advantages and challenges of different types of mouse models are summarized in Table 3.

Xenograft models

The xenograft model is established by implanting human cancer cell lines or tissues into immunodeficient mice, which can preserve genetic alterations of human cancers [25,26]. According to the location where those were implanted, xenograft models are further classified into ectopic (subcutaneous), orthotopic (thyroid bed), and metastatic (tail vein or intracardiac) [27,28]. The most commonly used immunocompromised mice are athymic nude, severely combined immune-deficiency (SCID) and NOD.*Cg-Prkdc*^{scid}*Il2rg*^{tm1Wjl}*/SzJ* (NSG) mice [19,22,27].

The flank xenograft model is relatively easy to generate and monitor tumor growth and treatment response. However, this

Table 2. Characteristics of Mouse Models of Cancer

Model	Host immune status	Implants	Location implanted
Xenograft model	Immunocompromised	Human cancer cell lines Patient-derived tissues	Ectopic or flank (subcutaneous) Orthotopic (thyroid bed)
Syngeneic model	Immunocompetent (same genetic background with implanted cells/tissues)	Mouse cancer cells or tissues	Metastatic (tail vein or intracardiac)
Genetically engineered model	Immunocompetent	None	None

Table 3. The Advantages and Challenges of Different Types of Mouse Models for Thyroid Cancer

	Advantages	Challenges
Ectopic (flank) xenograft model	Preserved genetic mutations of human thyroid cancers or *in vitro* tested condition Easy to generate Easy to monitor tumor growth and response	Lack of tumor microenvironment No distant metastases
Orthotopic (thyroid) xenograft model	Preserved genetic mutations of human thyroid cancers or *in vitro* tested condition Mimic innate immune microenvironment including stromal cells or vessels Rapid tumor expansion Reproducible distant metastasis	Lacking full immune system Cannot assess the effect of immunotherapies
Syngeneic (orthotopic) model	Fully functional immune system Can assess tumor immunology and the effect of immunotherapy Rapid tumor expansion	Not enough time for immune-editing process or development of distant metastasis because of rapid tumor growth
Genetically engineered mouse models (GEMMs)	Gradual tumor development model Best for assessing the interaction between tumor and microenvironmental cells Reproducible distant metastasis	Long latency to tumorigenesis Expensive

model lacks the local tumor microenvironment and distant metastasis [26]. The orthotopic xenograft model is more physiologic and tumorigenic than the flank models and has distant metastatic potential [25,27]. Nucera et al. [22] generated the 8505C orthotopic tumor which showed palpable neck tumors at 20 days post-transplantation and miliary multifocal lung micrometastasis. Morrison et al. [28] generated orthotopic tumors using various thyroid cancer cell lines and reported different take rates or tumor volumes. 8505C, T238, and K1 cell lines showed 100% take rate, but HTH7, C643, SW1736, MDA-T41, and TPC-1 cells failed to form orthotopic tumors. The orthotopic xenograft model also has an advantage in stimulating thyroid stromal cells, and the local innate immune system [25]. Some limitations of this model include rapid growth, which is different than many relatively slow growing advanced human cancers, the lack of an adaptive immune system and the deposition of cell not only in the thyroid, but around the thyroid, making assessment of extrathyroidal invasion difficult.

Patient-derived xenograft models

PDX models directly use patient-derived tissues, recreate human cancer gene expression, tumor heterogeneity, and microenvironment, and are expected to inform drug response better than standard xenograft models [19]. These models are successfully established in many cancers but are still limited in thyroid cancer research. Maniakas et al. [19] generated PDX model using six ATC tumors: the tumor implanted in the flank of immunodeficient athymic mice and expanded for at least four generations. Mutation, histopathological characterization of PDX models showed good fidelity with the original tumor. The take rate was about 30% [19]. This group of researchers reported the result of high-throughput screening multiple drugs using these models and matching cell lines and suggested the feasibility of using this systematic approach for preclinical *in vivo* drug testing or personalized therapeutics development [29].

Syngeneic models

The syngeneic model is established by implanting mouse cancer cells or tissues into immunocompetent mice of the same genetic background. Like the xenograft model, the syngeneic model can be ectopic (subcutaneous), orthotopic (thyroid bed), or metastatic (tail vein or intracardiac) [21]. Vanden Borre et al. [30] reported the development of syngeneic ATC orthotopic mouse model using cell lines derived from tumors arising in GEMM with thyroid-specific expression of mutant *BRAF* and deletion of either *TP53* or phosphatase and tensin homolog (*PTEN*), which were implanted into immunocompetent syngeneic B6129SF1/J mice. Caperton et al. [31] reported the development of syngeneic follicular thyroid cancer (FTC) ectopic mouse model using tumor cells from GEMM with mutant *HRas* and *PTEN* inactivation. These models showed very rapid tumor formation with significant immune cell infiltration in the tumor [30-32]. The syngeneic model has a significant advantage over the xenograft model in that researchers can study tumor growth with an intact immune system and investigate the immunemodulatory effect of therapies. The short latency of tumor development also makes this an attractive model [21,30]. The major drawback of this model is that the immunoediting process, so critical in human tumor development, may not proceed due to very rapid tumor formation, which introduces a model bias in the response of immunotherapeutic agents [33]. Because of very rapid tumor growth in most syngeneic mouse models, distant metastasis cannot be easily studied [30].

Genetically engineered models

The GEMM is established by genetic engineering tools and the Cre-loxP system is the most widely used [34]. The expression of oncogenes and/or loss of tumor suppressor genes can be spatially edited by controlling the expression of Cre recombinase using cell-specific regulatory elements such as thyroglobulin (Tg) or thyroid peroxidase (TPO), as well as temporally controlled by further engineering of Cre and exogenous inducers like tamoxifen (Table 4) [35].

As *BRAF* V600E mutations account for the majority in PTC and ATC tumors, many GEMMs contain *BRAF* V600E [2]. The first *BRAF* GEMM used the Tg promoter and constitutive *BRAF* V600E cDNA overexpression resulting PTC with high penetrance. However, the *BRAF* transgene led to dedifferentiation, which exhibited a loss of Tg expression, causing a negative feedback loop on the Tg-*BRAF* transgene [36]. To overcome this, a subsequent model used tamoxifen-inducible Cre ($CreER^{T2}$) under the control of the Tg promoter and Cre-activating *BRAF* V600E ($Braf^{CA}$) allele. PTCs were seen 6 months after tamoxifen injection and progressed to advanced DTC at 12 months. However, distant metastases were not detected [37]. Chakravarty et al. [38] also reported that conditional activation of *BRAF* V600E with doxycycline in the thyroid under the control of the Tg promoter resulted in mitogen-activated protein kinase kinase (MEK) and extracellular signal-regulated kinase (ERK) activation and PTC formation after 1 week of drug exposure. The treatment with a MEK inhibitor in this model restored tumor radioiodine uptake [38], leading to many human studies exploring

Table 4. Summary of Genetically Engineered Mouse Models for Follicular Cell-Derived Thyroid Cancer

Mouse alleles	Histopathology	Time of tumor development and/or survival
Tg-$CreER^{T2}$/$Braf^{CA}$ [37]	PTC	6 months post-induction
Tg-rtTA/tetO-BRAF [38]	PTC	1 week post-induction
TPO-$CreER^{T2}$/$Braf^{CA/+}$ [42]	PTC	12 weeks post-induction
TPO-$CreER^{T2}$/$Braf^{CA}$/$Trp53^{f/f}$ [42]	ATC	100 days post-induction
Tg-$CreER^{T2}$/$Braf^{CA}$/ $PIK3CA^{H1047R}$ [43]	PTC/ATC	PTC induced 3–6 months post-induction and progressed to ATC
Tg-$CreER^{T2}$/$Braf^{CA}$/$PTEN^{f/f}$ [43]	PTC/ATC	PTC induced 1.5 months post-induction and progressed to ATC
TPO-Cre/LSL-K-Ras^{G12D}/$PTEN^{f/f}$ [53]	FTC	50% of the mice died within 7 weeks from birth
TPO-Cre/$PRKAR1A^{f/f}$/$PTEN^{f/f}$ [54]	FTC	All showed tumor at 8 weeks of age
TPO-Cre/H-Ras^{G12V}/$PTEN^{f/f}$ [55]	FTC/PDTC	81% developed tumors by 1 year of age

Tg, thyroglobulin; $CreER^{T2}$, Cre/estrogen receptor ligand binding domain fusion; $Braf^{CA}$, Cre-activated BRAF V600E allele; PTC, papillary thyroid carcinoma; rtTA, reverse tetracycline transcription activator; tetO-BRAF, tetracycline resistant operator-MYC proto-oncogene tagged BRAFV600E; TPO, thyroid peroxidase; Trp53, transformation related protein 53; f/f, floxed/floxed; ATC, anaplastic thyroid carcinoma; PIK3CA, phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit alpha; PTEN, phosphatase and tensin homolog; LSL, lox-stop-lox; FTC, follicular thyroid cancer; PRKAR1A, protein kinase cAMP-dependent type I regulatory subunit alpha; PDTC, poorly differentiated thyroid carcinoma.

BRAF and MEK inhibition to improve radioiodine uptake in humans with DTC [39,40]. All these *BRAF* tumor models showed decreased thyroid function and elevated thyroid stimulating hormone (TSH) due to dedifferentiation [36-38,41]. Many investigators treat these mouse models with levothyroxine to mitigate the effect of high TSH on the model and better approximate the human condition. McFadden et al. [42] reported that *BRAF* V600E initiates PTC in the adult mouse thyroid and additional *TP53* loss enables the progression to ATC. PTC tumors developed in mice with TPO-CreER^{T2}/*Braf*$^{CA/+}$ at 12 weeks post-induction. Crossing this strain with the mice with homozygous deletion of p53 (Trp53$^{f/f}$) further accelerated to overt ATC. This model is referred to as TBP. An explosive tumor growth was seen approximately 100 days after tamoxifen treatment. They also showed that the selective BRAF inhibitor was not effective in controlling these ATC tumors and the combination of MEK/BRAF inhibitor was more effective [42]. Additional activation of phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit alpha (*PI3KCA*) (Tg-CreER^{T2}/Braf^{CA}/ PIK3CA^{H1047R}) or knock-out of *PTEN* (Tg-CreER^{T2}/Braf^{CA}/PTEN$^{f/f}$) also resulted in the development of ATCs [43].

RET/PTC or neurotrophic tyrosine receptor kinase (NTRK) rearrangement are also drivers of PTC [44,45]. The *RET/PTC1* [46,47] and *RET-PTC3* [48] transgenic mouse models using Tg promoter were reported, and both resulted in the development of PTC. However, these *RET/PTC* rearrangement in mice are germline, and PTC development and tumor progression may be more advanced in these mouse models compared with sporadic genetic alterations in humans. Buckwalter et al. [49] reported that mutation of major tyrosine signaling sites in *RET/PTC1* transgenic mice didn't block the development of the tumor. This suggests that other signaling pathways, or the microenvironment may play a major role in tumor formation [49]. The fusion of translocated promoter region (TPR) with NTKR1 (*TRK-T1*) developed follicular hyperplasia or PTC in about 50% of the mice [50].

RAS activation is a well-known driver in FTC. An early mouse model containing a mutant *K-Ras*^{G12V} gene under the control of rat Tg promoter showed no thyroid cancer development, and only one of these mice developed FTC following treatment with goitrogens (aminotriazol and sodium perchlorate in the drinking water) for 6 months [51]. This result suggests that *RAS* mutation alone or this degree of RAS signaling is insufficient to induce FTC and other alterations are required. One study using transgenic mice harboring the human *N-Ras*^{Q61K} under the control of the bovine Tg promoter reported that approximately 40% of the mice developed invasive FTC, in some with a mixed papillary morphology and about 25% showed de-differentiation with distant metastases [52]. These data suggest that secondary mutations may occur, or multiple copies of the transgene could be incorporated and lead to the abnormally high transcript expression [24]. These older transgenic mouse models of FTC have a limitation in that the cancer phenotype resulting from overexpression of a mutated *RAS* gene is different from the endogenous mutant *RAS* expressed at the physiological level. Miller et al. [53] crossed a mouse strain with TPO-Cre and an oncogenic *K-Ras*^{G12D} which is conditionally expressed through Cre-mediated deletion of a floxed STOP cassette preventing *K-Ras*^{G12D} expression (lox-stop-lox [LSL]-K-Ras), with a mouse strain with *PTEN* loss (PTEN$^{f/f}$). All of the double-mutant mice (TPO-Cre/LSL-K-Ras^{G12D}/PTEN$^{f/f}$) rapidly developed FTC and none survived over age 4 months [53]. Thyroid-specific double protein kinase cAMP-dependent type I regulatory subunit alpha (*PRKAR1A*)-*PTEN* knock-out mice also developed FTC [54]. Serum TSH levels were suppressed and thyroxine was elevated in these double-mutant mice, indicating development of thyrotoxicosis from functional FTC [53,54]. Thyroid-specific expression of *H-Ras*^{G12V} and *PTEN* inactivation (TPO-Cre/H-Ras^{G12V}/PTEN$^{f/f}$) also leads to the development of high grade FTCs or poorly differentiated thyroid cancer (PDTC) showing gross extrathyroidal extension and/or lymphovascular invasion, and lung metastasis [55].

A dominant negative mutation (PV) into the thyroid hormone nuclear receptor β (TRβ) generates another FTC mouse model. TRβ$^{PV/PV}$ mice reproduce human thyroid hormone resistance syndrome. T3 is unbale to bind mutant TRβ, and follicular cells become hyperplastic with elevated TSH levels. Invasive FTC was observed at 4 to 5 months of age and distant metastases to lung or heart was seen over 5 months of age. TRβ$^{PV/-}$ mice from crossing TRβ$^{PV/PV}$ mice with TRβ$^{-/-}$ mice also showed spontaneous FTC with lung metastasis [56]. The phosphoinositide 3-kinase (PI3K)-AKT signaling was activated in this model and PI3K inhibitor treatment inhibited FTC progression in these mice [57].

The GEMM model best interrogates the tumor microenvironment and the interaction between the tumor and immune system because the tumor is developed more slowly over time, not grafted [25,26,33]. Furthermore, this model helps to dissect the effect of each genetic alteration on tumor initiation and progression [2]. This model has limitations including long latency to tumor formation, complex breeding schemes, and high costs [25].

CONCLUSION AND FUTURE PERSPECTIVES

These different preclinical models have greatly improved our understanding of thyroid carcinogenesis and the development of new therapeutic strategies. Thyroid cancer cell lines and mouse models have different strengths and challenges, and any single model can't accurately reproduce all features found in human cancer. Understanding the characteristics and challenges of each model and applying the best model to specific research questions is important.

The humanized mouse model is one of the newer techniques in cancer research [58] and could be an important future preclinical model in thyroid cancer research. This model is established by transplanting human-derived peripheral polymorphonuclear cells or hematopoietic stem cells into severe combined immunodeficient mice to promote the development of the functional human immune system in mice. This model can reproduce the human tumor microenvironment, enabling a better to study immunotherapy. However, this model still faces many challenges, including frequent engraftment failure, MHC incompatibility between immune cells and tumors, the residual murine innate immunocytes, and the lack of specific cytokines [58]. These future models, including more advanced GEMM, PDX, and organoid models, will help bench-to-bedside studies in thyroid cancer research.

CONFLICTS OF INTEREST

No potential conflict of interest relevant to this article was reported.

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REFERENCES

1. Haugen BR, Alexander EK, Bible KC, Doherty GM, Mandel SJ, Nikiforov YE, et al. 2015 American Thyroid Association management guidelines for adult patients with thyroid nodules and differentiated thyroid cancer: the American Thyroid Association guidelines task force on thyroid nodules and differentiated thyroid cancer. Thyroid 2016;26:1-133.
2. Landa I, Knauf JA. Mouse models as a tool for understanding progression in BrafV600E-driven thyroid cancers. Endocrinol Metab (Seoul) 2019;34:11-22.
3. Bible KC, Kebebew E, Brierley J, Brito JP, Cabanillas ME, Clark TJ, et al. 2021 American Thyroid Association guidelines for management of patients with anaplastic thyroid cancer. Thyroid 2021;31:337-86.
4. Brose MS, Nutting CM, Jarzab B, Elisei R, Siena S, Bastholt L, et al. Sorafenib in radioactive iodine-refractory, locally advanced or metastatic differentiated thyroid cancer: a randomised, double-blind, phase 3 trial. Lancet 2014;384:319-28.
5. Schlumberger M, Tahara M, Wirth LJ, Robinson B, Brose MS, Elisei R, et al. Lenvatinib versus placebo in radioiodine-refractory thyroid cancer. N Engl J Med 2015;372:621-30.
6. Gianoukakis AG, Dutcus CE, Batty N, Guo M, Baig M. Prolonged duration of response in lenvatinib responders with thyroid cancer. Endocr Relat Cancer 2018;25:699-704.
7. Subbiah V, Kreitman RJ, Wainberg ZA, Cho JY, Schellens JH, Soria JC, et al. Dabrafenib and trametinib treatment in patients with locally advanced or metastatic BRAF V600-mutant anaplastic thyroid cancer. J Clin Oncol 2018;36:7-13.
8. Karapanou O, Simeakis G, Vlassopoulou B, Alevizaki M, Saltiki K. Advanced RAI-refractory thyroid cancer: an update on treatment perspectives. Endocr Relat Cancer 2022;29:R57-66.
9. Chintakuntlawar AV, Yin J, Foote RL, Kasperbauer JL, Rivera M, Asmus E, et al. A phase 2 study of pembrolizumab combined with chemoradiotherapy as initial treatment for anaplastic thyroid cancer. Thyroid 2019;29:1615-22.
10. Capdevila J, Wirth LJ, Ernst T, Ponce Aix S, Lin CC, Ramlau R, et al. PD-1 blockade in anaplastic thyroid carcinoma. J Clin Oncol 2020;38:2620-7.
11. Mehnert JM, Varga A, Brose MS, Aggarwal RR, Lin CC, Prawira A, et al. Safety and antitumor activity of the anti-PD-1 antibody pembrolizumab in patients with advanced, PD-L1-positive papillary or follicular thyroid cancer. BMC Cancer 2019;19:196.
12. Haugen B, French J, Worden FP, Konda B, Sherman EJ, Dadu R, et al., Lenvatinib plus pembrolizumab combination therapy in patients with radioiodine-refractory (RAIR), progressive differentiated thyroid cancer (DTC): results of a multicenter phase II international thyroid oncology group trial. J Clin Oncol 2020;38(15 suppl):6512.
13. Landa I, Ibrahimpasic T, Boucai L, Sinha R, Knauf JA, Shah RH, et al. Genomic and transcriptomic hallmarks of poorly differentiated and anaplastic thyroid cancers. J Clin Invest 2016;126:1052-66.

14. Pozdeyev N, Gay LM, Sokol ES, Hartmaier R, Deaver KE, Davis S, et al. Genetic analysis of 779 advanced differentiated and anaplastic thyroid cancers. Clin Cancer Res 2018;24:3059-68.
15. Landa I, Pozdeyev N, Korch C, Marlow LA, Smallridge RC, Copland JA, et al. Comprehensive genetic characterization of human thyroid cancer cell lines: a validated panel for preclinical studies. Clin Cancer Res 2019;25:3141-51.
16. Schweppe RE, Klopper JP, Korch C, Pugazhenthi U, Benezra M, Knauf JA, et al. Deoxyribonucleic acid profiling analysis of 40 human thyroid cancer cell lines reveals cross-contamination resulting in cell line redundancy and misidentification. J Clin Endocrinol Metab 2008;93:4331-41.
17. Gillet JP, Varma S, Gottesman MM. The clinical relevance of cancer cell lines. J Natl Cancer Inst 2013;105:452-8.
18. Henderson YC, Ahn SH, Ryu J, Chen Y, Williams MD, El-Naggar AK, et al. Development and characterization of six new human papillary thyroid carcinoma cell lines. J Clin Endocrinol Metab 2015;100:E243-52.
19. Maniakas A, Henderson YC, Hei H, Peng S, Chen Y, Jiang Y, et al. Novel anaplastic thyroid cancer PDXs and cell lines: expanding preclinical models of genetic diversity. J Clin Endocrinol Metab 2021;106:e4652-65.
20. Chen D, Tan Y, Li Z, Li W, Yu L, Chen W, et al. Organoid cultures derived from patients with papillary thyroid cancer. J Clin Endocrinol Metab 2021;106:1410-26.
21. Gu CY, Lee TK. Preclinical mouse models of hepatocellular carcinoma: an overview and update. Exp Cell Res 2022;412:113042.
22. Nucera C, Nehs MA, Mekel M, Zhang X, Hodin R, Lawler J, et al. A novel orthotopic mouse model of human anaplastic thyroid carcinoma. Thyroid 2009;19:1077-84.
23. Davis SN, Haugen BR. Ultrasound-guided orthotopic injections of thyroid cancer cell lines in mice. VideoEndocrinology 2020;7:ve.2020.0185.
24. Kirschner LS, Qamri Z, Kari S, Ashtekar A. Mouse models of thyroid cancer: a 2015 update. Mol Cell Endocrinol 2016;421:18-27.
25. Antonello ZA, Nucera C. Orthotopic mouse models for the preclinical and translational study of targeted therapies against metastatic human thyroid carcinoma with BRAF(V600E) or wild-type BRAF. Oncogene 2014;33:5397-404.
26. Jin Y, Liu M, Sa R, Fu H, Cheng L, Chen L. Mouse models of thyroid cancer: bridging pathogenesis and novel therapeutics. Cancer Lett 2020;469:35-53.
27. Zhang L, Gaskins K, Yu Z, Xiong Y, Merino MJ, Kebebew E. An in vivo mouse model of metastatic human thyroid cancer. Thyroid 2014;24:695-704.
28. Morrison JA, Pike LA, Lund G, Zhou Q, Kessler BE, Bauerle KT, et al. Characterization of thyroid cancer cell lines in murine orthotopic and intracardiac metastasis models. Horm Cancer 2015;6:87-99.
29. Henderson YC, Mohamed AS, Maniakas A, Chen Y, Powell RT, Peng S, et al. A high-throughput approach to identify effective systemic agents for the treatment of anaplastic thyroid carcinoma. J Clin Endocrinol Metab 2021;106:2962-78.
30. Vanden Borre P, McFadden DG, Gunda V, Sadow PM, Varmeh S, Bernasconi M, et al. The next generation of orthotopic thyroid cancer models: immunocompetent orthotopic mouse models of BRAF V600E-positive papillary and anaplastic thyroid carcinoma. Thyroid 2014;24:705-14.
31. Caperton CO, Jolly LA, Massoll N, Bauer AJ, Franco AT. Development of novel follicular thyroid cancer models which progress to poorly differentiated and anaplastic thyroid cancer. Cancers (Basel) 2021;13:1094.
32. Gunda V, Gigliotti B, Ashry T, Ndishabandi D, McCarthy M, Zhou Z, et al. Anti-PD-1/PD-L1 therapy augments lenvatinib's efficacy by favorably altering the immune microenvironment of murine anaplastic thyroid cancer. Int J Cancer 2019;144:2266-78.
33. Bertol BC, Bales ES, Calhoun JD, Mayberry A, Ledezma ML, Sams SB, et al. Lenvatinib plus anti-PD-1 combination therapy for advanced cancers: defining mechanisms of resistance in an inducible transgenic model of thyroid cancer. Thyroid 2022;32:153-63.
34. Kim H, Kim M, Im SK, Fang S. Mouse Cre-LoxP system: general principles to determine tissue-specific roles of target genes. Lab Anim Res 2018;34:147-59.
35. Kim CS, Zhu X. Lessons from mouse models of thyroid cancer. Thyroid 2009;19:1317-31.
36. Knauf JA, Ma X, Smith EP, Zhang L, Mitsutake N, Liao XH, et al. Targeted expression of BRAFV600E in thyroid cells of transgenic mice results in papillary thyroid cancers that undergo dedifferentiation. Cancer Res 2005;65:4238-45.
37. Charles RP, Iezza G, Amendola E, Dankort D, McMahon M. Mutationally activated BRAF(V600E) elicits papillary thyroid cancer in the adult mouse. Cancer Res 2011;71:3863-71.
38. Chakravarty D, Santos E, Ryder M, Knauf JA, Liao XH, West BL, et al. Small-molecule MAPK inhibitors restore radioiodine incorporation in mouse thyroid cancers with conditional BRAF activation. J Clin Invest 2011;121:4700-11.
39. Ho AL, Grewal RK, Leboeuf R, Sherman EJ, Pfister DG,

Deandreis D, et al. Selumetinib-enhanced radioiodine uptake in advanced thyroid cancer. N Engl J Med 2013;368:623-32.
40. Iravani A, Solomon B, Pattison DA, Jackson P, Ravi Kumar A, Kong G, et al. Mitogen-activated protein kinase pathway inhibition for redifferentiation of radioiodine refractory differentiated thyroid cancer: an evolving protocol. Thyroid 2019;29:1634-45.
41. Franco AT, Malaguarnera R, Refetoff S, Liao XH, Lundsmith E, Kimura S, et al. Thyrotrophin receptor signaling dependence of Braf-induced thyroid tumor initiation in mice. Proc Natl Acad Sci U S A 2011;108:1615-20.
42. McFadden DG, Vernon A, Santiago PM, Martinez-McFaline R, Bhutkar A, Crowley DM, et al. p53 constrains progression to anaplastic thyroid carcinoma in a Braf-mutant mouse model of papillary thyroid cancer. Proc Natl Acad Sci U S A 2014;111:E1600-9.
43. Charles RP, Silva J, Iezza G, Phillips WA, McMahon M. Activating BRAF and PIK3CA mutations cooperate to promote anaplastic thyroid carcinogenesis. Mol Cancer Res 2014;12:979-86.
44. Santoro M, Melillo RM, Fusco A. RET/PTC activation in papillary thyroid carcinoma: European Journal of Endocrinology prize lecture. Eur J Endocrinol 2006;155:645-53.
45. Greco A, Miranda C, Pierotti MA. Rearrangements of NTRK1 gene in papillary thyroid carcinoma. Mol Cell Endocrinol 2010;321:44-9.
46. Jhiang SM, Sagartz JE, Tong Q, Parker-Thornburg J, Capen CC, Cho JY, et al. Targeted expression of the ret/PTC1 oncogene induces papillary thyroid carcinomas. Endocrinology 1996;137:375-8.
47. Jhiang SM, Cho JY, Furminger TL, Sagartz JE, Tong Q, Capen CC, et al. Thyroid carcinomas in RET/PTC transgenic mice. Recent Results Cancer Res 1998;154:265-70.
48. Powell DJ, Russell J, Nibu K, Li G, Rhee E, Liao M, et al. The RET/PTC3 oncogene: metastatic solid-type papillary carcinomas in murine thyroids. Cancer Res 1998;58:5523-8.
49. Buckwalter TL, Venkateswaran A, Lavender M, La Perle KM, Cho JY, Robinson ML, et al. The roles of phosphotyrosines-294, -404, and -451 in RET/PTC1-induced thyroid tumor formation. Oncogene 2002;21:8166-72.
50. Russell JP, Powell DJ, Cunnane M, Greco A, Portella G, Santoro M, et al. The TRK-T1 fusion protein induces neoplastic transformation of thyroid epithelium. Oncogene 2000;19:5729-35.
51. Santelli G, de Franciscis V, Portella G, Chiappetta G, D'Alessio A, Califano D, et al. Production of transgenic mice expressing the Ki-ras oncogene under the control of a thyroglobulin promoter. Cancer Res 1993;53:5523-7.
52. Vitagliano D, Portella G, Troncone G, Francione A, Rossi C, Bruno A, et al. Thyroid targeting of the N-ras(Gln61Lys) oncogene in transgenic mice results in follicular tumors that progress to poorly differentiated carcinomas. Oncogene 2006;25:5467-74.
53. Miller KA, Yeager N, Baker K, Liao XH, Refetoff S, Di Cristofano A. Oncogenic Kras requires simultaneous PI3K signaling to induce ERK activation and transform thyroid epithelial cells in vivo. Cancer Res 2009;69:3689-94.
54. Pringle DR, Vasko VV, Yu L, Manchanda PK, Lee AA, Zhang X, et al. Follicular thyroid cancers demonstrate dual activation of PKA and mTOR as modeled by thyroid-specific deletion of Prkar1a and Pten in mice. J Clin Endocrinol Metab 2014;99:E804-12.
55. Jolly LA, Massoll N, Franco AT. Immune suppression mediated by myeloid and lymphoid derived immune cells in the tumor microenvironment facilitates progression of thyroid cancers driven by HrasG12V and Pten loss. J Clin Cell Immunol 2016;7:451.
56. Kato Y, Ying H, Willingham MC, Cheng SY. A tumor suppressor role for thyroid hormone beta receptor in a mouse model of thyroid carcinogenesis. Endocrinology 2004;145:4430-8.
57. Furuya F, Lu C, Willingham MC, Cheng SY. Inhibition of phosphatidylinositol 3-kinase delays tumor progression and blocks metastatic spread in a mouse model of thyroid cancer. Carcinogenesis 2007;28:2451-8.
58. Yin L, Wang XJ, Chen DX, Liu XN, Wang XJ. Humanized mouse model: a review on preclinical applications for cancer immunotherapy. Am J Cancer Res 2020;10:4568-84.