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Novel therapies identified in patient derived thyroid cancer xenografts

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Abstract

Introduction: The success of clinical trials for patients with radioiodine resistant aggressive thyroid cancer is dependent upon the use of successful pre-clinical models. Despite some success, there is still a need to develop improved pre-clinical models for these tumors that have the capability to translate more reliably from bench to bedside, thus allowing improved selection of novel therapeutic agents. Patient-derived tumor xenograft (PDTX) models are purported to most closely replicate a patient's response to therapy because of preservation of tumor heterogeneity and microenvironment. **Methods:** We have developed pre-clinical thyroid cancer PDTX models by implanting patient tumor tissue into immunocompromised mice. Each model is characterized and compared to the originating patient tumor tissue by short tandem repeat (STR) analysis for DNA fingerprinting, immunohistochemistry (IHC) for thyroid markers, and for oncogenic driver mutations. **Results:** We have developed 8 PDTX models that include insular thyroid carcinoma (ITC), poorly differentiated papillary thyroid carcinoma (PDTC), squamous cell thyroid carcinoma (SCTC), and anaplastic thyroid carcinoma (ATC). Each model demonstrates its own unique responses to radiation; cytotoxic therapies (doxorubicin, cisplatin, paclitaxel) or molecular targeted therapies such as tyrosine kinase inhibitors (sorafenib, sunitinib), and farnesyl transferase inhibitor (tipifarnib). Furthermore, in a HRAS mutant ATC PDTX model, remarkable responses are seen with combination therapy sunitinib plus paclitaxel. **Conclusions:** We expect that this might provide the rationale for some of these therapeutic strategies to move forward towards clinical trials.

Background

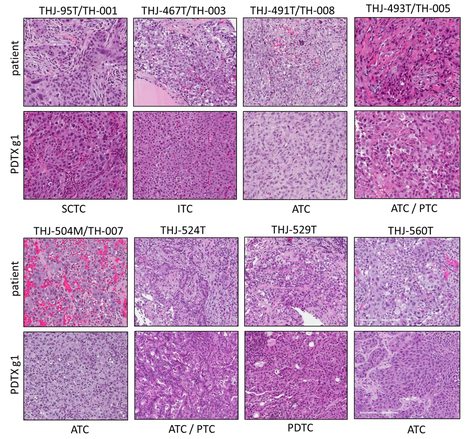
Thyroid cancer is the most common endocrine cancer and is categorized into 4 main subtypes: papillary, follicular, medullary and anaplastic. Papillary thyroid (PTC) and Follicular thyroid (FTC) are well-differentiated accounting for 80-90% of all thyroid cancers. Variants include tall cell, insular, columnar and Hurthle cell. Medullary thyroid (MTC) arises from neuroendocrine cells and accounts for 3-4%. Generally, PTC, FTC, and MTC are managed successfully with the current standard-of-care if detected early; however, up to 30% will have recurrence even decades later. Anaplastic thyroid (ATC) is rare (accounts for 1-2%) and is undifferentiated leading to its very aggressive nature and poor prognosis. Squamous cell (SCTC) is also very rare (<1%) with no known origin. SCTC is also highly aggressive with poor prognosis. For recurring or more aggressive thyroid cancers that are refractory to radioactive iodine, improved therapeutics need to be established.

The use of patient-derived tumor xenografts (PDTX) mouse models for therapeutic studies is thought to closely resemble therapeutic responses seen in the patients. Thus, the number the PDTX models available have readily increased. Creating PDTX models has its challenges and various success rates depending upon the tissue type.

Thyroid PDTX success rate = 15% (as of 10/6/2015)

Figure 1: Histology of thyroid PDTX models

A. Histological comparison of patient tissue and PDTX tissue



B. IHC of thyroid markers for degree of differentiation

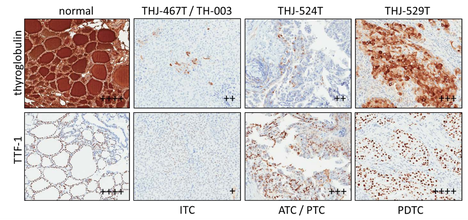


Table 1. DNA STR profile of validated PDTX models

	AMEL	D5S818	D13S317	D7S820	vWA	TH01	TPOX	CSF1PO	D18S51	D3S1358	D8S1179	FGA
patient 95	XY	12,13	9	10,11	16,17	6,9	8,11	12	15	14,17	13	22,23
THJ-95T / TH-001	XY	12,13	9	10,11	16,17	6,9	8,11	12	15	14,17	13	22,23
patient 467	XY	13	11	10	15,17	9,9.3	8,9	10,11	12,15	14,15	14	19,20
THJ-467T / TH-003	XY	13	11	10	15,17	9,9.3	8, 9	10,11	12,15	14,15	14	19,20
patient 491	XX	11,13	9,11	9,11	17,18	9.3	8,11	11	11,16	16	12,13	21,24
THJ-491T / TH-008	XX	11,13	9,11	9,11	17,18	9.3	8,11	11	11,16	16	12,13	21,24
patient 493	XY	12,14	11,12	12	14,15	7	8,11	10,13	12,19	16,17	11,14	19,24
THJ-493T / TH-005	XY	-----	11,12	12	14,15	7	8,11	10,13	12,19	16,17	11,14	19,24
patient 504	XY	11	11,12	10,11	15,17	9.3	10,12	10	12,15	15,17	10,13	19,21
THJ-504M / TH-007	XY	11	11,12	10,11	15,17	9.3	10,12	10	12,15	15,17	10,13	19,21
patient 524	XY	11,13	9,10	9,10	14,16	6,7	11	10,12	12,15	15,18	13	20,24.2
THJ-524T PDTX	XY	11,13	9,10	9,10	14,16	6,7	11	10,12	12,15	15,18	13	20,24.2
patient 529	XY	10,12	12	10,12	16,18	6,9	8	10,11	14,18	15,19	13,14	24,24.2
THJ-529T PDTX	XY	10,12	12	10,12	16,18	6,9	8	10,11	14,18	15,19	13,14	24,24.2
patient 560	XX	12	8,12	10,12	14	6,7	8	9,10	15,16	16,17	12,14	21,24
THJ-560 PDTX	XX	12	12	10,12	14	6,7	8	9,10	15	16,17	12,14	24

Table 2. Mutation summary of thyroid PDTX models

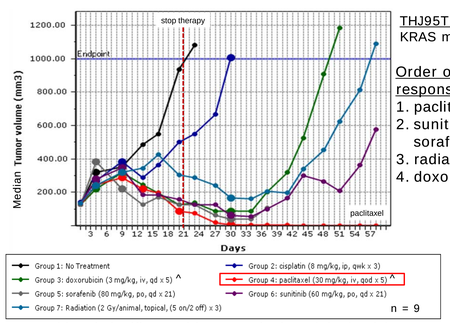
Cell line	Sex	Lesion	g0 incubation	Mouse strain	BRAF V600E	HRAS codon 12, 13, 61	KRAS codon 12, 13, 61	NRAS codon 12, 13, 61
THJ-95T / TH-001	M	SCTC	~5 months	Athymic nude	wt@	wt@	codon 12@ GGT→ GTT	wt@*
THJ-467T / TH-003	M	ITC	~2.5 months	Athymic nude	wt@	wt@	wt@	wt@*
THJ-491T / TH-008	F	ATC	~7 months	Athymic nude	wt@	wt@	wt@	wt@*
THJ-493T / TH-005	M	ATC/PTC	~1.5 months	Athymic nude	wt@	codon 61@ CCA→CCG	wt@	wt@*
THJ-504M / TH-007	M	ATC	~2.5 months	Athymic nude	mut@	wt@	wt@	wt@*
THJ-524T	M	ATC/PTC	~3 months	Athymic nude				
THJ-529T	M	PDTC	~4.5 months	NOD SCID Athymic nude				
THJ-560T	F	ATC	~2 months	NOD SCID Athymic nude				

@confirmed in patient tissue
*PDTX model had possible mutation at codon 62 (GAA→GAG), which may be due to artifact

Table 2. Summary of thyroid PDTX models.
g0 incubation indicates the number of months that the tumor was grown in the mouse host until collection.

BRAF V600E and RAS mutations were analyzed by PCR and confirmed by sequencing. PENDING mutation analysis includes PI3CKA, TERT promoter, and ALK exon 23

Figure 3. Squamous cell carcinoma PDTX monotherapy



THJ95T / TH-001
KRAS mutant

Order of responsiveness:
1. paclitaxel
2. sunitinib or sorafenib
3. radiation
4. doxorubicin

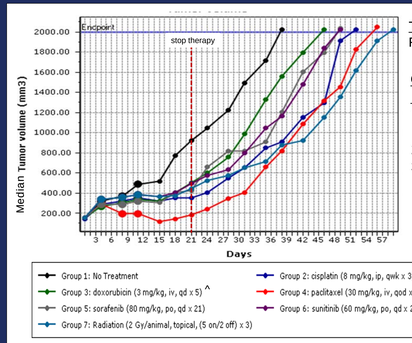
^ *considered significant with >5.0% body weight (BW) loss*

group	therapy	TTE	PR	CR	TFS
4	paclitaxel^	58+	1	8	7
6	sunitinib	58	3	0	0
5	sorafenib	55.4	3	1	1
7	radiation	56.1	1	1	1
3	doxorubucin^	49	2	1	0

PR = partial regression
CR= complete regression
TFS = tumor free survivor
TTE = time-to-endpoint

Figure 3. THJ-95T/TH-001 was very responsive to paclitaxel therapy (TTE=58+ days) with 1 PR, 8 CR and 7 TFS as compared to placebo (TTE=22.2 days) at endpoint. Other groups eventually resumed tumor growth without continued therapy. ^Significant weight loss observed in groups 3 & 4.

Figure 3. Follicular Insular carcinoma PDTX monotherapy

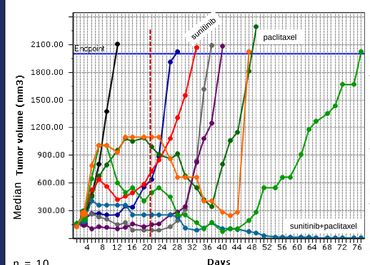


THJ467T / TH-003
RAS wildtype

Order of responsiveness:
1. paclitaxel or radiation
2. cisplatin
3. sunitinib or sorafenib

Figure 2. THJ-467T/TH-003 was slightly responsive to paclitaxel therapy (TTE=54.4 days) and radiation (TTE=56 days) as compared to placebo (TTE=37.5 days) at endpoint. *No PR, CR or TFS were observed*.

Figure 4. Anaplastic carcinoma PDTX combinatorial therapy



THJ493T / TH-005
HRAS mutant

Figure 4. THJ493T/ TH005 was most responsive to sunitinib+paclitaxel (TTE=77+ days, TFS=5) followed by radiation therapy (TTE=76.8 days, TFS=0) as compared to placebo (TTE=11.1 days).

group	therapy	TTE	PR	CR	TFS
4	sunitinib	31	0	1	1
3	paclitaxel^	47	1	1	1
7	combo^	77+	0	6	5

**No response was seen with cisplatin, pazopanib and carfilzomib.*

^Significant body weight loss was observed in groups 3, 5, 6, 7, 8, 9.

Summary

We have developed rare thyroid cancer PDTX models, some of which have no standard of care. These humanized models may be used to test new therapeutics predicting patient response to new therapies.