



Novel anaplastic thyroid cancer patient-derived xenograft models and cell lines: Expanding the molecular diversity of in vitro and in vivo models

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1. Introduction

- Anaplastic thyroid cancer (ATC) is a rare, aggressive, and deadly disease.
- Patient-derived xenograft (PDX) models have been shown to recreate human tumor biology, specifically in terms of gene expression, tumor heterogeneity, and drug responses, making them a robust *in vivo* pre-clinical model for new drug development.

2.1 Methods - PDX

- Six patients included (4F, 2M)
- Successful establishment of PDX model was defined as:
 - Expansion success from F0 to F3 (4 generations)
 - Ability to expand from a cryogenically frozen sample

2.2 Methods – Cell line

- Successful establishment of cell lines was defined as:
 - A total of at least 20 passages
 - A minimum of 6 months in culture
- Cell line doubling time was always calculated in triplicate
- Tumorigenesis was evaluated using an orthotopic model in athymic nude *nu/nu* mice

2.3 Methods – IHC+STR

- Immunohistochemistry (IHC) analyses were performed against human-specific pancytokeratin (CK), TTF-1, PAX8, BRAF^{V6002E}, and TP53
- Short tandem repeats (STR) analysis was performed for all PDX models, cell lines, and matching patient tissue using Promega PowerPlex® 16 HS

Table 1. Patient-derived xenograft (PDX) model characteristics.

	PDX model name/tumor ID	TNM	Disease stage	Diagnosis	Treatment prior to tissue collection	Last PDX generation	Expansion of PDX from frozen	Final generation-to-generation expansion time (days)	PDX tumor doubling time (days)
1	MDA-ATC1	T4bN1bM1	IVC	ATC	No	F5	F4	32	5-7
2	MDA-ATC2	T4aN1aM0	IVB	ATC	No	F4	F4	28	5-6
3	MDA-ATC3	T4aN1M0	IVB	Multifocal clusters of viable carcinoma consistent with residual ATC	Dabrafenib Trametinib	F4	F4	14	4-5
4	MDA-ATC4	T4aN1bM1	IVB	ATC arising associated with PTC	Atezolizumab Vemurafenib Cobimetinib	F5	F3	14	3-5
5	MDA-ATC5	T3aN0M0	IVC	ATC	No	F5	F2	14	3-4
6	MDA-ATC6	T4aN0M0	IVC	ATC focal associated PTC	No	F6	F6	14	3-4

3. Results

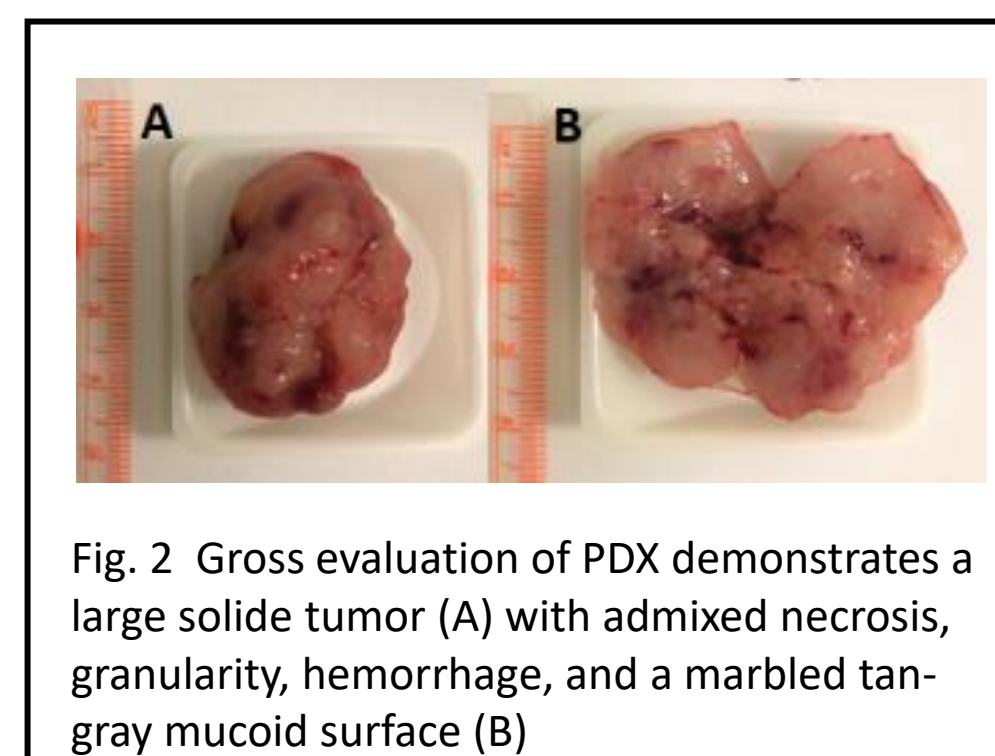
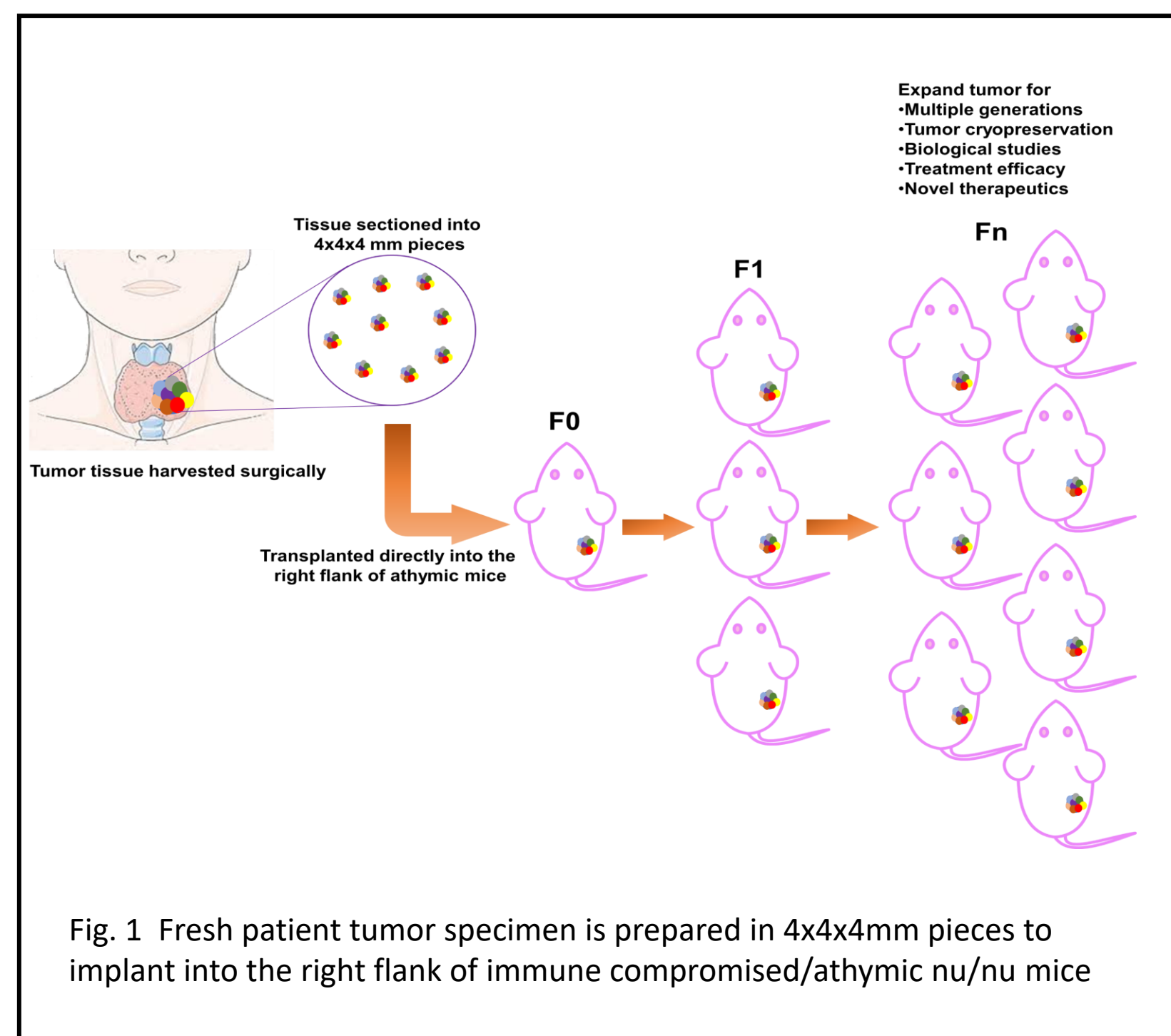


Fig. 2 Gross evaluation of PDX demonstrates a large solide tumor (A) with admixed necrosis, granularity, hemorrhage, and a marbled tan-gray mucoid surface (B)

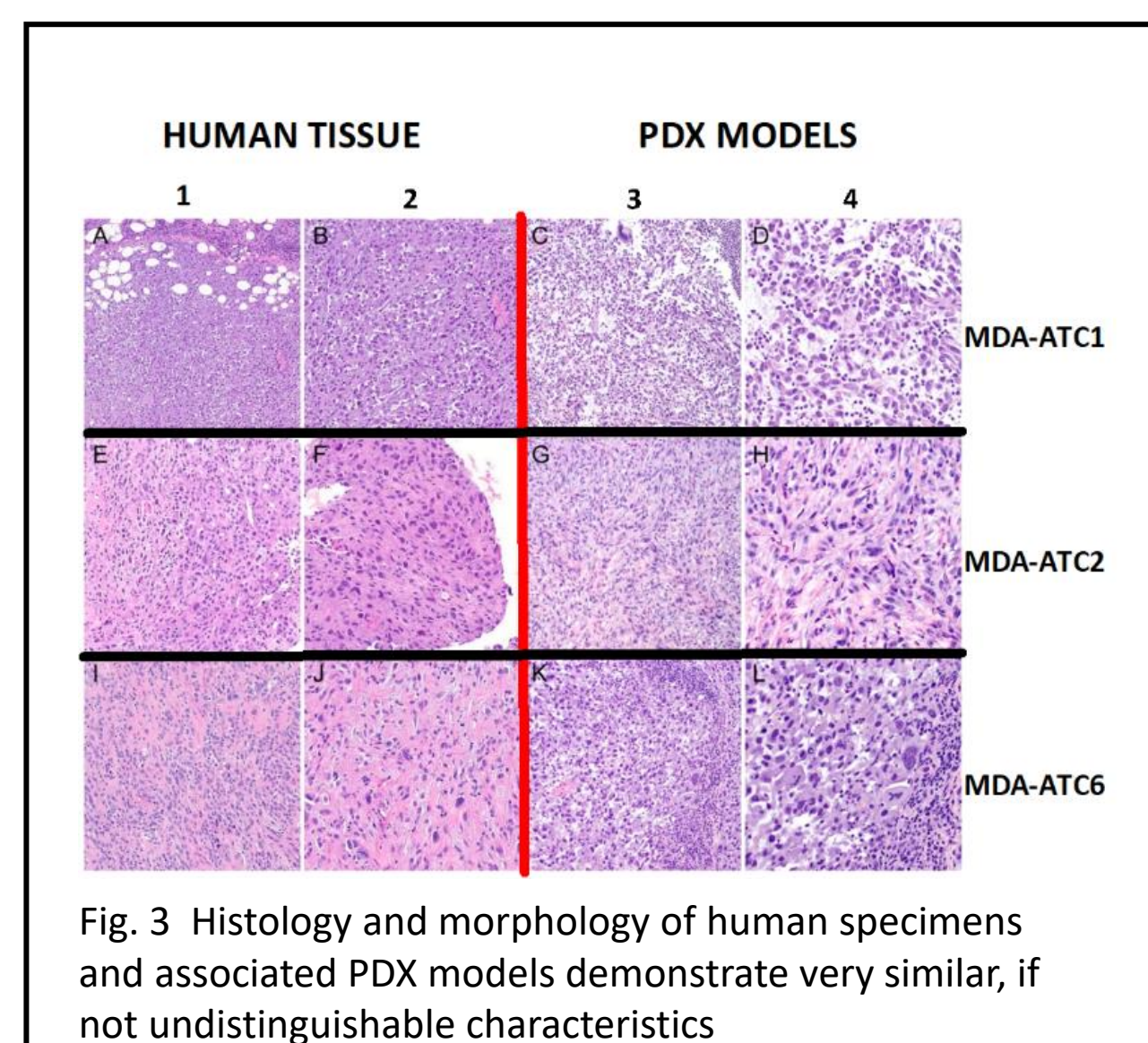


Fig. 3 Histology and morphology of human specimens and associated PDX models demonstrate very similar, if not undistinguishable characteristics

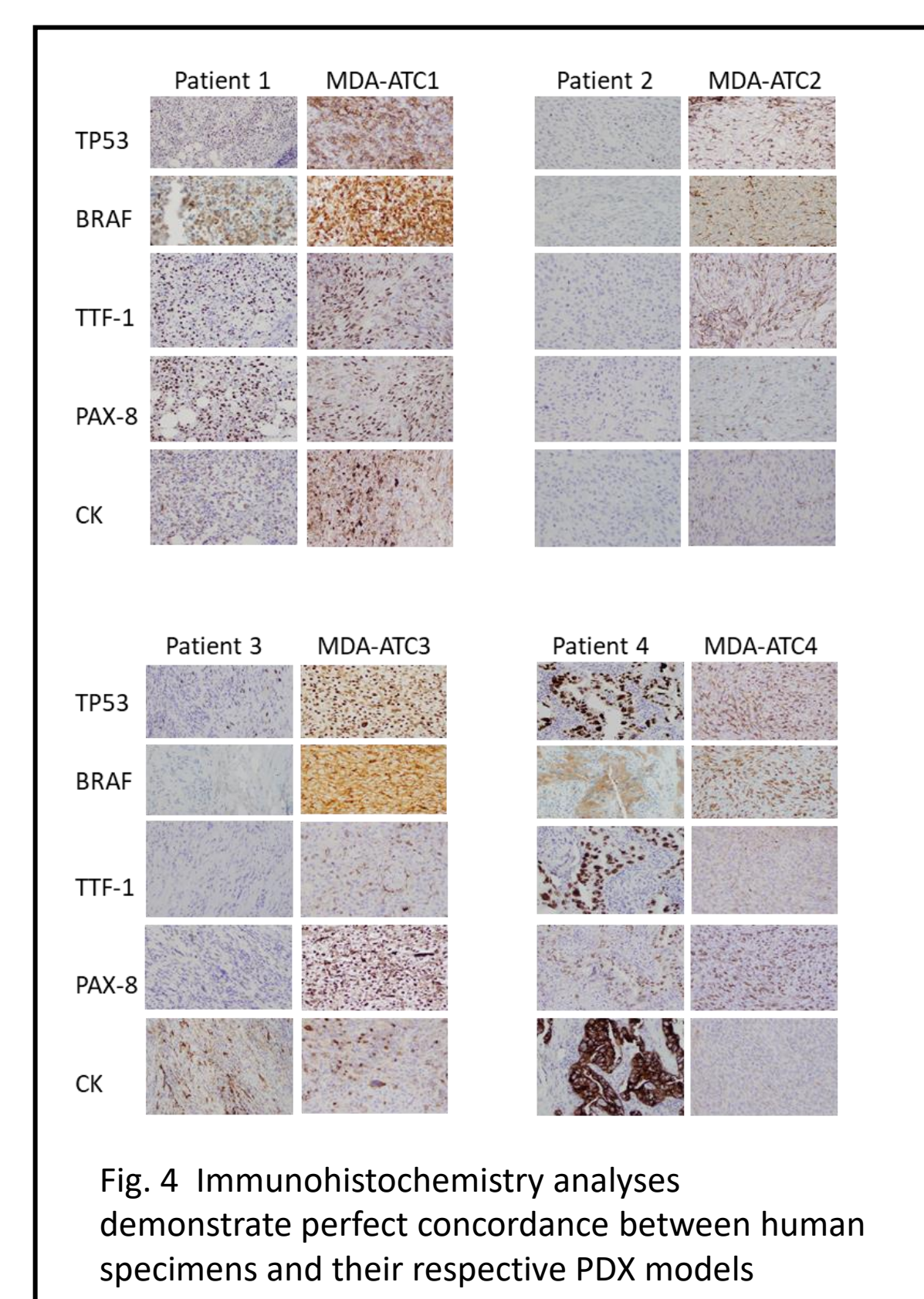


Fig. 4 Immunohistochemistry analyses demonstrate perfect concordance between human specimens and their respective PDX models

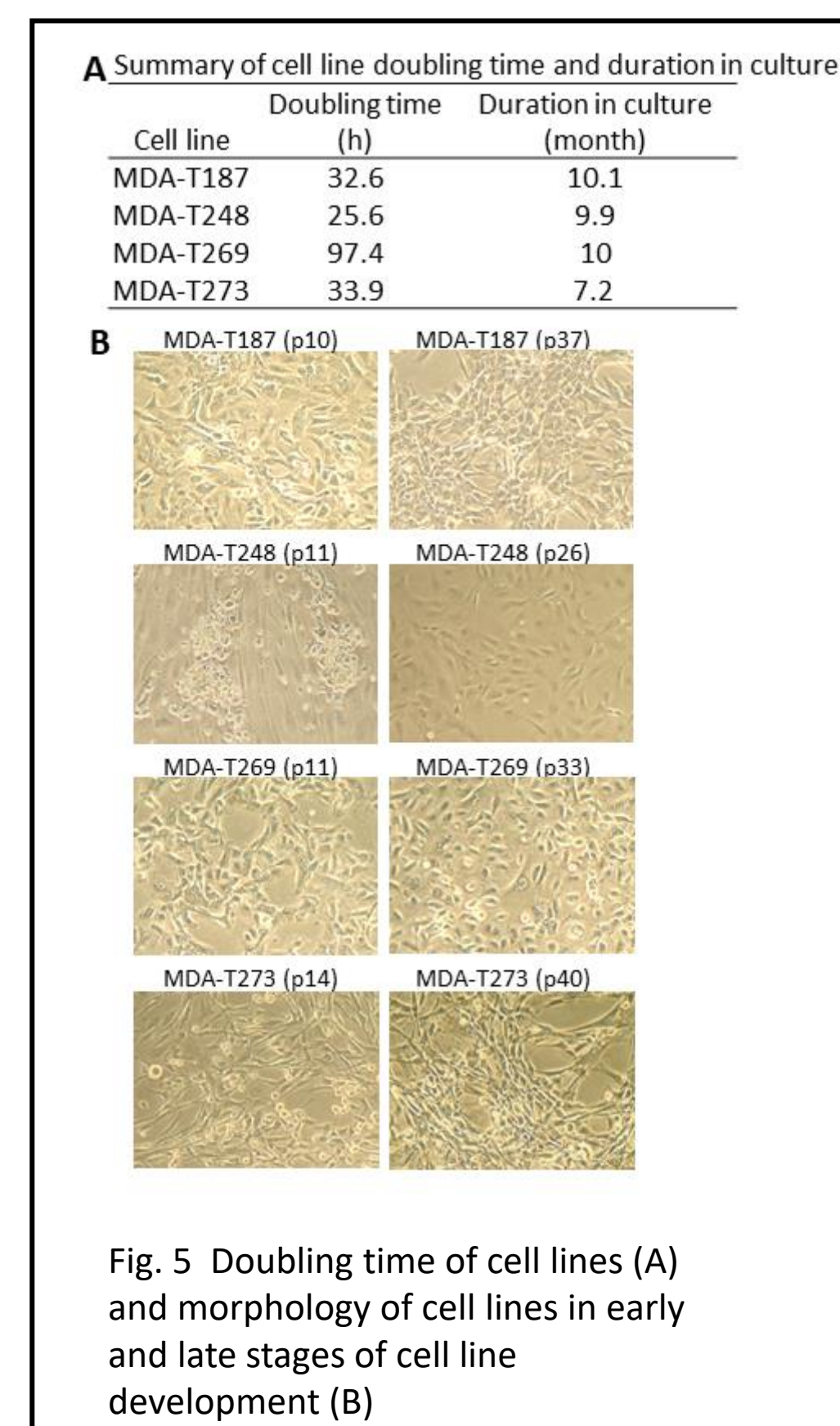


Fig. 5 Doubling time of cell lines (A) and morphology of cell lines in early and late stages of cell line development (B)

Table 2. Short-tandem repeats (STR) of patient tissues, cell lines, and patient-derived xenograft models (PDX).

Sample (Gender)	STR Source (passage or generation)	AM	CSF1PO	D5S818	D7S820	D13S317	D16S539	TH01	TPOX	vWA	Identity* (%)
MDA-ATC1	Frozen Tissue	X	10,12	11,14	9,12	11,12	11	9,3	8	16,18	100
(Female)	MDA-T187 cell line (p28)	X	12	14	9,12	12	11	9,3	8	16,18	88.5
	PDX (F1)	X	10,12	11,14	12	12	11	9,3	8	16,18	92.3
MDA-ATC2	Frozen Tissue	X,Y	12,13	9,12	10,12	11,12	11,12	6,9,3	8,12	17,18	100
(Male)	PDX (F3)	X,Y	12,13	9,12	10,12	12	11	6,9,3	8,12	17,18	92.3
MDA-ATC3	Frozen Tissue	X	10,12	12	9,12	11,12	8,10	6,9,3	8,12	14,15	100
(Female)	PDX (F3)	X	12	12	12	11	8,10	6,9,3	12	14	73.1
MDA-ATC4	Frozen Tissue	X	12	9,11	11	11,12	11,13	6,8	9,10	14,18	100
(Female)	MDA-T248 cell line (p25)	X	12	9,11	11	11	11,13	6,8	9,10	14,18	96.2
	PDX (F2)	X	12	9,11	11	11	11,13	6,8	9,10	14,18	96.2
MDA-ATC5	Frozen Tissue	X,Y	11,14	12	8,10	11,12	11,13	6	8,11	14,17	100
(Male)	MDA-T269 cell line (p20)	X	11,14	12	10	11,12	11,13	6	8,11	14,17	96.2
	PDX (F3)	X	11,14	12	10	11,12	11,13	6	8,11	14,17	96.2
MDA-ATC6	Frozen Tissue	X	11,12	12	10,11	9,11	11	7,9	8,11	14,17	100
(Female)	MDA-T273 cell line (p21)	X	11,12	12	10,11	9	11	7,9	8,11	14	92.3
	PDX (F3)	X	11,12	12	10,11	9	11	7,9	8,11	14	92.3

*Percent identity was determined by Tanabe algorithm based on 13 loci. Percent match = (number shared loci × 2)/(total number of loci in the questioned profile + total number of loci in the reference profile). Reference profile was frozen tissue.

4. Conclusions

- We have developed and characterized six novel ATC PDX models, and their cell line counterparts in four of six models
- These models contain unique histologic and genetic variations
- These models can serve as reliable pre-clinical models