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Characterization of novel patient derived melanoma xenografts and cell lines in response to targeted therapies

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Abstract

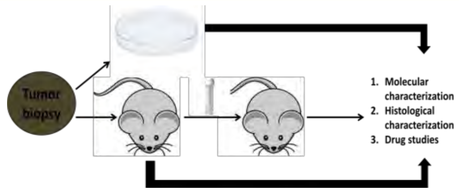
Purpose: Develop pre-clinical models that are applicable to clinical practice with more efficacious bench-to-bedside translation. One avenue proposed in this study is the use of patient derived xenograft (PDX) models. These models have previously been reported to have the ability to predict clinical activity as they are biologically stable in terms of gene expression pattern, tumor architecture, and mutation status (1). Additionally, as PDX models serve as individualized experimental models, there have been many reports of success stories in patient treatment and outcome (2-4). The detailed characterization of our pre-clinical melanoma models will represent a resource rich analysis for predictive biomarker discovery and drug activity, especially the design of novel combination therapeutic agents for metastatic melanoma.

Experimental Design: A panel of STR valid pre-clinical metastatic melanoma models were developed from human tumor tissue, including established cell lines, cell-line derived xenograft models and PDX models. Each model was molecularly characterized to confirm a match to that of the originating patient tumor tissue via protein immunohistochemistry (IHC), mRNA quantitative polymerase chain reaction (qPCR), DNA short tandem repeat (STR) analysis, and Sanger sequencing.

Results: All pre-clinical models were positive for the melanoma-specific marker Melan-A, (among others not shown) confirming a melanoma phenotype. BRAF, NRAS and KRAS mutations were identified in our models matching their respective patient tumor. Growth patterns among all models were established, resembling similar growth characteristics. Response to monotherapy treatments *in vivo* was demonstrated in the BRAF mutant model, Mela14.

Conclusions: Mutation status data (i.e. BRAF, RAS), patient therapeutic clinical history and a future analysis of oncogene overexpression (pERK, pAKT, PI3K, pmTOR, etc.) will provide the platform and rationale behind the use of specific therapeutic agents per model with the intent to design novel combination therapies that may reduce treatment related side effects, increase efficacy, and prolong survival in a patient setting.

Experimental Design



- Patient tumor biopsy tissues were harvested and collected for either cell culture (minced tissues), *in vivo* subcutaneous implantation into nude mice (4x4mm^3 sections), formalin fixed paraffin embedded (FFPE) (5x5 mm^3 sections), and cryogenically frozen
- Patient tumor tissues were subcutaneously implanted into the flanks of nude mice to monitor tumor growth. Established tumors were then cryogenically frozen before further mouse serial passages
- Established cell lines were subsequently passaged and used for *in vitro* drug proliferation assays and subcutaneous injections into nude mice to monitor tumor growth
- All model types derived from the initial patient tumor biopsy are analyzed for molecular and histological characterization, and various drug screens

Pre-clinical models exhibit melanoma-specific patient characteristics

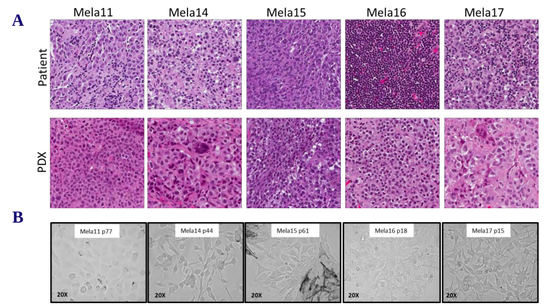


Figure 1. Pre-clinical melanoma model characterization. Hematoxylin and eosin stains show that pre-clinical melanoma PDX models exhibit both cellular pleomorphism and sheets of melanocytes similar to their matched patient tissue (Fig. 1A). These models also exhibit an epithelioid phenotype, a common feature of melanoma. Epithelial cells derived from patient tumor tissue were passaged to generate established cell lines (Fig. 1B). Melanin pigment can be seen in Mela15.

Cell line passage numbers: Mela11 p58; Mela14 p27; Mela15 p19; Mela16 p36; Mela17 p35

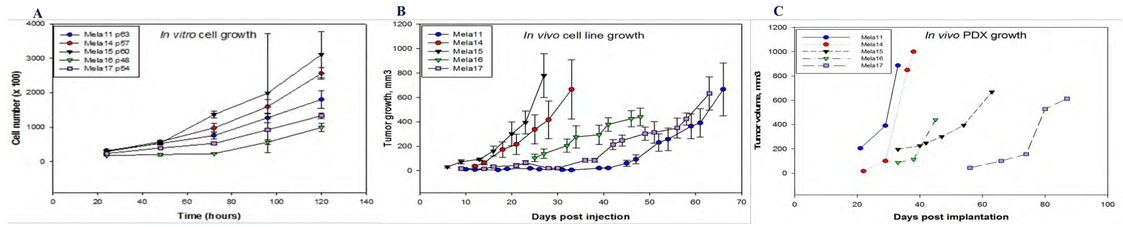
Figure 2. Pre-clinical models express the melanocytic lineage marker Melan-A. Melan-A is a cytoplasmic staining molecule specific for melanocyte lineage and is expressed in mature melanocytes (5). Deficiency of this protein in tumor samples is associated with poor prognosis. Patient and PDX tissue samples were FFPE and stained for Melan-A via IHC. Patient-derived cell lines were seeded 1x10^5 cells/well in 2-well poly d-lysine chambers and fixed in 2% paraformaldehyde 24 hours after incubation and additionally stained for Melan-A via ICC.

Pre-clinical models	Sex (Age)	Tumor source	Mutation	Oncogene protein expression	Patient clinical treatment(s)	Patient response to therapy
Mela11*	Male (54)	High inguinal lymph node	BRAF^{V600E}	BRAF (weak) pERK (high) pAKT (high)	1.Carbo/Abraxane/Avastin (11/2009) 2.Leukine immunotherapy (11/2009 to 11/2010) 3.Leukine immunotherapy (post-op) 4.Surgery 5.IPI (5/2014 to 7/2014)	1.---------- 2.Mets (surgically resected) 3.Mets (lymph nodes) 4.Mets (small bowel) 5.Disease free (8/2014)
Mela14*	Female (42)	Small bowel mesentery	BRAF^{V600E}	BRAF (high) pERK (high) pAKT (medium)	1.TMZ (12/2011) 2.VEM (5/2012) 3.Res of non-responding tumors and continues VEM 4.WBRT	1.Tumor progression 2.Mixed response to therapy 3.Brain mets (9/2012) 4.Deceased (10/2012)
Mela15*	Female (39)	Inguinal lymph node	NRAS Exon 61 Q→R	BRAF (absent pERK (medium) pAKT (medium)	1.Leukine immunotherapy 2.TMZ	1.---------- 2.Lung mets
Mela16*	Male (65)	Axillary lymph node	BRAF^{V600E}	BRAF (absent IHC) pERK (medium) pAKT (medium)	Axillary node resection after PET/CT (2/2013)	NED (8/2014)
Mela17*	Male (75)	Infraclavicular lymph node	KRAS Exon 61 Q→R	BRAF (absent) pERK (high) pAKT (medium)	Leukine immunotherapy	NED (7/2014)

*PDX and cell lines are DNA short tandem repeat (STR) validated to match the originating patient melanoma tissue
Carbo, carboplatin; IPI, ipilimumab; Mets, metastasis; NED, no evidence of disease; PET/CT, positron emission tomography-computerized tomography; Post, post-treatment; Pre, pretreatment; TMZ, temozolomide; VEM, vemurafenib; WBRT, whole brain tumor radiation

Figure 3. Pre-clinical models express mutations common in human melanoma. Sanger sequencing was used to assess genetic mutation statuses among our pre-clinical models to the patient matched tumor tissues. The oncogene protein expression profiles were obtained from IHC stains using molecular targeted antibodies. Patient's clinical treatment history along with response to therapy is also listed.

Growth and therapeutic response characteristics of pre-clinical models



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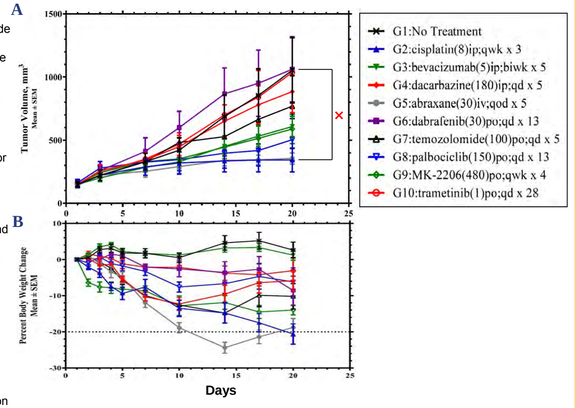
	Dabrafenib IC50	MK-2206 IC50	Trametinib IC50	Palbociclib IC50	Abraxane IC50
Molecular Target	BRAF^{V600E}	p-AKT	MEK1/2	CDK4/6	Antineoplastic Agent
Mela11	0.008uM	4.7uM	N/A	N/A	50.1nM
Mela14	0.25uM	4.5uM	2.5uM	6.6uM	70.8nM
Mela15	N/A	2uM	0.04uM	8.5uM	66.1nM
Mela16	N/A	N/A	N/A	N/A	N/A
Mela17	N/A	7uM	7.94uM	N/A	56.2nM

Figure 4. Growth and therapeutic characterization of pre-clinical models. Cells were plated 30x10^{3}cells/well in culture medium for a time course of five days and counted every 24 hours to determine an *in vitro* growth pattern (Fig. 4A). 5x10^6 established cell lines were injected subcutaneously into the left flanks of female nu/nu mice. Tumors volumes were measured twice a week until maximum tumor size was reached (1000-1500mm^3) (Fig. 4B). Majority of the models exhibit similar *in vitro* and *in vivo* growth patterns (Figs. 4A and 4B; Mela14, Mela15, and Mela17). Primary tumor tissues that produced initial tumors *in vivo* (G0), were cryogenically frozen and implanted again into female nu/nu mice to validate and confirm establishment of PDX models (Fig. 4C). Tumors were measured once a week until endpoint (1000-1500mm^3 tumor size) or once mice began to exhibit undesirable weight loss and/or frailty. Established cell lines were treated with various therapies to assess sensitivity and response. The above drugs (Fig. 4D) were chosen based on either mutation status (Fig. 3) and/or physician recommendations. IC50 data per therapy per cell line model is also listed (Fig. 4D).

Monotherapy *in vivo* dose response: Mela14 PDX

Figure 5. Athymic nude (nu/nu) mice bearing Mela14 xenograft were treated with multiple therapeutic agents individually and mean tumor volume (mm^3) was recorded. The p-value for differences between median tumor growth inhibition was measured at 20 days following treatment. Compared to no treatment, cisplatin and abraxane were statistically significant (*p-value<0.05) (Fig. 5A).

Body weight measurements were taken at various time points. Of the nine therapies abraxane appears to have the most negative affect on changes compared to body weight measured at day 1 treatment (Fig. 5B)



qwk, weekly treatment; ip, intraperitoneal injection; biwk, biweekly treatment; qd, every day treatment; qod, treatment every other day; iv, intravenous injection; po, by mouth

Future Directions

- Complete combination therapies in remaining pre-clinical models and assess sensitivity and response
- Validate antitumor synergy in PDX models
- Determine if PDX models mimic patient response to therapy
- Define novel mutations in pre-clinical models
- Validate biomarkers for response to therapy in tumor tissues

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