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The Melanoma Patient-Derived Xenograft (PDX) Model

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Ronald Myers, Ph.D.
JoVE Science Editor

Dear Ronald,

Attached please find our revised manuscript, "The Melanoma Patient-Derived Xenograft (PDX) Model." We wish this revised manuscript to be considered for publication in *JoVE*. We have addressed all of the editor and reviewer concerns, and we look forward to a timely review and appreciate the opportunity to share our work.

Sincerely,

Vito W. Rebecca, Ph.D.

TITLE:
A Melanoma Patient-Derived Xenograft Model

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KEYWORDS:
melanoma, patient-derived xenograft, therapy, resistance, in vivo models, metastasis

SUMMARY:
Patient-derived xenograft (PDX) models more robustly recapitulate melanoma molecular and biological features and are more predictive of therapy response compared to traditional plastic tissue culture-based assays. Here we describe our standard operating protocol for the establishment of new PDX models and the characterization/experimentation of existing PDX models.

ABSTRACT:
Accumulating evidence suggests that molecular and biological properties differ in melanoma cells grown in traditional two-dimensional tissue culture vessels versus in vivo in human patients. This is due to the bottleneck selection of clonal populations of melanoma cells that can robustly grow in vitro in the absence of physiological conditions. Further, responses to therapy in two-dimensional tissue cultures overall do not faithfully reflect responses to therapy in melanoma patients, with the majority of clinical trials failing to show the efficacy of therapeutic combinations shown to be effective in vitro. Although xenografting of melanoma cells into mice provides the physiological in vivo context absent from two-dimensional tissue culture assays, the melanoma cells used for engraftment have already undergone bottleneck selection for cells that could grow under two-dimensional conditions when the cell line was established. The irreversible alterations that occur as a consequence of the bottleneck include changes in growth and invasion properties, as well as the loss of specific subpopulations. Therefore, models that better recapitulate the human condition in vivo may better predict therapeutic strategies that effectively increase the overall survival of patients with metastatic melanoma. The patient-derived xenograft (PDX) technique involves the direct implantation of tumor cells from the human patient to a mouse recipient. In this manner, tumor cells are consistently grown under physiological stresses in vivo and never undergo the two-dimensional bottleneck, which preserves the molecular and biological properties present when the tumor was in the human

patient. Notable, PDX models derived from organ sites of metastases (i.e., brain) display similar metastatic capacity, while PDX models derived from therapy naive patients and patients with acquired resistance to therapy (i.e., BRAF/MEK inhibitor therapy) display similar sensitivity to therapy.

INTRODUCTION:

Preclinical models are critical for all aspects of translational cancer research, including disease characterization, discovery of actionable vulnerabilities unique to cancer versus normal cells, and the development of efficacious therapies that exploit these vulnerabilities to increase the overall survival of patients. In the melanoma field, tens of thousands of cell line models have been heavily utilized for drug screening, with >4,000 contributed by our group alone (WMXXX series). These cell line models were derived from melanoma patients with various forms of cutaneous melanoma (i.e., acral, uveal, and superficial spreading) and diverse genotypes (i.e., *BRAF*^{V600}-mutant and neuroblastoma RAS viral oncogene homolog [*NRAS*^{Q61R}-mutant]), which span the spectrum of disease present in the clinic[1,2].

Unequivocally, the most successful, targeted therapy strategy in the melanoma field has emerged from 1) the genomic characterization of patients' tumors identifying *BRAF* mutations in ~50% of melanomas[3] and from 2) preclinical investigation leveraging melanoma cell line models[4]. The BRAF/MEK inhibitor combination was Food and Drug Administration (FDA)-approved in 2014 for the treatment of patients whose melanomas harbor activating *BRAF*$^{V600E/K}$ mutations and boasts a >75% response rate[5]. Despite this initial efficacy, resistance rapidly arises in nearly every case due to multifarious intrinsic and acquired resistance mechanisms and intratumoral heterogeneity. Unfortunately, cell line models do not recapitulate representative biological heterogeneity when grown in two-dimensional culture in plastic vessels, which masks their clinically predictive potential when investigators attempt to experimentally determine therapies that might be effective in patients with a specific form or genotype of melanoma[6]. Understanding how to best model patient intratumoral heterogeneity will allow investigators to better develop therapeutic modalities that can kill therapy-resistant subpopulations that drive failure to current standard-of-care therapies.

Paramount to the limited predictive value of cell line models is how they are initially established. Irreversible alterations occur in the tumor clonal landscape when a single-cell suspension of a patients' tumor is grown on two-dimensional, plastic tissue culture vessels, including changes in proliferative and invasive potential, the elimination of specific subpopulations, and the alteration of genetic information[7]. Xenografts into mice of these melanoma cell line models represent the most frequently used in vivo platform for preclinical studies; however, this strategy also suffers from the poor recapitulation of complex tumor heterogeneity observed clinically. To overcome this shortcoming, there has been a growing interest in incorporating more sophisticated preclinical models of melanoma, including the PDX model. PDX models have been utilized for >30 years, with seminal studies in lung cancer patients demonstrating concordance between the patients' response to cytotoxic agents and the response of the PDX model derived from the same patient[8]. Recently, there has been a drive to utilize PDX models as the tool of choice for preclinical investigations both in the industry and in academic centers. PDX models, because of their

superior recapitulation of tumor heterogeneity in human patients, are more clinically relevant to use in therapy optimization efforts than cell line xenografts[9]. In melanoma, there are immense hurdles that blunt the therapeutic management of advanced disease[10]. Clinically relevant PDX models have been used to model clinical resistance and identify therapeutic strategies with clinically available agents to treat therapy-resistant tumors[11,12]. Briefly, the protocol presented here to generate PDX models requires the subcutaneous implantation of fresh tissue from primary or metastatic melanomas (collected by biopsy or surgery) into NOD/scid/IL2-receptor null (NSG) mice. Different variations in methodological approach are used by different groups; however, a fundamental core exists[13].

PROTOCOL:

The following animal protocols follow the guidelines of The Wistar Institute's humane ethics committee and animal care guidelines.

1. Melanoma tumor tissue collection

1.1. Collect tumor tissue (termed passage 0) from melanoma patients by one of the following surgery or biopsy methods.

1.1.1. For surgical excision tissue, maintain a minimum of 1 g of tissue (resect metastases and primary lesions) in transport storage media (RPMI 1640 + 0.1% fungizone + 0.2% gentamicin) at 4 °C or on ice.

1.1.2. For surgical biopsy tissue, maintain less than 1 g of tissue (often punch biopsies of subcutaneous [s.c.] metastases and lymph node [LN] metastases) in transport storage media at 4 °C or on ice.

1.1.3. For core biopsy tissue, wash out a cylinder (core) of tissue of approximately 10 mm x 1 mm (often, liver biopsies) into a 15 mL tube containing 5 mL of transport storage media at 4 °C or on ice.

1.1.4. For fine needle aspirate (FNAs) tissue, keep a very small amount of tissue (less than 1 mm in size) taken directly from the patient in a needle and syringe at 4 °C or on ice.

1.2. Deliver the tissue in transport storage media at 4 °C or on ice on the same day or with overnight shipping after surgical excision or biopsy. Process the tissue within 1–2 h of delivery.

2. Tumor tissue processing for mouse implantation

2.1. Surgical excision or surgical biopsy tissue processing

2.1.1. Transfer the tissue to a sterile Petri dish and separate the tumor tissue from surrounding normal tissue as much as possible.

2.1.2. Remove necrotic tissue (usually identified as pale-whitish tissue located centrally within the tumor) from the remaining tumor as much as possible.

2.1.3. Use a scalpel to subdivide an initial tumor chunk into approximately equal pieces (~3 mm x 3 mm) for surgical mouse implantation (**Figure 2**).

2.1.4. Optionally, if enough tumor tissue is available, snap-freeze the tissue for downstream assays (RNA sequencing [RNASeq], whole exome sequencing [WES], etc.).

2.1.5. Make a tumor slurry by mincing the tumor tissue using a cross blade technique with two scalpel blades. Mince the tumor chunks as finely as possible to form a slurry, which is now ready for surgical mouse implantation.

2.1.6. Alternatively, if the tumor tissue is too hard for mechanical dissociation, use a digestion dissociation procedure to form gel-like slurry and a single-cell suspension for implantation and/or injection.

2.1.6.1. Mince the tumor chunks as finely as possible to form slurry.

2.1.6.2. Put the slurry in a 50 mL tube with cold Hank's balanced salt solution $(HBSS)^{-/-}$ (without Ca^{++} and Mg^{++}); then, centrifuge and pellet at 220 x *g* for 4 min at 4 °C.

2.1.6.3. Resuspend the slurry in 10 mL of warmed fresh digest media (200 U/mL collagenase IV + 5 mM $CaCl_2$ + 50 U/mL DNase in $HBSS^{-/-}$) per 1 g of tumor tissue.

2.1.6.4. Place the tube in a 37 °C water bath for 20 min and mix vigorously every 5 min with a disposable pipette.

2.1.6.5. Wash with up to 50 mL of $HBSS^{-/-}$; then, centrifuge at 220 x *g* for 4 min at 4 °C.

2.1.6.6. Add 5 mL of prewarmed TEG (0.025% trypsin + 40 µg/mL ethylene glycol-bis(β -aminoethyl ether)-N,N,N',N'-tetraacetic acid [EGTA] + 10 µg/mL polyvinyl alcohol [PVA]) per 1 g of tumor tissue, gently resuspend/shake, and place the tube at 37 °C for 2 min without mixing.

2.1.6.7. Add at least 1 equal volume of cold staining media (1% bovine serum albumin [BSA] + 10 mM 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid [HEPES] + 1x penicillin-streptomycin in L15 media) to quench the trypsin and centrifuge at 220 x *g* for 4 min at 4 °C.

2.1.6.8. Resuspend the sample in 10 mL of staining media per 1 g of tumor tissue and filter it through a 40 µm cell strainer to get a single-cell suspension for mouse injection (**Figure 2**).

2.1.6.9. Slurry remaining on top of the cell strainer can also be collected for surgical mouse implantation.

2.2. Core biopsy tissue processing

2.2.1. Pour the core cylinder tissue in tissue-transport tube into a 5 cm Petri dish.

2.2.2. Remove excess liquid, scrape tissue to the edge of the Petri dish, finely mince the tumor tissue, add ~100–150 µL of $HBSS^{-/-}$ on top of the tumor tissue, and quickly draw the HBSS/tumor tissue suspension into the 1 mL syringe.

2.2.3. Attach a 23 G needle to the 1 mL syringe, pass the HBSS/tumor tissue suspension through the needle into a 1.5 mL spin tube.

2.2.4. Redraw the tumor suspension into the syringe with the needle still on until it can pass smoothly through the needle.

2.2.5. Finally draw the tumor suspension back into the syringe and detach the 23 G needle.

2.2.6. Attach a 27 G needle and draw an equal volume (~100–150 µL) of artificial extracellular matrix (see **Table of Materials**) into the syringe.

2.2.7. Add an equal volume of artificial extracellular matrix slowly into the 1.5 mL spin tube with the tumor/$HBSS^{-/-}$ suspension, carefully avoiding the formation of bubbles.

2.2.8. Draw one last time back into the syringe and the core biopsy tumor tissue is now ready for mouse injection.

2.3. FNA tissue processing

2.3.1. Place the syringe containing the FNA samples (trapped in the needle) on ice.

2.3.2. Separate the needle (containing FNA tissue) from the syringe and remove the plunger from the syringe, add ~150–200 µL of $HBSS^{-/-}$ to the top of the syringe.

2.3.3. Re-insert the plunger into the syringe and the needle (containing FNA tissue), and push out the $HBSS^{-/-}$ through the needle into a 1.5 mL spin tube. This step removes the FNA tumor from the needle.

2.3.4. Repeat step 2.3.3 with the $HBSS^{-/-}$/FNA suspension twice using the same syringe to maximize the retrieval of tumor tissue from the needle.

2.3.5. Add an equal volume (~100–150 µL) of artificial extracellular matrix to the 1.5 mL spin tube containing the HBSS/FNA suspension.

2.3.6. Adequately mix by slowly drawing up the artificial extracellular matrix/HBSS$^{-/-}$/FNA suspension back into the 23 G needle and repeating twice.

2.3.7. Draw the entire artificial extracellular matrix/HBSS$^{-/-}$/FNA volume back into the syringe, replace the 23 G needle with a 27 G needle, and the FNA sample is now ready for mouse injection.

3. Tumor implantation and injection in mice

3.1. Implantation of surgical excision or surgical biopsy tissue

NOTE: Ensure all surgical instruments are sterile by autoclaving or the use of pre-sterilized disposable instruments.

3.1.1. Shave hair from the lower back of NSG 6-8 week male or female mice leaving an approximately 1.5 cm x 3 cm area with no hair. Anesthetize mice using isoflurane, and confirm by gently squeezing the foot as a test of responsiveness. Use vet ointment on their eyes to prevent dryness.

3.1.2. Place individual mice on a heat pad in the nose cone of the anesthesia machine, scrub the shaved area with chlorhexidine. Then douse with 70% ethanol and allow to evaporate.

3.1.3. Prepare chunks or divide tumor slurry in a Petri dish into individual mounds for surgical implantation (i.e., into three equal mounds if to be implanted into 3 mice).

3.1.4. Using the scalpel blade, make an incision of approximately 5 mm long on the center of the back of the mouse, take one pair of forceps and lift up skin on the side of the incision opposite of operator.

3.1.5. Take the scissors into the other hand and separate the skin from the muscle layer by gently cutting the fascial membrane with small scissor cuts, thereby creating a “pocket” for the tumor tissue.

3.1.6. Pick up one tumor chunk or one individual mound of tumor slurry tissue with the scalpel blade and gently place tissue into the created pocket.

3.1.7. Administer 100 µL of artificial extracellular matrix on the tumor tissue mound in the pocket.

3.1.8. Using two pairs of forceps, pull up the incision on both ends so that the wound edges come close together, and close the wound by applying one or two wound clips.

3.1.9. Subcutaneous inject 1–5 mg/kg meloxicam as an analgesic in mice after surgery.

3.1.10. Take the mouse out of the nose cone and place it back into its original cage, observe the mouse while waking up. Do not return to a cage until fully recovered.

3.1.11. Remove wound clips after approximately 7 days. If healing is not complete after 7 days, leave the wound clip in for an additional one or two days.

NOTE: If using a single cell suspension from surgical excision or surgical biopsy tissue processing, it will mix with artificial extracellular matrix (at 1:1 ratio) for mice injection.

3.2. Injection of FNA or core biopsy tissue

3.2.1. Place an NSG mouse on a steel grid rack, hold the mouse firmly by the tail, and gently pull the mouse back. It will grasp the grid with its front legs firmly. Alternatively, restrain a mouse in the non-dominant hand and let the flank area visible.

3.2.2. Disinfect the skin of the flank with alcohol prep swabs, slowly and steadily inject the contents of the syringe under the skin of the mouse.

3.2.3. Pull out the needle and place the mouse back into its cage.

4. Monitor tumor growth

4.1. Monitor mice once weekly to check for palpable tumors.

4.2. Once tumors are at a measurable size (approximately 50 mm^3), use a caliper to record tumor dimensions. Use the following formula to calculate tumor volumes: (width x width x length) / 2.

4.3. Harvest tumor once the tumor volume reaches around 1.5 cm^3 (approximately 4–10 weeks). The tumor is now called mouse passage 1 (MP1).

5. Harvest tumor for banking tissue, reimplantation, and experiment/characterization

5.1. Euthanize mouse in a CO_2 chamber, check vital signs to confirm death, and then submerge the mouse in a Virkon solution to sterilize the skin for 30 s in the bio-cabinet.

5.2. Use curved scissors and surgical forceps to lift skin adjacent to the tumor and make a horizontal cut.

5.3. Use a blunt separation technique to mobilize the skin on both sides of the tumor and over the tumor, exposing the tumor.

5.4. Use scissors or a scalpel blade to separate the tumor from the fascia.

5.5. Resect the tumor and transfer the tumor to a sterile Petri dish, cut the tumor into small pieces and remove necrotic tissue from the tumor.

5.6. Bank tumor tissue for future implantation.

5.6.1. Take 2-3 small tumor pieces smaller than ~10 mm x 10 mm and mince them into pieces smaller than ~1 mm x 1 mm.

5.6.2. Transfer all minced tissue to a 2 mL cryogenic vial, add 1 mL of freezing media (10% DMSO + 90% FBS).

5.6.3. Mix well and place cryogenic vials into a pre-cooled isopropanol-based cell-freezing container on dry ice.

5.6.4. Store container in -80 °C freezer overnight and then transfer cryogenic vials to liquid nitrogen (LN_2) storage.

5.7. Snap-freeze tissue for downstream assays (RNASeq, WES, etc.).

5.7.1. Place tumor tissue pieces (~3 mm x 3 mm) into a cryogenic vial and put the cryogenic vial in LN_2 immediately. Store vials in -80 °C freezer.

6. PDX therapy trials

NOTE: It will take two expansion phases to grow enough tumor tissue to generate the necessary number of PDX bearing mice for the therapy trial.

6.1. Pick up a cryovial containing banked PDX tissue from LN_2, and place the cryovial in a 37 °C water bath until the freezing media containing the tumor tissue is just starting to melt.

6.2. Empty the contents of the cryovial into pre-warmed $HBSS^{-/-}$ in a 50 mL tube, and wash tissue.

6.3. Pellet tissue by centrifugation for 5 min at 1,200 rpm.

6.4. Remove $HBSS^{-/-}$ from the tumor sample by vacuum aspiration with a Pasteur pipet.

6.5. Slide the PDX tissue from the 50 mL tube into a 5 cm or 10 cm Petri dish and place on wet ice.

6.6. Follow the above implantation protocol to implant banked tissue from a cryogenic vial into 5 NSG mice for the first round of PDX tumor expansion.

6.7. Once tumors reach ~600–800 mm^3, harvest 1-2 tumors to get a single cell suspension with the above protocol for tumor tissue processing: mechanical dissociation, collagenase digestion dissociation procedure.

6.8. Pellet cells and resuspend in 6 mL of $HBSS^{-/-}$/artificial extracellular matrix (1:1 ratio), subcutaneous inject ~50 NSG mice, with 100 µL of cell mixture per mouse for the second round of PDX tumor expansion.

6.9. Measure tumor size with calipers biweekly.

6.10. Wait for a tumor size of ~100 mm^3 in 3-5 weeks, randomize mice into groups, and start treatment.

6.11. When tumor size reaches the maximum IACUC-approved volume, stop treatment.

6.12. Follow the above harvest tumor protocol to collect snap frozen tumor pieces, tumor pieces for paraffin blocks.

REPRESENTATIVE RESULTS:

Tumor tissue for melanoma PDX models can come from a variety of different sources and can also be processed per the growth dynamics of individual models and the desired use of the PDX tissue. The priority when establishing a PDX model is to have sufficient material to bank for future use and DNA for characterization (**Figure 1**).

Once sufficient material is banked, tumor tissue can be expanded in one of three main methods to grow enough tumor to perform a formal therapy study (**Figure 2A**). Each of the methods described herein will allow for the expansion of tumor from PDXs (**Figure 2B**). It is our experience that creating a single-cell suspension of tumor cells with the use of enzymatic digestion (collagenase IV) can allow for more rapid tumor growth, and can allow one initial tumor to be expanded into 10 – 20 mice, whereas the tumor chunk and slurry method can only be expanded into 5 – 10 mice (**Figure 2C**). As has been previously demonstrated in other tumor types, melanoma PDX models often reflect the drug sensitivity the patient displayed when on therapy. Shown here is a representative therapy curve from a melanoma patient with *BRAF*^{V600E} mutant melanoma who initially responded to a BRAF inhibitor but ultimately relapsed. The PDX derived from this patient also displayed initial sensitivity to BRAF inhibition (Rx1) plus an additional inhibitor (Rx2); however, the tumors ultimately relapsed (**Figure 3**).

FIGURE AND TABLE LEGENDS:

Figure 1: PDX model generation workflow for banking tumor tissue and performing therapy studies.

Figure 2: Alternative implantation methods. (**A**) Tumors can be processed into either chunks, a slurry suspension, or as a single-cell suspension. (**B**) All three methods will allow for growth of

tumors in vivo. Shown here are mice subcutaneously implanted with tumor and imaged 12 days after implantation. (**C**) Shown are tumor growth curves for the mice injected with one of the three implantation methods. N = 5 per arm; error bars are standard error.

Figure 3: Representative data for a PDX therapy trial. Mice were implanted with PDX tumors and treated with either a vehicle control or a two-inhibitor combination of a BRAF inhibitor and a MEK inhibitor. N=6 per arm. Randomization was used to place mice into study groups. Of note, although ~500 mm^3 was used in this example as the starting tumor volume for therapy initiation, the routine volume to begin PDX studies is 100–200 mm^3 as PDX tumors are aggressive and their growth is difficult to inhibit once they too big a size (>300 mm^3).

DISCUSSION:

We have herein described generating PDX models of melanoma with patient tissue derived from primary and metastatic tumors, core biopsies, and FNAs. When directly engrafted into NSG mice, tumors present similar morphologic, genomic, and biologic properties to those observed in the patient. In the case when only a small quantity of tissue is available to investigators, as often occurs with FNAs, the PDX technique allows for the expansion of the tumor tissue for DNA, RNA, and protein characterization, as well as for therapy trials to allow preclinical drug development.

Critical to the success of PDX engraftment is the quality of material investigators begin with. Care must be taken to ensure tumor tissue is appropriately preserved as much as possible in sections 1 and 2. Importantly, the response to therapy of PDX melanoma models better recapitulates the sensitivity of the donor patient, allowing for robust preclinical investigations to develop improved therapeutic strategies to combat therapy resistance and improve the durability of response. Most metastatic melanoma patients do not experience cures with existing standard of care (SOC) therapies[5]. Our PDX melanoma collection contains more than 500 distinct models, including those derived from patients who relapsed on targeted therapy and immunotherapy[1,2]. This resource will be critical for the development of therapeutic modalities that overcome resistance to current SOC. Future applications for the PDX model will rely on cost-effective, high throughput approaches that leveraging PDX models in drug screens and CRISPR-Cas9 screens to identify novel effective strategies for different genotypes (i.e., *BRAF*^{V600E}, *NRAS*^{Q61R}) and subtypes (i.e., uveal, acral) of melanoma[14].

One limitation of the PDX technique is the necessity that tumor material is engrafted into mice without an immune system to ensure engraftment success[1]. Therefore, PDX studies optimizing therapeutic strategies to combat therapy resistance do not address how new therapy strategies may positively or negatively impact the immune system and/or anti-tumor immune responses. Fortunately, advances in the field of mouse humanization with a human immune system have been made and will allow for more ideal PDX studies in mice that better recapitulate the human microenvironment[15].

In summary, PDX models allow for preclinical investigations of melanoma cells that better recapitulate the tumor heterogeneity and melanoma aggressiveness observed in the clinic (versus other two-dimensional and standard xenograft approaches). PDX models allow for a

deeper understanding of which genes are involved in therapy resistance and provide a more clinically-relevant model from which more effective therapies can be developed to increase the overall survival of patients with metastatic melanoma.

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DISCLOSURES:

The authors have nothing to disclose.

REFERENCES:

1. Garman, B. et al. Genetic and Genomic Characterization of 462 Melanoma Patient-Derived Xenografts, Tumor Biopsies, and Cell Lines. *Cell Reports*. **21** (7), 1936-52 (2017).
2. Krepler, C. et al. A Comprehensive Patient-Derived Xenograft Collection Representing the Heterogeneity of Melanoma. *Cell Reports*. **21** (7), 1953-67 (2017).
3. Davies, H. et al. Mutations of the BRAF gene in human cancer. *Nature*. **417** (6892), 949-54 (2002).
4. Paraiso, K.H. et al. Recovery of phospho-ERK activity allows melanoma cells to escape from BRAF inhibitor therapy. *British Journal Of Cancer*. **102** (12), 1724-30 (2010).
5. Long, G.V. et al. Long-Term Outcomes in Patients With BRAF V600-Mutant Metastatic Melanoma Who Received Dabrafenib Combined With Trametinib. *Journal of Clinical Oncology.* **36** (7), 667-73 (2018).
6. Hidalgo, M. et al. Patient-derived xenograft models: an emerging platform for translational cancer research. *Cancer Discovery*. **4** (9), 998-1013 (2014).
7. Hausser, H.J., Brenner, R.E. Phenotypic instability of Saos-2 cells in long-term culture. *Biochemical and Biophysical Research Communications*. **333** (1), 216-22 (2005).
8. Fiebig, H.H. et al. Development of three human small cell lung cancer models in nude mice. *Recent Results In Cancer Research*. **97**, 77-86 (1985).
9. Izumchenko, E. et al. Patient-derived xenografts effectively capture responses to oncology therapy in a heterogeneous cohort of patients with solid tumors. *Annals of Oncology*. **28** (10), 2595-605 (2017).
10. Shi, H. et al. Acquired resistance and clonal evolution in melanoma during BRAF inhibitor therapy. *Cancer Discovery*. **4** (1), 80-93 (2014).
11. Monsma, D.J. et al. Melanoma patient derived xenografts acquire distinct Vemurafenib resistance mechanisms. *American Journal of Cancer Research*. **5** (4), 1507-18 (2015).
12. Das Thakur, M. et al. Modelling vemurafenib resistance in melanoma reveals a strategy to forestall drug resistance. *Nature*. **494** (7436), 251-5 (2013).
13. Meehan, T.F. et al. PDX-MI: Minimal Information for Patient-Derived Tumor Xenograft Models. *Cancer Research*. **77** (21), e62-e6 (2017).
14. Gao, H. et al. High-throughput screening using patient-derived tumor xenografts to predict clinical trial drug response. *Nature Medicine*. **21** (11), 1318-25 (2015).

15. De La Rochere, P. et al. Humanized Mice for the Study of Immuno-Oncology. *Trends in Immunology.* **39** (9), 748-63 (2018).

Figure 1

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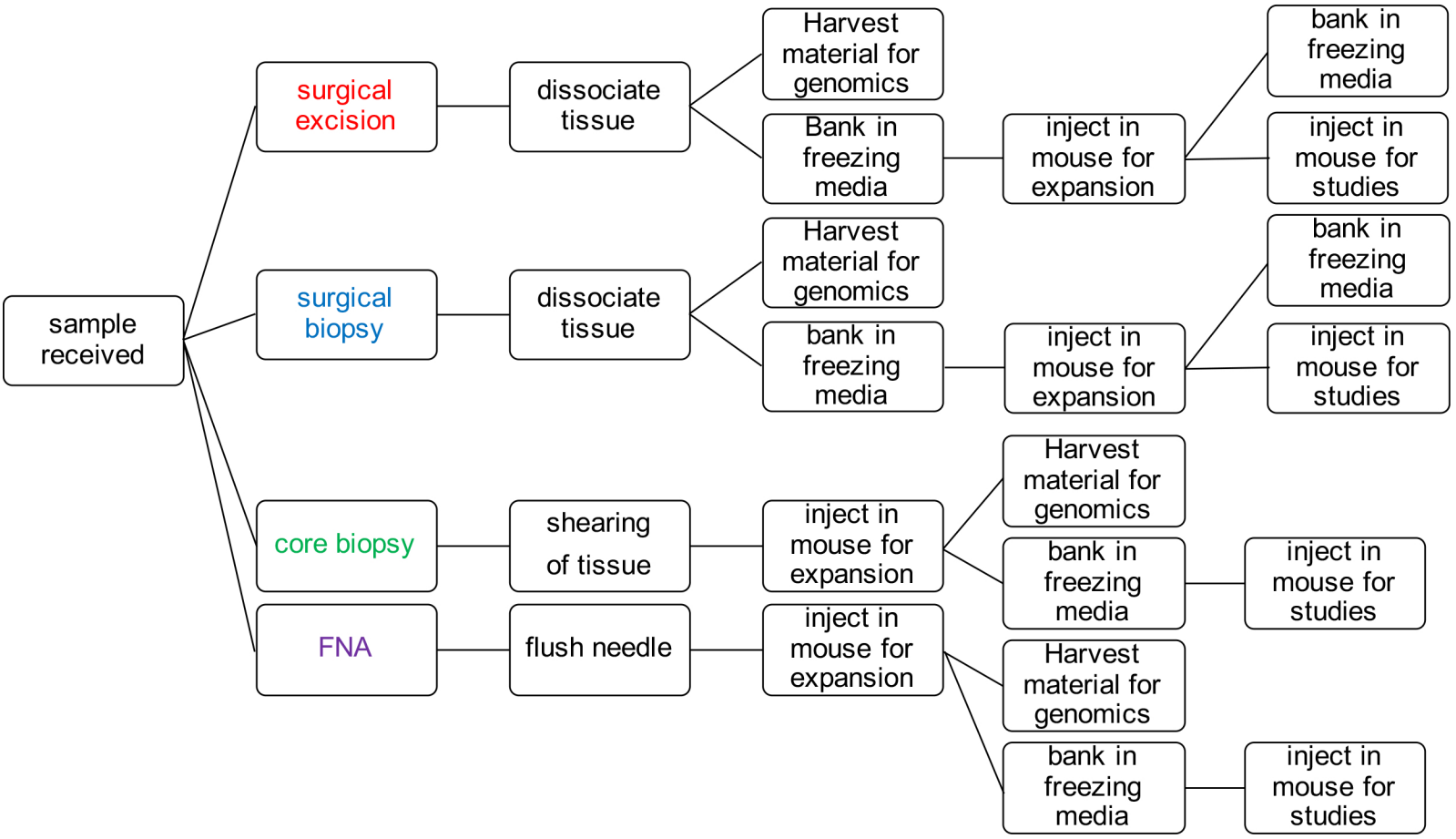


Figure 2

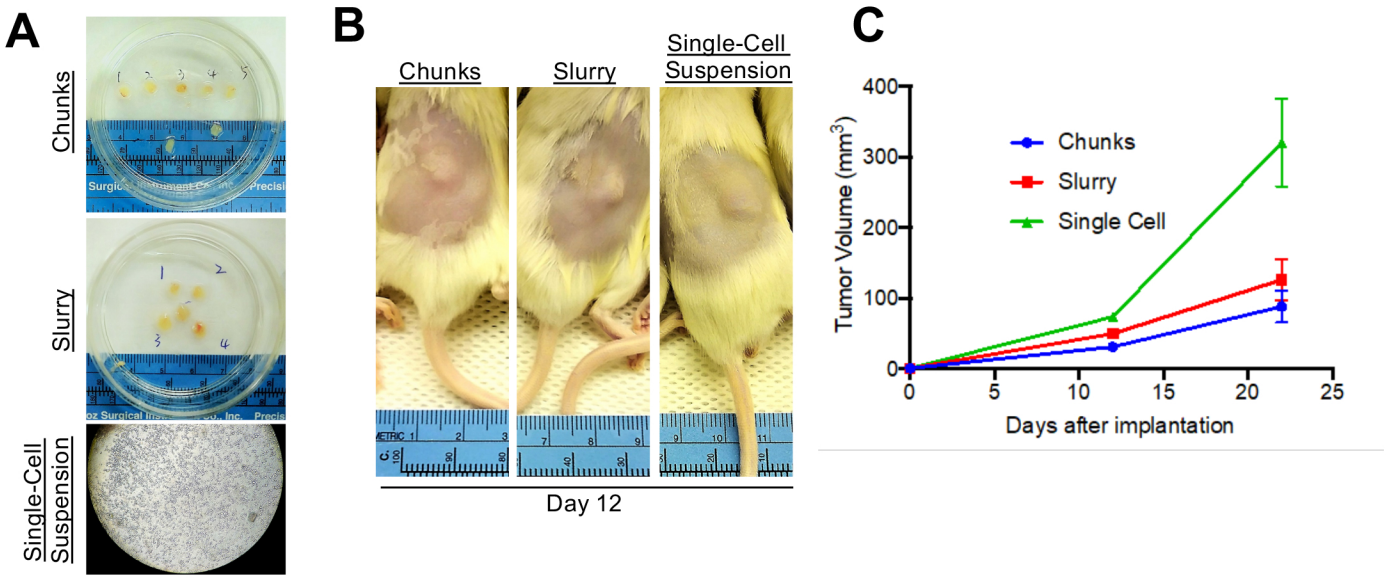
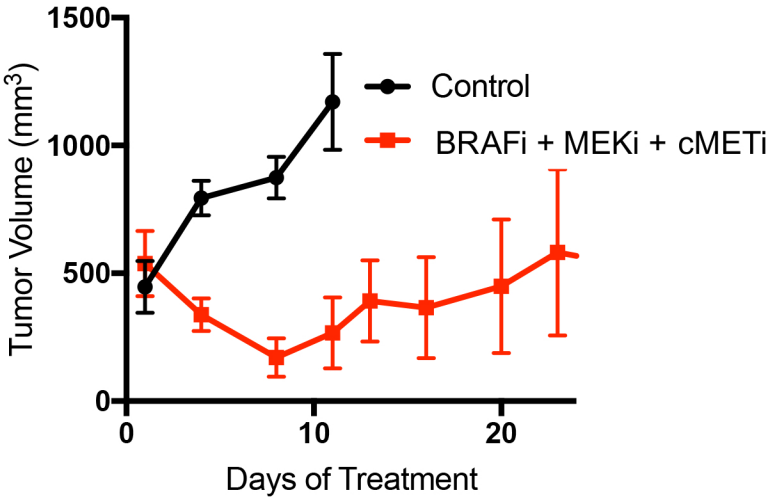


Figure 3



Name of Material/ Equipment	Company
1 M Hepes	SIGMA-ALDRICH CORPORATION
100x PenStrep	Invitrogen
1x HBSS-/- (w/o Ca++ or Mg++)	MED
2.5% Trypsin	SIGMA-ALDRICH CORPORATION
BSA	SIGMA-ALDRICH CORPORATION
Chlorhexidine	Fisher Scientific
Collagenase IV (2,000 u/mL)	Worthington
DMSO	SIGMA-ALDRICH CORPORATION
DNase	SIGMA-ALDRICH CORPORATION
EGTA (ethylene glycol bis(2-aminoethyl ether)-N,N,N’N’-t	Merck
FBS	INVITROGEN LIFE TECHNOLOGIES
Fungizone	INVITROGEN LIFE TECHNOLOGIES
Gentamicin	FISHER SCIENTIFIC
Isoflurane	HENRY SCHEIN ANIMAL HEALTH
Leibovitz's L15 media	Invitrogen
Matrigel	Corning
Meloxicam	HENRY SCHEIN ANIMAL HEALTHRequisi
NOD/SCID/IL2-receptor null (NSG) Mice	The Wistar Institute, animal facility
PVA (polyvinyl alcohol)	SIGMA-ALDRICH CORPORATION
RPMI 1640 Medium (Mod.) 1X with L-Glutamine	Fisher Scientific
Scalpel	Feather
Virkon	GALLARD-SCHLESINGER IND
Wound clips	MikRon

Catalog Number	Comments/Description
Cat # H0887-100ML	
Cat # 15140163	
Cat # MT21-023-CV	
Cat # T4549-100ML	10 mL aliquots stored at –20oC
Cat # A9418-500G	
Cat# 50-118-0313	
Cat #4189	make up in HBSS-/- from Collagenase
Cat # C6295-50ML	
Cat # D4527	
Cat # 324626.25	
Cat # 16000-044	
Cat # 15290-018	
Cat # BW17518Z	
Cat # 050031	
Cat # 21083027	
Cat # 354230	Artificial extracellular matrix
Cat # 025115	1-5mg/kg, as painkiller breeding
Cat # P8136-250G	
Cat# MT10041CM	
Cat # 2976-22	
Cat # 222-01-06	
Cat #427631	

IV powder stock (Worthington #4189, u/mg indicated on bottle and varies with each lot); freeze 1

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Author(s): Min Xiao, Vito W. Rebecca, Meenhard Herlyn

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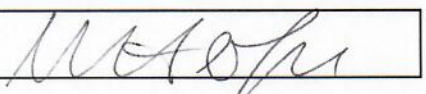
Signature:	
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Your manuscript, JoVE59508 "The Melanoma Patient-Derived Xenograft (PDX) Model," has been editorially and peer reviewed, and the following comments need to be addressed. Note that editorial comments address both requirements for video production and formatting of the article for publication. Please track the changes within the manuscript to identify all of the edits.

After revising and uploading your submission, please also upload a separate rebuttal document that addresses each of the editorial and peer review comments individually. Please submit each figure as a vector image file to ensure high resolution throughout production: (.svg, .eps, .ai). If submitting as a .tif or .psd, please ensure that the image is 1920 x 1080 pixels or 300 dpi.

Your revision is due by **Jan 21, 2019**.

To submit a revision, go to the JoVE submission site and log in as an author. You will find your submission under the heading "Submission Needing Revision".

Best,

Phillip Steindel, Ph.D.
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Editorial comments:

General:

1. Please take this opportunity to thoroughly proofread the manuscript to ensure that there are no spelling or grammar issues.

Confirmed

2. For in-text formatting, corresponding reference numbers should appear as numbered superscripts after the appropriate statement(s).

Fixed

3. Please format text according to JoVE guidelines:12 pt Calibri font, all text aligned to the left margin (including in the protocol), spaces between all paragraphs and protocol steps/substeps.

We have fixed this issue.

4. Please include at least 6 keywords or phrases.

We have fixed this concern.

5. Please define all abbreviations before use (e.g, BRAF/MEK, NRAS).
We have fixed this issue. BRAF and MEK are not typically defined in review and research articles.

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For example: Matrigel, Eppendorf.
We have fixed this issue. However, we are not sure what to call Matrigel, can you please advise.

Abstract:
1. Abstract: Please do not include references in the Abstract
We have fixed this issue.

Protocol:
1. Please adjust the numbering of the Protocol to follow the JoVE Instructions for Authors. For example, 1 should be followed by 1.1 and then 1.1.1 and 1.1.2 if necessary. Please refrain from using bullets or dashes.
We have fixed this issue.

2. Please ensure that all text in the protocol section is written in the imperative tense. The actions should be described in the imperative tense in complete sentences wherever possible.
We have fixed this issue.

3. There is a 10-page limit for the Protocol, but there is a 2.75 page limit for filmable content. After editing and reformatting (see above), please highlight 2.75 pages or less of the Protocol (including headers and spacing) that identifies the essential steps of the protocol for the video, i.e., the steps that should be visualized to tell the most cohesive story of the Protocol. Remember that non-highlighted Protocol steps will remain in the manuscript, and therefore will still be available to the reader.

4. Being a video based journal, JoVE authors must be very specific when it comes to the humane treatment of animals. Regarding animal treatment in the protocol, please add the following information to the text:
b) Please specify the euthanasia method.
We have addressed this issue.

c) Please mention how proper anesthetization is confirmed.
We have addressed this issue.

d) Please specify the use of vet ointment on eyes to prevent dryness while under anesthesia.

We have addressed this issue.

e) For survival strategies, discuss post-surgical treatment of animal, including recovery conditions and treatment for post-surgical pain.
N/A

f) Discuss maintenance of sterile conditions during survival surgery.
N/A

g) Please specify that the animal is not left unattended until it has regained sufficient consciousness to maintain sternal recumbency.
We have addressed this issue.

h) Please specify that the animal that has undergone surgery is not returned to the company of other animals until fully recovered.
We have addressed this issue.

i) Please do not highlight any steps describing euthanasia.
We have addressed this issue.

5. Please include an ethics statement before the numbered protocol steps, indicating that the protocol follows the guidelines of your institution's human research ethics committee.
We have addressed this issue.

6. Please include an ethics statement before your numbered protocol steps, indicating that the protocol follows the animal care guidelines of your institution.
We have addressed this issue.

7. Please add more details to your protocol steps. Please ensure you answer the "how" question, i.e., how is the step performed? Alternatively, add references to published material specifying how to perform the protocol action. If revisions cause a step to have more than 2-3 actions and 4 sentences per step, please split into separate steps or substeps.
We have added more detail to the protocol steps.

Specific Protocol steps:
1. 1: Please include more details or references for each of these surgery/biopsy methods.
Surgery/biopsy was performed by the hospital that contributed the material for PDX establishment.

2. 3.i.1: Please specify the age and sex of the mice. Also, please include a reference describing how this strain is produced (or list them in the Table of Materials).
Fixed in line 173, 174, Table of Materials

3. 5.iv.1: How big is a ‘small tumor piece’ and how small are tumors minced?
Fixed in line 225-226

4. 6.i.1: How is implanting done?
Described in Section 3 Tumor implantation and injection in mice.

5. 6.i.7: Around how long does it take for tumors to reach this size?
Fixed in line 256

6. 6.i.8: How is the tumor harvested?
Fixed in line 258, can find in Section 5. Harvest tumor for banking tissue, reimplantation and experiment/characterization.

Figures:
1. Please remove titles and legends from the Figures themselves-the legends in the manuscript are sufficient.
We have addressed this issue.

2. Figure 1: As there is only one panel here, please remove the ‘A’.
We have addressed this issue.

3. Figure 2C, Figure 3: Please explain the error bars in the legends.
We have addressed this issue.

Discussion:
1. Discussion: As we are a methods journal, please revise the Discussion to explicitly cover the following in detail in 3–6 paragraphs with citations:
a) Critical steps within the protocol
b) Any modifications and troubleshooting of the technique
c) Any limitations of the technique
d) Any future applications of the technique
Discussion has been expanded.

References:
1. Please ensure references have a consistent format. If an article has more than 6 authors, please use only the first author followed by ‘et al.’
We have fixed this.

Table of Materials:
1. Please ensure the Table of Materials has information on all materials and equipment used, especially those mentioned in the Protocol.

Fixed in Table Materials

Reviewers' comments:
Reviewer #1:

Manuscript Summary:
This manuscript describes methods for the development of Melanoma PDX models from fresh human tumor tissue. The authors detail various methods that can be used dependent upon the type of tumor tissue sample provided.

Reviewers comments:

Introduction (additional references).

The Authors need to expand/rewrite the introduction to better highlight the clinical relevance of PDX compared to cell line xenografts (1). Focusing in on melanoma, for which there are many articles describing the challenges of treating melanoma and the development of resistance in patients (2), and how clinically relevant melanoma PDX models have been used to model clinical resistance and identify appropriate (clinically translatable) therapies to treat resistant tumors (3,4). (Suggestions of relevant articles are provided below).
We thank the reviewer for providing these excellent references and have included them in the introduction.

1. Izumchenko E. et al. Patient-derived xenografts effectively capture responses to oncology therapy in a heterogeneous cohort of patients with solid tumors. Ann Oncol. 2017 Oct 1;28(10):2595-2605. (Improved correlation with preclinical drug response and clinical response)
2. Shi H. et al. Acquired resistance and clonal evolution in melanoma during BRAF inhibitor therapy. Cancer Discov. 2014;4:80-93. (Article discussing the clinical manifestation of BRAF inhibitor resistance which can be model in PDX)
3. Monsma DJ. et al. Melanoma patient derived xenografts acquire distinct Vemurafenib resistance mechanisms. Am J Cancer Res. 2015 Mar 15;5(4):1507-18.
4. Das Thakur M. et al. Modelling Vemurafenib resistance in melanoma reveals a strategy to forestall drug resistance. Nature. 2013;494:251-255. (Both articles describe using Melanoma PDX to identify clinically relevant mechanisms of resistance to mutant BRAF inhibitors and therapeutic strategies to overcome.)

Protocol
Please provide as statement regarding whether this work has been approved by your institute IACUC and IRB committees.

Fixed in line 87, 88

Section 1
1. Which RPMI 1640 is used e.g. High or low glucose what other additives?
Fixed in Table of Materials

2. Please provide an appropriate time that the tissue is held in transport storage media between surgical resection and tissue processing.
Fixed in Table of Materials

Section 2
1. Please provide information on how a decision is made between implanting tumor fragments or making a slurry. Is the slurry the preferred method for larger tissue samples?
Fixed in 103-105

2. Line 125 - should this say "tumor chunks" rather than "slurry"?
Fixed in 121, 122. Chunk is more than 3 mm tissue pieces. Slurry is from mincing chunks finely.

Section 3 Part i
1. Please comment on why the authors prefer invasive surgery rather than using a tumor implant needle (Trocar) to implant the tumor fragments.
Our implantation procedure is minor surgery. We use a scalpel to make a minor incision on the skin, use blunt scissors to make “pocket” under skin, then insert tumor chunk/slurry in it. Refer to the NIH PDX protocol, they use scissors to make a cut on the skin, use trocar to make a tunnel under skin, then insert chunk. Our procedure is very similar to trocar procedure.

2. 70% ethanol alone is not enough to sterilize a surgical site. Please provide more details.
Fixed in line 177

3. Did you use analgesics? Please indicate their use which I am sure is a requirement of you IACUC.
Fixed in line 191

Section 3 Part ii
1. Not clear how the mouse is restrained for tumor injection, the current text is inadequate.
Fixed in line 199-201

2. How is the skin sterilized prior to tumor injection?
Fixed in line 202

Section 5

1. How are the mice euthanized prior to tissue collection?

Fixed in line 214

2. I have concerns as to the quality of the cryo-preserved tissue with a rapid freeze on dry ice. A rate-controlled freeze down to -80°C, is better for the recovery of viable tissue.

Fixed in line 229. We put vials in a pre-cooled container on dry ice, not directly place vials on dry ice. This container is isopropanol-based cell-freezing container for slow rate freezing.

Section 6

1. Once sufficient material is obtained from "seed" mice, which is the authors preferred method for tissue transplant? It seems to be that the authors prefer to dissociate rather than transplant tumor chunks, this needs to be clarified. If disassociation is the authors preferred method, then I question why they feel the need to describe the transplant of chunks in this manuscript. PDX chunks are the quickest and easiest method to transplant the tumor (especially if a Trocar is used).

When we bank PDX tissue, we prefer to transplant chunk or slurry. When we perform PDX therapy trials after the second round of expansion, we prefer a single cell suspension from dissociated tumor tissue for subcutaneous injection by using 27 gauge needles. It is an easier and quicker way to generate ~50-60 PDX implanted mice in a short time. We also find it can have more uniform tumor to decrease tumor size variation amongst the groups.

Figure 1.

1. This figure needs to be re-drawn as it doesn't indicate what is described in the figure title. The workflow seems to be more focused on sample collection. According to the figure as drawn, surgical excision and biopsy samples are not implanted into mice and just used for sample collection! This is in disagreement with the protocol as written.

We have redrawn Figure 1 to show our workflow for both sample collection and therapy studies.

2. Can the authors indicate if their PDX workflow also included multiple in vivo passages to bank sufficient tissue.

We do bank material from multiple *in vivo* passages to bank sufficient material.

3. Please clarify what "for DNA" means. Does this include RNA also? If so it might be better to state "for Genomics" or "RNA and DNA samples"

We have changed the phrasing to “Harvest material for genomics” in the Figure.

Figure 2

1. Can the authors address if they see any morphological differences between the different transplant methods?

We do not observe any significant morphological differences between the different transplant methods. We have added this in the discussion.

2. Please provide a graph in Fig C showing the full growth characteristics of the tumors from the different methods. It is clear from the protocol that tumors are not harvested at 100mm3, the figure only shows Slurry and Chunk growth out to 100mm3.
We did not extend the study shown in Figure 2C beyond 22 days of implantation. The study was performed to determine whether there were significant differences in variability and speed of tumor growth between the different implantation methods.

Figure 3
1. Please provide more information regarding this study, including, drugs and treatment schedules, what is an appropriate starting tumor volume for PDX (500mm3 is too large) and whether randomization was used to place mice into study groups.
We have added the additional detail.

Table of materials
1. Include Info regarding type of RPMI 1640 used.
Fixed in Table of Materials

2. Other reagents are mentioned in the protocol and not listed here e.g. DNase, FBS, DMSO etc.
Fixed in Table of Materials

Reviewer #2:

Manuscript Summary:

PDX and organoids represent recently the most relevant preclinical models in cancer research.

Authors described method for melanoma PDX establisment from tumor tissue obtained by several methods. The protocol is unambiguously written, it is possible to recapitulate the method by following it.

Minor Concerns:
only minor typos occur in the manuscript
row 139 - why is medium for trypsin neutralization is named 'staining' medium?
We once used this media to label antibodies to cells for flow cytometry. We called it staining buffer.

row 263 the method how to harvest cryopreserved tissue prior mouse injection should be described in more detail
Fixed in line 240-245

Reviewer #3:

Manuscript Summary:
The manuscript is a detailed description of the generation of patient-derived xenografts (PDX) from melanoma samples of various origin (surgical excision and biopsy, core biopsy and fine needle aspirate). The authors describe at length how to best approach the generation of PDX starting from abundant or scarce tumor tissue, how to process it, transplant it into immune-compromised animals and bio-bank it. The procedure is well explained, as the different options to take when the sample is very small. The protocol is easy to follow and reagents clearly specified. The second part of the manuscript is dedicated to drug treatment of a representative PDX, where
The protocol stems from previous work of the authors, where

Major Concerns:
None

Minor Concerns:
The authors state that they have generated PDXs from either primary or metastatic melanoma samples, but they don't mention which is the take rate of their melanoma samples and if it is different in the case of a primary or metastatic tumor. Moreover they don't specify which is the variability among the injected animals when transplanting small samples derived from aspirates. Did all injected animals engraft? This information is particularly relevant if one wants to start a colony of melanoma PDXs.
In our previous PDX paper, we showed that the overall success rate for establishing melanoma PDX was 83% when we corrected adverse events like sample contamination, unexpected death of a primary recipient animal, etc. We found no significant difference in tumor growth latency with a mean ranging from 8.3 to 9.5 weeks between FNA, core, and excisional biopsies. We observed consistent tumor engraftment in mice at 1000, 100, 10, and 1 cell(s)/mouse if we injected tumor cells enzymatically digested from patients' tumor tissue. Most of our samples were metastases. We didn't compare the take rate between primary and metastatic samples.

On the same line, genetic variability over serial passages in the animal should be mentioned. Are the tumor stable enough to give reproducible results also at late passages? This is relevant for the treatment schedule.
We didn't compare genetic variability over serial passages. We also didn't have enough data to address reproducible results at late passages.

When describing the tissue processing for implantation, the authors claim they mix tumoral cells with Matrigel when dissociating cells from core biopsy and needle aspirates, but not when cells are coming from surgical excision. If the Matrigel is used as a matrix support to embed

cells and partially recreate the tumor-surrounding environment, why is not used in the case of dissociated cells? It should be clarified.
Fixed in line 201. We also use Matrigel when using single cell suspensions from surgical excision.

Seeing that therapy trial is indicated in the protocol, it would be helpful to indicate how many mice are required per treatment group. If 6 are treated (figure 3), how many mice are injected? Only 6 or more to then choose the animals where the tumors are growing similarly both in term of latency and size?
This is an excellent question. For every treatment arm, we implant 8 mice with PDX if we want to have 6 enrolled in the study. Therefor in the study shown in Figure 3, 16 mice were implanted to ensure 12 mice would have equivalently sized tumors in an effort to reduce variation.

Figure 1 is not mentioned at the beginning of the protocol, as expected, but only at the end (line 287). Both Figure 1 and 3 contain only one panel, it is unnecessary to indicate A at the top of the figures, being no B or C to show.
We have removed the letters.