Published in final edited form as:

Methods Mol Biol. 2018 ; 1786: 1–27. doi:10.1007/978-1-4939-7845-8_1.

Generation of Prostate Cancer Patient-Derived Xenografts to Investigate Mechanisms of Novel Treatments and Treatment Resistance

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Abstract

Treatment advances lead to survival benefits of patients with advanced prostate cancer. These treatments are highly efficacious in a subset of patients; however, similarly to other cancers, after initial responses the tumors develop resistance (acquired resistance) and the patients succumb to the disease. Furthermore, there is a subset of patients who do not respond to the treatment at all (de novo resistance). Preclinical testing using patient-derived xenografts (PDXs) has led to successful drug development, and PDXs will continue to provide valuable resources to generate clinically relevant data with translational potential. PDXs demonstrate tumor heterogeneity observed in patients, preserve tumor-microenvironment architecture, and provide clinically relevant treatment responses. In view of the evolving biology of the advanced prostate cancer associated with new treatments, PDXs representing these new tumor phenotypes are urgently needed for the study of treatment responses and resistance. In this chapter, we describe methodologies used to establish prostate cancer PDXs and use of these PDXs to study de novo and acquired resistance.

Keywords

Prostate cancer; Abiraterone; Enzalutamide; Resistance; Testosterone

1 Introduction

Advanced prostate cancer is a heterogeneous disease and there is no curative treatment available at this time. Treatment advances have led to the approval of new agents that extend the median survival of patients with this disease for only 3–6 months [1, 2]. However, within the population there are patients with tumors that do not respond to these therapies exhibiting de novo resistance, and patients with tumors that initially respond but after the initial response develop acquired resistance. Patients demonstrating de novo or acquired resistance ultimately succumb to the disease.

Preclinical research is needed to better understand the complex biology of advanced prostate cancer and molecular signatures of responses and resistance to therapies. With few exceptions, preclinical studies in advanced prostate cancer used cell lines to generate xenografts and in many cases these studies provided results that were hardly replicable in clinical trials. Reasons for such discrepancies are that (1) the in vivo use of cell lines does not necessarily represent the high level of cellular heterogeneity observed in the original tumors in patients, (2) xenografts from cell lines do not retain the epithelial-stromal

interaction and the tumor architecture of patient tumors, and (3) the design of preclinical or clinical trials that may not mirror each other. In order to generate clinically relevant data, preclinical research has to be focused on the generation and the use of patient-derived, clinically predictive models—patient-derived xenografts (PDXs).

For studies of advanced prostate cancer, locally advanced and metastatic tumors obtained from patients represent a valuable source for PDXs establishment. To establish PDX models, fresh tissues harvested from patients are directly implanted into immunocompromised mice. Established PDXs retain the 3D tumor-microenvironment architecture of the original tumor and also replicate the intra-tumoral heterogeneity of the patient's tumor. Our and others data show that the morphology and heterogeneity of PDXs are maintained over repeated passages [3, 4]. However, it is well recognized that prostate cancer PDXs are very difficult to establish with a very low take rate, and in many cases after initial growth, the PDXs fail to grow after the first transplant or are lost after serial passaging—leading to the high cost of developing and sustaining serially passaged models. Nonetheless, once established prostate cancer PDXs provide reliable preclinical models to study the biology of this disease and to evaluate treatment responses that predict tumor resistance and tumor sensitivity up to 97% and 90% accuracy, respectively [5]. However, one has to be aware that when designing preclinical studies and in order to generate clinically relevant results, it is absolutely critical to use multiple prostate cancer PDXs that exhibit the wide range of characteristics mimicking the notoriously heterogeneous character of this disease in patients. Despite the difficulty of prostate cancer PDX establishment, our group (LuCaP xenograft series; *see* Table 1) and other groups (*see* Table 2) have demonstrated success in establishing, characterizing, and conducting preclinical studies using prostate cancer PDXs [3, 4, 6–22]. To make these models available to the research community, The Movember Foundation—the leading global organization committed to changing men's health—funded the GAP1 Global Prostate Cancer Xenograft Initiative, which focused on making tissue microarrays of all existing prostate cancer PDXs for molecular and histological characterization.

It is well accepted that treatment resistance is associated with phenotypic changes of the tumors with alternative pathways being involved in tumor survival and progression. The emergence of new drug resistance urgently prompts the development of new PDXs that capture the biology underlying treatment responses and resistance of these most recent tumors. For example, PDXs of tumors from patients that do not respond to the most recent second-generation antiandrogens abiraterone and/or enzalutamide are needed to investigate mechanisms of *de novo* resistance, while PDXs of tumors from patients that originally responded but subsequently developed resistance are needed to investigate mechanisms of acquired resistance. In addition, with more vigorous inhibition of androgen receptor signaling, it appears that prostate cancers in some cases undergo trans-differentiation to neuronal phenotype that displays minimum to no androgen receptor signaling activity [3, 23]. Currently, very limited number of these neuronal-like PDXs is available [3, 24–26], and very little is known about the biology behind and the potential treatment targets of this very aggressive disease. In this chapter, we will provide methods to establish PDXs and methods how to address *de novo* and acquired drug resistance.

2 Materials

2.1 Chemicals and Kits

1. Sterile 0.9% sodium chloride for injection.
2. Ketaset (Ketamine, 100 mg/mL).
3. Xyla-Ject (Xylazine, 20 mg/mL).
4. 1× GIBCO Dulbecco's Phosphate Buffered Saline (DPBS), stored at 4 °C.
5. Gentamicin (40 mg/mL), injection, USP, stored at 20–25 °C.
6. Betadine surgical scrub, 7.5% povidone iodine.
7. 70% ethanol.
8. BrdU (5-bromo-2'-deoxy-uridine), 97%. 80 mg/kg solution is prepared in sterile saline just before injection.
9. Buprenex (Buprenorphine 0.3 mg/mL).
10. Architect Total PSA Assay (Abbott Laboratories, Abbott Park, IL).

2.2 Surgical and Injection Materials

1. 4/0 Coated visorb polyglycolic acid suture.
2. 13-Gauge cancer implant needle.
3. Deltaphase isothermal pad.
4. Deltaphase operating board.
5. Microdissecting forceps, 4", serrated.
6. Tungsten carbide iris scissors, 4.5".
7. Autoclip applier, 9 mm autoclips, and autoclip remover.
8. Sterile petri dish.
9. Rib-back carbon steel surgical blade, 22, sterile.
10. Calibrated micropipet.
11. Tailveiner restrainer.
12. 26-Gauge needle.
13. 1.0 mL Syringe.
14. Feeding needle, 20 gauge 1 ½ in.
15. Electric clippers.
16. Sterile gauze.

2.3 Mice

Xenotransplantation of human cancer tissues is mostly performed in immunocompromised mice and multiple strains of mice are available.

1. NU/NU nude mouse (Crl:NU-*Foxn1^{nu}*) (*see* Note 1).
2. NMRI/NU nude mouse (Crl:NMRI-*Foxn1^{nu}*) [27] demonstrates enhanced xenotransplantation take rate [28].
3. Fox Chase SCID mouse (CB17/Icr-*Prkdc^{scid}*/IcrIcoCrl) is a severely combined immune deficient (SCID) mutant mouse that is deficient for T and B lymphocytes [29]. The mice exhibit normal NK cell and myeloid cell functions [30–32]. Up to 25% of CB17 SCID mice are “leaky” (i.e., spontaneously produce both T and B cells) [33, 34] and these mice in some occasions develop T-cell lymphoma.
4. Hairless SCID mouse, SHO^{TM} Mouse (Crl:SHO-*Prkdc^{scid}Hr^{hr}*) has been recently developed by Charles River Laboratories. This SCID mouse might be beneficial to use when one considers in vivo imaging because of the autofluorescence and luminescence of CB17 SCID mice are high.
5. *Rag2* knock-out mouse (B6.129S7-*Rag1^{tm1Mom}/J*) displays a SCID phenotype which completely lacks functional T and B cells and does not display a leaky phenotype [35].
6. BALB/c *Rag2* and the X-linked *Il2rg* double knock-out mouse (*Rag2^{tm1Flv}Il2rg^{tm1Flv}*) lacks T, B, and NK cells [36].
7. Fox Chase SCID beige mouse (CB17. Cg-*Prkdc^{scid}Lyst^{bg-J}*/Crl) is a SCID mouse (without functional T and B cells) with <2% incidence of “leakiness.” This mouse produces NK cells but the cytolytic activity of NK cells is reduced [37].
8. Non-obese diabetic SCID (NOD-SCID) mouse [38] is a relatively popular mouse for the study of human hematopoiesis and on occasion for xenotransplantation. These mice present markedly reduced NK cell activity and functionally less mature macrophages when compared to those in SCID CB17 mice. These SCID mice often develop T-cell lymphoma at old ages (*see* Note 2).
9. IL-2 NOD-SCID mice (NOD.Cg-*Prkdc^{scid}Il2rg^{tm1Wjl}*/SzJ, Jackson Laboratory, Bar Harbor, ME) are also used for xenografting. However, these SCID mice in some occasions develop T-cell lymphoma (*see* Note 2).

3 Methods

We follow universal safety precautions and all procedures are performed in certified ASBL2/BSL2 biosafety cabinets. All animal procedures are in compliance with and approved by the Institutional Animal Care and Use Committee and National Institutes of Health guidelines prior to initiation of studies. This methods section has been updated from our previously published protocols [39].

3.1 Anesthesia

1. Inject Ketamine/Xylazine solution (130 mg/8.8 mg/kg) intraperitoneally prior to surgery.
2. Wait approximately 3–5 min for mice to reach the surgical plane of anesthesia in which they will remain for approximately 15–20 min.

3.2 Preparation for Surgery

1. Autoclave and sterilize all surgical instruments prior to surgeries.
2. Sterilize all instruments during surgical procedures between cages using a glass bead sterilizer.
3. Once the mouse reaches the surgical plane of anesthesia, shave the surgical site (if necessary, e.g., SCID mice), and sterilize the site with a betadine solution and 70% ethanol by swabbing three times alternatively.
4. Place mice on a heated sterile stainless steel surgical plate in a biosafety cabinet.
5. Perform surgery (see below for details).

3.3 Castration

The majority of advanced prostate cancer tumors are castration-resistant. Therefore, in many cases, the animals used for preclinical evaluation of therapy efficacy for advanced prostate cancer are performed in castrated mice in order to mimic the human situation (see Notes 1 and 3).

1. Male mice are castrated once they reach 6–8 weeks of age (see Note 4).
2. Anesthetize animals and allow them to reach the surgical plane of anesthesia.
3. Swab the testicular region with betadine solution and 70% ethanol three times alternatively.
4. Make a small incision on the scrotal sac using a sterile scalpel or surgical scissors.
5. Apply slight pressure to the abdomen to move testicles to the scrotum.
6. Gently pull the testicle and attached fat pad through the scrotal incision.
7. Cut the vas deferens and associated blood vessels to remove the fat pad and testicle.
8. Repeat the procedure to remove the second testicle, using the same incision.
9. Once castration is complete, the scrotum is pulled out and away from the body and closed with a 9 mm autoclip.
10. Allow mice to recover on an isothermal heat pad until ambulatory.
11. A 2-week period is allowed for the mouse to recover and for the androgens to subside prior to introducing a castration-resistant tumor to the mouse.

3.4 Tumor Sources

Multiple sources of tumors can be used for prostate cancer PDX establishment, including biopsies of primary cancer and metastases, primary cancers harvested at radical prostatectomy or transurethral resection of the prostate, as well as metastases harvested at surgery (very rare) and autopsy of patients who died of advanced prostate cancer (*see* Tables 1 and 2, Notes 5 and 6) [3, 6–17]. Tumor bits are implanted into mice with a condition recapitulating the clinical situation (*see* Notes 7–11). For preclinical studies, implantation of PDX tumor bits generated from stock tumors growing in mice are used, or alternatively the PDX tissues are dissociated to single cell suspensions for injections to femur or tibia. When designing a preclinical study, the principal biological question has to be identified before proceeding to the selection of models (*see* Notes 12–15).

Despite the high maintenance costs of PDX colonies, PDXs are still considered a clinically preferable resource for preclinical testing among academic institutions and industries (*see* Notes 16 and 17). PDXs established in mice can either be tested on the first passage for personalized therapy (which likely captures the most heterogeneity and complexity of the original patient's tumor) or passaged to produce more of the same tumor to set up a larger study for basic cancer research or preclinical drug testing (natural selection may occur to enrich a certain population of cells and therefore less heterogeneous than the first passage) [40]. Larger preclinical study can be set up by transplanting the tumor bits as follows:

1. Extract tumors under sterile conditions from the host and section into ~20 mm slices in a sterile Petri dish using dissecting scissors or a sterile surgical blade.
2. Immediately place tissue sections in ~10 mL 1× DPBS containing 4 mg/mL gentamicin for 5 min and then rinse with 1× DPBS and cut into 20 mg pieces (about 3–4 mm per cubed side).
3. These tissue pieces are ready for implantation and should be introduced to the host as soon as possible.

3.5 Subcutaneous Implantation

The subcutaneous implantation is very simple to perform and in majority of cases used for PDX establishment and in preclinical studies (*see* Tables 1 and 2). It allows easy monitoring of tumor take and growth rates due to the easy access to the tumor. It also generates relatively large amounts of tumor tissue for analyses. We and others have successfully used subcutaneous implantation for PDX generation [4, 9–12, 16, 26] and for evaluation of multiple new agents, as well as to study the biology of prostate cancer and AR signaling [41–48]. In our hands, the original take rate of patient tissues is ~20% and the rate of transplantable PDXs (for more than three passages) is ~10% with the time to initial growth within 3–20 months. In our efforts to establish PDXs from metastatic tumors obtained from rapid autopsy, we have not observed any significant differences in the establishment rate or the latency period prior to the growth among different metastatic sites (lymph node, liver, bladder, bone metastases). We have generated 22 stable prostate cancer PDXs that mirror multiple phenotypes of patient tumors (Table 1). For a more complete characterization of each PDX model, please refer to <http://depts.washington.edu/uroweb/uploads/FileUpload/>

[aacr2014preclinicalmodelsofpros-tatecancerlucapdxs.pdf](#). The procedure for developing subcutaneous PDXs is as follows:

1. Prepare the mouse for surgery (*see* Subheading 3.2). Shave, if the mouse has hair, the area from the hind leg to foreleg for the subcutaneous implantation of tumor.
2. A trocar is a hollow stainless steel tube with a sharp tip which is combined with a blunt plunger that is used to implant solid tumor bits subcutaneously.
3. Draw a 20 mg tumor bit into a 13-gauge sterile trocar and insert the trocar subcutaneously closer to the hind leg.
4. Inject the tumor bit around the rib cage area and remove the trocar.
5. The site of the puncture heals quickly and no other postoperative care is necessary.
6. Subcutaneous tumor volumes are measured twice weekly using handheld digital calipers and calculated using the formula: Length $\times$ Width $\times$ Height $\times$ 0.5236.
7. If the tumor produces prostate-specific antigen (PSA), blood is collected once weekly to correlate the tumor growth with the PSA response (*see* Subheading 3.10).

3.6 Subrenal Capsule Implantation

Subrenal implantation is also used by investigators when attempting establishment of new PDXs because it is a heavily vascularized site that supports tumor growth (*see* Table 2) [14, 18, 19]. While PDX establishment is still feasible provided that the mouse is sacrificed at a certain time frame, it is impossible to trace the tumor volume for investigation of drug responses by calipers. Serum PSA levels (if the tumor produces PSA) or *in vivo* imaging techniques (if tumor cells have been previously labeled with luciferase or GFP; not applicable to tumor bits) may be applied for tumor measurement. The procedure for developing PDXs in the subrenal capsule is as follows:

1. Anesthetize the mouse (e.g., NOD-SCID male mouse, *see* Table 2), shave and sterilize the right side (*see* Subheading 3.2).
2. Once the mouse reaches the surgical plane of anesthesia, make a small incision using tungsten carbide scissors in the cutaneous layer just below the last rib. Cut the muscle and slip the kidney out. Keep the kidney moist with sterile 0.9% sodium chloride flushes.
3. Make a small opening in the renal capsule using sterile forceps and a 5–10 mg tumor bit is inserted into the pocket.
4. Gently push the kidney back into the incision and suture the muscle with a 4/0 coated visorb polyglycolic acid suture.
5. Clip the cutaneous layer with a 9 mm autoclip.

6. Allow recovery of the mice on heat pads. Once the animal is awake, buprenorphine (0.05 mg/kg) is subcutaneously injected; the second dose will be administered 4–6 h later, and additional doses every 8–12 h for a total of 48 h, then as needed. Remove autoclips 7–10 days later.
7. For tumors producing PSA, blood for serum PSA levels is collected once weekly to monitor tumor growth in the subrenal capsule (*see* Subheading 3.10). Alternatively, for cells pre-labeled with luciferase or GFP, tumor growth can be monitored by *in vivo* imaging.
8. Excise and weigh tumors after sacrifice.

3.7 Intra-tibial Injection

Prostate cancer autopsy results from ours and others showed that bone is one of the main sites of prostate cancer metastasis [49–54]. To mimic the tumor cell interaction with the bone microenvironment, prostate cancer bone PDXs can be established by injecting single cell suspension of metastasis from patients directly into the bone [8]. More often, direct intra-bone injection of PDX cells derived from other organs is used to investigate biology of tumor-bone interaction and evaluation of efficacy of new agents against tumors growing in the bone (tibia or femur; [55–62]). The main purpose of this intra-bone injection of tumor cells is to study the tumor-bone interaction, rather than to study the biological process of bone metastasis.

Similarly to growth under the subrenal capsule, tumors growing in the bone cannot be measured directly. Therefore, serum PSA, radiography, or *in vivo* imaging is used to evaluate/correlate tumor take and growth of PDXs in the bone. Since bone can only hold a limited number of cells ($\sim 2 \times 10^5$ cells), PDX establishment is relatively difficult. The procedure for developing PDXs in the tibia for drug testing is as follows:

1. Four-to-six weeks old mice are used for intra-tibial injections. It is important to use young mice to ensure that the tibiae are not yet fully calcified. Injection into tibiae of older mice often results in breakage of the tibiae after injection and tumor growth occurs in the muscle instead of the bone (*see* Note 4).
2. Lay mice in the supine position on an isothermal heat pad and plate and the right leg is soaked with 70% ethanol after the surgical plane of anesthesia is reached.
3. Inject 2×10^5 cells in 20 μ L volume using a 1 mL syringe fitted with a 26-gauge needle. We do not recommend injecting larger volumes or larger number of cells since that often results in overfilling the tibia cavity and bone breakage.
4. Stabilize the right foot of the mouse with one hand while the other hand moves the syringe and needle in a drill like motion on the proximal end of the tibia.
5. Inject the cells into the tibial cavity once the needle is inserted approximately 3 mm into the tibia.
6. Allow mice to recover on heat pads and once animal is awake, buprenorphine (0.05 mg/kg) is subcutaneously injected; the second dose will be administered 4–6 h later, and additional doses every 8–12 h for a total of 48 h, then as needed.

7. For tumors producing PSA, blood for serum PSA levels is collected once weekly to monitor tumor growth in the bone (*see* Subheading 3.10).

3.8 Drug Administration

Multiple routes of administrations are used to deliver drugs to mice. The choice depends on bioavailability, solubility, frequency, and length of administration needed [63]. Standard routes of systemic drug delivery in preclinical models include oral gavage, intraperitoneal (IP) injection, intravenous (IV) injection, and subcutaneous injection (Table 3; [63–65]). We use oral gavage whenever possible, because of the clinical relevance of this drug delivery method and the drug can be administered either as solution or as suspension. Procedures of different routes of drug administrations are listed below.

3.8.1 Oral Gavage

1. Gently, yet firmly restrain the mouse to temporarily immobilize the head and the body.
2. Draw the drug into a syringe. Using a gavage needle attached to a syringe, follow the roof of the mouth down to the esophagus using a single smooth movement. If resistance is felt, remove the gavage needle entirely and start over again.
3. Once the tip of the gavage needle is immediately below the last rib, deliver the dose (maximum 200 μ L per mouse) and slowly remove the gavage needle.
4. If gasping, coughing, or sputtering is observed, the mouse must be immediately and humanely euthanized as the dose most likely was incorrectly administered.
5. Frequency of gavage is recommended to be once daily. However, if double dosing is required per day, IACUC usually limits the treatment period to 4 weeks.

3.8.2 IP Injection

1. Animals are gently restrained and tipped backwards.
2. Imagine a straight line from knee to knee and about 1 cm from the mid-line. At that spot, insert the needle approximately $\frac{3}{4}$ cm into the abdomen and deliver the dose (maximum volume generally recommended is 10 mL/kg per mouse).
3. Withdraw needle and dispose of sharp safely.

3.8.3 IV Injection

1. Restrain mouse in a Tailveiner Restraint for mice.
2. Heat tail briefly to help enlarge veins then wipe with 70% ethanol.
3. Find the lateral tail vein and insert the needle running parallel with the vein.
4. Dose animal slowly (maximum volume generally recommended is 10 mL/kg per mouse), withdraw needle, apply pressure to injection site with sterile gauze, and release mouse from restrainer.

3.8.4 Subcutaneous Injection

1. Pull the skin from the shoulder.
2. Inject the drugs into the pocket under the skin (maximum volume generally recommended is 10 mL/kg per mouse).

3.9 Drug Resistance

Drug resistance includes *de novo* (intrinsic) or acquired resistance. *De novo* resistance refers to a tumor where growth does not respond to a drug, while acquired resistance refers to a tumor where growth responds to a drug for a certain period of time and then tumor growth resumes.

Prostate cancer is heterogeneous—tumor morphology and phenotypes vary among patients or within an individual [66, 67]. Abiraterone and enzalutamide are the two most recent second-generation antiandrogens that are used to treat castration-resistant prostate cancer; however, *de novo* resistance (based on the PSA response rate) was observed in 71% and 46% of patients, respectively [1, 2]. Furthermore, all patients who initially responded to the drugs developed acquired resistance and the disease progressed. Therefore, it is imperative to develop new PDXs to study these two types of resistance. In our preclinical testing, we have observed that some PDXs exhibit *de novo* resistance (Fig. 1a) to a treatment while others respond followed by acquired resistance (Fig. 1b). Furthermore, we have also observed that some PDXs exhibit inter-tumor heterogeneity: from mice implanted with the same donor tumor, tumors in some mice showed *de novo* resistance while others originally responded but developed acquired resistance (Fig. 2). The advantage of using transplantable PDXs to study drug resistance is that one can generate a larger study to harvest the tumor samples at different time points to evaluate the mechanisms underlying responses and resistance.

To establish new treatment-resistant PDXs, direct implantation of fresh treatment-resistant tumor extracted from patients will be ideal. However, these tumors are difficult to obtain and usually the establishment of the PDXs takes months, and with low probability of success (see Note 5). An alternative approach is to generate resistant tumors from existing PDXs. For example, when PDXs progress on treatment, the resistant tumors can be harvested and propagated in mice under constant drug-selective pressure. In our LuCaP PDX series, we have models that exhibit *de novo* resistance as well as those that developed acquired resistance to specific treatments (e.g., castration, docetaxel, abiraterone, and enzalutamide).

3.10 Retro-orbital Bleed

Retro-orbital bleeds are performed once a week or every other week to collect blood for interim analyses including pharmacokinetics, pharmacodynamics, or levels of PSA (to evaluate tumor burden in Subheadings 3.5–3.7). In accordance with the IACUC's guidelines, no more than 1% of the animal's body weight should be collected within a 2-week period. The procedure for retro-orbital bleed is as follows:

1. Place a narrow pipette or a micro-hematocrit tube in the medial or lateral canthus of the eye.

2. Using a slight rotation and slight pressure, apply the tube in the canthus to bleed from the retro-orbit of the mouse.
3. Once blood is acquired, sterile gauze is applied with gentle pressure to obtain hemostasis.

3.11 Euthanasia

Animals are euthanized once tumor volumes (subcutaneous tumors that can be measured) reach 1000 mm³, if animals lose 20% or more of body weight, or if animals become otherwise compromised (hunched in posture, piloerected, rapid respiration, lethargic, etc.). The procedure for euthanasia is as follows:

1. Anesthetize mice by intraperitoneal injection with 130 mg/8.8 mg/kg Ketamine/Xylazine solution.
2. Exsanguinate mice under the surgical plane of anesthesia via cardiac puncture then cervically dislocate.

3.12 Cardiac Puncture

1. Cardiac puncture can be performed for terminal blood collection (~0.5–1 mL) and is performed only after the mouse is anesthetized.
2. A 1 mL slip tip syringe fitted with a 26-gauge needle is used for this procedure and the mouse is placed in the supine position.
3. Insert the needle at the distal end of the rib cage and slowly move it upwards towards the left ventricle of the heart while the syringe is very slightly drawn back to enable the observation of blood entering the hub.
4. Slowly pull the plunger once blood is observed while keeping the syringe and needle in place.

3.13 Cervical Dislocation

1. Cervical dislocation is a rapid and humane approach to euthanizing mice once anesthetized.
2. Secure the left forefinger and thumb around the base of the mouse's skull while the right hand is grasping the base of the tail.
3. In one smooth, secure, and quick motion, both hands are pulled apart and the cervical vertebrae in the neck of the mouse are separated.

4 Notes

1. In our laboratory, the prostate tumor take rates in SCID mice are better than those in NU/NU nude mice.
2. NOD-SCID mice often develop metastatic thymic lymphoma at older ages, and invasion of the lymphoma into grafts affects data analysis and subsequent grafting [19, 38]. Therefore, NSG or NOD-SCID gamma mice (NOD.Cg-

Prkdc^{scid} Il2rg^{tm1Wjl/SzJ}) which are not known to develop thymic lymphoma and are more severely immunocompromised may be a better alternative for in vivo study and passage [19].

3. Orthotopic implantation of castration-resistant tumor for PDX establishment is only feasible in intact mice because in castrated mice the mouse prostate is very small making such implantation impossible. We do not routinely use orthotopic implantation to generate PDX because mouse prostate is not highly vascularized (when compared to the subrenal capsule, *see* Subheading 3.6) and not easily accessible for tumor measurement by calipers (when compared to subcutaneous implantation, *see* Subheading 3.5).
4. For setting up studies of castration-resistant prostate cancer, we usually castrate mice at 6–8 weeks of age. However, for intra-tibial studies, mice are castrated at a young age (4–6 weeks of age) before the bone is calcified and too hard for injection of tumor cells.
5. Despite the high clinical relevance, prostate cancer PDXs are notoriously difficult to be established in the laboratory. Based on our experience and the literature, the take rates for primary tumors and for metastatic tumors are ~10% and 15%, respectively. However, some laboratories report over 90% take rate of primary prostate cancer tumors under subrenal capsule [3] and take rate of 100% within 4–6 weeks using tissue recombination with mouse seminal vesicle mesenchyme grafted under subrenal capsule with testosterone (T) supplementation ([19], *see* Table 2).
6. Primary tumor samples usually offer tissues of better viability, but the primary tumor cells are expected to be less aggressive in comparison to metastatic tumor cells, resulting in potentially lower take rates. Furthermore, samples from primary prostate cancer also contain benign epithelial cells and stroma as well as tumor cells. However, it was reported that tumor cells outgrow benign cells resulting in a readily enriched tumor population upon serial passage in vivo [19]. In contrast, metastatic samples are expected to contain more aggressive tumor cells but biopsy of bone is very difficult because the tumor is usually osteoblastic and even when successfully acquired only limited sample volume is available. When obtained from rapid autopsy, the tumor cells may show compromised viability depending on the patient's prior treatments and time between death and tumor harvest. One good source of metastatic tumor samples that provide cells of good viability and volume for PDX establishment are tumors from bone fractures of patients with metastases [68].
7. There are significant differences in serum testosterone (T) and dihydrotestosterone (DHT) between human and mouse males. Human males have sustained levels of T (160–920 ng/dL) and the primary androgen deprivation therapy decreases serum T to a castrated level (<50 ng/dL). A recent article demonstrated that serum T level in intact mice are more similar to hypogonadal/castrated male and levels present in castrated mice are closer to levels in men on abiraterone [69]. In view of the lower level of serum T in mice

vs. men, we have to ensure the host T environment is comparable to that of the patient from whom the tumor is extracted. That is, primary prostate cancer extracted from hormone-naïve patients might establish and grow better when inoculated in intact mice supplemented with T (e.g., silastic tube filled with T or T pellet and implant them subcutaneously to mice to achieve ~500 ng/dL serum total T) for optimal tumor take and growth [3, 14, 18, 19, 69]. For castration-resistant prostate cancer biopsies, tumor bits can be implanted into intact mice with no T supplementation. These castration-resistant tumors may or may not subsequently respond to further androgen ablation upon host castration. For abiraterone-resistant prostate cancer (either from biopsies or autopsies), it would be logical to implant the tumor bits into castrated mice because intact mice have 30–13,800-fold higher total serum T than that in the abiraterone-treated patients [69]. However, we did not see a decline in the tumor take rate when putting the abiraterone-treated patient tumor into an intact host.

8. Total T fluctuation in intact male mice is high when compared to that in human. One of the major reasons is that in human there is steroid hormone binding globulin (SHBG) that binds a majority of T to regulate the bioavailability of free T. Mice do not express and secrete SHBG, resulting in a high fluctuation of total T in the blood stream [70].
9. Human adrenal gland produces considerable amounts of the androgens dehydroepiandrosterone (DHEA) and androstenedione which are precursors for T. In the past it was published that mouse adrenal gland lacks the enzyme 17 alpha-hydroxylase and does not produce these adrenal precursors for T synthesis [71]. However, with a development and the use of more sensitive methodology to evaluate androgen levels (such as mass spectrometry) it has been shown that adrenal gland in mice produces androgens.
10. It has recently been discovered that prostate cancer tumors can produce T, DHT, and other androgens themselves—a process called intra-tumoral androgen synthesis [72].
11. While supplementation of T to normal levels (~500 ng/dL) can support prostate cancer growth, it has been shown that supraphysiological levels of T (~2000 ng/dL) can inhibit tumor growth [73].
12. To demonstrate a proof-of-principle study that inhibition/activation of a specific protein will inhibit/promote tumor progression in vivo, one can test the candidate drug using a single PDX with a high expression/low expression of the protein of interest. Nonetheless, proof-of-principle experiments will require careful interpretation to address the translational relevance to the mixed clinical population.
13. Prostate cancer in patients is a very heterogeneous disease and like other cancers, no single PDX model will ever represent this heterogeneous population. Therefore, use of multiple PDXs that span characteristics of the patient tumor

population will increase translational relevance of results generated in preclinical settings.

14. Preclinical testing using PDXs can provide novel information to identify biological pathways involved in tumor progression, the role of a specific target, and efficacy of drug combinations. However, dosage of drugs may not be projected from mouse to human and it has to be specifically titrated.
15. PDXs are generated in immunocompromised mice. While maintaining an intact innate immune system, the mice are deficient in the adaptive immune system that presents a limiting factor in studying immune interaction with tumor progression or immunotherapy against tumors. In an attempt to reconstitute the immune system, transplantation of human hematopoietic stem cells is an option (e.g., *Rag2^{tm1Flv} Il2rg^{tm1Flv}* mouse in Method 2.3, [74]).
16. PDXs need to be established and maintained in vivo in mice with only limited numbers that can be frozen down and regrown after thawing. It is also difficult to grow PDXs in vitro. PDX maintenance is an expensive and labor demanding endeavor.
17. PDXs demonstrate lower take rate and more variable growth rates when compared to xenografts generated from relatively homogeneous cell lines [3]. Therefore, a larger number of animals per group (resulting in a higher cost of studies) would be needed to power sufficiently the preclinical studies using PDXs when compared to studies using conventional xenografts derived from cell lines. But the belief is that PDXs better mimic tumor growth and drug responses in patients [5].
18. There are only few groups that succeeded in developing prostate cancer PDXs and to our knowledge these models are not commercially available at present. However, researchers who developed these models, in a majority of cases, share the models in collaborative fashion upon execution of appropriate material transfer agreements.
19. It is recommended that the preclinical studies be designed that mirror what can be done in patients, followed by a clinical trial that reflects the preclinical study design [75]. In this way, one can hope that the success rate of such clinical trial might be higher and the biological mechanism underlying a certain new treatment will be better associated with the prior preclinical characterization. It has been shown that use of PDXs in preclinical testing can generate data of highly translational potential mimicking what has been observed in clinical trials (PMID: 26479923).
20. PDXs that spontaneously metastasize in a reproducible manner are lacking—development of PDXs with a high frequency of bone metastasis (the major site of prostate cancer metastasis) is highly desired [75].

Acknowledgements

We would like to thank the Richard M. Lucas Foundation and the Prostate Cancer Foundation for their long-term support for the generation and characterization of prostate cancer PDX models. The work is also supported by NIH PO1 CA085859 (to Robert L. Vessella) and the PNW Prostate Cancer SPORE NIH P50 CA097186 (to Peter S. Nelson). We very much appreciate Dr. Robert Vessella and Dr. Paul H. Lange for their continuous support of our work. We extend our gratitude to the patients who donated their tissues for research and the assistance of the many clinicians who facilitated this process.

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Fig. 1.
Growth response curves in two different PDX models showing (a) de novo resistance and (b) acquired resistance to docetaxel

Fig. 2.

Growth response curves of individual animals of the same PDX showing *de novo* resistance and acquired resistance to a treatment

Table 1

Sources and characteristics of LuCaP prostate cancer PDXs (PMID: 28156002)

	Number
Patients	156
Samples implanted	277
Tumor sources	
Lymph node metastasis	8
Bone metastasis	2
Liver metastasis	5
TURP	4
Ascites	1
Bladder metastasis	1
Omental fat	1
Characteristics	# of PDXs
AS vs CR pairs	11
Mutated AR	8
AR v5,6,7	1
TMPRSS2/ERG	10
PTEN-negative	13
Rb Deletion	2
Osteoblastic	7
Neuroendocrine	4

Table 2

Summary of existing prostate cancer PDXs in addition to the LuCaP series

Group	Xenograft name	Type and source	Procedure	Tumor type	Graft site	Mouse strain	Take rate	T supplement?	Refs.
Navone	MDA-PCa-118 series	CRPC	Biopsy		Subcutaneous	CBI17SCID	12%	Yes	[9, 10]
	MDA-PCa-118a	Left superior pubic ramus							
	MDA-PCa-118b	Left hemipelvis							
	MDA-PCa-114 series	CRPC	Pelvic exenteration				100%	Yes	[4, 11, 12]
	MDA-PCa-114-11	Bladder neck		LCNEC					
	MDA-PCa-114-13	Bladder neck		SCPC					
	MDA-PCa-114-20	Rectal wall		SCPC					
	MDA-PCa-114-23	Rectal wall		SCPC					
	MDA-PCa-114-4	Prostate		LCNEC					
	MDA-PCa-114-9	Prostate							
	MDA-PCa-114-2	Prostate		LCNEC					
	MDA-PCa-114-6	Prostate		LCNEC					
	MDA-PCa-79	CRPC-rectal wall	Pelvic exenteration	Adenocarcinoma					[11–13]
	MDA-PCa-117-9	CRPC-prostate	Cystoprostatectomy	Adenocarcinoma with NE differentiation					
	MDA-PCa-130	CRPC-prostate	Cystoprostatectomy	Adenocarcinoma					
	MDA-PCa-170 series	CRPC	Cystoprostatectomy						
	MDA-PCa-170-1	Prostate							
	MDA-PCa-170-4	Prostate		Adenocarcinoma					
	MDA-PCa-180 series	CRPC	Cystoprostatectomy						
	MDA-PCa-180-11	Bladder wall							
	MDA-PCa-180-14	Bladder wall		Adenocarcinoma with NE differentiation					

Group	Xenograft name	Type and source	Procedure	Tumor type	Graft site	Mouse strain	Take rate	T supplement?	Refs.
Wang	MDA-PCa-180-18	Bladder wall		Adenocarcinoma with NE differentiation					
	MDA-PCa-180-21	Bladder wall		Adenocarcinoma with NE differentiation					
	MDA-PCa-180-30	Seminal vesicle		Adenocarcinoma with NE differentiation					
	MDA-PCa-146 series	CRPC	Cystoprostatectomy						
	MDA-PCa-146-10	Bladder wall		SCPC					
	MDA-PCa-146-17	Bladder wall							
	MDA-PCa-146-20	Bladder wall							
	MDA-PCa-155 series	CRPC	Pelvic exenteration						
	MDA-PCa-155-2	Bladder wall		SCPC					
	MDA-PCa-155-9	Bladder wall		SCPC					
	MDA-PCa-155-12	Bladder wall		SCPC					
	MDA-PCa-155-16	Prostate		SCPC					
	MDA-PCa-149-1	CRPC-ureter		Adenocarcinoma					
	LTL (Living Tumor Laboratory) 310	Primary prostate cancer	Biopsy		Renal capsule	NOD-SCID		Yes	[3]
	LTL311	Primary prostate cancer	Biopsy						http://www.livingtumorlab.com/PDC_Prostate.html
	LTL313 series	Primary prostate cancer	Biopsy						
	LTL313A								
	LTL313B								
	LTL313BR	CR line of LTL313B generated in the mouse							
	LTL313C								
	LTL313D								
	LTL313H								

Group	Xenograft name	Type and source	Procedure	Tumor type	Graft site	Mouse strain	Take rate	T supplement?	Refs.
	LTL331 series	Primary prostate cancer	Prostatectomy						
	LTL331								
	LTL331R	CR line of LTL331 generated in the mouse							
	*LTL352	Ureteral metastasis	Surgery to remove metastasis	NE					
	*LTL370	Penile metastasis	Surgery to remove metastasis	NE					
	LTL412	Lymph node metastasis	Surgery to remove metastasis						
	LTL418	Primary prostate cancer	Prostatectomy						
	PCal	Primary prostate cancer, treatment naïve	Prostatectomy					Yes	[14]
	**LTL220M	Primary prostate cancer, treatment naïve	Prostatectomy					Yes	[15]
	**LTL220N								
	**LTL221N								
Jamieson	PCSD1 (Prostate Cancer San Diego 1)	CRPC-femur	Hemiarthroplasty		Intra-femoral	(RAG2) ^{-/-} γC ^{-/-}	66–100%	No	[8]
					Subcutaneous		100%	No	
Risbridger		Localized prostate cancer (moderate grade)	Prostatectomy		Renal capsule	NOD-SCID	100%	Yes	[19]
		Dissociated cells	Prostatectomy				27%	Yes	
Williams	UCRU-PR-2	Localized prostate cancer (poorly differentiated)	TURP	Adenocarcinoma with NE differentiation	Subcutaneous	NU/NU	10%	Yes	[16]
	UCRU-PR-4	CRPC-prostate	Biopsy	Adenocarcinoma	Subcutaneous	NU/NU			
	BM18	Metastatic prostate cancer-femur	Bone surgery (intramedullary fixation for a pending fracture)		Subcutaneous	SCID			[17]
Pheel		Localized prostate cancer (grade 4), treatment naïve	Prostatectomy		Subcutaneous	(RAG2) ^{-/-} γC ^{-/-}		Yes	[6]

Group	Xenograft name	Type and source	Procedure	Tumor type	Graft site	Mouse strain	Take rate	T supplement?	Refs.
Loda		Localized prostate cancer (grade 7–9)	Prostatectomy		Renal capsule	NU/NU, NOD-SCID	56%	Yes	[7]
Weerden	PC295	Lymph node metastasis	Surgery to remove metastasis	Adenocarcinoma with NE trans-differentiation upon castration of mice	Orthotopic Subcutaneous		72%		
	PC310	Primary prostate cancer, treatment naïve	Prostatectomy	Adenocarcinoma with NE trans-differentiation upon castration of mice	Subcutaneous	NRMI/NU, NU/NU		Yes/no	[20,24]
	PC324	CRPC-prostate	TURP						
	PC339	CRPC-prostate	TURP						
	PC374	CRPC-scrotal skin	Surgery to remove metastasis						[20]
	*PC346	Primary prostate cancer, treatment naïve	TURP						
	*PC346B	Primary prostate cancer, treatment naïve	TURP						
	PC82	Primary prostate cancer							[21,22]
	PCI 3 3	Metastasis-bone							
	PCI35	Primary prostate cancer							

CRPC castration-resistant prostate cancer, *LCNEC* large-cell neuroendocrine carcinoma, *NE* neuroendocrine, *SCPC* small-cell prostate carcinoma, *T* testosterone, *TURP* transurethral resection of the prostate; * or ** denotes model from different tissues from the same patient

Table 3

Advantages and disadvantages of different routes of drug administrations

Route	Advantages	Disadvantages
Oral gavage	Clinically preferred, administered in the form of aqueous solution or homogeneous suspension	First-pass effect by hepatic metabolism leads to low bioavailability
IP injection	Technically easy when compared to oral gavage and IV injection	First-pass effect by hepatic metabolism (primary route of absorption is into the mesenteric vessels, which drain into the portal vein and pass through the liver for metabolism before entering systemic circulation), may cause painful ileus and peritonitis in rodents
IV injection	Clinically relevant, no first-pass effect, maximum bioavailability	Low injection volume, technical difficulty, the bioavailability may be high at the beginning but non-sustainable
Subcutaneous injection	Clinically relevant, no first-pass effect, sustained bioavailability, can be administered in the form of aqueous or oily solutions or homogeneous suspension	Absorb at a lower rate