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# Intratibial implantation of tumor cells

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[dx.doi.org/10.17504/protocols.io.bvrsn56e](https://dx.doi.org/10.17504/protocols.io.bvrsn56e)

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This protocol describes the steps required for the successful implantation of small cell neuroendocrine prostate cancer patient-derived xenograft (PDX) cells in the bone. Bone is one of the most common sites for the development of metastatic prostate cancer and its study is important for evaluating the tumor characteristics and response to therapy.

This protocol can be used for the implantation of any tumor cell line in the bone.

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<https://dx.doi.org/10.17504/protocols.io.bvrsn56e>



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Species: Mouse, Male.

Strain: NOD.Cg-Prkdcscid Il2rgtm1Wjl/SzJ

Age: 6-8 weeks.

Vender: Jackson Laboratory

Housing: House animals within the university-specific IACUC approved housing mouse colony facility at a weight ranging 14-19 grams.

Material	Source	Catalog Number
Povidone-Iodine Prep Pads	MEDLINE	MDS093918
Phosphate buffered saline	Gibco	14040117
Ophthalmic ointment	Akorn	59399-162-35
Lidocaine Hydrochloride 2% w/v Solution	ADVANZ Pharma	N01BB02
Buprenorphine	Covetrus	059122
Ice bucket	Corning	1167U68
Disposable sterile pads	Medline	MSC281224
Alcohol Prep Pads	WEBCOL	6818
70% Ethanol	Carolina	861261
1 cc syringe	BD	309628
Isoflurane	Piramal Critical Care(RxElite)	66794001725
BD Insulin Syringes 31 G, 0.3 ml	BD	BD-25150
Gentamicin	Gibco	15750060
DMEM	Gibco	11965092
Fetal bovine serum (FBS)	Global Life Sciences Solutions	SH3039603

Equipment

1. Isoflurane small animal anesthesia machine



Small animal anesthesia system

2. Water circulating system



3. Heating pad/Circulating water blanket/pad



4. Weighing Scale

5. Bead Sterilizer

6. [Bain breathing tube](#)

7. [Mouse nose cone](#)

8. [Mouse knock down box](#)

9. Nair or any hair removal product/instrument

## Preparation before surgery

1 **Preparation of surgical instruments and supplies:** All of the instruments and supplies should be sterilized.

2 Surgery record sheets

A	B	C
PI		
Personnel		
Date		
Procedure name	Intratibial Implantation	
Protocol #		
Type	Survival Surgery	
Species	Mouse	
Experimental agents administered	Tumor cells	
Anesthetics	Isoflurane	Dosage: (1-5 % or mg/kg; mL)
Analgesics	(1) Lidocaine	Dosage
	(2)Buprenorphine	Dosage

A	B	C
Mouse ID		
Mouse weight		
Anesthesia start time		
Analgesics	(1) Lidocaine	Time administered:
	(2) Buprenorphine	Time administered:
Tumor cells administration time		
Anesthesia end time		

This is a template for surgery records and every user should use the template according to their institute's IACUC regulations.

### 3 Preparation of the PDX cells for implantation

#### Preparation of fresh PDX cells for implantation

Note: Follow the steps till step number 24 from the following protocol to prepare single-cell digestion from fresh tumor tissue: ([dx.doi.org/10.17504/protocols.io.bvrn56w](https://dx.doi.org/10.17504/protocols.io.bvrn56w))

#### Preparation of cells for implantation from frozen biobank

- 3.1 Prepare DMEM medium: Prepare fresh DMEM media supplemented with **10 % volume** FBS and **100 ug/ml** Gentamicin.
- 3.2 Retrieve cryovials containing cells from liquid nitrogen storage. Thaw the cell-containing cryovial by placing it in 37 degrees water bath. Move the vial into a BSL2 hood and transfer the contents of the vial in a 15 ml conical tube

containing fresh DMEM media ( **9 mL** DMEM per **1 mL** of the cell mixture) and mix gently.

- 3.3 Perform live/dead assay using trypan blue and note the live and total cell count.
- 3.4 Centrifuge the 15 ml tube containing cells at **300 x g , 5 mins** and aspirate the supernatant. Resuspend the cells in fresh DMEM to a final concentration of 1M live cells/10 ul and transfer this mixture into an eppendorf tube.

Keep the Eppendorf tube on ice for the remainder of the procedure.

- 3.5 Transfer the cell suspension into a 31G 0.3 ml syringe right before beginning the surgery.

Keep the syringe on the ice at all times.

#### Preparation of surgical space

#### 4 Station 1: Fur removal



Station 1, Fur removal station with anesthesia tubing and nose cone, clippers for hair removal, sterile cotton tips for removal of left-over fur and kimwipe for cleaning.

4.1 Place a disposable sterile pad on a heating pad.

4.2 Aseptically sterilize the surgical area by spraying with 70% ethanol.

## 5 Station 2: Surgery



Station 2, Surgery station with 1) sterilized surgery instruments, 2) sterile dissolvable sutures, 3) lidocaine, 4) buprenorphine 5) 70% ethanol prep pad, 6) povidone-iodine prep pads, 7) disposable sterile pad, 8) eye ointment, 9) sterile cotton tipped applicators, 10) ice box with ice, 11) syringe on ice for intrahepatic injection of cells, 12) eppendorf tube containing cell suspension, 13) weighing scale, 14) kimwipes, 15) bead sterilizer and 16) nose cone and bair tubing.

- 5.1 Place a disposable sterile pad on a heating pad.
  - 5.2 Aseptically sterilize the surgical area by spraying with 70% ethanol.
  - 5.3 Place all the autoclaved surgical instruments within the sterilized surgical area.
- 6 **Heat:** Animal should be kept on a heating pad or circulating water blanket/pad during the entire procedure and, after the surgery is over for approx. 2-4 hrs.

#### Anesthetization and fur removal of mouse

- 7 Place the animal in a knock-down box circulating with a gas mixture of Isoflurane @ 1.4-2.0% and O<sub>2</sub> @ 1-1.2 lt/min inhalant, maintained via a bain-closed system.
- 8 Note the start time for anesthesia.
- 9 Move the animal to the station 1 nose cone and apply ophthalmic ointment on the animal's eyes to prevent them from drying out during the procedure.
- 10 Determine the anesthetic depth by pinching the animal's foot for a reflex response.
- 11 Depilate right or left knee.
- 12 Administer buprenorphine subcutaneously. Note the time of administration.

The concentration of buprenorphine should be kept as mentioned in the institute's IACUC



protocol.

- 13 Prep the shaved area with povidone-iodine prep pads, rubbing in a circular motion. Apply 70% alcohol pads to the surgical area to remove the betadine and loose hair. Repeat this process 2 or more times.
- 14 The animal is now ready to be moved to station 2 for surgery.

#### Surgical procedure (Station 2)

- 15 Gently grasp lateral malleolus, medial malleolus, and lower half of tibia with forefinger and thumb, then bend leg such that the knee is visible and accessible.
- 16 Wet the skin with 70% ethanol to increase the visibility of the underlying patellar ligament, which should be visible as a distinct, thick, white line.
- 17 Transfer the cell suspension into a syringe

Alternatively, cells can be transferred into the syringe before step 14. Make sure to mix the cells in the syringe before injecting them into the mouse.

When the cells are in the syringe, especially if you are injecting multiple mice, you must make sure the cells are resuspended and not settled to the bottom of the syringe each time you inject to inject the correct number of cells per mouse.

- 18 While firmly grasping the ankle/leg of the mouse insert the syringe with cells suspension under the patella, through the middle of the patellar ligament, and into the anterior intercondylar area in the top of the tibia.

Campbell JP, Merkel AR, Masood-Campbell SK, Elefteriou F, Sterling JA (2012). Models of bone metastasis.. Journal of visualized experiments : JoVE.  
<https://doi.org/10.3791/4260>

The pre-loaded syringe with cells might get blocked due to cells being lodged into the needle, making it impossible to inject the cells. One way to avoid it would be to use an empty needle to make the insertion and then use the pre-loaded syringe to inject the cells using the same path. Relocating the same path might be difficult.

- 19 When inserting the needle into the tibia, guide carefully through the growth plate using steady, firm pressure with slight drilling action.
- 20 The needle will encounter markedly less resistance upon penetration of the tibial growth plate.
- 21 Use a gentle, lateral movement of the needle to ensure the needle is in the tibia and through the growth plate. The movement will be limited if the needle is in the proper place within the tibia.
- 22 Slowly depress the plunger to inject 10 µl of cell solution. Little to no resistance should be felt at this point.
- 23 Slowly extract the needle.
- 24 Administer lidocaine in the flank. Note the time of administration

The concentration of lidocaine should be kept as mentioned in the institute's IACUC protocol. For this protocol, 0.5% v/v solution of lidocaine was administered.

25 Start prepping the next mouse for surgery.

#### Post-Op care and monitoring

26 Place the animal in a clean cage on a warm heating pad to aid in regaining its body temperature.

27 Observe the animal until it has regained full consciousness and is walking around in the cage

28 Observe the animal on a daily basis until sacrificed.