Intrahepatic implantation of tumor cells

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This protocol describes the steps required for successful implantation of small cell neuroendocrine prostate cancer patient-derived xenograft (PDX) cells in the liver. Liver is one of the most common sites for 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 implantation of any tumor cell line in the liver.

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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.

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Materials	Source	Catalog Number
Surgical Instruments	Kent Scientific	INMOUSEKIT
Sterile cotton tipped applicators	Puritan	25-806 10WC
Povidone-Iodine Prep Pads	MEDLINE	MDS093918
Phosphate buffered saline	Gibco	14040117
Petri dishes	Millipore Sigma	P5481
Pasteur pipette	Corning	7095D-9
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
Disposable scalpels	Exel international	29550
Buprenorphine		
Alcohol Prep Pads	WEBCOL	6818
Absorbable sutures	CP Medical	421A – VISORB® 5/0 FS-2 30"
70% Ethanol	Carolina	861261
1 cc syringe	BD	309628
Isoflurane	Piramal Critical Care(RxElite)	66794001725
27 G Needle	BD	BD-305109
Gentamicin	Gibco	15750060
DMEM	Gibco	11965092
Fetal bovine serum (FBS)	Global Life Sciences Solutions	SH3039603

quipment

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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

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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	Hepatic Tumor 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

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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.bvrun56w)

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.

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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/10-20 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 syringe right before beginning the surgery.

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.

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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 and 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 bain tubing.

5.1 Place a disposable sterile pad on a heating pad.

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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.

Anesthetization and fur removal of mouse

6 Place the animal in a knock-down box circulating with a gas mixture of Isoflurane @ 1.4-2.0% and O_2 @ 1-1.2 lt/min inhalant, maintained via a bain-closed system.

Heat: Animal should be kept on a heating pad or circulating water blanket/pad during the entire procedure and, until the animal has fully recovered from anesthesia/is mobile

7 Note the start time for anesthesia.

8 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.

9 Determine the anesthetic depth by pinching the animal's foot for a reflex response.

10 Depilate the ventral side to remove fur from the abdomen.

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11 Disinfect the surgical area by rubbing the area in a circular motion with a povidone-Iodine prep pad followed by a 70% alcohol pad. Repeat this process 2 more times.

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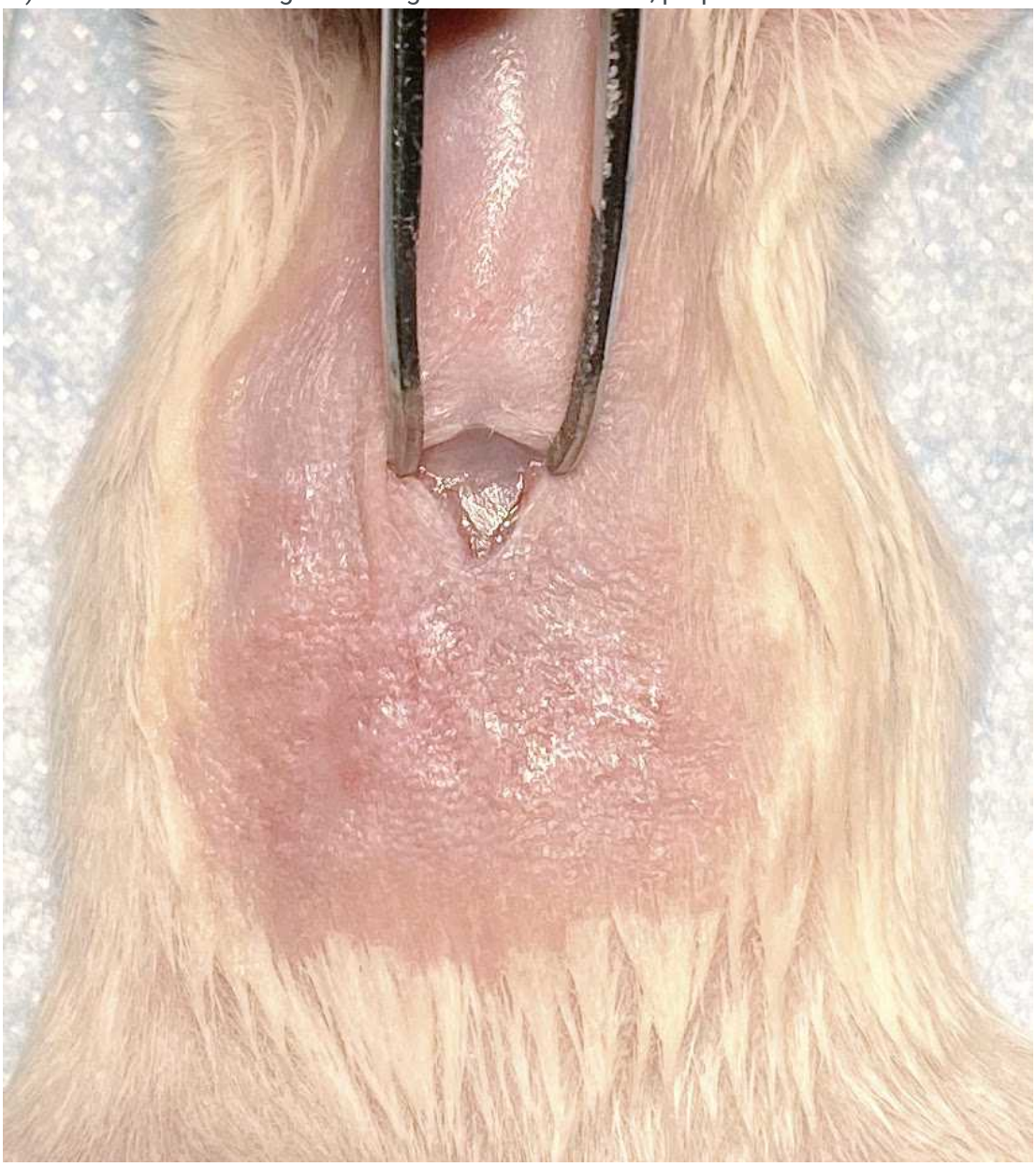
12 The animal is now ready to be moved to station 2 for surgery.

Surgical procedure (Station 2)

13 Gently grab the skin with iris forceps 1 cm under the xiphoid process and make a small (1–2 cm) vertical incision using dissecting scissors in the skin, perpendicular to the linea alba.



14 Gently grab the peritoneum muscle layer and make an incision to expose the liver.

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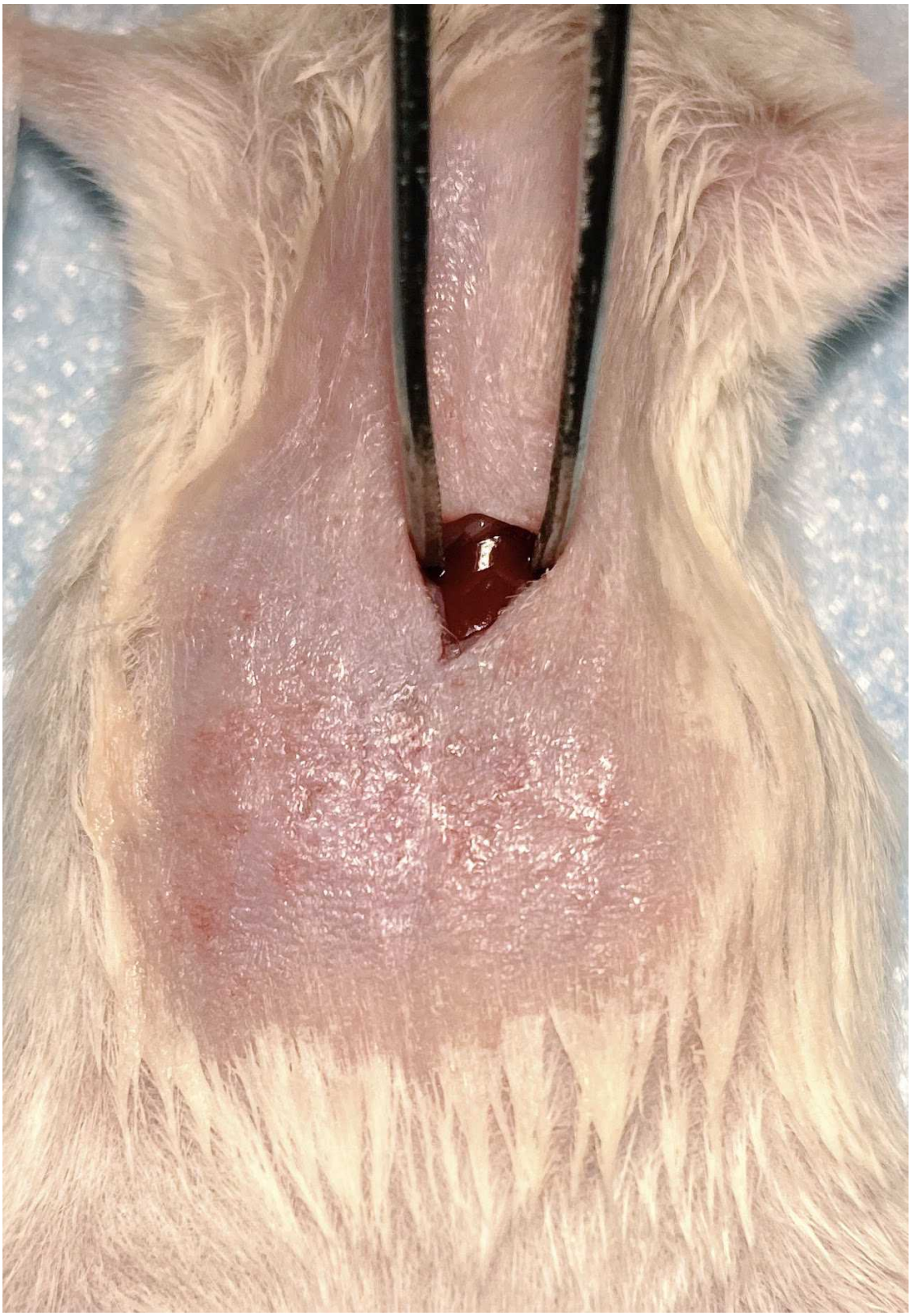
15 Pull the muscle layer with iris forceps to adequately expose the liver for needle access. Two hemostats instead of forceps can be used to hold both sides of the muscle layer apart.



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16 Transfer the cell suspension into a 1 cc syringe with a 27 G needle.

Do not leave the liver exposed for too long. This step should be completed in less than 30 secs. Alternatively, cells can be transferred into the syringe before step 13.

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.

17 While keeping the liver in view with forceps or hemostats, puncture the liver with the pre-loaded syringe and advance the needle a few millimeters along the subcapsular place.

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Inject 10-20 ul of cells suspension from the syringe into the liver. Hold the syringe in place for 20-30 secs to minimize any leakage of cells from the liver.

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19 Pull the needle slowly while applying pressure with sterile cotton-tipped applicators in order to minimize leakage of the cells. The injection site should turn white upon successful injection.

20 Using absorbable sutures, suture the muscle layer and then suture the outer skin layer of the incision site.

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21 Upon completion of the sutures administer buprenorphine and lidocaine subcutaneously. Note the time of administration.

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

22 Sterilize the surgical instruments using the bead sterilizer by placing them inside for ~ 10 secs.

23 Start prepping the next mouse for surgery.

Post-Op care and monitoring

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

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

26 Observe the animal on a daily basis until sacrificed as per your IACUC guidelines.

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