**Mayo Clinic Brain Tumor Patient-Derived Xenograft (PDX) National Resource**

**Instructions for Intracranial Tumor Implantation Using PDX Explant Cultures**

**A few important notes:**

* Users are responsible for acquiring the appropriate institutional approvals before engraftment.
	+ These institutional approvals will likely influence how the below protocol can be executed. It is the user’s responsibility to modify the below generic protocol to meet their institution’s requirements.
* Engraftment must be done in immunodeficient mice.
	+ Short-term explant cultures should be used within approximately two weeks.
		- More indolent lines may be kept for longer periods, but the maximum culture time should be four weeks.
	+ We do not recommend passaging short-term explant cultures *in vitro* prior to injection.
* After implantation, mice should be closely monitored for overall body condition.
* Each PDX line provided will have a unique lineage which is highlighted and explained below. You should commit the lineage(s) you receive from us to your records somewhere should there be a need to refer back to this in the future.
	+ Example: 12, 16, 14, 10
		- 12: The GBM PDX line. Came from the 12th patient tumor xenografted.
		- 16: Mouse number with the GBM flank tumor
		- 14: Previous mouse flank number. #14 flank tumor was passaged to #16 flank.
		- 10: Tumor generation. Number of times the tumor was passed from mouse-to-mouse.
		- VF: Virus-Free. At one point, this line was cleared of the LDEV mouse virus.
			* We no longer test for this as the virus does not affect our studies.
		- G: Our abbreviation for Glioblastoma or GBM

Materials:

* Triple antibiotic (Bacitracin, Neomycin, Polymyxin B sulfate, G&W Laboratories)
* Children's liquid ibuprofen
* Dremel drill
* Dremel engraving bit (#113; 1.16 mm diameter)
* Needle Drivers
* Forceps
* Autoclip Applicator
* Harvard Apparatus #34-0554
* 9mm Autoclips (package of 500)
* Harvard Apparatus #34-0555
* Autoclip Removal Forceps
* Harvard Apparatus #34-0556
* Stainless steel surgery tray w/ cover
* Cidex
* Sterile PBS
* Mouse Anesthetic
* Ketamine (100 mg/ml)
* Xylazine (20 mg/ml)
* Heating pads
* Scale
* Weigh bowl (blue desiccator bottom)
* Providine-Iodine Swab sticks
* Artificial tears (Petrolatum opthalmic ointment, Puralub Vet Ointment, Dechra)
* Sterile Gauze
* Sterile 1.5ml tubes (Eppendorf)
* Bone wax
* Scalpels
* 10-μL Hamilton syringe with a 26-G needle
* Sterile cotton swabs
* Cells on wet ice
* 0.5-cc tuberculin syringes
* 50 mL sterile conical (Falcon)
* 50 ml conical tube Styrofoam holder or like
* 4-0 vicryl with rb-1 needle (Ethicon J30 4H)
* Paper towels

Prior to Injections:

* Make sure sufficient mouse numbers are in house for your planned experiment.
	+ Select only healthy animals for injection.
* Make sure your tools for injection are prepared according to your institutional IACUC guidelines.
* Make sure your mice are placed on analgesic prior to surgery according to your institutional IACUC guidelines.

Cell Line Preparation:

Cells should be used within four hours of preparation.

1. Warm the appropriate media that will be needed for the cells.
* Pay close attention to the type of cultures you are using for injections: FBS or stem cell cultures.
1. Gather PBS, trypsin (for FBS cell cultures) or TrypLE trypsin replacement (for stem cell cultures), and media into a clean, biological hood.
2. Carefully aspirate media out the flask, add 5-10ml of sterile PBS into each flask, and rock to wash off excess media.
* Dispense PBS down the side of the flasks to keep cells adherent.
1. Remove PBS and add 5-10ml of trypsin or TrypLe to each flask. Incubate at room temperature for 3-10 minutes. Once cells begin rounding up and can be dislodged from the plate when tapping the outside of the flask, proceed to the next step.
* If cells are not releasing from the plastic, the flasks can be incubated at 37°C with careful monitoring of cell lifting.
* Avoid over-trypsinizing cells.
1. Add Complete DMEM (FBS cell cultures) OR Complete StemPro (Stem cell cultures) to inactivate the trypsin. Use media to rinse any adherent cells off the flask and transfer cell/media mix to a conical tube, and centrifuge the cells at 320 RCF for three minutes.
* Keep the volume at an even number (e.g., 10 ml) to help with math.
1. Spin cells down in the centrifuge at 1200rpm for 3 minutes before resuspending cell pellet in 10-30ml of PBS depending on how many flasks were trypsinized.
2. Mix 10ul of resuspended cells with 10ul of Trypan blue in a 1.5ml tube. Mix with pipette and transfer 10ul of the mixture to a hemocytometer. Count cells in the top left and lower right quadrant. Multiply the total number of cells counted in both quadrants by 10,000. That calculation will give you cells/ml.
3. Calculate the number of cells and volume needed for the injections.
* We typically use 300,000 cells in 3ul (100,000 cells/ul or 100 million cells/ml) per mouse.
	+ Some aggressive lines – such as GBM12 and GBM43 – are injected at 100,000 cells in 3ul per mouse.
* A typical way to do the calculation would be as follows:
	+ How many mice needed for the study? N=10 mice.
		- Always mix up enough cells for at least 5 extra mice, so in this case, calculate for 15 mice.
	+ 15 mice x 3ul = 45ul. We need 45ul of cells at 100,000 cells/ul or 4.5 million cells total.
	+ If our count was 300 using the hemocytometer, 300 \* 10,000 = is 3 million cells/ml (3000 cells/ul).
	+ Since we need 4.5 million cells and we have 3 million cells per ml, we can spin down 1.5ml of cells and resuspend to a total volume of 45ul with PBS.
1. Aspirate off the PBS from the cells, making sure not to disturb the cell pellet, and resuspend cells in the appropriate volume of PBS. Place cells on wet ice until ready to be injected.
	* Given the small volume of these injections, assume there will be some volume trapped in the cell pellet. Add a little less PBS than is required and then see where you are at volume wise and adjust as necessary.

Surgery:

Given your setup, prepare the room at the same time as cells are being prepared. Once ready, begin the below protocol.

1. Anesthetize mice using your approved method.
* We use ketamine/xylazine mix and adjust dose by individual mouse weights.
* When injecting anesthetic (IP), insert needle on anatomical right side of the mouse to avoid injecting into bladder or cecum.
	+ Insert needle about half way into the mouse and lift up slightly to insure that you are not in any organs. Remove and reinsert, if needle is observed in the gut, other organ, or subcutaneously.
1. Once mice are down, apply artificial tears to the mice.
2. Clean the surgical sites with an alcohol wipe or 70% alcohol and then clean with a Providine-Iodine swab. Use a toe pinch to make sure that the mouse is completely down before moving on.
3. Apply pressure to the skin of the skull and make a midline incision from the back of the skull to just behind the back of the eyes using a scalpel.
* Make the incision as small as possible to allow for drilling of the burr hole and insertion of the needle.
1. Start the drill and adjust the RPM to 20K, if desired.
2. Dip the drill bit into the Sterile PBS and apply pressure to the skin of the skull. Locate the bregma and move the bit to the injection site:
* 1.0mm forward (about 0.5 of the bit diameter) and
* 2.0mm lateral (about 1.5 bit diameter to the right)
1. Applying slight pressure to allow for cutting and control of the bit, drill a burr hole.
2. Once the bit “pops” through the skull, immediately remove pressure. Wipe the bit clean with sterile gauze wet with sterile PBS, and set aside.
3. While someone is drilling the burr hole, start loading cells into needles.
* We use a stereotactic frame and autoinjector for this part. Our autoinjector control unit settings are:
	+ Syringe I.D. 0.460mm
	+ Volume measure ul
	+ Volume 3.000 ul
	+ Rate per minute 1.000 ul
	+ Mode Infuse
1. Grab the 1.5ml Eppindorf tube containing the cells from the ice bucket, and flick tube to mix. Make sure cells are a homogeneous suspension.
2. Use the pump to load 3.1 ul of cells into syringe.
* Solution should be cloudy. If clear, you likely have a clog. Flush the needle with sterile PBS or replace.
* Don’t touch the tip of the needle to the bottom of the Eppendorf tube as this will dull the needle.
1. Repeat step 11 until all needles are loaded. Verify that each needle has a volume of 3.1ul.
2. Recap the Eppendorf and place them back in the wet ice bucket.
* Cells should only be kept for, at maximum, 4 hours on ice. If additional time is needed, prepare cells in multiple batches.
1. Load the mice into the bite blocks on the jigs and place ear pins. Adjust as needed so the skull is level in both planes.
* If needed, use a cotton swab to move the skin out of the way so both the Bregma and burr hole are visible.
1. Place the tip of the needle over the bregma, just above the skull and dial in the below coordinates:
* From the bregma move the bit to the injection site:
	+ 1.0 mm forward and
	+ 2.0 mm lateral (right)
1. Lower the needle tip so the bevel is at the top of the skull. Dial in a depth of 3 mm from the top of the skull, and start the pump.
2. When the pump stops injecting cells, wait at least 1 minute before removing the needle from the brain.
3. Once injections are completed and the mice are ready to be closed, break off a piece of BoneWax and apply to each mouse by separating the skin of the mouse to expose the burr hole.
4. Apply 1-2 wound clips per mouse to the head wound, depending on the size.
* Make sure the clips are secure and that the mice can easily close its eyes.
1. Apply a generous amount of triple antibiotic ointment to the wounds, and return mice to cage.
* Use of a warming pad under the cage will provide thermal support until the mice are awake.
1. Actively monitor mice until they have regained consciousness.
2. Monitor the mice daily for 10-14 days following surgery.