Standard Operating Procedure: Intravitreal injections for Retinoblastoma PDX Models

Objective: This is a standard procedure for giving medications or tumor cells to create a retinoblastoma orthotopic xenograft.

Materials:

- 16G Needle
- 5ul Hamilton Syringe
- CB17 SCID mouse (Nude or NSG can also be injected but is harder to see sclera)
- Resuspended cells in media (*Tip*: with such small amounts, it is easier to keep cells in a 1ml slip tip syringe – use a micropipette tip to place in the syringe –
- do not pull up through the needle cells will go into the dead space)

Procedure:

- Put animal in isoflurane chamber and anesthetize animal to the surgical plane of anesthesia (use toe pinch method to confirm)
- Place the animal in lateral recumbency and push back the eyelids of the upward facing eye with the index finger and thumb to proctose the eye and expose the sclera. Be cautious not to obstruct breathing by pressing on the trachea.
- Take a 16G needle and using the tip of the bevel make a small incision between the sclera and cornea. Some fluid/blood may start to exit once the incision has been made, this is normal. If profuse bleeding occurs, do not proceed with injection.
- Keeping an eye on where the incision has been made, take the Hamilton syringe loaded with 5ul of cells and place the end of the needle into the incision. Avoid pushing needle too far into the eye as you do not want to puncture/damage the lens or hit any intraocular vessels.
- Carefully, inject the cells into the vitreous. You will see the eye bubble with the injected media.
- Once cells have been injected, gently remove the needle from the incision and take pressure off of the eye to cover the incision.
- Mild bleeding or fluid leakage may be observed. Make sure the animal recovers from anesthesia and if the animal shows any signs of pain or discomfort follow up.
- For bilateral injections, make sure that the animal remains in the surgical plane of anesthesia. Change the 16G needle after multiple incisions to ensure a sharp tip.