

SOP 4

Obtaining Post-Mortem Samples for Cell Culture and Xenografts

Parents of children with relapsed high-risk neuroblastoma and other cancers may ask whether donated materials from their child can be used to help research efforts. Post-mortem blood (via a central catheter) obtained from children who have died of cancer frequently provides sufficient tumor cells for cell line or xenograft generation. The blood may be obtained up to 12 hours after death, but success at growing tumor is higher with samples obtained within an hour or 2 of death. Post-mortem tumor tissue can also be submitted. Heparinized bone marrow aspirates are especially valuable samples if they can be obtained.

As samples obtained post-mortem are not considered as from human subjects, therefore use of a standard biobanking protocol to collect the specimens is not applicable. If the patient was entered onto a biobanking protocol and consented for such prior to death, the latter provides ready access to clinical annotation for the post-mortem specimens. In general, one can obtain the blood specimen with a family consent for a limited post-mortem (that only lists the samples to be obtained, with obtaining blood not requiring incisions etc). For questions please contact Dr. C. Patrick Reynolds at the Texas Tech University Health Sciences Center School of Medicine (patrick.reynolds@TTUHSC.edu; Lab 806-743-2707 or Office 806-743-1558). Post-mortem samples can be received by the Reynolds laboratory if obtained with consent as required by the submitting institution. The specimens should be obtained as follows:

EDTA Blood. Please obtain 5 to 10 mls of blood using EDTA as an anticoagulant (no heparin) which can be used to extract cell-free DNA.

Heparinized Blood. Blood can often be drawn, especially within ~ 1 hour after death from the central venous catheter. To obtain the blood it is best to use 50 cc syringes wet with ~ ½ ml of heparin (preservative free preferred) and a 14 ga or 18 ga needle. Aspiration of 50 to 100 cc is possible and submitting as much as is feasible is requested. However, even as few as 5 to 10 mls is worth sending. A few external chest compressions may be considered if aspirating the blood is difficult. Percutaneous or direct (at autopsy) aspiration from the left ventricle has also been successful.

Bone Marrow Aspirates. Aspiration with standard bone marrow aspirate needles, with multiple pulls and advancement of the needle, from anterior or posterior iliac crest and/or sternum.

Tumor Tissue. Aseptic biopsy of tumor can generate cell lines and PDXs, but only if obtained relatively soon after death. Tissue should be minced to fragments of about 200 mg and placed in cell culture medium containing some fetal bovine serum and (importantly) 100 mcg/ml gentamicin. Tumor tissue should also be snap-frozen and stored at -80 (and shipped on dry ice) for genomics studies.

If the materials and facilities to transfer the blood aseptically to 50 ml polypropylene tubes or to a 100 to 300 cc blood or platelet bag are available, that is the preferred container for shipping. Addition of 100 micrograms per ml tissue culture gentamicin is strongly preferred but not necessary if unavailable. If death prevents shipping for 1-2 days due to holiday or weekend addition of gentamicin (100 mcg/ml final) and 10 to 20% by volume sterile cell culture medium is requested. Samples not shipped the same day should be stored at 4 deg C until shipment/ If an autopsy is performed within 6 hours of death, it is requested that tumor and or marrow aspirates also be submitted in addition to the blood sample. The most likely sample to generate cell lines and xenografts post-

mortem is the blood or marrow aspirates. The heparinized blood should be sent in aseptic secure containers, at 4 deg C or room temperature but in an insulated Styrofoam container to prevent temperature fluctuations.

Time Limits on Specimen. The closer to time of death the better, but samples can be obtained up to a maximum of 24 hours after death for blood and bone marrow samples and 6 hours after death for tissue. The major limitation in time for blood appears to be clotting of the central line, which can sometimes be overcome with vigorous aspiration via 14 ga needle.

Shipping Address (weekdays):

Dr. C Patrick Reynolds
Texas Tech Univ Health Sciences Center
3601 4th Street STOP 9445
Lubbock, TX 79430-9445

For shipments *arriving* on a weekend or holidays, please contact the lab by email for alternate address.

Phone (for Fed Ex): 806-743-1558

Email: COGcell@TTUHSC.edu Copy also: Patrick.Reynolds@TTUHSC.edu