MOLECULAR CHARACTERIZATION INITIATIVE (MCI) OF PEDIATRIC TUMORS THROUGH COG STUDY APEC14B1

FACT SHEET FOR SITE PATHOLOGISTS

MCI Overview

The goal of the Molecular Characterization Initiative (MCI) of Pediatric Tumors is to provide exome sequencing (**ES**) of paired tumor/germline samples (25-500 ng DNA), tumor classification by Illumina methylation array analysis (100-250 ng DNA) and Archer solid fusion panel analysis (250 ng RNA) for a subset of pediatric tumors. The analysis will be conducted in the CAP/CLIA accredited Nationwide Children's Institute for Genomic Medicine Clinical Laboratory (**IGM**) with return of results to patients and treating physicians.

MCI was Activated March 21st, 2022 with APEC14B1 (Project:EveryChild) Amendment #3A.

MCI will add eligible diseases sequentially

What should the pathologist know before submitting tissue for MCI?

- Submit surgical pathology report. A final diagnosis is not required but a descriptive and/or differential diagnosis is essential for issuing complete molecular reports. A draft report is acceptable.
- Tissue will ship to the Biopathology Center (**BPC**) at Nationwide Children's Hospital. Allow 4-5 business days for pathology QA and nucleic acid extraction.
- Nucleic acids will be transferred to IGM to perform sequencing studies. Return of clinical reports is expected within 2 weeks of receipt of nucleic acids.

How to maximize efficiency and turn-around times

1) Encourage proceduralists to obtain adequate tissue for ancillary studies through education around volume requirements for successful sequencing.
2) *Strongly* consider sending frozen tissue for sequencing rather than FFPE tissue (see 5aiii below).
   Note: If provisional or final diagnosis is a low [1] grade glioma, FFPE is preferred over frozen tissue for adequate pathology QA review.
   If provisional or final diagnosis is papillary thyroid carcinoma, FFPE is strongly preferred over frozen tissue for adequate pathology QA review.
   a. Frozen tissue yields less fragmented DNA and substantially higher quality RNA that together result in significantly better data quality.
   b. Submit 50-100 mg of tissue (a minimum of 30 mg of frozen tissue is required for molecular characterization assays.
      i. For resection specimens or open biopsies, approximately 4-5 mm^3 is requested
      ii. For needle biopsies, a minimum of *three* 16-18g cores, each 0.8-1cm in length, is requested
         1. The Biopathology Center prefers tissue frozen in foil

2. If submitting frozen tissue in cryovial, they request use of the following procedure:
    a. Prior to tissue collection:
        i. Label cryovial(s) according to instructions below.
        ii. Place cryovial(s) on dry ice to freeze. The vials should appear frosty when ready.
    b. Place tissue in foil and allow to completely freeze (using either direct contact with dry ice, or liquid nitrogen vapor).
    c. Gently remove the frozen tissue from the foil. If the tissue is sticking to the foil, then gently run a finger over the back of the foil to loosen the tissue.
    d. Using clean forceps place each tissue core in a pre-chilled cryovial. Tissue should move freely in the vial.

3) If frozen tissue cannot be provided, then an FFPE tissue block is preferred
    a. Consider putting aside a block specifically for submission for molecular characterization
    b. For resections or open biopsies, the tissue in this block should be 0.5 - 1 cm^3
    c. For needle biopsies, place at least *three* 16-18g cores, each 0.8-1cm in length, in a single block (to approach a roughly dime-sized cut surface)
4) If cannot send frozen tissue or a block, send slides from the most representative block to include
    a. 1 H&E (first slide) and 20, 5 μm unstained uncharged air-dried slides cut and numbered sequentially after the H&E stained slide.
    b. For specimens requiring decalcification use EDTA. Acid decalcified specimens (including Formical) cannot be accepted.
    c. The CUSA aspirate may serve as a good source of tumor tissue for many brain tumor resections and should be sent to pathology as a specimen, if possible.
5) Maximize tumor tissue
    a. Methylation testing requires at least 60% viable tumor content in the block
        i. Tumor content is determined based on overall cellularity (e.g., at least 60% of nuclei in the sample should belong to tumor v. inflammation/stroma, etc.).
        ii. Necrotic tissue doesn't yield analyzable nucleic acids, thus less than 10-20% necrosis is ideal.
        iii. Some tumors may have a high volume of normal tissue (eg. infiltrative low-grade gliomas, lymph node metastases, tumors with abundant inflammatory cells, etc). For these cases, and particularly for low-grade gliomas and papillary thyroid carcinomas, FFPE tissue should be prioritized for submission to ensure adequate QA review and assessment of tumor content.
    b. Tumor can be macrodissected from FFPE block/slides at the BPC.

Return of results

Clinical reports for ES, RNA Fusion and Methylation analysis will be made available via a secure web portal hosted by IGM (methylation analysis will be returned for CNS only). Authentication to the portal is handled by a direct integration to the CTEP single sign-on system (SSO). Institutional PIs and Lead CRAs may access the IGM portal by logging in with their CTEP credentials and have immediate access to download PDF clinical reports only associated with their institution. Each institution should develop their own system to return these results to pathology for incorporation into an integrated report. If the results of molecular testing influence the pathologic diagnosis, please provide a revised pathology report to be uploaded to the APEC14B1 CRF.

Questions about tissue submission, contact BPCBank@nationwidechildrens.org

Questions about timing of return of results, contact IGMMCI@nationwidechildrens.org

Questions about results, contact COG disease committee representative (refer to APEC14B1 FAQs for disease liaisons)