impact genetics inc.
advancing genetic diagnostics

The Importance of Tumor Analysis in Identifying *RB1* Mutations

Bilateral Retinoblastoma (hereditary RB): Tumor cells carry two *RB1* gene mutations. Blood cells carry one mutation. At Impact, we detect an *RB1* mutation in blood in 96% of bilateral cases.

Unilateral Retinoblastoma: Tumor cells carry two *RB1* gene mutations. Blood cells carry only

- One *RB1* mutation 15% of the time (hereditary RB), and
- No *RB1* mutations 85% of the time (non-hereditary RB).

At Impact, we detect both tumor mutations in 96% of cases.

Tumor is important:

1. **Mosaicism:** In unilateral and bilateral retinoblastoma, identifying both tumor mutations reduces the risk of the lab missing a mosaic mutation in blood. Without knowing the tumor mutation, we detect down to 10-20% mosaicism in blood. Knowing the tumor mutation allows us to identify mutations in blood down to 1-10% mosaicism.
2. **Metastatic Monitoring:** The second tumor mutation can be used to monitor for metastatic disease, if indicated.
3. **Undetectable Mutation:** In unilateral retinoblastoma, if one or both *RB1* mutations are not detectable in tumor (4% of cases), we know that they cannot be detected by our test methods (e.g. deep intronic splice mutations) and therefore, risk for carrying a germline mutation remains 15%. If tumor is not available, and no mutation is found in blood, we assume risk for carrying a germline mutation is reduced to 0.6% (4% of 15%).

In some cases, live tumor may be collected for molecular testing after chemotherapy. Instructions for collecting live tumor are attached.

Case Study: 9-year girl with bilateral refractory retinoblastoma

Background: The child had recurring retinoblastoma. No tumor had been available for genetic testing and no mutation had been identified in blood. The risk of other tumors was unknown. The risk to parents, siblings and offspring was unknown.

Genetic Testing: The child's eye was removed because of the recurring tumors. Vitreous seeds from the recurrent tumor, undamaged by chemotherapy, were sent for genetic analysis. DNA was extracted and the *RB1* tumor mutations were identified. Blood could then be tested for the mutations. One of the tumor mutations was found to be mosaic, at a very low level, in blood.

Impact:

- Because of mosaicism, this patient has less risk for her offspring to be affected and less risk of future cancers
- Parents and siblings are no longer considered at risk and do not require any further clinic visits
- Offspring can be tested for the specific mutation
  - A child carrying the mutation will be monitored
  - A child who does not carry the mutation will not require monitoring
- Metastatic monitoring, using the second tumor mutation, is possible, if needed in the future