

Liquid Biopsy in Retinoblastoma Research

Traditional biopsy of retinoblastoma has long been contraindicated due to the risk of extraocular spread. But in a new application of liquid biopsy research, aqueous humor is showing promise as a surrogate marker for retinoblastoma.

In a groundbreaking study published in 2017, researchers found that the aqueous humor of eyes with retinoblastoma can carry enough tumor-derived DNA to perform genetic analysis of the tumor.¹ A second study, published in 2018, identified a potential biomarker for the disease in aqueous humor.²

The Clinical Challenge

At present, retinoblastoma diagnosis and treatment decisions are based on clinical findings alone. “For advanced retinoblastoma, the prediction of whether our treatment will save these eyes is about 50/50—and flipping a coin in front of a parent is really frustrating both for me as a surgeon and for the parents,” said Jesse L. Berry, MD, at Children’s Hospital in Los Angeles.

Moreover, she said, “If two kids with retinoblastoma come in with the same clinical features, they’re going to get the same treatment, often intra-arterial chemotherapy or intravenous chemotherapy, but these are general [not personalized] chemotherapy regimens.”

She added, “Why do two kids look identical clinically, but one child responds really well to our current therapeutic

regimen while the other won’t at all? How can we prognosticate better for these children?”

Novel Hypothesis

During her search for a better diagnostic and prognostic tool, Dr. Berry hypothesized that while the tumors form in the back of the eye, the aqueous humor in the front of the eye might carry tumor-derived DNA. “They’re very necrotic tumors, so they undergo a lot of cell lysis, releasing their DNA into the eye,” she said.

Proof of principle. Initially, Dr. Berry and her colleagues analyzed six aqueous humor samples from three children aged 7 to 28 months, looking for genetic material in these eyes, which were undergoing salvage therapy.¹ They found that tumor DNA could be detected in the aqueous humor—and that it could be found not only in cases of large, active tumors but also in those that involved much smaller tumors that had already been treated, Dr. Berry said. “This opened the door for us to investigate this whole broader realm of aqueous humor as a liquid biopsy for retinoblastoma.”

The concept underpinning aqueous sampling is that some tumor cells die and rupture; the DNA then diffuses



ULTIMATE GOAL. This line of research opens the door to *in vivo* diagnosis of retinoblastoma, said Dr. Berry (shown here).

across the vitreous face and makes its way into the aqueous humor—and some of that DNA material floats into the anterior chamber, where it is then accessible for a biopsy, said J. William Harbour, MD, at Bascom Palmer Eye Institute in Miami. “Previously, that was just a theory, but Dr. Berry has proved that this is possible.”

Molecular analysis. In a second study, Dr. Berry and her colleagues evaluated the tumor DNA in eyes that had been removed and in those that were saved.² For this study, the researchers evaluated 63 samples of aqueous humor from 29 eyes. “After comparing the groups, we found a potential biomarker of aggressive tumors: a gain of 6p,” Dr. Berry said.

With a 6p gain, there’s an extra copy—or copies—of part of the small arm

BY REBECCA TAYLOR, CONTRIBUTING WRITER, INTERVIEWING JESSE L. BERRY, MD, DAN S. GOMBOS, MD, FACS, AND J. WILLIAM HARBOUR, MD.

of chromosome 6. “In our study, the 6p gain was associated with nearly 10 times increased odds of that eye needing to be removed,” said Dr. Berry.

These results raise the possibility that researchers might finally have a biomarker that would allow them to tell parents which eyes contain an aggressive tumor and thus have a far lower likelihood of responding to therapy—and which eyes may experience better outcomes, Dr. Berry said.

Giant Steps Forward

What’s really novel about aqueous humor sampling, Dr. Berry said, is that it allows for in vivo diagnosis. “Before, if the eye was saved, we never saw what was happening at the molecular level of the tumor,” she said. “We’re allowing researchers—and hopefully, one day, doctors—to discover information about the tumor while the child still has the eye and is being treated.”

And it’s only when studies identify biomarkers that researchers are able to begin moving toward targeted therapy and precision medicine. As with so much current cancer research, the ultimate aim in retinoblastoma research is to find targetable biomarkers that promote tumorigenesis.

This avenue of research moves these quests forward. “In some cases where Dr. Berry had to remove the eye, she did the liquid biopsy and compared it to analysis of the tumor and found very similar results,” Dr. Harbour said. “That was critical as well, to show that not only can we do a liquid biopsy and get genetic information, but that that genetic information reflects what is in the tumor. These are both very important breakthroughs.”

Elsewhere in the Eye

While Dr. Berry is focusing on retinoblastoma, Dr. Harbour is looking at the potential of liquid biopsy for uveal melanoma. “Unlike retinoblastoma, melanomas do not spread very easily” from the point of fine-needle biopsy into the tumor, he said.

“We currently look at genetic markers from tumor biopsy and can estimate whether the patient is at low, medium, or high risk of spread of their melano-

ma to other parts of the body, but we would eventually like to incorporate a liquid biopsy platform,” he said.

This would be of particular benefit in cases that involve small tumors, “where biopsy can be challenging, and we may want to biopsy at multiple times,” Dr. Harbour said. In these instances, he said, “It would be ideal if we could just take a sample of fluid from the anterior chamber.”

Other researchers are investigating the use of liquid biopsy in vitreoretinal lymphoma.³

Looking Ahead

Precision medicine. “This genetic testing is for what we’re now calling ‘precision medicine,’ which is staging the patient in terms of prognosis and then predicting which treatment will be the best for the patient,” Dr. Harbour said. “If we could find that certain genetic markers can guide us as to which eyes need to be removed and which can be safely treated with chemotherapy, that would be a powerful way to use this liquid biopsy technology.”

He added, “What’s exciting is that the current technology we have for sequencing genetic material is so exquisitely sensitive that it allows us to detect and analyze genetic material from cancers in much smaller quantities than we would have ever imagined in the past. We can make progress in treating patients, using liquid biopsy techniques, in a way that minimizes harm and risk to the patients while getting sufficient material to guide their therapy.”

Cautious optimism. Despite the promise of aqueous sampling, Dr. Berry cautioned, many questions remain to be answered. Dan S. Gombos, MD, FACS, at MD Anderson Cancer Center in Houston, agreed: “These advances have enormous potential in further stratifying prognosis and therapy, but there is lack of uniformity in the management of retinoblastoma and many variables influencing therapy.”

As Dr. Gombos pointed out, “Even within the same center, an eye with a particular retinoblastoma grouping may receive a different modality, with different agents, cycles, and doses. So it may be challenging to identify a

particular modality or agent correlating with [effective treatment of] a specific genetic fingerprint.”

Moreover, he said, aqueous sampling has potential risk for infection and damage to ocular structures. “The hope is that this research would serve as a bridge to a noninvasive or purely hematogenous biomarker.”

Next steps in retinoblastoma. Dr. Berry has three research initiatives underway, including a multicenter trial to collect aqueous humor samples from patients with retinoblastoma across the United States. “For parents who are willing, we’ll also take aqueous humor samples at diagnosis, to gather critical data about what the tumor profile in the aqueous looks like at diagnosis and what biomarkers we can find,” she said. “In the future, I hope to see a child at diagnosis, take aqueous humor, and have that be informative to me and the parents.” She’ll also begin evaluating the potential of blood as another form of liquid biopsy for retinoblastoma.

1 Berry JL et al. *JAMA Ophthalmol.* 2017;135(11):1221-1230.

2 Berry JL et al. *Mol Cancer Res.* 2018;1-12.

3 Cani AK et al. *Oncotarget.* 2017;8(5):7989-7998.

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