



## International Research Journal of Ophthalmology

Review Article

Open Access

### Usefulness of Liquid Biopsy for Intraocular Malignancies

Syed Mehabub Ul Kadir

Department of Oculoplasty and Ocular Oncology, Bangladesh Eye Hospital and Institute, 78-Satmosjid Road, Dhanmondi, Dhaka-1205, Bangladesh

**\*Corresponding Author:** Syeed Mehabub Ul Kadir, Assistant Professor and Consultant, Department of Oculoplasty and Ocular Oncology, Bangladesh Eye Hospital and Institute, 78-Satmosjid Road, Dhanmondi, Dhaka-1205, Bangladesh, Email: [mehbubkadir@gmail.com](mailto:mehbubkadir@gmail.com)

 **ORCID ID:** <https://orcid.org/0000-0002-2077-6784>

**Received Date:** Dec 24, 2021 / **Accepted Date:** Dec 31, 2021 / **Published Date:** Jan 03, 2022

**Cite this article as:** Syeed Mehabub Ul Kadir. 2021. Usefulness of Liquid Biopsy for Intraocular Malignancies. Int Res J Optha. 4: 01-06.

**Copyright:** This is an open-access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited. Copyright © 2021; Syeed Mehabub Ul Kadir

#### Introduction

Retinoblastoma (RB) is the most common primary intraocular malignancy in children, whereas Uveal melanoma (UM) is the most common intraocular malignancy in adults [1,2]. Tissue biopsy is the standard gold technique for diagnosing the malignant neoplasm but an incisional tissue biopsy or fine needle aspiration biopsy (FNAB) is contraindicated for the intraocular malignancy [3]. Clinical diagnosis and imaging study are the only way to diagnose the intraocular malignancy due to the risk and fear of extraocular spread [4]. Recently, liquid biopsy has gained in popularity in the ophthalmic field. Liquid biopsy allows retinoblastoma diagnosis and a better understanding of the metastatic spread of uveal melanoma. Recently, the USA Food and Drug Administration (FDA) approved the make use of liquid biopsy (LB) as an appropriate diagnosis, prognosis, and also for monitoring tool in non-small cell lung carcinoma to keep away from invasive tissue biopsy in designated

cases [5-7]. Liquid biopsy (LB) utilizes biofluid to evaluate for tumor-derived cells or cell-free DNA. LB is a relatively non-invasive technique rather than a tissue biopsy. In LB, material collected from multiple body fluids such as aqueous humor (AH), blood, cerebrospinal fluid, urine, and saliva for molecular diagnosis [8] and detecting of cancer biomarkers such as circulating tumor cells (CTC), tumor derived cell free DNA (ct-DNA), circulating tumor RNA (ct-RNA), microRNA (miRNA), tumor-related exosomes (TREs), and extracellular vesicles (EVs) [7]. Aqueous humor samples for RB (Ocular LB) and Venous blood samples for UM (systemic LB) are utilizing for analyzing the molecular characteristics [8]. In others ophthalmic malignancies like conjunctival melanoma or squamous cell carcinoma, the role of LB is still not studied because tissue biopsy is routinely done for confirming the diagnosis and also for mutational status [9-11].

**Retinoblastoma (RB)**

Retinoblastoma (RB) is the most common primary ocular malignancy in infants and young child from 8 months to 4 years, and most patients are diagnosed before the age of 2 years [1]. RB may be inherited and non-inherited. Unilateral RB is usually a non-inherited form, and bilateral or trilateral RB is associated with the inherited condition. RB is the cause of a mutation of the tumor suppressor gene RB1 located on chromosome 13q14 [12]. Leukocoria (white spot in the eye) and strabismus are the most common clinical features. Tissue biopsy is not indicated for the chance of extraocular dissemination. Sometimes, it may mimic Coats disease, persistent fetal vasculature, and retinopathy of prematurity despite using the diagnostic tools optical coherence tomography (OCT) and B scan ultrasonography [1]. With the advancement of the multimodality treatment protocol, the survival rate and globe salvage are excellent and reduced the rate of enucleation. Localized ocular RB can be treated by cryotherapy or laser therapy or brachytherapy. Intravitreal chemotherapy is using for vitreous seeding. More advanced cases may be treated by intravenous chemotherapy (IVC), or intraarterial chemotherapy (IAC) or enucleation [13,14]. In neglected or untreated cases, RB may be associated locally invasive to the orbit through extraocular spread and also spread to the brain through the optic nerve [1]. LB is relevant for two reasons; firstly, for diagnosis to establish the mutational status of the RB1 gene and secondly for monitoring the prognosis [15]. In RB, the aqueous humor (AH) sample is used as a liquid biopsy, and analyzes genetic biomarkers, and collects AH from the anterior chamber by an aqueous puncture. It is an easy, relatively non-invasive, and safe procedure performed under general anesthesia in combination with thorough eye examination or intravitreal application of chemotherapy. A higher number of AH somatic chromosomal copy alteration, including 6p gain, is associated with a predictive of more advanced and aggressive RBs [16,17]. A study found that AH ct-DNA is concordant with ct-

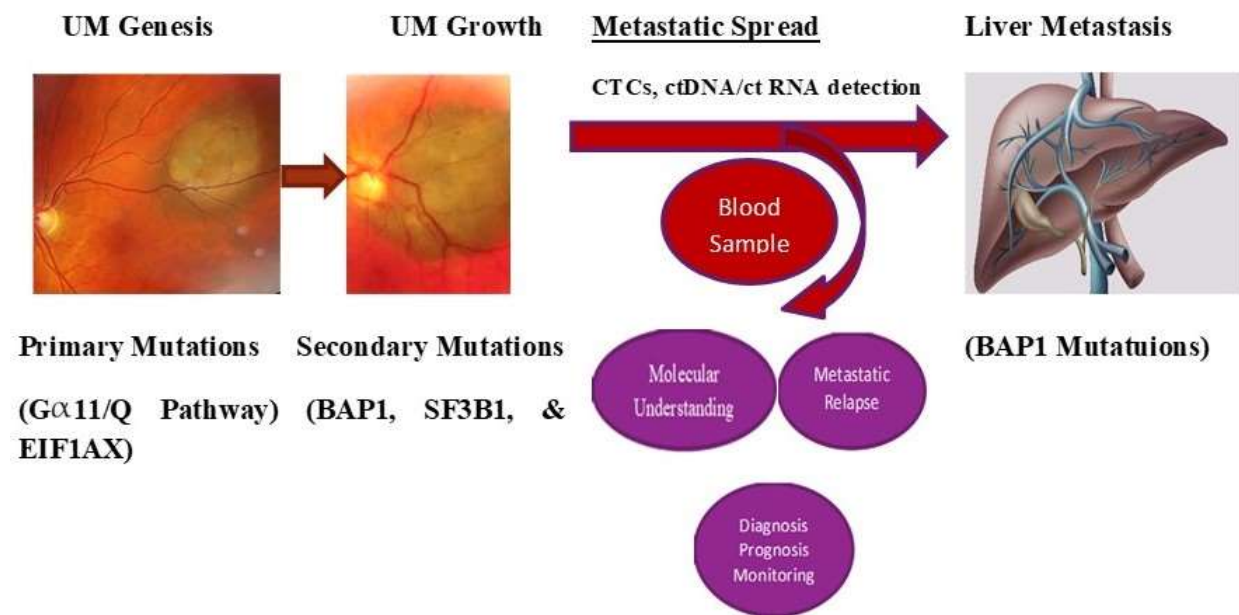
DNA providing from a tissue sample of enucleated patients, and AH provided a higher ct-DNA sensitivity compared to the blood sample [8]. AH LDH is reported to be increased in locally advanced RB. The AH Neuron Specific Enolase (NSE) is found higher in enucleated RB eyes compared to controls [18]. Serum biomarkers such as miRNA-17, miRNA-18a, and miRNA-20a may also be considered for potential biomarkers [19]. Still, incisional tissue biopsy is contraindicated, RB research is now directed to Aqueous humor sample analysis for establishing diagnosis, prognosis, and monitoring of treatment response. Further studies on large sample size are warranted to assess AH puncture indications and determine the best biomarkers.

**Uveal Melanoma (UM)**

The diagnosis of UM is depended on clinical and imaging studies like B-scan ultrasonography or Magnetic resonance imaging (MRI) findings. Transscleral and intravitreal biopsies can be done, but they are technically challenging and may be associated with intraocular complications and extraocular tumor dissemination [20]. The main indication of a tissue biopsy is to assess whether the patient is at metastatic risk or monitoring after primary treatment [21]. External beam radiation therapy (EBRT), proton beam therapy, and brachytherapy are helpful to treat the small to medium-sized tumors, and enucleation is applicable for larger tumors. Local disease control is achieved in up to 95% of cases. Contemporary, metastatic spread is a miraculous finding at the time of the primary tumor treatment, and remain in dormancy for a spell. About 33% to 50% of the patients will occurrences a metastatic spread within the ten years of primary treatment [4]. UM metastasizes through the hematogenous route and the liver is the primary metastatic site of UM [22]. Very recently, the classification of UM patients as low or high metastatic risk is based on chromosomal and genetic abnormalities. LB aims to assess the molecular profiling and find earlier the metastatic spread

because UM cells spread through the bloodstream many months to years before the primary treatment. LB play important role for early detection of UM spread that ultimately improving the overall patient management and early referral to clinical study [23]. For analyzing the molecular characteristics of UM, arterial or venous blood is using for LB to identify CTCs, ct-DNA or ct-RNA), non-coding miRNA, TREs, and TEPs [24]. UM, genetics is different from the skin and conjunctival melanomas, and about 80% of UM harbour mutual exclusivity of GNAQ and GNA11 primary driver mutations, and PLCB4 and CYSLR2 mutations have also been reported [21]. UM, dissemination and prognosis are usually related to secondary driver mutations (BAP1, SF3B1 and EIF1AX) occurring later in the carcinogenesis. The BAP1 mutation is associated with a poorer UM

prognosis. SF3B1 and EIF1AX are associated with a better prognosis [25]. Chromosomal abnormalities, like as loss of chromosome 3 and 8q, are reported in high metastatic risk group of patients [26]. Gene expression profiles are helpful for distinguishing low, intermediate and severe metastatic risk UM [27]. Low to intermediate risk UM is associated with SF3B1 and EIF1AX mutations, whereas high-risk UM is associated with BAP1 mutations [28]. Both CTCs and ct-DNA detection are associated with liver metastatic spread, with reduced Progression-Free Survival (PFS) and Overall Survival (OS) [29]. Blood LB appears to be a non-invasive and useful technique for detecting and studying UM, and have a great relevance for distinguishing benign from malignant pigmented uveal tumours, when a tissue biopsy is not available.



**Figure 1:** Flowchart of Liquid biopsy from blood sample for diagnosing the metastatic spread of uveal melanoma.

**Conclusions**

LB is a relatively non-invasive, easy up and coming technique for diagnosing, and detection of recurrence. It has a prognostic value for overall survival and progression. LB may also

allow early detection of metastatic spread of UM.



### References

1. AlAli A, Kletke S, Gallie B. 2018. Retinoblastoma for Pediatric Ophthalmologists. *Asia Pac J Ophthalmol.* 7: 160-168. Ref.: <https://pubmed.ncbi.nlm.nih.gov/29737052/> DOI: <https://doi.org/10.22608/apo.201870>
2. Singh AD, Turell ME, Topham AK. 2011. Uveal melanoma: Trends in incidence, treatment, and survival. *Ophthalmology.* 118: 1881-1885. Ref.: <https://pubmed.ncbi.nlm.nih.gov/21704381/> DOI: <https://doi.org/10.1016/j.ophtha.2011.01.040>
3. Scarlotta M, Simsek C, Kim AK. 2019. Liquid Biopsy in Solid Malignancy. *Genet Test Mol Biomark.* 23: 284-296. Ref.: <https://pubmed.ncbi.nlm.nih.gov/30916594/> DOI: <https://doi.org/10.1089/gtmb.2018.0237>
4. Bensoussan E, Thariat J, Maschi C. 2016. Outcomes after Proton Beam Therapy for Large Choroidal Melanomas in 492 Patients. *Am J Ophthalmol.* 165: 78-87. Ref.: <https://pubmed.ncbi.nlm.nih.gov/26940166/> DOI: <https://doi.org/10.1016/j.ajo.2016.02.027>
5. León ML, Vieito M, Anido U. 2016. Clinical Application of Circulating Tumour Cells in Prostate Cancer: From Bench to Bedside and Back. *Int J Mol Sci.* 17. Ref.: <https://pubmed.ncbi.nlm.nih.gov/27657044/> DOI: <https://doi.org/10.3390/ijms17091580>
6. Payne K, Brooks J, Spruce R. 2019. Circulating Tumour Cell Biomarkers in Head and Neck Cancer: Current Progress and Future Prospects. *Cancers.* 11. Ref.: <https://pubmed.ncbi.nlm.nih.gov/31387228/> DOI: <https://doi.org/10.3390/cancers11081115>
7. Hofman P. 2017. Liquid biopsy for early detection of lung cancer. *Curr Opin Oncol.* 29: 73-78. Ref.: <https://pubmed.ncbi.nlm.nih.gov/27906860/> DOI: <https://doi.org/10.1097/cco.0000000000000343>
8. Berry JL, Xu L, Polski A. 2020. Aqueous Humor Is Superior to Blood as a Liquid Biopsy for Retinoblastoma. *Ophthalmology.* 127: 552-554. Ref.: <https://pubmed.ncbi.nlm.nih.gov/31767439/> DOI: <https://doi.org/10.1016/j.ophtha.2019.10.026>
9. Pandiani C, Béranger GE, Leclerc J. 2017. Focus on cutaneous and uveal melanoma specificities. *Genes Dev.* 31: 724-743. Ref.: <https://pubmed.ncbi.nlm.nih.gov/28512236/> DOI: <https://doi.org/10.1101/gad.296962.117>
10. Finger PT, Pavlick AC. 2019. Checkpoint inhibition immunotherapy for advanced local and systemic conjunctival melanoma: A clinical case series. *J Immunother Cancer.* 7: 83. Ref.: <https://pubmed.ncbi.nlm.nih.gov/30909967/> DOI: <https://doi.org/10.1186/s40425-019-0555-7>
11. Scholz SL, Cosgarea I, Süßkind D. 2018. NF1 mutations in conjunctival melanoma. *Br J Cancer.* 118: 1243-1247. Ref.: <https://pubmed.ncbi.nlm.nih.gov/29559732/> DOI: <https://doi.org/10.1038/s41416-018-0046-5>
12. Nahon ES, Martel A, Maschi C. 2019. The Molecular Pathology of Eye Tumors: A 2019 Update Main Interests for Routine Clinical Practice. *Curr Mol Med.* 19: 632-664. Ref.: <https://pubmed.ncbi.nlm.nih.gov/31418658/> DOI: <https://doi.org/10.2174/1566524019666190726161044>
13. Munier FL, Gaillard MC, Balmer A. 2012. Intravitreal chemotherapy for vitreous disease in retinoblastoma revisited: From prohibition to conditional indications. *Br J Ophthalmol.* 96: 1078-1083. Ref.: <https://pubmed.ncbi.nlm.nih.gov/22694968/> DOI: <https://doi.org/10.1136/bjophthalmol-2011-301450>
14. Soliman SE, Racher H, Zhang C. 2017. Genetics and Molecular Diagnostics in Retinoblastoma-An Update. *Asia Pac J Ophthalmol.* 6: 197-207. Ref.: <https://pubmed.ncbi.nlm.nih.gov/28399338/> DOI: <https://doi.org/10.22608/apo.201711>





15. Soliman SE, Wan MJ, Heon E. 2017. Retinoblastoma versus advanced Coats' disease: Is enucleation the answer? *Ophthalmic Genet.* 38: 291-293. Ref.: <https://pubmed.ncbi.nlm.nih.gov/27435805/> DOI: <https://doi.org/10.1080/13816810.2016.1199715>
16. Berry JL, Xu L, Kooi I. 2018. Genomic cfDNA Analysis of Aqueous Humor in Retinoblastoma Predicts Eye Salvage: The Surrogate Tumor Biopsy for Retinoblastoma. *Mol Cancer Res.* 16: 1701-1712. Ref.: <https://pubmed.ncbi.nlm.nih.gov/30061186/> DOI: <https://doi.org/10.1158/1541-7786.mcr-18-0369>
17. Xu L, Polski A, Prabakar RK. 2020. Chromosome 6p Amplification in Aqueous Humor Cell-Free DNA Is a Prognostic Biomarker for Retinoblastoma Ocular Survival. *Mol Cancer Res.* 18: 1166-1175. Ref.: <https://pubmed.ncbi.nlm.nih.gov/32434859/> DOI: <https://doi.org/10.1158/1541-7786.mcr-19-1262>
18. Ghiam BK, Xu L, Berry JL. 2019. Aqueous Humor Markers in Retinoblastoma, a Review. *Transl Vis Sci Technol.* 8: 13. Ref.: <https://pubmed.ncbi.nlm.nih.gov/31019846/> DOI: <https://doi.org/10.1167/tvst.8.2.13>
19. Beta M, Venkatesan N, Vasudevan M. 2013. Identification and Insilico Analysis of Retinoblastoma Serum microRNA Profile and Gene Targets towards Prediction of Novel Serum Biomarkers. *Bioinform Biol Insights.* 7: 21-34. Ref.: <https://pubmed.ncbi.nlm.nih.gov/23400111/> DOI: <https://doi.org/10.4137/bbi.s10501>
20. Sellam A, Desjardins L, Barnhill R. 2016. Fine Needle Aspiration Biopsy in Uveal Melanoma: Technique, Complications, and Outcomes. *Am J Ophthalmol.* 162: 28-34. Ref.: <https://pubmed.ncbi.nlm.nih.gov/26556006/> DOI: <https://doi.org/10.1016/j.ajo.2015.11.005>
21. Onken MD, Worley LA, Char DH. 2012. Collaborative Ocular Oncology Group report number 1: Prospective validation of a multi-gene prognostic assay in uveal melanoma. *Ophthalmology.* 119: 1596-1603. Ref.: <https://pubmed.ncbi.nlm.nih.gov/22521086/> DOI: <https://doi.org/10.1016/j.ophtha.2012.02.017>
22. Goh AY, Layton CJ. 2016. Evolving systemic targeted therapy strategies in uveal melanoma and implications for ophthalmic management: A review. *Clin Exp Ophthalmol.* 44: 509-519. Ref.: <https://pubmed.ncbi.nlm.nih.gov/26601795/> DOI: <https://doi.org/10.1111/ceo.12688>
23. Shoushtari AN, Carvajal RD. 2016. Treatment of Uveal Melanoma. *Cancer Treat Res.* 167: 281-293. Ref.: <https://pubmed.ncbi.nlm.nih.gov/26601868/> DOI: [https://doi.org/10.1007/978-3-319-22539-5\\_12](https://doi.org/10.1007/978-3-319-22539-5_12)
24. Bande R, Fernandez M, Lago B. 2020. Blood Biomarkers of Uveal Melanoma: Current Perspectives *Clin Ophthalmol.* 14: 157-169. Ref.: <https://pubmed.ncbi.nlm.nih.gov/32021081/> DOI: <https://doi.org/10.2147/opth.s199064>
25. Nahon ES, Martel A, Maschi C. 2019. The Molecular Pathology of Eye Tumors: A 2019 Update Main Interests for Routine Clinical Practice. *Curr Mol Med.* 19: 632-664. Ref.: <https://pubmed.ncbi.nlm.nih.gov/31418658/> DOI: <https://doi.org/10.2174/1566524019666190726161044>
26. Rodrigues M, Ait RK, Salviat F. 2020. Association of Partial Chromosome 3 Deletion in Uveal Melanomas with Metastasis-Free Survival. *JAMA Ophthalmol.* 138: 182-188. Ref.: <https://pubmed.ncbi.nlm.nih.gov/31895446/> DOI: <https://doi.org/10.1001/jamaophthalmol.2019.5403>
27. Onken MD, Worley LA, Ehlers JP. 2004. Gene expression profiling in uveal melanoma reveals two molecular classes and predicts metastatic death. *Cancer Res.* 64: 7205-7209. Ref.: <https://pubmed.ncbi.nlm.nih.gov/15492234/> DOI: <https://doi.org/10.1158/0008-5472.can-04-1750>
28. Smit KN, Jager MJ, de KA. 2020. Uveal melanoma: Towards a molecular



understanding. Prog Retin Eye Res. 75: 100800. Ref.:

<https://pubmed.ncbi.nlm.nih.gov/31563544/>

DOI:

<https://doi.org/10.1016/j.preteyeres.2019.10.0800>

29. Metz CH, Scheulen M, Bornfeld N. 2013. Ultradeep sequencing detects GNAQ and GNA11 mutations in cell-free DNA from plasma of patients with uveal melanoma. Cancer Med. 2: 208-215. Ref.:
- <https://pubmed.ncbi.nlm.nih.gov/23634288/>  
DOI: <https://doi.org/10.1002/cam4.61>