Childhood Cancer Data Initiative Webinar Series

From Discovery to Translation: Developing Aqueous Humor Liquid Biopsy for Childhood Eye Cancer

Liya Xu

NIH NATIONAL CANCER INSTITUTE

December 9, 2025

Today's Speaker

Liya Xu, Ph.D.

- Assistant Professor of Research Ophthalmology at the University of Southern California (USC) / Children's Hospital Los Angeles (CHLA)

Agenda

1. *Clinical Background and Challenges*
2. *Discovery*
3. *Molecular Insights*
4. *Translational Potential and Future Directions*

Clinical Background and Challenges

Retinoblastoma as a cancer biology model and the unmet needs in patient caret

Retinoblastoma (RB): A Childhood Eye Cancer

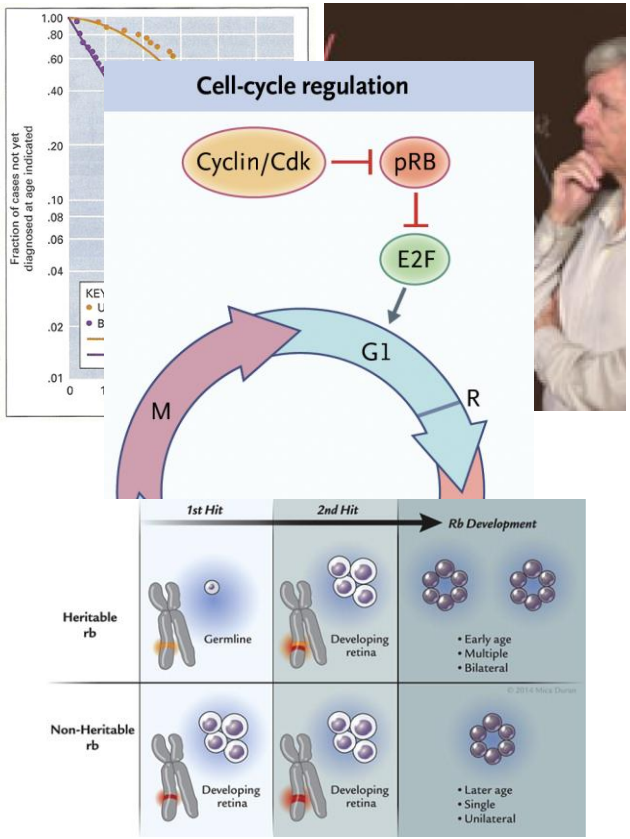
- Rare tumor: ~1 in 15–20,000 live births worldwide
- Presents in infants/toddlers, median age ~18 months
- Can happen in one eye (~60%) or both (~40%)
- Today: ~98% survival in high-income countries
- Major ongoing challenges: global equity & vision/eye preservation

Photos courtesy of Dr. Jerry A. Shields, Wills Eye Hospital

Tomar et al., Ophthalmology 2021

RB as a Foundational Cancer Model

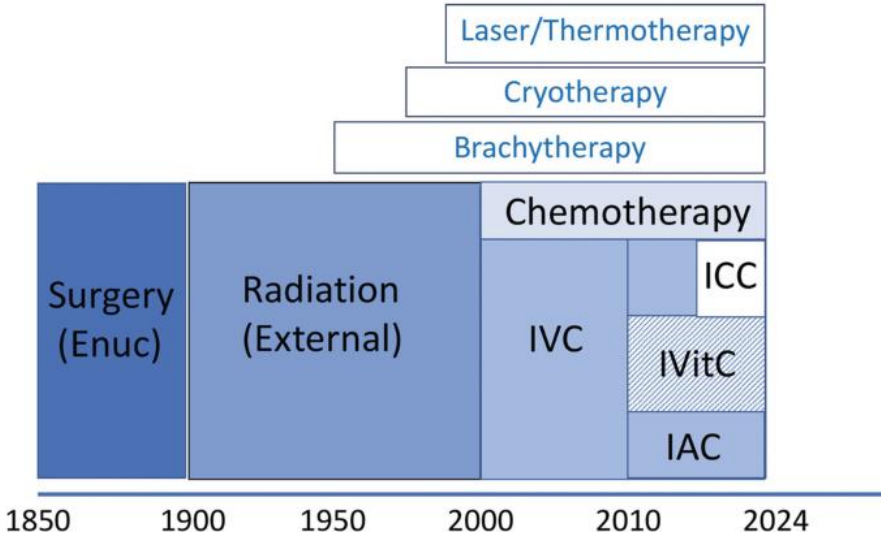
- One of the most intensively studied cancers
- Knudson's 1971 **two-hit hypothesis** → tumors arise after both alleles of a tumor suppressor gene are inactivated (cornerstone of cancer genetics)
- *RB1* = first tumor suppressor gene identified
- pRB → key regulator of the cell cycle clock in all vertebrate cells
- Framework from RB now underpins cancer genetics across oncology



Retinoblastoma 2017

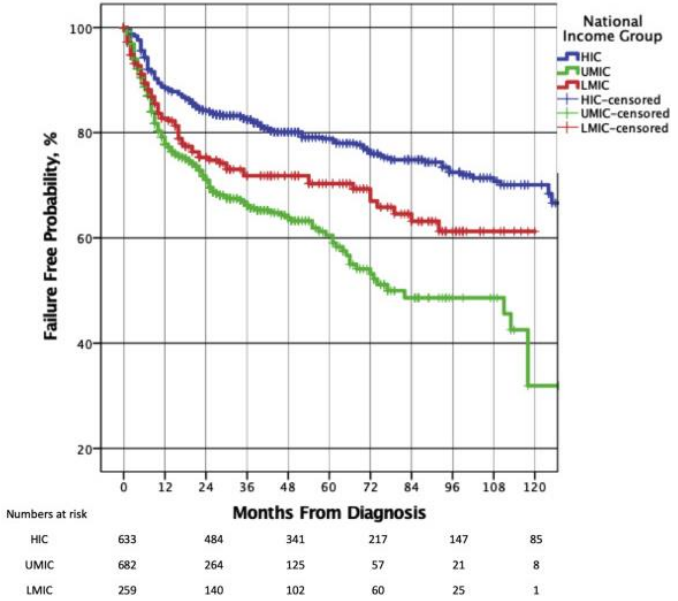
The Paradox of Treatment

Evolution of Treatment



Vishal et al., Clinical Ophthalmic Oncology

Cumulative Proportion of Salvaged RB eyes

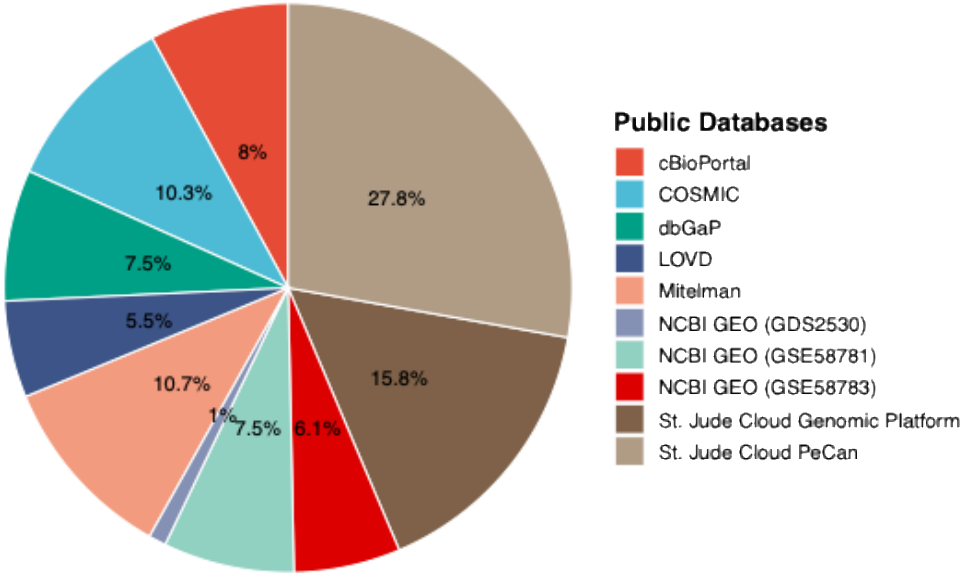


Tomar et al., Ophthalmology 2021

The Knowledge – Care Gap in RB

- Decades of RB biology discoveries → minimal direct benefit for patients
- Still no molecular tools to guide diagnosis, risk stratification, or therapy
- Precision oncology has transformed many cancers… but not RB
- It is the only solid tumor where there is a global, across-the-board prohibition of biopsy, written into all guidelines

Public Data Banks: All From Enucleated Eyes



Public Database	Type of Data	Number of Samples
cBioPortal for Cancer Genomics	Mutations, CNA, Structural Variants	95 patients, 97 samples
St. Jude Cloud Genomic Platform	RNAseq, WGS, WES	193 samples
St. Jude Cloud PeCan	Variants, mutational signatures, expression, histology	339 samples, 256 subjects
Mitelman	Copy Number data	130 samples
COSMIC	Overview of mutations	126 samples
NCBI GEO (Dataset: GSE58783)	Methylation arrays	74 samples
NCBI GEO (Dataset: GSE58781)	SNP array	94 samples
NCBI GEO (Dataset: GDS2530)	Transcriptome of tumors with LOH of 16q	12 samples
dbGaP	WGS, RNAseq, SNP array	9, 4, 91 samples
LOVD	Variants	67 samples

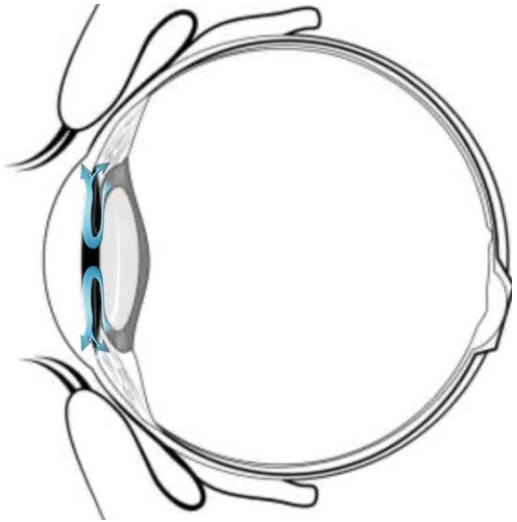
How can we safely access tumor-genomics from *In Vivo* RB?

Discovery

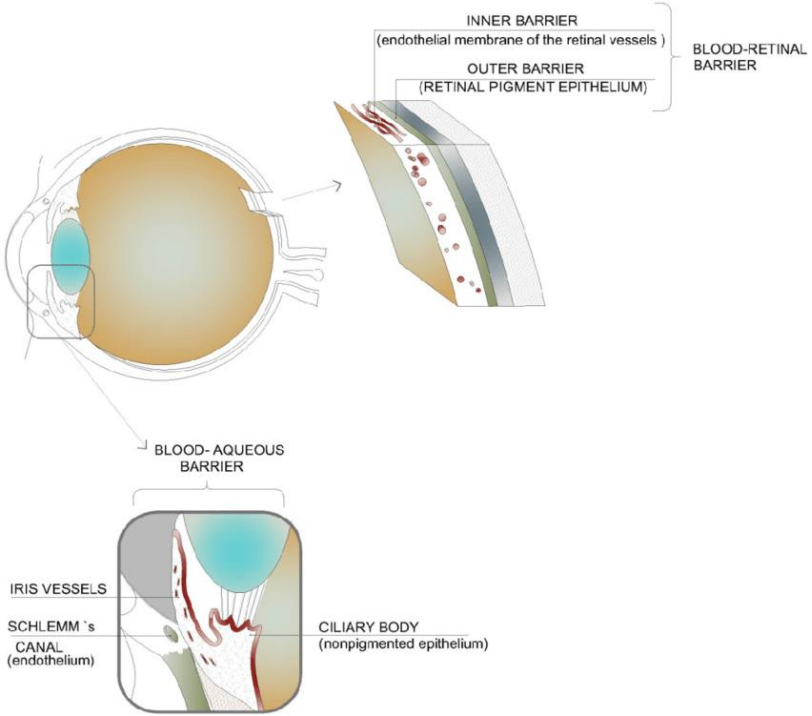
Identifying aqueous humor as a novel source of tumor-derived cfDNA

The Hypothesis: Aqueous Humor (AH) is Ideal Ocular LBx Platform

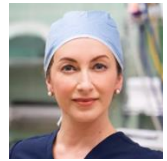
- AH as a Proximal Fluid



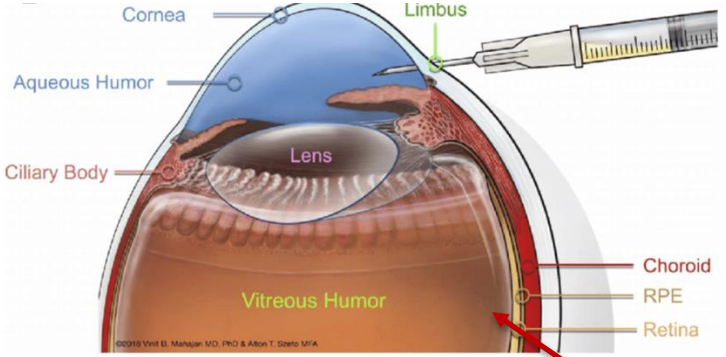
- AH as a Pure Source



Aqueous Paracentesis

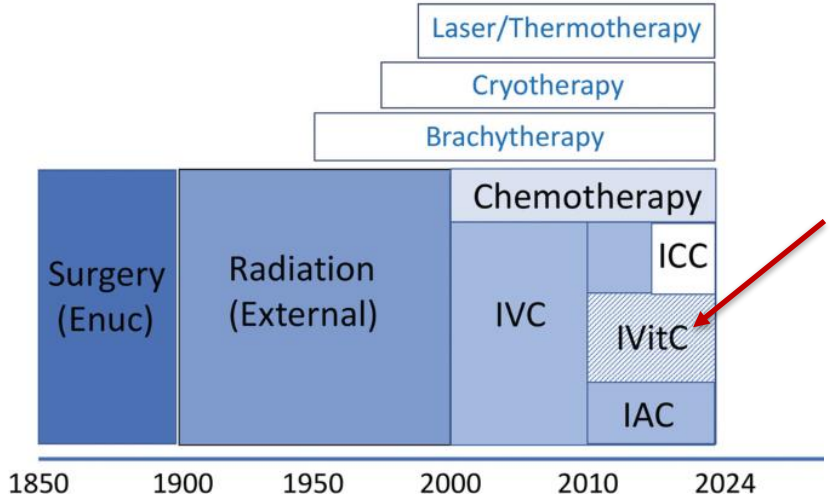


Jesse L. Berry, M.D.



Velez et al. Translational Vision Sci. & Tech. 2018

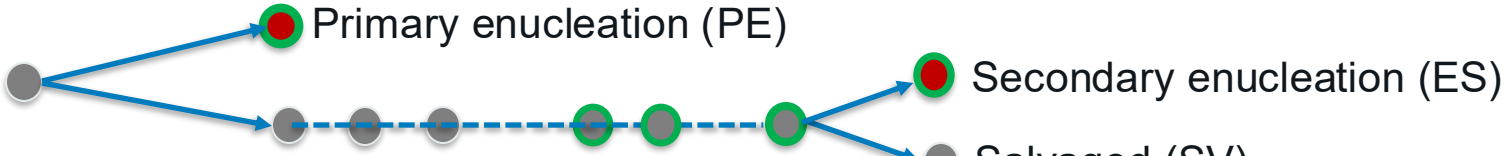
- ~ 50 years practice
- Every trained ophthalmologist

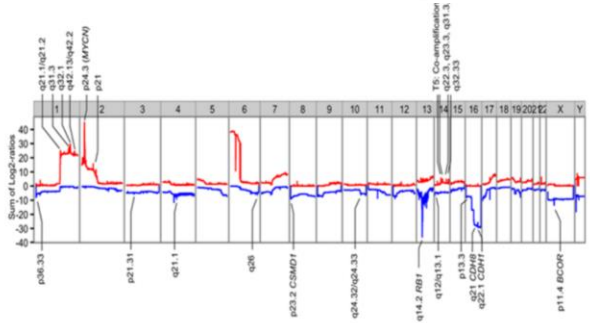


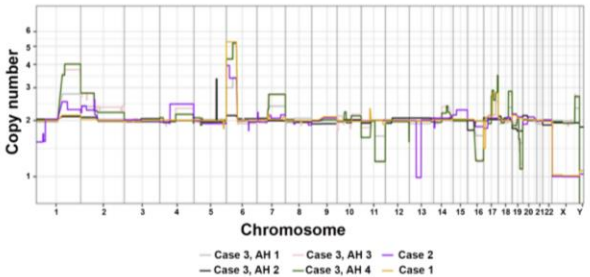
Vishal et al., Clinical Ophthalmic Oncology

Intravitreal chemotherapy

Proof of Concept







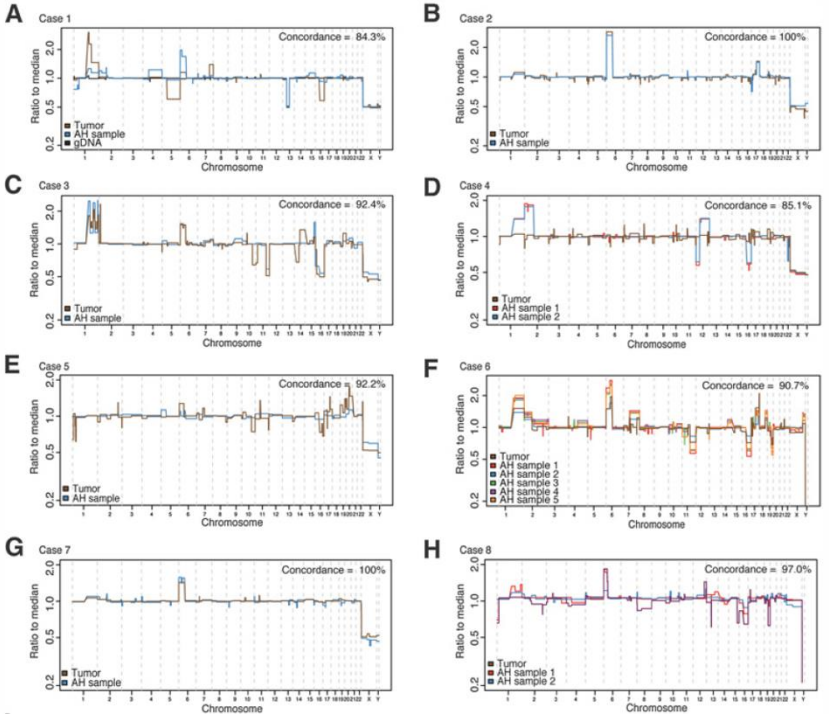
- Highly recurrent RB-related somatic copy number alterations (SCNAs RB-SCNAs) (1q,2p,6p gains; 13q,16q loss and a focal MYCN amp on 2p).
- We identified tumor-derived cfDNA in the AH with RB-SCNAs.

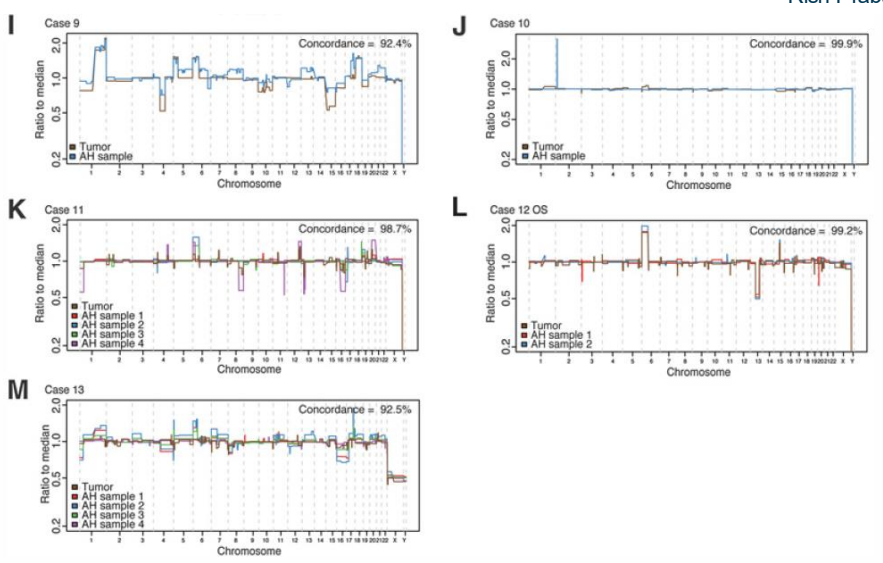
JAMA Ophthalmology | Original Investigation

Aqueous Humor as a Surrogate Tumor Biopsy for Retinoblastoma

Proof of Concept

Rish Prabakar, Ph.D.



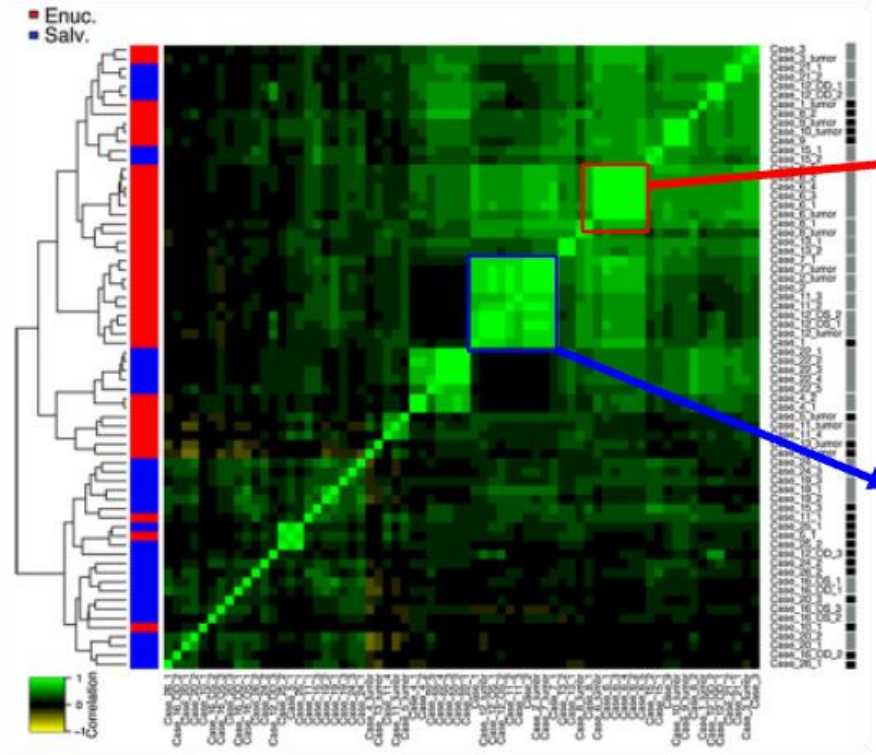


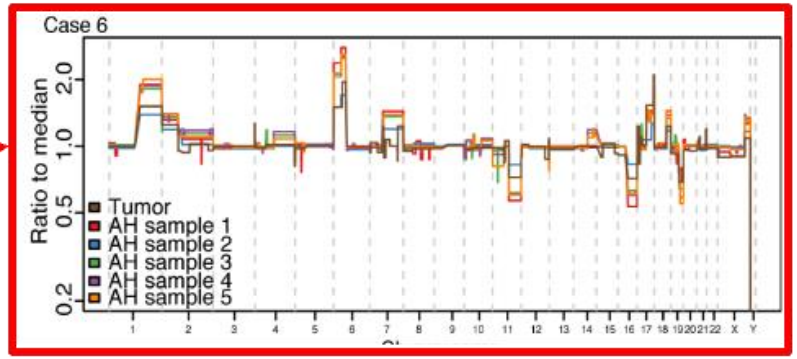
- **11/13 with >90% concordance**
- **2 cases with 85% concordance with multiple tumors**

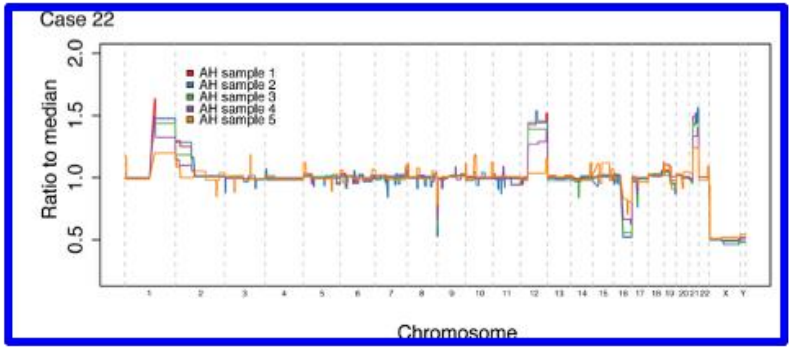
Berry et al., Mol. Cancer Res., 2018

Proof of Concept

Rish Prabakar, Ph.D.







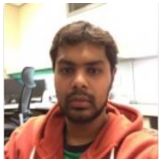
Berry et al., Mol. Cancer Res., 2018

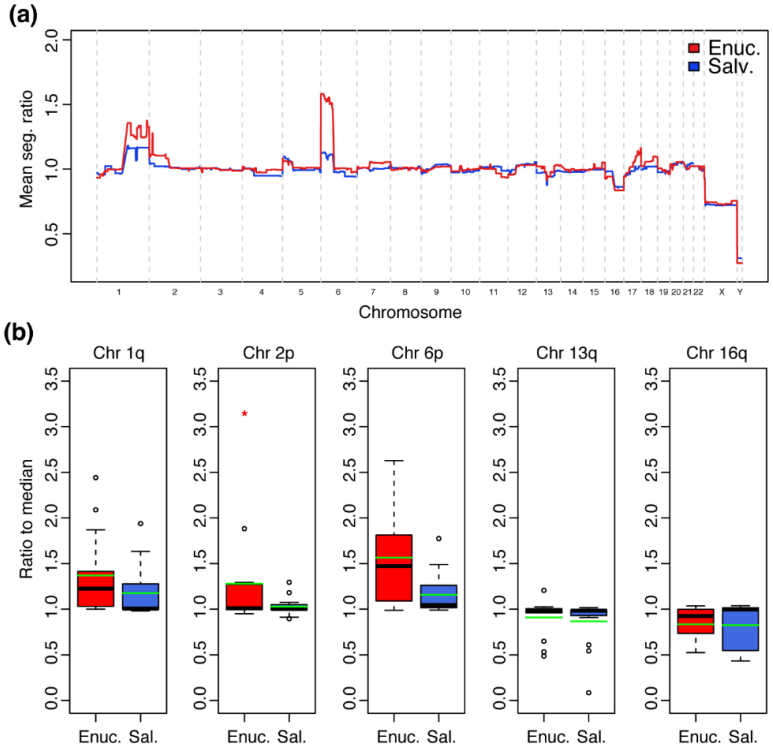
Molecular Insights

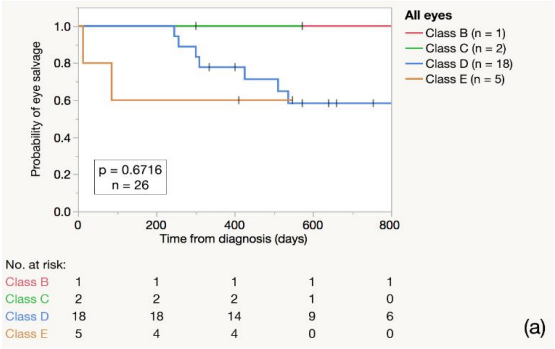
Genomic and methylation landscapes revealed through aqueous humor liquid biopsy

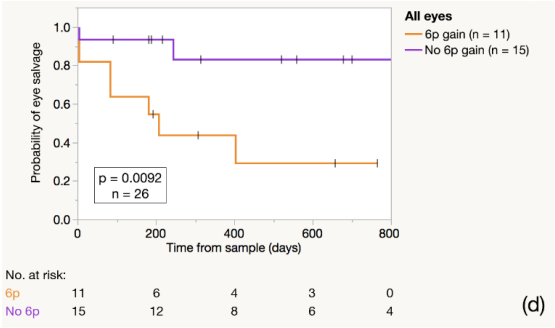
Chr6p Gain as Prognostic Biomarker


Ashley Polski, M.D.


Rish Prabakar, Ph.D.







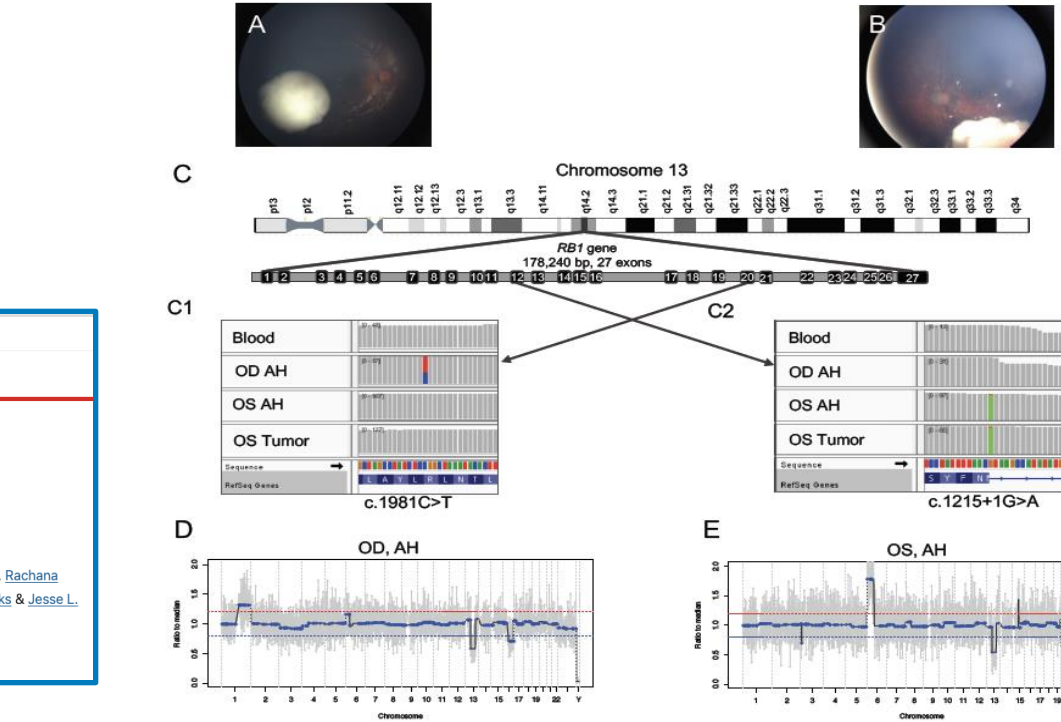
Berry et al., & Xu et al., Mol. Cancer Res., 2018, 2020

6p gain was associated with a 10x increased odds of enucleation (OR 10, 95% CI: 1.8-55.6)

Eye Specific AH Profiling is Necessary


Xiaowu Gai, Ph.D.


Elyssa Wong, M.D.



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Case Report | Open Access | Published: 27 July 2021

Inter-eye genomic heterogeneity in bilateral retinoblastoma via aqueous humor liquid biopsy

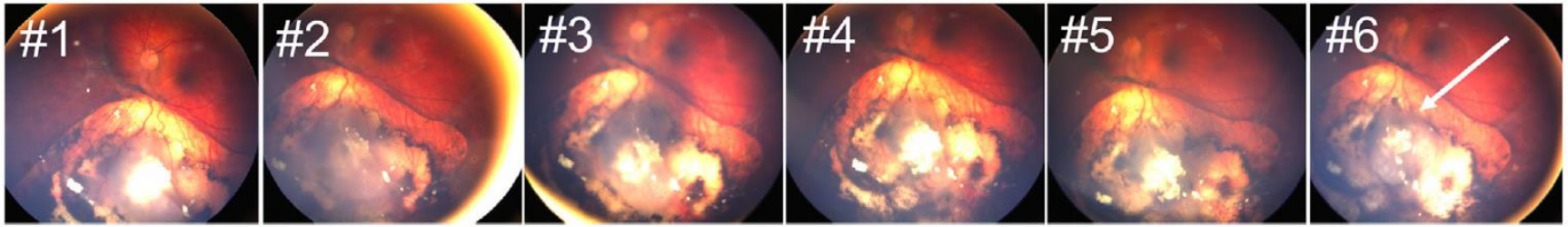
Elyssa Y. Wong, Liya Xu, Lishuang Shen, Mary E. Kim, Ashley Polski, Rishvanth K. Prabakar, Rachana Shah, Rima Jubran, Jonathan W. Kim, Jaclyn A. Biegel, Xiaowu Gai, Peter Kuhn, James Hicks & Jesse L. Berry

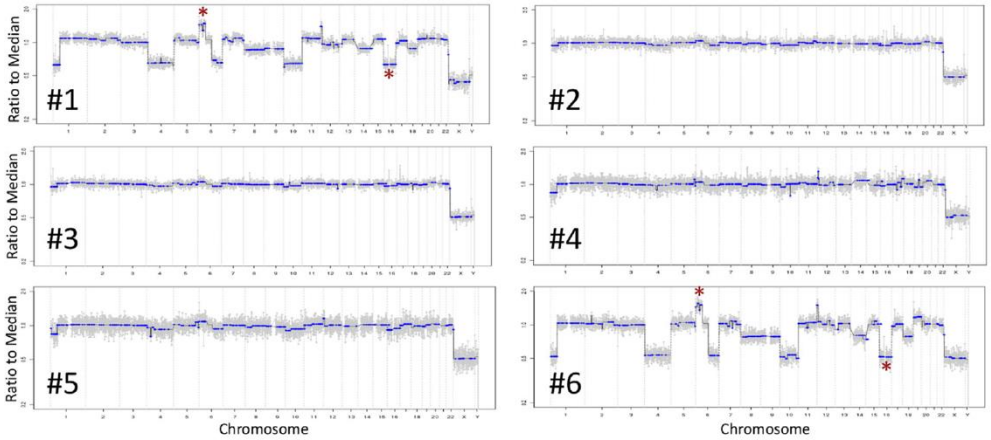
npj Precision Oncology **5**, Article number: 73 (2021) | Cite this article

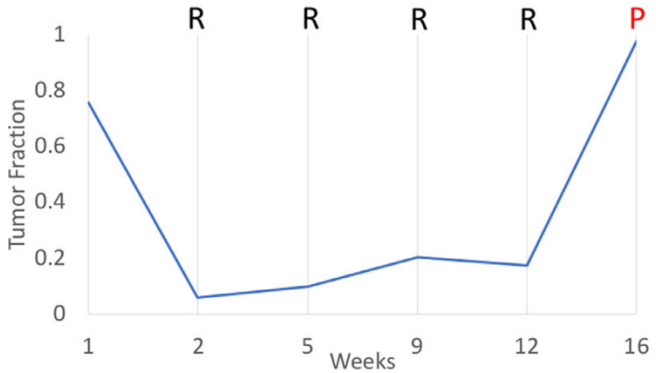
Wong et al., NPJ Precis. Oncol. 2021

Tumor Fractions (TFx) for Longitudinal Monitoring

Ashley Polski, M.D.



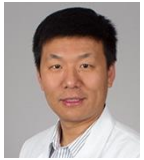




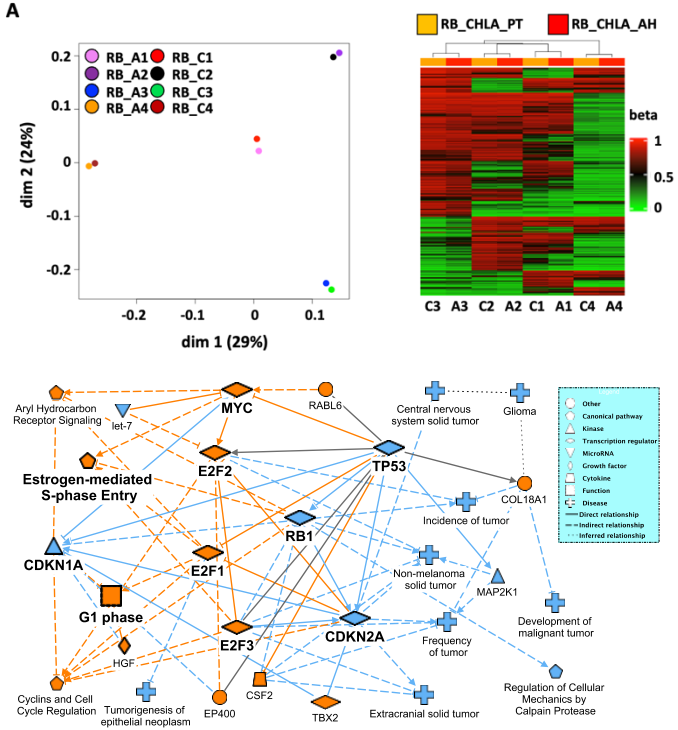
Polski et al., TVST 2020

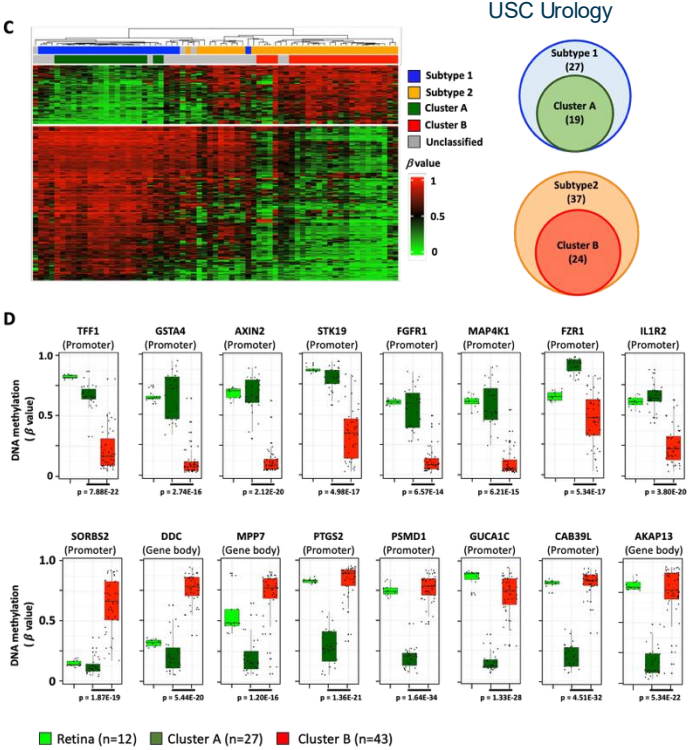
AH methylation pattern for RB subtyping

Gangning Liang, Ph.D.
USC Urology



Hongtao Li, Ph.D.
USC Urology



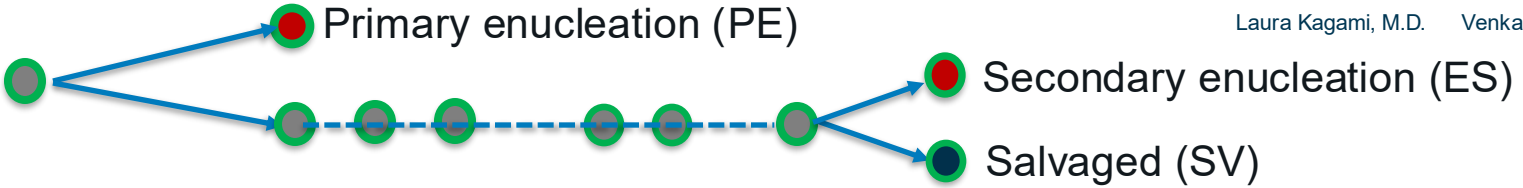


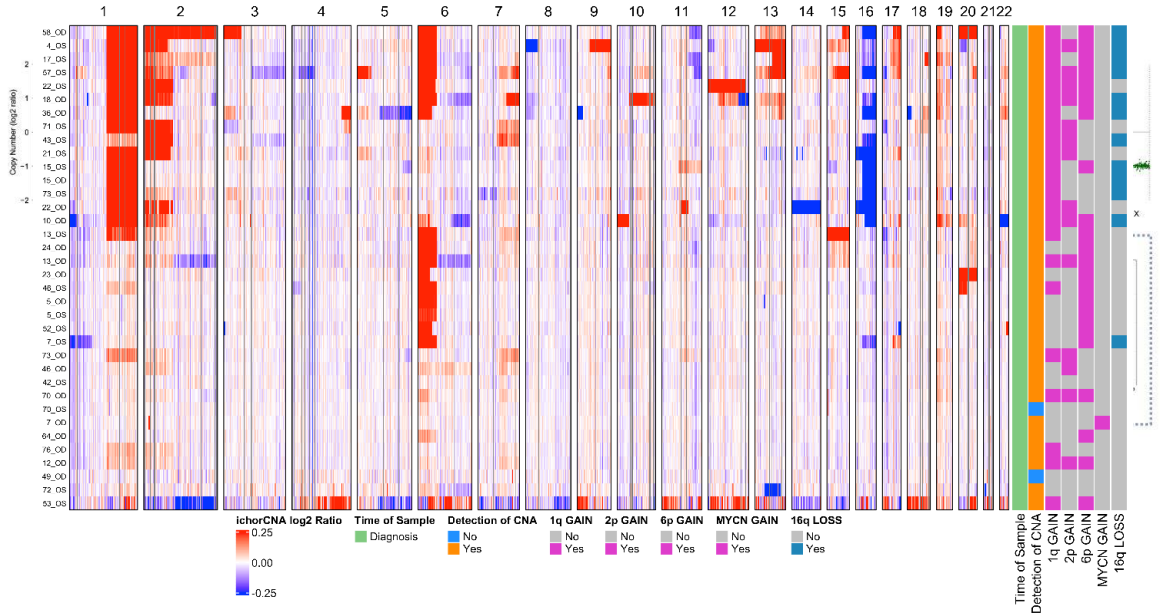
Li et al., Nature Communication 2022

Diagnostic Aqueous Humor Sampling

Laura Kagami, M.D.

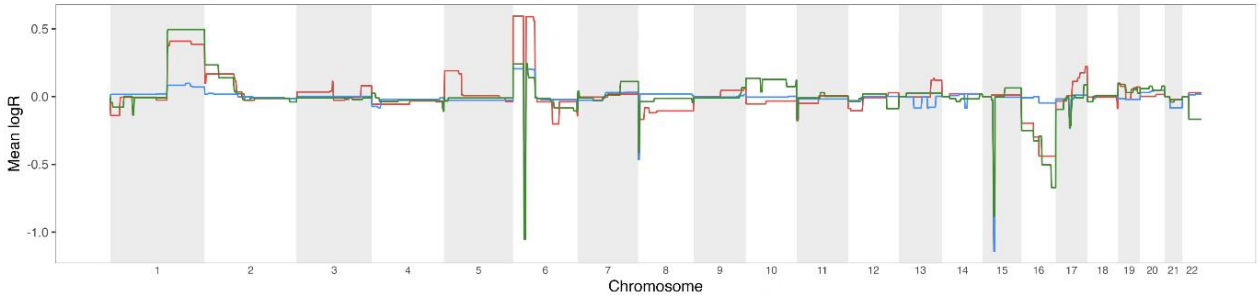
Venkata Yellapantula, Ph.D.





Diagnostic AH Genomics For Risk Stratification

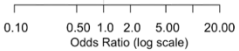
Venkata Yellapantula, Ph.D.



Progressed (n=12)
Regressed (n=31)
Slow Responder (n=14)

Univariate Marker Association (Firth's OR + Permutation P)

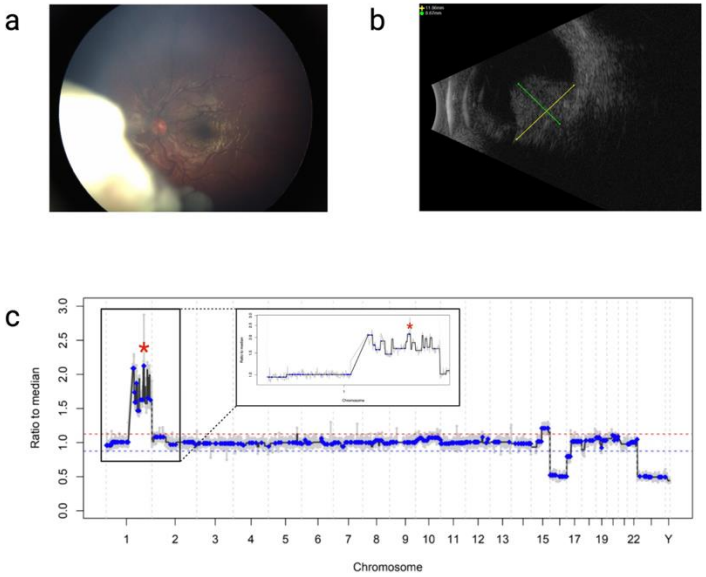
Marker	OR (95% CI)	Adj P (Perm)
chr16q-	9.60 (2.91 - 37.60)	0.001
chr1q+	6.10 (1.98 - 21.33)	0.017
chr6p++	6.04 (1.70 - 26.75)	0.035
TFx (High vs Low)	5.32 (1.80 - 17.18)	0.022
chr17q+	5.18 (1.44 - 23.02)	0.087
chr1q++	4.42 (1.21 - 19.75)	0.159
AJCC Stage (High vs Low)	3.28 (0.92 - 14.43)	0.543
chr2p++	3.02 (0.66 - 18.11)	0.868
chr2p+	2.80 (0.99 - 8.32)	0.394
chr6p+	1.96 (0.69 - 5.76)	0.898
chr17p-	1.23 (0.32 - 4.70)	1.000

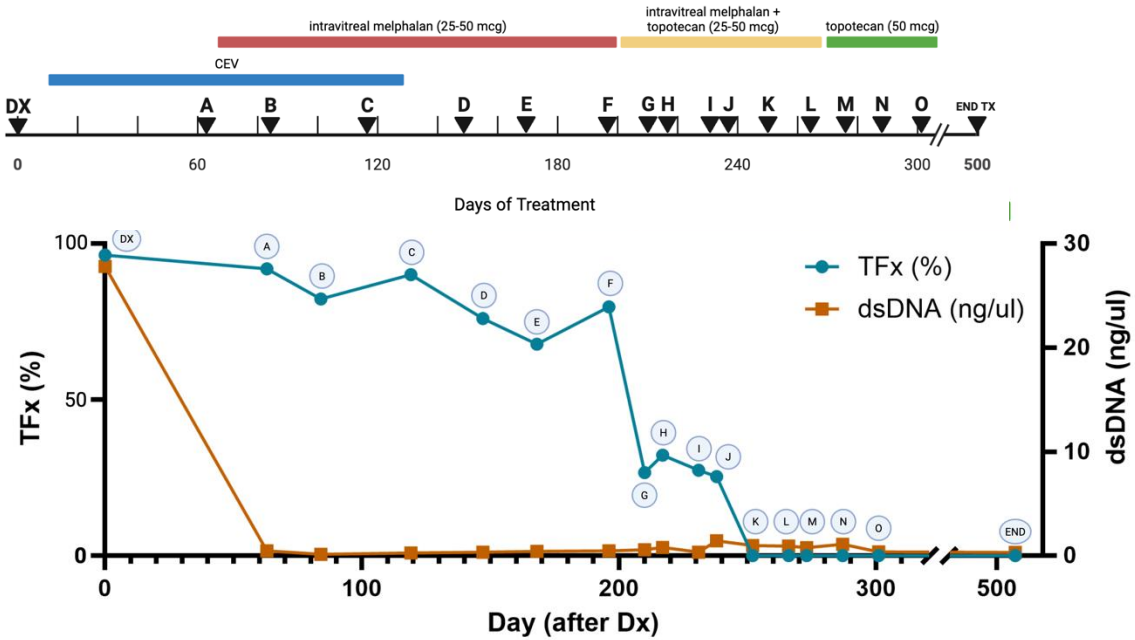


- 16q-, 1q+, 6p++, high TFx → high odds of treatment failure
- AH genomics at diagnosis provides actionable prognostic information

MDM4 Segmental Gain Associated with Melphalan Resistance

Elain Huang, M.D. Candidate



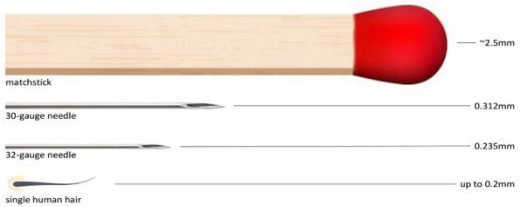


Huang et al., JCO Precision Oncology 2025

Translational Potential and Future Directions

From molecular discovery to clinical application with LBSeq4Kids

RB Eye AH Paracentesis Safely



Safety Assessment of Aqueous Humor Liquid Biopsy in Retinoblastoma: A Multicenter Study of 1203 Procedures

Douglas Chigane [1], Drishti Pandya [1], Muskaan Singh [2], Brianne Brown [1], Michelle Lin [1], Liya Xu [3], Andrew W Stacey [4], Alyssa C Bonnell [4], G Baker Hubbard 3rd [5], Hans Grossniklaus [5], Alison H Skalet [6], Kellyn N Bellsmith [6], Sara E Lally [7], Margarida Simão-Rafael [8], Cristina Jou Muñoz [8], Jaume Català-Mora [8], Denis Malaise [9], Livia Lumbroso-Le Rouic [9], Alexandre Matet [10], Guillermo L Chantada [8], Nathalie Cassoux [10], Carol L Shields [7], Jesse L Berry [11]

Conclusions: Aqueous humor liquid biopsy via anterior chamber paracentesis is a safe and well-tolerated procedure in pediatric patients with retinoblastoma when performed by trained ocular surgeons under general anesthesia. This large-scale analysis supports the procedure's use as a minimally invasive diagnostic tool with minimal risk, providing valuable molecular insights for retinoblastoma management. These findings offer reassurance to clinicians and parents regarding the safety of AH liquid biopsy for retinoblastoma.

Chigane et al., Ophthalmology 2025

Grade of Anterior Chamber Paracentesis Complications
Mild (self-limited)
Subconjunctival hemorrhage
Conjunctival hyperemia
Air bubble in anterior chamber
Self limited iris incarceration
Small self limited Descemet's tear
Corneal abrasion with no needed intervention
Mycrohyphema
Conjunctivitis
Allergy to surface sterilizers
Moderate (need of intervention)
Ocular hypotony
Keratitis
Visual significant corneal scarring
Descemet's membrane tear requiring intervention
Peripheral located corneal abscess
Iris trauma
Iritis
Iris incarceration
Anterior chamber fibrin formation
Self limited hyphema
Cataract with no precluded view
AH leak at paracentesis site
Severe (vision, eye, or life threatening)
Complicated corneal abscess
Hypopyon
Complete hyphema
Endophthalmitis
Suprachoroidal hemorrhage
Violation of the lens capsule
Cataract precluding fundus view
Tumor spread

CLIA-LDT LBseq4Kids

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Alexander R. Judkins, MD
Department of Pathology & Laboratory Medicine
Pathologist–in-Chief and Laboratory Director
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California State License CDF-00347990

Children's Hospital LOS ANGELES

Ship To:
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Children's Hospital Los Angeles
4650 Sunset Blvd.
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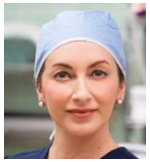
Los Angeles, CA 90027

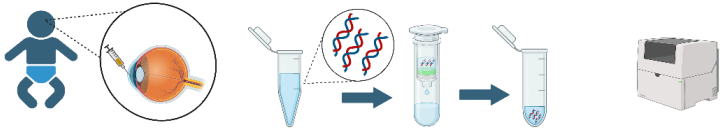


Jesse L. Berry, M.D.

Jaclyn Biegel, Ph.D.

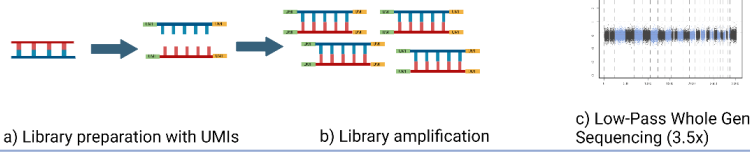
CPM Liquid Biopsy (LBSeq4Kids) test requisition

1. Sample collection and processing



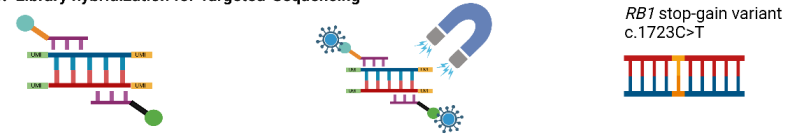
a) Aqueous humor collection b) cfDNA extraction c) cfDNA abundance (TapeStation)

2. Library preparation for Low-Pass Whole Genome Sequencing



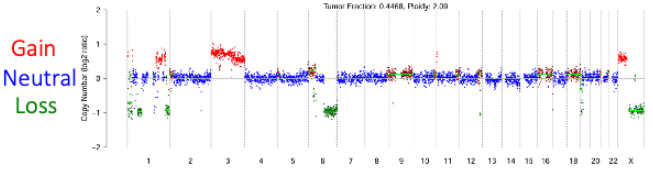
a) Library preparation with UMIs b) Library amplification c) Low-Pass Whole Genome Sequencing (3.5x)

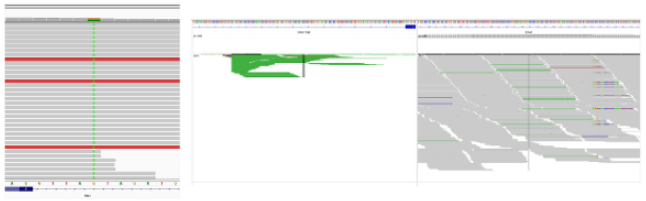
3. Library hybridization for Targeted-Sequencing



a) Hybridize capture probes b) Streptavidin bead binding c) Targeted Sequencing (1000x)

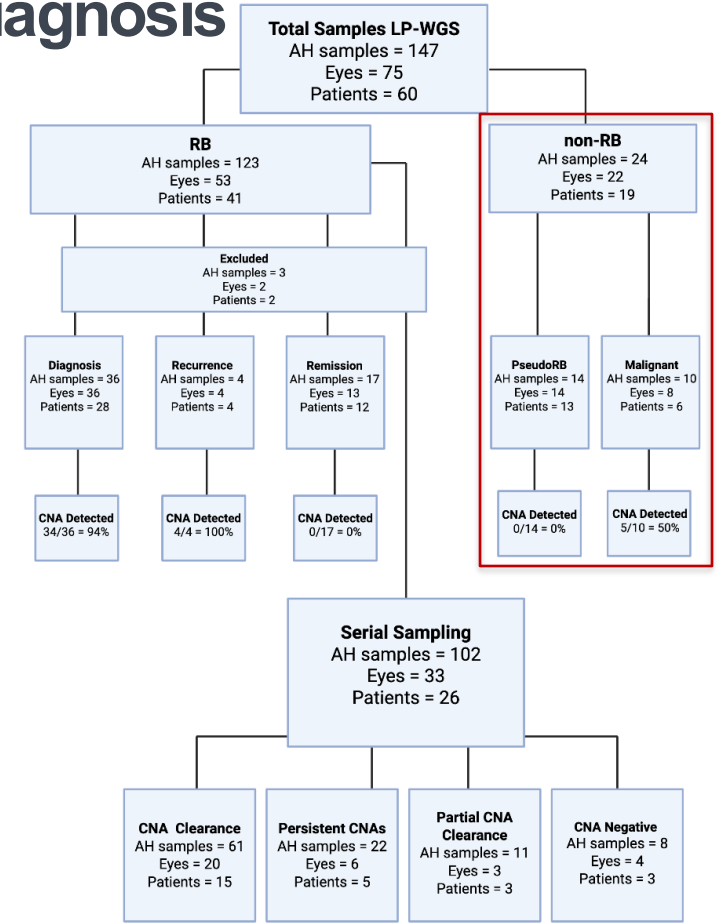
RB1 stop-gain variant
c.1723C>T





RB1 variant *KIAA1549::BRAF* fusion

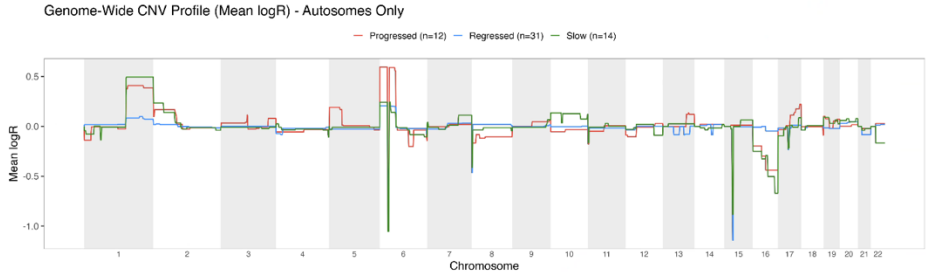
Diagnosis

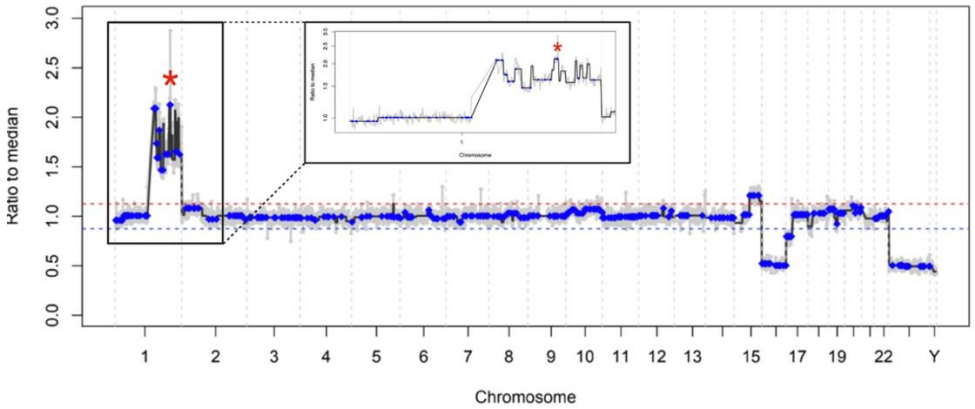


	Patients	Eyes	AH Samples	CNA detected
Retinoblastoma				
Diagnosis	28	36	36	34/36 = 94%
Recurrence	4	4	4	4/4 = 100%
In remission	12	13	17	0/17 = 0%
Active disease	22	30	63	39/63 = 62%

- Accurately distinguishes active RB from remission or benign disease
- Molecular confirmation supports timely treatment decisions
- Non-invasive testing could spare non-RB eyes from unnecessary enucleation.

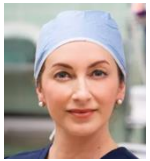
Prognosis/Risk Stratification – In Development





- Goal: Identify molecular markers predicting progression, regression, or treatment response.
- Major challenge: treatment heterogeneity and lack of standardized NCCN guidelines
- Require multi-center collaboration to unify data across treatment settings

International RB AH Consortium



Jesse L. Berry, M.D.

- **Fully Executed:** Sample + Data: 7 US + 1 Canada
- **Fully Executed:** Data Sharing:13 European Sites
- **Pending Activation:** Sample + Data: 4 US + 1 International (India)
- **Pending Center Start Up:** 22 centers

Closing

- RB is the cancer we cannot biopsy, but AH liquid biopsy changes that.
- We now have a safe, validated, CLIA-certified platform to access in vivo tumor biology.
- We are finally connecting RB genomics to real-time treatment decisions, something impossible for 150 years.
- And now, with an expanding international consortium, we have the scale needed to build true prognostic and predictive models.
- CCDI is the bridge that can help us share these data safely, responsibly, and equitably, so no child with RB is left behind, anywhere in the world.

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PATIENTS AND FAMILIES

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EV Core @CHLA
FACS Core @ CHLA

Q&A

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Thank you for attending!

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