# Non-invasive Diagnosis of Retinoblastoma using cell-free DNA from aqueous humour

Amy Gerrish – R&D Scientist
Birmingham Women's and Children's NHS Foundation Trust

# Retinoblastoma

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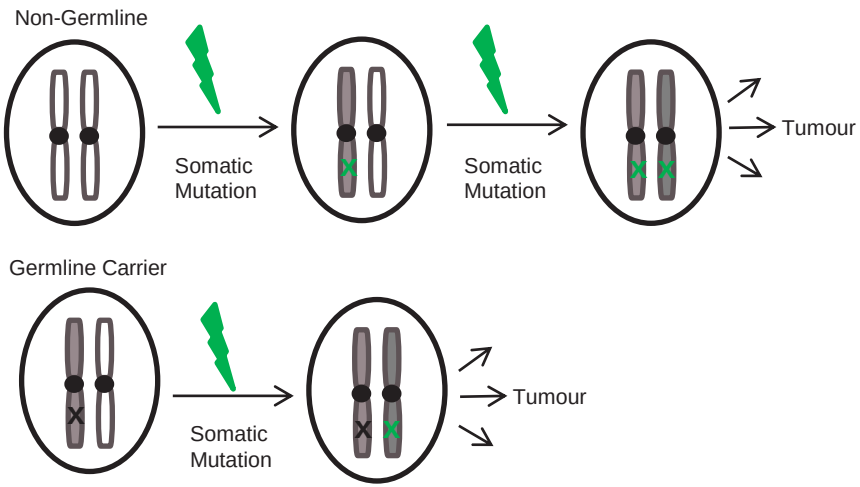
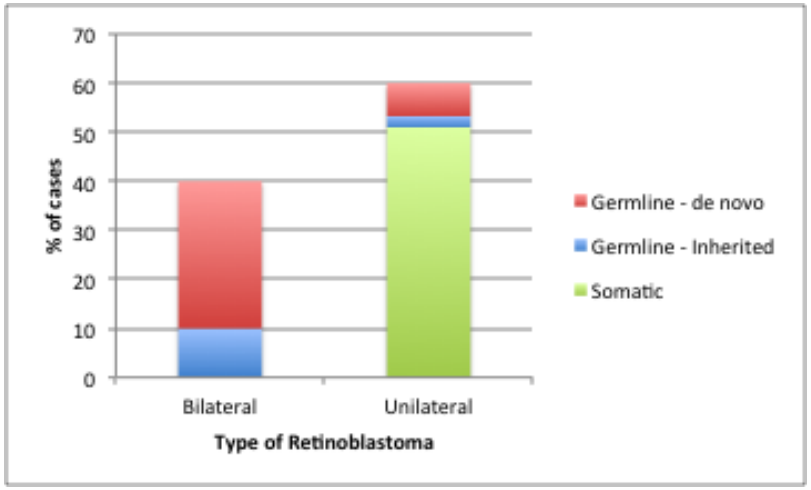
- Most common childhood eye cancer
- Can affect one eye (unilateral) or both eyes (bilateral)

- Majority of retinoblastoma caused by inactivation of both copies of the *RB1* gene
  - Germline and/or Somatic Mutations




# Genetic Diagnosis of Somatic Variants

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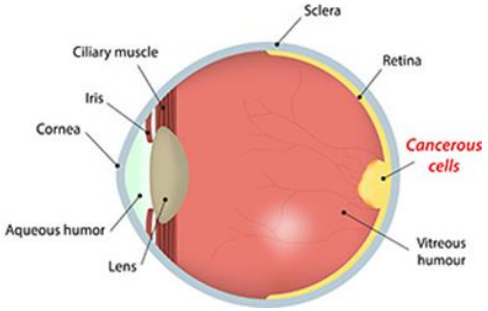
- Unilateral germline carriers can be identified from genomic DNA
- Identifying true somatic unilateral patients requires both blood and tumour sample
- Tumour only available where eye is removed (enucleation)

- Potential for circulating tumour DNA
- Possible source of ctDNA → aqueous humour (a bi-product of treatment)

- Intravitreal Chemotherapy (IViC)
  - Drugs injected directly into the eye
  - Includes the removal ~100ul aqueous humour prior to drug injection




# Wellcome Trust Innovation Grant

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1. Determine whether aqueous humour (AH) is an adequate source of cell-free DNA

2. Develop genetic test on cell-free DNA from AH taken from enucleated eyes (where tumour DNA is available)

3. Validate test on cell-free DNA on AH from eyes undergoing IViC

wellcome

# Assay Design

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- Next Generation Sequencing (NGS)

- Targeted Capture of *RB1* and Chromosome 13 using Nimblegen SeqCap
- Designed probes to detect
  - Single Nucleotide Variants (SNVs)
  - Loss Of Heterozygosity (LOH)
  - Copy Number Variants (CNVs) in *RB1* and *MYCN*
- Sequenced cfDNA, gDNA and tumour DNA (where available)
- Developed bioinformatic pipeline

# Results – Enucleated Patients

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| Patient | cfDNA conc ng/ul |
|---|---|
| E1 | 2.12 |
| E2 | 228 |
| E3 | 0.183 |
| E4 | 394 |
| E5 | 0.169 |
| E6 | 0.141 |
| E7 | 244 |
| E8 | 1.96 |
| E9 | 1.01 |
| E10 | 1.38 |

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# Results – Enucleated Patients

NHS
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| Patient | cfDNA conc ng/ul | *RB1* Germline Mutation | *RB1* Somatic Mutation |
|---|---|---|---|
| | | | Tumour DNA |
| E1 | 2.12 | Negative | c.1363C>T p.(Arg455*) |
| | | | LOH |
| E2 | 228 | Negative | c.751C>T p.(Arg251*) |
| | | | LOH |
| E3 | 0.183 | Negative | c.1959dupA |
| | | | LOH |
| E4 | 394 | Negative | c.763C>T p.(Arg255*) |
| | | | LOH |
| E5 | 0.169 | Negative | c.1251_1252delAA |
| | | | LOH |
| E6 | 0.141 | Negative | Deletion 1-17 RB1 |
| | | | Deletion of 25-27 RB1 |
| E7 | 244 | c.1496_97dup mosaic (9%) | c.1496_97dup |
| | | | LOH |
| E8 | 1.96 | c.1072 C to T p.(Arg358*) mosaic (21%) | c.1072 C to T p.(Arg358*) |
| | | | LOH |
| E9 | 1.01 | Negative | c.958C>T p.(Arg320*) |
| | | | c.1981C>T p.(Arg661*) |
| E10 | 1.38 | Negative | c.147delT |
| | | | c.1330C>T p.(Gln444*) |

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# Results – Enucleated Patients

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| Patient | cfDNA conc ng/ul | *RB1* Germline Mutation | *RB1* Somatic Mutation | | |
|---|---|---|---|---|---|
| | | | Tumour DNA | Detected in cfDNA? | SNV MUT Allele % |
| E1 | 2.12 | Negative | c.1363C>T p.(Arg455*) | Yes | 91% |
| | | | LOH | Chr 13 LOH | |
| E2 | 228 | Negative | c.751C>T p.(Arg251*) | Yes | 99% |
| | | | LOH | Chr 13 LOH | |
| E3 | 0.183 | Negative | c.1959dupA | Yes | 87% |
| | | | LOH | RB1 LOH | |
| E4 | 394 | Negative | c.763C>T p.(Arg255*) | Yes | 90% |
| | | | LOH | RB1 LOH | |
| E5 | 0.169 | Negative | c.1251_1252delAA | Yes | 94% |
| | | | LOH | RB1 LOH | |
| E6 | 0.141 | Negative | Deletion 1-17 RB1 | Deletion exons 2-17 | |
| | | | Deletion of 25-27 RB1 | Deletion exons 24-27 | |
| E7 | 244 | c.1496_97dup mosaic (9%) | c.1496_97dup | Yes | 94% |
| | | | LOH | RB1 LOH | |
| E8 | 1.96 | c.1072 C to T p.(Arg358*) mosaic (21%) | c.1072 C to T p.(Arg358*) | Yes | 100% |
| | | | LOH | RB1 LOH | |
| E9 | 1.01 | Negative | c.958C>T p.(Arg320*) | Yes | 42% |
| | | | c.1981C>T p.(Arg661*) | Yes | 47% |
| E10 | 1.38 | Negative | c.147delT | Yes | 45% |
| | | | c.1330C>T p.(Gln444*) | Yes | 44% |

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# CNV Detection

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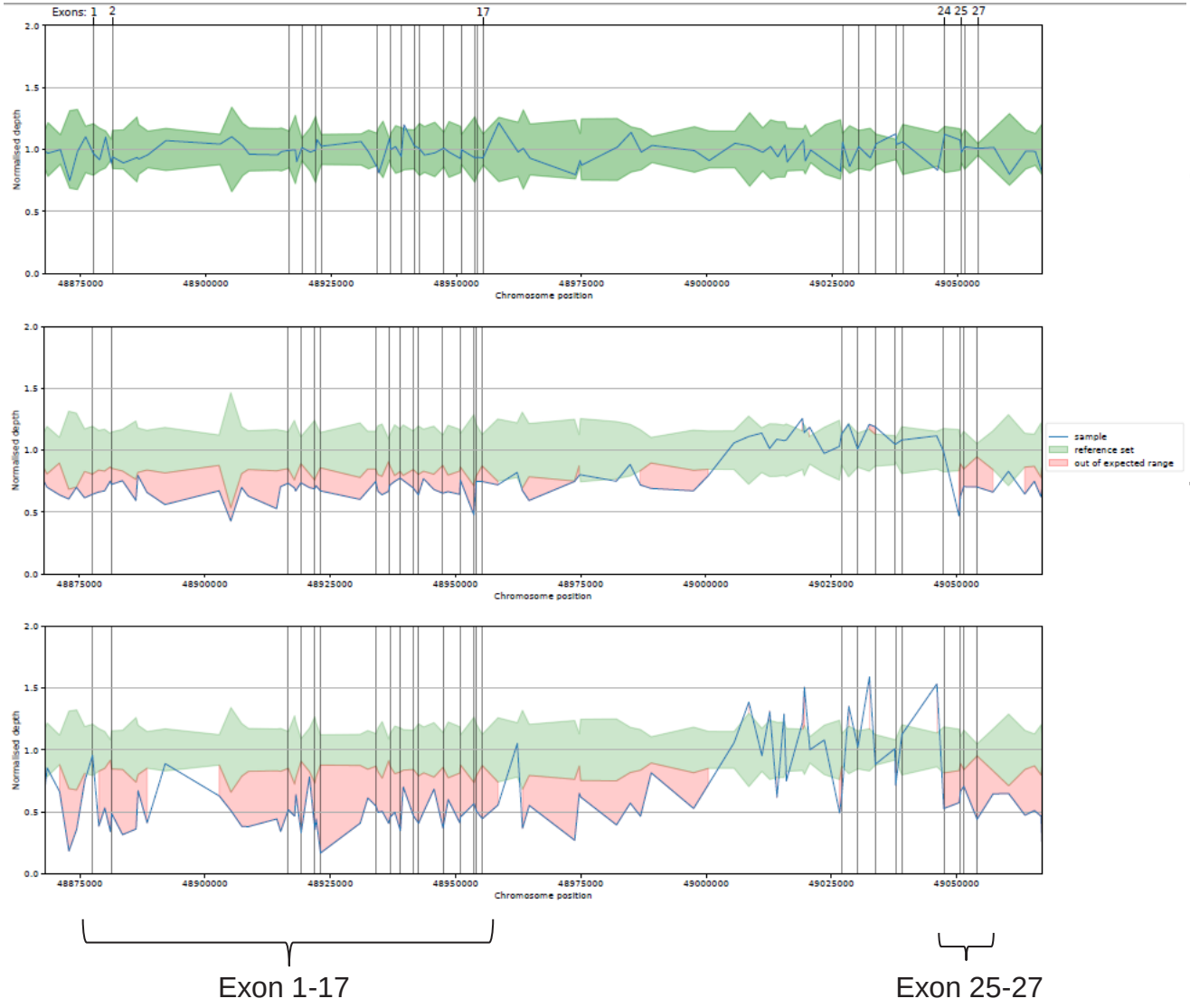


Genomic DNA

Tumour DNA

cfDNA DNA

# LOH Detection

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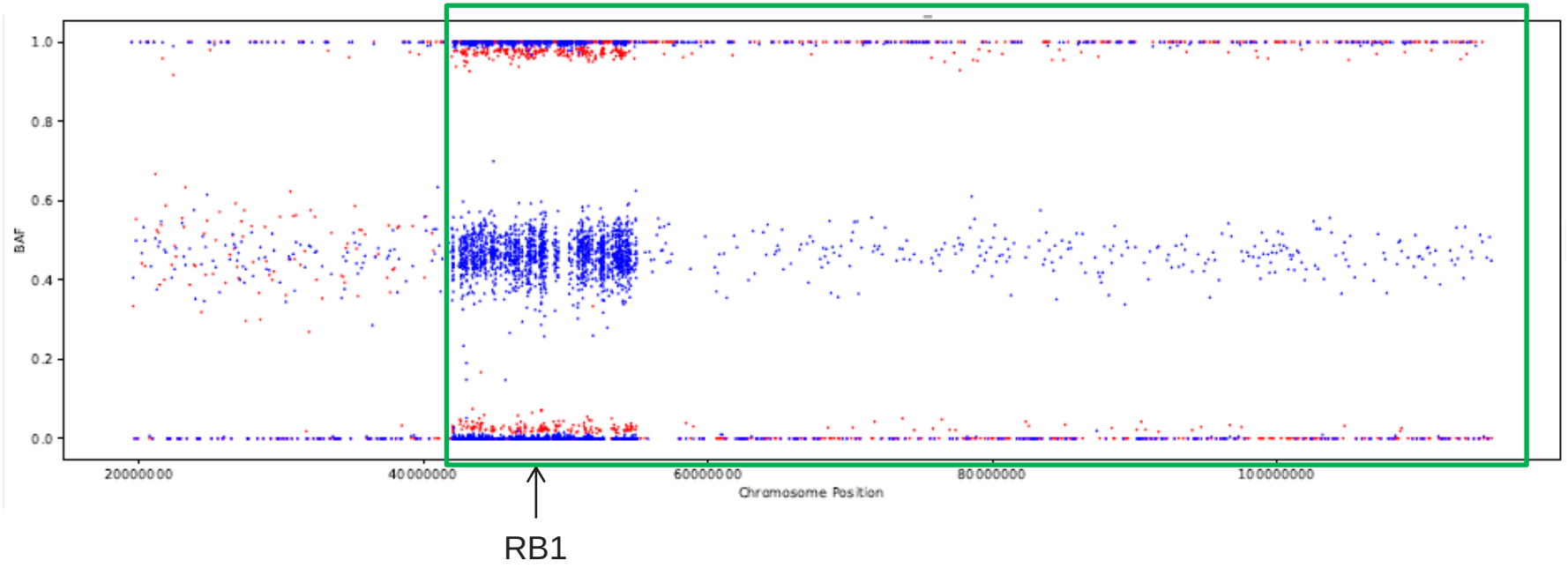
- Beta allele frequency plots




- cfDNA
- gDNA

# Results – Enucleated Patients

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| Patient | cfDNA conc ng/ul | *RB1* Germline Mutation | *RB1* Somatic Mutation | | |
|---|---|---|---|---|---|
| | | | Tumour DNA | Detected in cfDNA? | SNV MUT Allele % |
| E1 | 2.12 | Negative | c.1363C>T p.(Arg455*) | Yes | 91% |
| | | | LOH | Chr 13 LOH | |
| E2 | 228 | Negative | c.751C>T p.(Arg251*) | Yes | 99% |
| | | | LOH | Chr 13 LOH | |
| E3 | 0.183 | Negative | c.1959dupA | Yes | 87% |
| | | | LOH | RB1 LOH | |
| E4 | 394 | Negative | c.763C>T p.(Arg255*) | Yes | 90% |
| | | | LOH | RB1 LOH | |
| E5 | 0.169 | Negative | c.1251_1252delAA | Yes | 94% |
| | | | LOH | RB1 LOH | |
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| | | | LOH | RB1 LOH | |
| E9 | 1.01 | Negative | c.958C>T p.(Arg320*) | Yes | 42% |
| | | | c.1981C>T p.(Arg661*) | Yes | 47% |
| E10 | 1.38 | Negative | c.147delT | Yes | 45% |
| | | | c.1330C>T p.(Gln444*) | Yes | 44% |

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# Results – IViC Patients

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| Patient | cfDNA conc ng/ul |
|---|---|
| IViC 1 | 0.012 |
| IViC 2 | 0.015 |

# Results – IViC Patients

Birmingham Women's and Children's NHS Foundation Trust

| Patient | cfDNA conc ng/ul | *RB1* Germline Mutation | *RB1* Somatic Mutation |
|---|---|---|---|
| | | | Tumour DNA |
| IViC 1 | 0.012 | c.1666C>T p.Arg556* | NA |
| IViC 2 | 0.015 | Negative | NA |

# Results – IViC Patients

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| Patient | cfDNA conc ng/ul | *RB1* Germline Mutation | *RB1* Somatic Mutation | | |
|---|---|---|---|---|---|
| | | | Tumour DNA | Detected in cfDNA? | SNV MUT Allele % |
| IViC 1 | 0.012 | c.1666C>T p.Arg556* | NA | c.1666C>T p.(Arg556*) | 100% |
| | | | | Chr 13 LOH | |
| IViC 2 | 0.015 | Negative | NA | c.1078dupA | 80% |
| | | | | RB1 LOH | |

- Four additional IViC patients produced unquantifiable levels of cfDNA
- Patient clinical history likely to be a factor

# Future Plans

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1. Determine clinical and technical factors to maximise cfDNA levels in AH from IViC patients
   - Tumour burden
   - Treatment history
   - Sample storage and processing

2. Expand IViC cohort for diagnostic testing
   - Have >40 samples from 11 additional patients to analyse
   - Collaboration with other RB centres

3. Investigate potential of prognostic testing
   - Potential for monitoring of causal mutations and additional somatic changes
   - Serial IViC samples available

Birmingham Women's & Children's Hospital Charity

# Acknowledgments

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