# Unusual Clinical Behavior of a Very Late Retinoblastoma Relapse in a Patient with a Germline RB Mutation



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**Abbreviations:**

| **Abbreviation** | **Full Term** |
|---|---|
| PET/CT | Positron Emission Tomography-Computed Tomography |
| FDG | fluorodeoxyglucose |
| CNS | Central Nervous System |
| RB | Retinoblastoma gene |
| COG | Children's Oncology Group |
| IIRC | International Intraocular Retinoblastoma Classification |
| CBC | Complete Blood Count |
| LDH | Lactate Dehydrogenase |
| MRI | Magnetic Resonance Imaging |
| TMA | Thrombotic Microangiopathy |

| | |
|---|---|
| EFS | Event Free Survival |
| LOH | Loss of Heterozygosity |
| ATP | Adenosine Triphosphate |
| DNA | Deoxyribonucleic acid |

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**To the editor:**

Retinoblastoma is the most common pediatric intraocular malignancy with survival rates exceeding 95% utilizing therapies including systemic chemotherapy, intra-arterial chemotherapy, and local therapies (e.g. focal laser), and in advanced cases, eye enucleation [1,2]. High-risk features for extra-ocular spread include tumor invasion of the post laminar optic nerve, choroid, anterior segment or sclera with relapses occurring typically within two years of diagnosis [1,3, 4].

We report a patient with bilateral intraocular retinoblastoma who suffered a systemic relapse after a long-term remission (Supp. Fig S2). At the age of two months, he was diagnosed with group B (IIRC) bilateral retinoblastoma harboring a germline intronic *RB1* mutation (Supp. Table S1). He was treated on the Children's Oncology Group (COG) ARET0331 protocol with six cycles of systemic carboplatin, vincristine, and focal laser therapy. Due to disease progression, his right eye was enucleated at the age of 12 months. Based on the lack of high-risk histopathology, no additional therapy was administered.

After eight years of remission, he presented with vague wrist pain and found to have minimal soft tissue swelling of the wrist. His complete blood count (CBC) and lactate dehydrogenase (LDH) level were normal. He was evaluated by rheumatology, diagnosed with idiopathic arthritis and started on naproxen therapy with pain relief.

Three months later, he developed worsening leg pain. His physical exam demonstrated a 2 cm left axillary lymph node. His hemoglobin was 10.9 g/dL, platelets 136,000/cumm and LDH >6000 U/L (undiluted). His bone marrow demonstrated diffuse tumor infiltration by metastatic retinoblastoma confirmed by immunophenotyping and synatophysin staining. Cytogenetics demonstrated an abnormal karyotype including gains of chromosome 1q, 2p, 6p, and 13q32-34. A PET/CT demonstrated diffuse bony disease with liver and axillary lymph node metastases (Fig. 1B). A brain/orbits MRI were negative for CNS involvement.

He began treatment following the COG ARET0321 protocol for metastatic retinoblastoma with the intention to ultimately proceed to stem cell transplant. This cycle was complicated by tumor lysis syndrome and acute renal failure requiring hemodialysis. His LDH normalized to 275 U/L after peaking at 22,331 U/L (Supp. Fig. 1A). Tumor restaging demonstrated no evidence of bone marrow disease and near resolution of his metastatic disease on PET scan (Fig. 1C). Following the third chemotherapy cycle there continued to be

no residual tumor. Stem cell transplantation was postponed due to the development of cisplatin-induced thrombotic microangiopathy (TMA).

Eight months following his initial relapse, he developed left eye deviation with a right cerebellar mass found on MRI imaging (Figure 1D) (confirmed to be retinoblastoma following a gross total resection) with spinal metastasis. There continued to be no metastatic disease outside the CNS.

He received craniospinal proton radiation therapy (40Gy) with daily carboplatin and weekly vincristine which were ultimately discontinued due to worsening TMA, was stopped. Palliative therapy was started, and he expired 4 weeks later due to progressive disease. At autopsy, there was no evidence of disease outside the CNS.

Over the past 25 years, 4 patients at our institution developed metastatic retinoblastoma within two year of diagnosis (supplement Table S2). An observational study reported that 402 patients with low-risk features did not develop metastatic disease [9], which makes this case unusual as he had low-risk disease at diagnosis.

The marked LDH level in our patient (Supp. Fig. 1A) reflected the rapid proliferation of the tumor which likely contributed to its chemosensitivity, resultant tumor lysis syndrome and acute renal failure.

To characterize the genomic landscape and tumor progression, targeted sequencing was performed on the eye (September 2009), bone marrow (February 2018), and brain (October 2018) (Supp. Table S1). The normal DNA from the eye tissue revealed a germline pathogenic variant in the RB1 gene, and in all three tumors, biallelic inactivation of RB1, acquired by somatic inactivation of the remaining allele through copy-neutral LOH. A set of high-confidence somatic mutations and copy number changes were present in each of the three

tumor samples (Fig. 1A, supp. Table S1) Similar to many pediatric neoplasms including retinoblastomas [11,12,13], the overall somatic mutation burden in these tumors were low and all the mutations were non-recurrent with unknown functional impact. Copy number analysis of the primary cancer, biopsied in 2009, demonstrated copy neutral LOH of 13q and losses on 3q. Two metastatic samples, biopsied in 2018, shared these events in the primary cancer, with additional gains on chromosomes 1q, 2p, 6p, 12, and 15. The brain biopsy showed further gains of chromosome 19 and copy loss on 17p (Fig. 1A). This is consistent with prior reports of several highly recurrent copy number alterations in retinoblastoma [12,13].

One theory to explain tumor dormancy, posits that cancer cells, may acquire the ability to manipulate the tumor microenvironment and evade the immune system, metastasizing to sanctuary tissues and enter a state of cellular senescence where they are metabolically active, but do not proliferate until stimulated to "reawaken" and divide [14-17].

Of the mutations found (Supp. Table S1), *CYP21A2, CD276, FANC2D*, are associated with PolyPhen scores predicting for pathogenicity and have been previously reported in tumor migration, invasion, and cell cycle control [18-26], possibly playing a role in this patient's tumor dormancy.

The low burden of somatic mutations supports the notion that tumorigenesis/evasion may be related to another mechanism, however, the observed mutations and their role in tumor dormancy should be further investigated.



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**Supplement:**

METHODS

Patient enrollment

Sequencing of clinical samples was performed under our Institutional Review Board (IRB)–approved studies at the University of Michigan (Michigan Oncology Sequencing Protocol, MI-ONCOSEQ, IRB # HUM00046018, HUM00067928, HUM00056496).

Integrative Clinical Sequencing

Two archival formalin-fixed paraffin-embedded (FFPE) samples and a frozen bone marrow sample were obtained for sequencing. A section of FFPE blocks were cut for evaluation, and remaining portions of each specimen were retained for nucleic acid extraction. Hematoxylin and eosin (H&E)-stained sections were reviewed by a board certified pathologist to identify areas with highest tumor content and an area of normal tissue. Tumor genomic DNA and total RNA from the frozen bone marrow were extracted using the AllPrep DNA/RNA/miRNA kit (QIAGEN). DNA from FFPE samples was extracted using the DNeasy Blood and Tissue Kit (QIAGEN), and total RNA was extracted using the miRNeasy

FFPE kit (QIAGEN). RNA integrity was measured on an Agilent 2100 Bioanalyzer (Agilent Technologies).

Integrative clinical sequencing was performed in a Clinical Laboratory Improvement Amendments (CLIA) compliant sequencing lab as described before (Refs 4-5). Paired-end target-captured exome libraries from tumor and normal samples, and tumor transcriptome libraries were sequenced using the Illumina HiSeq2500. Aligned exome and transcriptome sequences were analyzed to detect somatic mutations, copy-number alterations, gene fusions, and gene expression as described before (Ref 4).

**Figure legends:**

**Figure 1:**

**Panel A:** Copy number assessment was performed by targeted exome sequencing of matched tumor and normal samples with chromosomal changes at the different stages of relapse compared to the initial tumor. The most notable copy number aberration was uniparental disomy (UPD) of chromosome 13. Germline RB1 mutation in combination with UPD 13 resulted in biallelic inactivation of RB1. On array CGH, there was aneuploidy with additional chromosomal alterations during disease progression.

**Panel B:** Abnormal FDG uptake on PET/CT scan demonstrating diffuse bony involvement and uptake in liver, left axillary and infraclavicular regions.

**Panel C:** After one cycle of chemotherapy, no abnormal FDG uptake except for minimal left axillary uptake.

**Panel D:** 3 x 3.2 x 2.5 cm mass lesion in the posteromedial aspect of the right cerebellar hemisphere with associated edema and mass effect.



**Figure 1**