151 Retinoblastoma: From Genetics and Pathology to Therapy
Sunday, May 07, 2017 3:15 PM–5:00 PM
Room 321 Paper Session
Program #/Board # Range: 854–859
Organizing Section: Anatomy and Pathology/Oncology

Program Number: 854
Presentation Time: 3:15 PM–3:30 PM
Retinoblastoma and Next-Gen-Sequencing Era: Challenges and Opportunities at Clinics and Counseling in India
Govindasamy Kumaramanickavel[1], Sivasankar Malaichamy[2], Rohit Shetty[1], Arkasubhra Ghosh[1], Somasekar Seshagiri[3], Vedam L. Ramprasad[2]. [1]Narayana Nethralaya, Bangalore, India; [2]MedGenome, Bangalore, India; [3]Genentech, South San Francisco, CA.
Purpose: India accounts for the highest, about 20%, of the global burden of retinoblastoma (RB). We assessed how current molecular genetic technology is assisting and changing the landscape of RB clinical practice and counseling in a tertiary eye clinic in India.
Methods: In the present study, 51 children with RB underwent complete ophthalmic examination, clinical management and genetic analysis for germline status of the RB1 gene using targeted next generation sequencing (NGS) and multiplex ligation-dependent probe amplification (MLPA) techniques. The RB1 gene DNA libraries, from peripheral blood, were sequenced to mean >80-100X coverage on Illumina sequencing platform (HiSeq 2500). We used SALSA MLPA kit P047 RB1 (Amsterdam, Netherlands) as per manufacturer's recommendations. Mutation and non-mutaion groups were analysed for disease recurrence, disease progression, histopathology types, clinical management and other clinical parameters. Appropriate ethical consents were obtained.
Results: Thirty patients had bilateral RB (BLRB), twenty one had unilateral RB (ULRB) and two had familial history of RB. Average age of presentation was 1.8 years in BLRB and 2.3 years in ULRB. The genetic analysis revealed twenty nine variations (57%) in RB1 gene, including a novel mutation (7%), three variants with unknown significance (10%) and four heterozygous whole gene deletions (14%). The detection rates were 86% in BLRB and 18% in ULRB, on par with recent robust studies. Genotype–phenotype correlation showed an association of disease recurrence in genetic mutation group compared to the non-mutation group (p= 0.001). In a prenatal counseling, whole RBI gene maternal allele was detected in an unborn fetus.
Conclusions: In our study, mutation group had increased recurrence and mutation knowledge influenced clinical decisons like enucleation, increased frequency of periodic examination and radiotherapy was not advised for them. Clinical management and counseling (including prenatal) were influenced by the presence or absence of germline mutation, detected by the current NGS and MLPA molecuar genetic techniques. These techniques were important tools and the results were very useful to convice parents during clinical management and counseling.
Commercial Relationships: Govindasamy Kumaramanickavel, None; **Sivasankar Malaichamy**, None; **Rohit Shetty**, None; **Arkasubhra Ghosh**, None; **Somasekar Seshagiri**, None; **Vedam L. Ramprasad**, None
Support: SciGenom Research Foundation, Cochin, India

Program Number: 855
Presentation Time: 3:30 PM–3:45 PM
Vitreous and Subretinal Seeds in Retinoblastoma: Clinicopathologic Correlation
Alec L. Amram[1], Grecia Rico[2], Jonathan W. Kim[3, 4], Dan Gombos[5, 6], Patricia Chevez-Barrios[2, 5]. [1]Ophthalmology & Visual Sciences, University of Texas Medical Branch, Galveston, TX; [2]Department of Pathology and Genomic Medicine, Houston Methodist Hospital, Houston, TX; [3]Department of Ophthalmology, University of California, Los Angeles, Los Angeles, CA; [4]Children's Hospital Los Angeles, Los Angeles, CA; [5]Retinoblastoma Center of Houston, Houston, TX; [6]Section of Ophthalmology, University of Texas, MD Anderson Cancer Center, Houston, TX.
Purpose: Vitreous seeding has been recognized as the strongest predictor of retinoblastoma treatment failure. Recently, intravitreal chemotherapy injection has led to improved outcomes for these patients. Munier et al recently proposed a new clinical classification scheme for retinoblastoma with vitreous seeds; Francis et al was then able to demonstrate a significant difference in regression between seed classes. Here we describe the first correlation of this clinical classification scheme with histopathological features.
Methods: We reviewed enucleated eyes with retinoblastoma from the Retinoblastoma Center of Houston that had clinical and macroscopic photographs and routinely processed, hematoxylin and eosin stained slides from 2010-2015 to identify those with vitreous and subretinal seeds. Immunohistochemistry with CD68 was used to evaluate for the presence of macrophages. Eyes were classified by clinical and macroscopic tumor seed type and cellular components were correlated within each category.
Results: 14 of 138 eyes reviewed had adequate vitreous or subretinal seeds and clinical/macroscopic photos. Clinically identified "dust" seeds (Type 1) represent individual viable tumor cells and macrophages. Clinically "sphere" seeds (Type 2) represent two histological types: 1. Macroscopically gray/translucent spheres are composed of non-necrotic, mitotically active retinoblastoma cells. 2. Macroscopically gray spheres with a white/yellow center are composed of an outer rim of viable cells with a center of necrotic material. Both sphere types contain a pole of dispersing single, viable cells. "Cloud" seeds (Type 3) are composed of necrotic debris with few scattered macrophages and rare viable cells.
Conclusions: Spheres with translucent centers may represent the most aggressive vitreous seed subtype as they contain multiple layers of viable tumor cells and shed single cells. In contrast, the overall lack of response to treatment seen in "cloud" seeds is likely due to an absence of replicating tumor cells. Knowledge of the composition of retinoblastoma seed types should help guide treatment and anticipate clinical response in trials that focus on the safety, efficacy, and outcomes of novel retinoblastoma therapy.
Commercial Relationships: Alec L. Amram, None; **Grecia Rico**, None; **Jonathan W. Kim**, None; **Dan Gombos**, None; **Patricia Chevez-Barrios**, None

Program Number: 856
Presentation Time: 3:45 PM–4:00 PM
Aqueous Humor as a Surrogate Liquid Tumor Biopsy in Retinoblastoma
Jesse L. Berry[1, 2], Liya Xu[3], Kevin Stachelek[2], A. Linn Murphree[2, 1], Emily Zolfoghari[2], Kathleen McGovern[2], Thomas Lee[2, 1], Anders Carlsson[3], Peter Kuhn[4, 3], Jonathan Kim[1, 2], David Cobrinik[2, 1], James Hicks[4, 3]. [1]USC Roski Eye Institute, Los Angeles, CA; [2]Ophthalmology, Children's Hospital Los Angeles, Los Angeles, CA; [3]University of Southern California, Department of Biological Sciences, Dornsife College of Letters, Arts, and Sciences, Los Angeles, CA; [4]University of Southern California, Norris Comprehensive Cancer Center, Keck School of Medicine, Los Angeles, CA.
Purpose: The aim of this study is to determine whether tumor-derived nucleic acids and tumor DNA copy number alterations can be detected in the aqueous humor (AH) of retinoblastoma (RB) eyes.
Methods: AH was analyzed for DNA, RNA and miRNA using Qubit HS kits. Circulating cell-free DNA (cfDNA) isolation and sequencing library protocols were optimized to retain cfDNAs from the AH and these optimized methods were applied to AH samples from RB patients. Shallow whole genome sequencing was performed on Illumina platform followed by genome-wide copy number variation (CNV) profiling to assess the presence of tumor DNA fractions in AH cfDNA.
Results: Eighteen AH samples from 8 patients (6 patients pre-intravitreal injection and 2 post-enucleation) were examined. All had measurable DNA, RNA and miRNA, with miRNA having the highest concentrations. Whole genome sequencing of AH cfDNA from 2 primarily enucleated eyes and sequential AH cfDNAs obtained from 2 eyes undergoing intravitreal melphalan injections for treatment of tumor seeding revealed tumor-derived cfDNA based on CNV profiles that demonstrated tumor copy number alterations.
Conclusions: This is the first study to evaluate AH from RB eyes undergoing salvage therapy with intravitreal injection of melphalan. We were able to assay quantifiable levels of nucleic acids in treated and untreated eyes and found that AH cfDNA had retinoblastoma-related DNA copy number alterations in all four tested RB patients. This suggests that AH can serve as a 'surrogate tumor biopsy' when tumor tissue is not available.
Commercial Relationships: Jesse L. Berry, None; **Liya Xu**, None; **Kevin Stachelek**, None; **A. Linn Murphree**, None; **Emily Zolfoghari**, None; **Kathleen McGovern**, None; **Thomas Lee**, None; **Anders Carlsson**, None; **Peter Kuhn**, None; **Jonathan Kim**, None; **David Cobrinik**, None; **James Hicks**, None
Support: Nautica Foundation

Program Number: 857
Presentation Time: 4:00 PM–4:15 PM
Identifying Invasion-Promoting Molecular Pathways In Retinoblastoma
Laura Asnaghi[1], Alka Mahale[2], Hind Alkatan[4], Deepak P. Edward[3, 2], Wayne Yu[5], Saleh Al Mesfer[2], Azza Maktabi[2], Leen Abu Safieh[2], Charles Eberhart[1, 3]. [1]Dept of Pathology, Sch of Med, Johns Hopkins University, Baltimore, MD; [2]King Khaled Eye Specialist Hospital, Riyadh, Saudi Arabia; [3]Department of Ophthalmology, Johns Hopkins University, Baltimore, MD; [4]Department of Ophthalmology, King Saud University, Riyadh, Saudi Arabia; [5]Microarray Core Facility, Sidney Kimmel Cancer Center, Johns Hopkins University, Baltimore, MD.
Purpose: Retinoblastoma is the most frequent malignant intraocular cancer in children. In advanced cases, dissemination in the CNS or metastasis to distant organs can lead to death. Our goal was to identify the molecular drivers and signaling pathways associated with tumor invasion and metastatic spread into the optic nerve and choroid in order to develop new prognostic markers and therapeutic targets.
Methods: RNA extracted from eleven snap frozen retinoblastoma specimens was analyzed by RNA-seq (low input, non-strand specific). Samples were divided between invasive (retrolaminar, n=4; intralaminar, n=1) and non-invasive (prelaminar, n=4, no optic nerve invasion, n=2). Four cases with optic nerve invasion and two without also showed focal (<3mm) choroidal invasion, but none had massive choroidal invasion.
Results: We found 267 genes whose expression was modified more than two-fold in invasive versus non-invasive retinoblastomas: 27 upregulated and 240 downregulated. In the invasive cohort, we observed about 28-fold induction of *DLX6*, a transcription factor known to enhance migration and invasion by upregulating Twist1, and 11-fold induction of the matrix metallo-proteinase *MMP12*, which promotes invasion by degrading the extracellular matrix. We also found 24-fold reduction in *WIF1* (WNT Inhibitory Factor 1), a tumor suppressor epigenetically silenced in various cancers, 16-fold reduction in *CLUSTERIN*, associated with apoptosis, and 11-fold decrease in *GLI3*, an inhibitor of Sonic hedgehog signaling.
Conclusions: Our gene expression profile analysis showed that several genes and pathways were differentially expressed in invasive versus non-invasive retinoblastomas. In particular WNT, Sonic hedgehog, and MMP signaling might be responsible for driving CNS dissemination in retinoblastoma. Functional studies are in progress to confirm these data in retinoblastoma lines.
Commercial Relationships: Laura Asnaghi, None; **Alka Mahale**, None; **Hind Alkatan**, None; **Deepak P. Edward**, None; **Wayne Yu**, None; **Saleh Al Mesfer**, None; **Azza Maktabi**, None; **Leen Abu Safieh**, None; **Charles Eberhart**, None
Support: Financial support: King Khaled Eye Specialist Hospital (KKESH) – Wilmer Eye Institute (WEI) Collaborative Research Grant

Program Number: 858
Presentation Time: 4:15 PM–4:30 PM
Novel STAT3 Inhibitors with Michael Acceptor from In-House Chemical Library as Therapeutics for Retinoblastoma
Eunoo Bak[1, 2], Dong Hyun Jo[1, 3], Jin Hyoung Kim[1, 3], Kyeojin Kim[4], Seungbeom Lee[4], Young-Ger Suh[5], Jeong Hun Kim[1, 6]. [1]Fight against Angiogenesis-Related Blindness (FARB) Laboratory, Clinical Research Institute, Seoul National University Hospital, Seoul, Korea (the Republic of); [2]Department of Ophthalmology, Seoul National University College of Medicine, Seoul, Korea (the Republic of); [3]Tumor Microenvironment Research Center, Global Core Research Center, Seoul National University, Seoul, Korea (the Republic of); [4]College of Pharmacy, Seoul National University, Seoul, Korea (the Republic of); [5]College of Pharmacy, Cha University, Seongnam, Gyeonggi-do, Korea (the Republic of); [6]Department of Biomedical Sciences, Seoul National University College of Medicine, Seoul, Korea (the Republic of).
Purpose: Our previous studies demonstrated that STAT3 inhibition could be a treatment option for retinoblastoma and chemicals with a Michael acceptor from our in-house library exerted inhibitory action on STAT3 activation. This study aims to develop potent STAT3 inhibitors from a library of 27 different derivatives with a Michael acceptor for the treatment of retinoblastoma.
Methods: To figure out cellular toxicity of 27 different chemicals with a Michael acceptor, SNUOT-Rb1 and Y79 cells from 2 different retinoblastoma cell lines were treated with STAT3 inhibitors. Cell viability was measured using WST-1 assay and the level of STAT3 phosphorylation was estimated by ELISA measurement. Quantitative

real-time polymerase chain reaction was performed to measure the expression levels of target genes of STAT3 upon treatment with STAT3 inhibitors. From a library of 27 STAT3 inhibitors, the 2 most potent STAT3 inhibitors were selected. Then, *in vivo* therapeutic efficacy of them was investigated in the mouse orthotopic transplantation model. The toxicity of STAT3 inhibitors on normal cells and tissues was investigated at the levels of gene expression, cellular viability, and histologic integrity.
Results: Differential cellular toxicity, STAT3 phosphorylation, and expression of target genes were investigated in retinoblastoma cells upon treatment with 27 different chemicals with a Michael acceptor. The 2 most potent STAT3 inhibitors effectively inhibited the formation of tumors in the mouse orthotopic transplantation model. Interestingly, these STAT3 inhibitors did not significantly affect the cellular viability, the expression of genes regarding cell survival, and histologic integrity of the retina with a sufficient range of therapeutic window.
Conclusions: Novel STAT3 inhibitors identified from screening of an in-house library of chemicals with a Michael acceptor demonstrated potent *in vitro* and *in vivo* efficacy without definite toxicity on normal tissues. We expect that these STAT3 inhibitors can be utilized for the treatment of retinoblastoma.
Commercial Relationships: Eunoo Bak, None; **Dong Hyun Jo**, None; **Jin Hyoung Kim**, None; **Kyeojin Kim**, None; **Seungbeom Lee**, None; **Young-Ger Suh**, None; **Jeong Hun Kim**, None

Program Number: 859
Presentation Time: 4:30 PM–4:45 PM
Pharmacokinetics, Toxicities, and Vascular Variations in a Small Animal (Rabbit) Model of Intra-arterial Chemotherapy
Anthony B. Daniels[1, 2], Michael T. Froehler[3], Janene M. Pierce[4], Amy H. Nunnally[4], M. W. Calcutt[5], Thomas M. Bridges[6], David C. LaNeve[7], Phillip E. Williams[7], Kelli L. Boyd[8, 2], Michelle L. Reyzer[5], Craig W. Lindsley[6, 9], Debra L. Friedman[10, 2], Ann Richmond[11, 12]. [1]Departments of Ophthalmology, Cancer Biology, and Radiation Oncology, Vanderbilt University Medical Center, Nashville, TN; [2]Vanderbilt-Ingram Cancer Center, Vanderbilt University Medical Center, Nashville, TN; [3]Cerebrovascular Program, Vanderbilt University Medical Center, Nashville, TN; [4]Department of Ophthalmology and Visual Sciences, Vanderbilt University Medical Center, Nashville, TN; [5]Department of Biochemistry, Vanderbilt University, Nashville, TN; [6]Vanderbilt Center for Neurosciences Drug Discovery, Department of Pharmacology, Vanderbilt University, Nashville, TN; [7]Department of Surgery, Vanderbilt University Medical Center, Nashville, TN; [8]Department of Pathology, Microbiology and Immunology, Vanderbilt University Medical Center, Nashville, TN; [9]Department of Chemistry, Vanderbilt University, Nashville, TN; [10]Department of Pediatrics, Vanderbilt University Medical Center, Nashville, TN; [11]Tennessee Valley Healthcare System, Department of Veterans Affairs, Nashville, TN; [12]Department of Cancer Biology, Vanderbilt University, Nashville, TN.
Purpose: We recently described the first small animal model of intra-arterial chemotherapy (IAC) in rabbits. Here, we determine the pharmacokinetics of IAC melphalan in this model, and the systemic and ocular toxicities associated with IAC melphalan. We also describe, for the first time, the vascular variations in ocular blood supply in rabbits.
Methods: Ocular vascular supply was determined angiographically in 79 eyes of 47 3.0kg-New Zealand white rabbits. The dominant ophthalmic artery (OA) of each eye was selectively catheterized. Melphalan 0.4mg/mL (up to 1.2mg/kg) was infused in pulsatile fashion. For pharmacokinetic studies, 18 rabbits were sacrificed at serial time-points. Retina, bilateral vitreous, and blood were collected. Toxicity was assessed by fluorescein angiography, electroretinography, and histopathology, prior to and 5-weeks post-treatment. Complete blood counts were obtained weekly.
Results: The OA was successfully catheterized for 79/79(100%) eyes in 47/47(100%) rabbits. Melphalan was delivered in 31/31(100%) eyes. External OA-dominant vascular variation was present in >75% of eyes, and dual internal/external supply in ~5%, with no correlation between a rabbit's two eyes. In treated eyes, maximum melphalan concentration (C_{max}) in retina was 4.95μM (30-minutes post-infusion) vitreous C_{max} was 2.24μM (1-hour), and areas-under-the-curve ($AUC_{0\rightarrow\infty}$) were 5.26μM*hr for retina and 4.19μM*hr for vitreous. Peripheral blood C_{max} was 1.04μM. Drug half-life was ~1 hour. Treated eye vitreous C_{max} was >100-fold higher, and $AUC_{0\rightarrow\infty}$ was ~50-fold higher, than untreated eye. No angiographic or histopathologic evidence of vascular occlusion, emboli, or retinal damage were seen, even with 1.2mg/kg melphalan. Electroretinographic reductions were not seen 5 weeks following IAC melphalan treatment. With 0.8-1.2mg/kg melphalan, transient neutropenia occurred at 1-week, which was not seen with 0.4mg/kg doses.
Conclusions: This is the first small animal model of IAC. Ocular vascular supply in the rabbit is variable, and is independent for each eye. IAC melphalan delivery in rabbits leads to excellent ocular penetration and pharmacokinetics, with peak vitreous drug concentrations and areas-under-the-curve that are significantly better than in previous large animal models of IAC. IAC melphalan did not lead to significant ocular, vascular, or systemic toxicities in our rabbit model system.
Commercial Relationships: Anthony B. Daniels, None; **Michael T. Froehler**; **Janene M. Pierce**, None; **Amy H. Nunnally**, None; **M. W. Calcutt**, None; **Thomas M. Bridges**, None; **David C. LaNeve**, None; **Phillip E. Williams**, None; **Kelli L. Boyd**, None; **Michelle L. Reyzer**, None; **Craig W. Lindsley**, None; **Debra L. Friedman**, None; **Ann Richmond**, None
Support: This work was supported by a Career Starter Grant from the Knights Templar Eye Foundation [ABD], by the Vanderbilt Faculty Research Scholars program [ABD], by a Career Development Award from the Research to Prevent Blindness Foundation [ABD], and by an unrestricted departmental grant from Research to Prevent Blindness to the Vanderbilt Department of Ophthalmology and Visual Sciences. This work was also supported by grant NCI CA 116021 [AR] and by a Department of Veterans Affairs Career Scientist Award [AR]. It was also supported by P41 GM103391-06 and by the National Center for Research Resources, Grant UL1RR024975-01, now at the National Center for Advancing Translational Sciences (2 UL1 TR000445-06).