

**SCIENTIFIC SESSION 5  
RETINOBLASTOMA 1**

Shilpakalavedika Convention Center  
Sunday, January 25, 2004  
8:30 AM – 10:30 AM

*Chair: Brenda Gallie*  
*Co-chair: Supriyo Ghose*  
*Moderator: Laurence Desjardins*  
*Session Summary: Laurence Desjardins*

	<b>Presenter</b>	<b>Title of Presentation</b>	<b>Time</b>
1	Arun Singh	Introduction to Alfred Knudson	8:30 AM
2	Alfred Knudson	Guest Lecture: The Retinoblastoma Gene, Development, and Cancer	8:32 AM
3	Linn Murphree	Key Note Lecture: Classification of Retinoblastoma	8:50 AM
4	Domenico Mastrangelo	The Abberant Methylation in Retinoblastoma	9:05 AM
5	Chitra Kannabiran	RB1 Gene Mutations in Indians with Retinoblastoma	9:15 AM
6	Raman Mittal	Atypical Presentations of Retinoblastoma	9:25 AM
7	Doris Hadjistilianou	Bilateralization of Early Unilateral Retinoblastoma : Personal Experience	9:35 AM
8	Vasudha Erranguntla	Intraoperative Serial RetCam Fluorescein Angiography in the Management of Retinoblastoma	9:45 AM
9	Mark Duffy	MRI, Chemotherapy, and Optic Nerve Involvement - Case Studies and a New Possible Risk for Systemic Spread of Retinoblastoma	9:55 AM
10	Nilutpol Borah	Transpupillary Thermotherapy in Retinoma	10:05 AM
11	Andreas Schuler	Beta Ray Brachytherapy of Retinoblastoma	10:15 AM
12	Laurence Desjardins	Session Summary	10:25 AM

## GUEST LECTURE THE RETINOBLASTOMA GENE, DEVELOPMENT, AND CANCER

Alfred Knudson

Fox Chase Cancer Center, Philadelphia, PA, USA

## KEY NOTE ADDRESS CLASSIFICATION OF RETINOBLASTOMA

A Linn Murphree

The Retinoblastoma Center, Children's Hospital Los Angeles, University of Southern California, Los Angeles, CA, USA

## THE ABERRANT METHYLATION IN RETINOBLASTOMA

Domenico Mastrangelo, Theodora Hadjistilianou, Hisayuki Shigematsu, Andrea Zern, Adi Gazdar

Department of Ophthalmology, University of Siena, Siena, Italy

**PURPOSE:** The methylation profile of retinoblastoma has not been studied in detail. To clarify the epigenetic changes of retinoblastoma, we analyzed the expression pattern and the methylation status of multiple genes in retinoblastoma cell lines (n = 2) and primary tumors (n = 62). **METHODS:** We examined the promoter methylation status of 15 genes using methylation-specific PCR and mRNA expression of frequently methylated genes using RT-PCR. **RESULTS:** RASSF1A, DcR2, HIN-1, Caspase 8, and DcR1 were frequently methylated (> 65%), while some genes (p16, DR5, 3-OST-2, CyD2, RUNX3, TMS1, SOCS1, and CRBP1) were not methylated. DR4 and RB1 were infrequently methylated (7- 8%). The genes were rarely methylated in non-malignant tissues (lung, liver, kidney, and muscle) from pediatric cases. Loss of mRNA expression of RASSF1A, DcR2, HIN-1, Caspase 8, and DcR1 was concordant with promoter methylation but was restored after treatment with demethylating agent, 5-Aza-2'-deoxycytidine in the retinoblastoma cell lines. **CONCLUSION:** Promoter hypermethylation of some genes may play an important role in the pathogenesis of retinoblastoma. These findings may be of relevance for a better understanding of tumorigenesis and new therapeutic approaches for retinoblastoma.

## RB1 GENE MUTATIONS IN INDIANS WITH RETINOBLASTOMA

Chitra Kannabiran, Kalyan Chakravarthy, Saroj Kiran Velamakanni, Md Ata-ur-Rasheed, Geeta Vemuganti, Santosh Honavar

LV Prasad Eye Institute, Hyderabad, India

**PURPOSE:** To determine the range of mutations in the RB1 gene in Indian patients with retinoblastoma. **METHODS:** Blood samples from 68 patients, clinically diagnosed as retinoblastoma, were studied for mutations. In 35 patients, who underwent enucleation, tumor tissue was retrieved from the freshly enucleated eyeballs. Exons and promoter of the RB1 gene were amplified by PCR using genomic DNA isolated from peripheral blood leukocytes and tumors. PCR products were screened for mutations either by direct sequencing (21 patients) or by single-strand conformation polymorphism (SSCP) followed by sequencing (47 patients). PCR products were sequenced bidirectionally by automated sequencing. LOH analysis was done on tumors by using microsatellite markers flanking the RB1 locus. **RESULTS:** Out of 68 patients, 44 had bilateral and 24 had unilateral retinoblastoma. Only 4 patients had familial disease. Mutations were detected in 29 of 68 patients, 22 with bilateral and 7 with unilateral disease. 16 patients had nonsense mutations, 4 had frameshifts, 8 had

splice site mutations and 1 patient had a missense mutation. All mutations found in patients with bilateral disease were constitutional whereas all mutations found in unilateral retinoblastoma were somatic. **CONCLUSION:** Mutations could be detected in 43% of patients by the methods used. The data suggest that more sensitive techniques of mutation screening as well as methods designed to detect large deletions and epigenetic changes are probably required to identify the full range of mutations.

## ATYPICAL PRESENTATIONS OF RETINOBLASTOMA

Raman Mittal, Santosh Honavar, Geeta Vemuganti, Vijay Anand Reddy, Milind Naik, Ramesh Murthy

LV Prasad Eye Institute, Hyderabad, India

**PURPOSE:** Retinoblastoma usually presents with leucocoria or strabismus, however there are certain unusual presenting features where high index of suspicion for retinoblastoma should be entertained. The purpose of this study is to review such atypical presentations of retinoblastoma encountered in a tertiary care center. **METHODS:** Retrospective review of records of patients with retinoblastoma from January 1998 to September 2003. **RESULTS:** Twenty-seven of 289 patients with retinoblastoma during the study period presented with atypical features. Mean age at presentation was 4.5 years (range 1 month - 7 years). The most common atypical presenting feature was orbital cellulitis in 10 patients (37%). The other manifestations included secondary glaucoma in 9 (34%), hyphema in 3 (11%), endophthalmitis in 2 (7%), spontaneously ruptured globe in 1 (4%), cataract in 1 (4%) and ciliary body mass in 1 (4%). B-scan ultrasonography showed an intraocular mass and calcification in only 17 patients (63%). Twelve patients (44%) had extraocular extension. **CONCLUSION:** A significant number of patients present with atypical manifestations of retinoblastoma. In a child presenting with orbital cellulitis, secondary glaucoma, hyphema, endophthalmitis, spontaneously ruptured globe and even unexplained cataract, retinoblastoma should be ruled out. Delayed diagnosis in these patients' results in extraocular extension and poor prognosis.

## BILATERALIZATION OF EARLY UNILATERAL RETINOBLASTOMA: PERSONAL EXPERIENCE

Doris Hadjistilianou, Sonia, Domenico Mastrangelo, Silvia Marconcini, Gianluca Martone, Aldo Caporossi

Retinoblastoma Referral Center, Department of Ophthalmology, University of Siena, Siena, Italy

**PURPOSE:** To demonstrate that a close follow-up of unilateral retinoblastoma diagnosed before the age of 12 months is strictly necessary in order to detect bilateralization of the disease and improve the quality of life of affected patients. **METHODS:** A retrospective investigation has been performed on a series of 170 unilateral retinoblastoma referred to our Institution between 1980 and 2003. **RESULTS:** 61 out of 170 (36%) unilateral retinoblastomas were diagnosed before the age of 12 months. 7 out of 61 (11%) showed bilateralization. Familiarity was not a risk factor of bilateralization. New tumors could be easily controlled by focal treatment. Further interesting features are also reported. **CONCLUSION:** Early unilateral retinoblastoma represents a special category of disease, which deserves close follow-up for early intervention. It may also reveal unexpected features that should be further analyzed and discussed.

## INTRAOPERATIVE SERIAL RETCAM FLUORESCIN ANGIOGRAPHY IN THE MANAGEMENT OF RETINOBLASTOMA

Vasudha Erraguntla, Leslie MacKeen, Helen Chan, Elise Heon, Brenda Gallie

*The Hospital for Sick Children, University of Toronto, Toronto, Canada*

**PURPOSE:** To assess the usefulness of intraoperative fluorescein angiography (FA) in the management of retinoblastoma. **METHODS:** Intravenous fluorescein angiograms were performed at each visit on 17 anaesthetized retinoblastoma patients using the RetCam (Massie Laboratories Inc, Pleasanton, CA). **RESULTS:** 1. Tumor recurrence was detected prior to becoming evident on color RetCam images (4 children). 2. Residual tumor activity was detected in treated tumors (3 children). 3. Ischemic neovascularisation was distinguished from tumor activity (1 child). 4. Peripheral retinal vasculitis, not detected clinically or by fundus photography, was observed in 7 children who were treated with chemotherapy within a median interval of 4 weeks (range: 3 to 96 weeks). 5. New tumors in infants were not detected earlier than with standard RetCam images (2 children). **CONCLUSION:** Wide field digital FA with the RetCam plays an important role in the management of retinoblastoma. FA was particularly useful in assessing tumor activity, distinguishing tumor activity from a benign complication and discovery of a sub-clinical potential complication of chemotherapy. Real-time viewing of the FA at the examination under anesthesia facilitated decisive intervention. FA did not detect new tumors earlier than standard examination or RetCam images.

#### **MRI, CHEMOTHERAPY AND OPTIC NERVE INVOLVEMENT: CASE STUDIES AND A NEW POSSIBLE RISK FOR SYSTEMIC SPREAD OF RETINOBLASTOMA**

*Mark Duffly, Michael Shapiro, Charles Blake, Audrey Chen, Deepak Edward, Richard Labotka*

*University of Illinois, Chicago, IL, USA*

We present two patients with unilateral retinoblastoma (RB) tumors and optic nerve involvement. Patient 1 is a South-Asian male with a large right eye RB and significant optic nerve involvement (9mm by MRI). After 6 cycles of chemotherapy, MRI showed a marked decrease of the right optic nerve tumor to just past the lamina. Patient 2 was a Hispanic female diagnosed with RB and optic nerve involvement (just past lamina). The patient underwent chemotherapy to lessen the optic nerve involvement before enucleation. Histology of both patients showed no intra- or extra-scleral extension. Patient 2 showed no residual optic nerve tumor cells but did show focal arachnoid hyperplasia. The optic nerve of patient 1 showed an isolated 'skip' lesion of viable cells approximately 6 mm posterior to the lamina surrounded by arachnoid hyperplasia. The resection margin was free and no cells were seen in the CSF space. Patient 1 is doing well while patient 2 died of rapid disseminated disease 6 months later despite aggressive therapy. We present two conclusions for consideration: 1) MRI is limited in ability to differentiate viable optic nerve tumor from arachnoid hyperplasia, and 2) this previously undescribed optic nerve RB skip lesion may represent a significant risk factor systemic spread and mortality.

#### **TRANSPUPILLARY THERMOTHERAPY IN RETINOMA**

*Nilutpol Borah, Jayanta Das, Bal Agarwal, Jogendra Mazumdar*

*Sri Sankaradeva Nethralaya, Guwahati, India*

**PURPOSE:** Retinoma are rare retinal lesions found in retinoblastoma patients, previously termed as "spontaneous regression of retinoblastoma". Retinoma are translucent elevated retinal mass with calcification and pigment epithelial changes. We report three patients of retinoma without family history who were followed up for mean 21 months and treated with transpupillary thermotherapy (TTT) upon progression of

tumor size. **METHODS:** TTT was delivered via slit lamp using diode laser (810nm) in 3 unilateral retinoma (3 patients) posterior to equator. None had associated vitreous or subretinal seeds. Mean age -13.3 years (11-15). Average follow up period following TTT - 10 months (3-16). Fundus examination, fluorescein angiography, A and B scan ultrasonography, CT scan, systemic examinations were routinely done. Mean tumor height - 2.3 mm, base - 7 mm. Treatment parameters: power 250 mW (220-300), spot size-4000 micron, and duration-14-24 minutes. **RESULTS:** All tumors (n=3) were successfully treated in average 2 sessions achieving calcific, gliotic or flat scars. Recurrence of treated retinoma did not occur during follow up period. Complications were: lens opacity, choroidal atrophy, retinal traction and pigment hypertrophy. **CONCLUSION:** TTT for retinomas without associated vitreous or subretinal seeding was found to be effective in this small series. Frequent follow up for an indefinite period and genetic counseling is necessary in patients with retinoma and their family members.

#### **BETA RAY BRACHYTHERAPY OF RETINOBLASTOMA**

*Andreas Schüler, Wolfgang Sauerwein; Christine Jurklies, Gerasimos Anastassiou, Norbert Bornfeld*

*University of Essen, Essen, Germany*

**PURPOSE:** Evaluation of brachytherapy with <sup>106</sup>Ru/<sup>106</sup>Rh-plaques in the therapy of retinoblastoma. **METHODS:** Retrospective non-comparative case observation study. Patients with uni- or bilateral retinoblastoma treated with <sup>106</sup>Ru/<sup>106</sup>Rh-brachytherapy between 1979 and 2001 in the University of Essen. Included were 136 patients with 142 affected eyes and 178 retinoblastoma treated with local irradiation. Survival, eye salvaging rate, local tumor recurrence and complication rate. **RESULTS:** Mean age of the patients was 1.89 years (SD 1.7 years). Brachytherapy was primary treatment in 60 cases (34%). The majority was secondary brachytherapy after laser- and cryocoagulation, brachytherapy, chemotherapy or EBRT without sufficient tumor control. Tumor height was 3.7 mm (SD 1.4mm) with a mean diameter of 5.0 mm (SD 2.8mm) and a distance to the optic disk of 4.8 disk diameter (SD: 3.6). Mean dose at the sclera was 346 Gy (SD 168 Gy) with a mean dose at the apex of 80 Gy (SD 41 Gy). All dose values in this paper are based on the former BEBIG dosimetry and are marked as Gy therefore. Recurrence after <sup>106</sup>Ru/<sup>106</sup>Rh-brachytherapy occurred in 8 cases (5 year survival: 93%) and enucleation was necessary in 15 eyes (5 year survival: 24.3 %). Radiation retinopathy occurred in 22 eyes (15.5%) and radiation optic neuropathy in 12 eyes (8.5%). Cox regression revealed lower radiation dose at the sclera (risk ratio (RR)=0.99, p=0.014) or vitreous tumor seeding (RR=6.3, p=0.01) as risk factors for recurrence. Factors predicting enucleation were preceded brachytherapy (RR=91, p=0.0002), EBRT (RR=23.7, p=0.002), chemotherapy (RR=8.9, p=0.009) and larger tumor diameter (RR=1.4, p=0.0004). Predictors for retinopathy were preceding EBRT (RR 4.9, p=0.0005) and retinal detachment (RR=1.3, p=0.02). Optic neuropathy was significant more frequent after prior brachytherapy (RR=16.2 p=0.018) and after EBRT (RR=5.6, p=0.015). Kaplan-Meier analysis identified larger plaque diameters as significant risk factors for development of optic neuropathy (Wilcoxon p=0.015) and final enucleation (Wilcoxon p<0.0001). **CONCLUSION:** <sup>106</sup>Ru/<sup>106</sup>Rh-brachytherapy allows local tumor control rates up to 93% at 5 years. Recurrence must be expected in cases with vitreous seeding. Irradiation dose (based on old BEBIG dosimetry) should not remain below 50 Gy. Incidence of complications is increased by preceded irradiation or chemotherapy.

#### **SESSION SUMMARY**

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