Notice of Award

CLINICAL INVESTIGATOR AWARD
Department of Health and Human Services
National Institutes of Health

NATIONAL EYE INSTITUTE

Federal Award Date: 02/23/2017

Grant Number: 1K08EY027464-01
FAIN: K08EY027464

Principal Investigator(s):
Anthony Brent Daniels

Project Title: Developing alternative approaches to reduce retinal toxicity and prevent vision loss in the treatment of intraocular retinoblastoma

D CLINTON BROWN
DIRECTOR OFFICE OF SPON PROG
VANDERBILT UNIVERSITY MEDICAL CTR
3319 WEST END AVE, STE 970
NASHVILLE, TN 372031059

Award e-mailed to: sponsoredprograms@vanderbilt.edu

Period Of Performance:
Budget Period: 03/01/2017 – 02/28/2018
Project Period: 03/01/2017 – 02/28/2022

Dear Business Official:

The National Institutes of Health hereby awards a grant in the amount of $191,925 (see "Award Calculation" in Section I and "Terms and Conditions" in Section III) to VANDERBILT UNIVERSITY MEDICAL CENTER in support of the above referenced project. This award is pursuant to the authority of 42 USC 241 42 CFR 52 and is subject to the requirements of this statute and regulation and of other referenced, incorporated or attached terms and conditions.

Acceptance of this award including the "Terms and Conditions" is acknowledged by the grantee when funds are drawn down or otherwise obtained from the grant payment system.

Each publication, press release, or other document about research supported by an NIH award must include an acknowledgment of NIH award support and a disclaimer such as "Research reported in this publication was supported by the National Eye Institute of the National Institutes of Health under Award Number K08EY027464. The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health." Prior to issuing a press release concerning the outcome of this research, please notify the NIH awarding IC in advance to allow for coordination.

Award recipients must promote objectivity in research by establishing standards that provide a reasonable expectation that the design, conduct and reporting of research funded under NIH awards will be free from bias resulting from an Investigator's Financial Conflict of Interest (FCOI), in accordance with the 2011 revised regulation at 42 CFR Part 50 Subpart F. The Institution shall submit all FCOI reports to the NIH through the eRA Commons FCOI Module. The regulation does not apply to Phase I Small Business Innovative Research (SBIR) and Small Business Technology Transfer (STTR) awards. Consult the NIH website http://grants.nih.gov/grants/policy/coi/ for a link to the regulation and additional important information.

If you have any questions about this award, please contact the individual(s) referenced in Section IV.

Sincerely yours,

Karen Robinson-Smith
Grants Management Officer
NATIONAL EYE INSTITUTE

Additional information follows

SECTION I – AWARD DATA – 1K08EY027464-01

Award Calculation (U.S. Dollars)

Salaries and Wages	$138,825
Fringe Benefits	$13,883
Personnel Costs (Subtotal)	$152,708
Other	$25,000
Federal Direct Costs	$177,708
Federal F&A Costs	$14,217
Approved Budget	$191,925
Total Amount of Federal Funds Obligated (Federal Share)	$191,925
TOTAL FEDERAL AWARD AMOUNT	$191,925
AMOUNT OF THIS ACTION (FEDERAL SHARE)	$191,925

SUMMARY TOTALS FOR ALL YEARS		
YR	**THIS AWARD**	**CUMULATIVE TOTALS**
1	$191,925	$191,925
2	$191,925	$191,925
3	$191,925	$191,925
4	$191,925	$191,925
5	$191,925	$191,925

Recommended future year total cost support, subject to the availability of funds and satisfactory progress of the project

Fiscal Information:

CFDA Name: Vision Research
CFDA Number: 93.867
EIN: 1352528741A1
Document Number: KEY027464A
PMS Account Type: P (Subaccount)
Fiscal Year: 2017

IC	CAN	2017	2018	2019	2020	2021
EY	8472448	$191,925	$191,925	$191,925	$191,925	$191,925

Recommended future year total cost support, subject to the availability of funds and satisfactory progress of the project

NIH Administrative Data:
PCC: 1 /NXA / **OC**: 415L / **Released**: [redacted] 02/17/2017
Award Processed: 02/23/2017 12:14:22 AM

SECTION II – PAYMENT/HOTLINE INFORMATION – 1K08EY027464-01

For payment and HHS Office of Inspector General Hotline information, see the NIH Home Page at http://grants.nih.gov/grants/policy/awardconditions.htm

SECTION III – TERMS AND CONDITIONS – 1K08EY027464-01

This award is based on the application submitted to, and as approved by, NIH on the above-titled project and is subject to the terms and conditions incorporated either directly or by reference in the following:

a. The grant program legislation and program regulation cited in this Notice of Award.
b. Conditions on activities and expenditure of funds in other statutory requirements, such as those included in appropriations acts.
c. 45 CFR Part 75.
d. National Policy Requirements and all other requirements described in the NIH Grants Policy Statement, including addenda in effect as of the beginning date of the budget

period.

e. Federal Award Performance Goals: As required by the periodic report in the RPPR or in the final progress report when applicable.
f. This award notice, INCLUDING THE TERMS AND CONDITIONS CITED BELOW.

(See NIH Home Page at http://grants.nih.gov/grants/policy/awardconditions.htm for certain references cited above.)

Research and Development (R&D): All awards issued by the National Institutes of Health (NIH) meet the definition of "Research and Development" at 45 CFR Part§ 75.2. As such, auditees should identify NIH awards as part of the R&D cluster on the Schedule of Expenditures of Federal Awards (SEFA). The auditor should test NIH awards for compliance as instructed in Part V, Clusters of Programs. NIH recognizes that some awards may have another classification for purposes of indirect costs. The auditor is not required to report the disconnect (i.e., the award is classified as R&D for Federal Audit Requirement purposes but non-research for indirect cost rate purposes), unless the auditee is charging indirect costs at a rate other than the rate(s) specified in the award document(s).

An unobligated balance may be carried over into the next budget period without Grants Management Officer prior approval.

This grant is subject to Streamlined Noncompeting Award Procedures (SNAP).

This award is subject to the requirements of 2 CFR Part 25 for institutions to receive a Dun & Bradstreet Universal Numbering System (DUNS) number and maintain an active registration in the System for Award Management (SAM). Should a consortium/subaward be issued under this award, a DUNS requirement must be included. See http://grants.nih.gov/grants/policy/awardconditions.htm for the full NIH award term implementing this requirement and other additional information.

This award has been assigned the Federal Award Identification Number (FAIN) K08EY027464. Recipients must document the assigned FAIN on each consortium/subaward issued under this award.

Based on the project period start date of this project, this award is likely subject to the Transparency Act subaward and executive compensation reporting requirement of 2 CFR Part 170. There are conditions that may exclude this award; see http://grants.nih.gov/grants/policy/awardconditions.htm for additional award applicability information.

In accordance with P.L. 110-161, compliance with the NIH Public Access Policy is now mandatory. For more information, see NOT-OD-08-033 and the Public Access website: http://publicaccess.nih.gov/.

In accordance with the regulatory requirements provided at 45 CFR 75.113 and Appendix XII to 45 CFR Part 75, recipients that have currently active Federal grants, cooperative agreements, and procurement contracts with cumulative total value greater than $10,000,000 must report and maintain information in the System for Award Management (SAM) about civil, criminal, and administrative proceedings in connection with the award or performance of a Federal award that reached final disposition within the most recent five-year period. The recipient must also make semiannual disclosures regarding such proceedings. Proceedings information will be made publicly available in the designated integrity and performance system (currently the Federal Awardee Performance and Integrity Information System (FAPIIS)). Full reporting requirements and procedures are found in Appendix XII to 45 CFR Part 75. This term does not apply to NIH fellowships.

Treatment of Program Income:
Additional Costs

SALARY CAP:
None of the funds in this award shall be used to pay the salary of an individual at a rate in excess of the applicable salary cap. Therefore this award and/or future years are adjusted accordingly, if applicable. Current salary cap levels can be found at the following URL:
http://grants1.nih.gov/grants/policy/salcap_summary.htm

REMINIDER – REQUIREMENTS FOR THE APPROPRIATE SIGNATURES ON NIH FORMS AND OFFICIAL DOCUMENTATION:
NIH no longer accepts forms or other documentation bearing generic departmental signatures or their electronic equivalent (e.g., Department of Sponsored Research). All forms and documentation submitted to the NIH must reflect the name of the individual, electronic, or otherwise, with the appropriate institutional authority to submit such information (i.e., Authorized Organizational Official (AOR), Signing Official (SO), Business Official (BO), Principal Investigator (PD/PI). See NIH Guide Notice: NOT-OD-16-071.

PRIOR APPROVAL:
Requests which require the prior approval of the NEI must be submitted in writing to the Grants Management Specialist. All requests should reference the complete grant number and must be signed by the authorized official of the business office of the grantee organization and by the principal investigator.

GM ROLES & RESPONSIBILITIES:
If you need assistance from the National Eye Institute (NEI) during the course of this grant, please contact the grants management and program staff listed on the Notice of Grant Award (NGA). The telephone numbers of these individuals, as well as other extramural staff members of the NEI, can be located on the NEI web site, http://www.nei.nih.gov. The grants management and program staff members work closely with one another through all phases of the project to facilitate the award and the administration of the grant. Their functions are defined as follows:

GRANTS MANAGEMENT CONTACT: The Grants Management Specialist is responsible for all business management matters associated with the review, negotiation, award, and administration of grants. Grants Management Specialists serve as the focal point for receiving and responding to all questions and correspondence related to business management and policy matters, such as correspondence giving or denying any prior approval required by Public Health Service (PHS) policy or special Terms and Conditions of Award, transfer of the grant to another institution, a change in the period of support, or any action which commits, or may result in committing the NEI to a change in the amount of funding.

PROGRAM CONTACT: The Program Director is responsible for all scientific and technical matters associated with the grant. The program official reviews and monitors scientific progress of the project and provides advice and assistance relative to all technical problems to ensure that the scientific objectives of the research program can be pursued effectively and successfully. All questions or correspondence dealing with research progress, changes in research direction, unique scientific opportunities, or any other scientific needs should be addressed to the Program Director.

STAFF CONTACTS

The Grants Management Specialist is responsible for the negotiation, award and administration of this project and for interpretation of Grants Administration policies and provisions. The Program Official is responsible for the scientific, programmatic and technical aspects of this project. These individuals work together in overall project administration. Prior approval requests (signed by an Authorized Organizational Representative) should be submitted in writing to the Grants Management Specialist. Requests may be made via e-mail.

Grants Management Specialist: Sylvia Braxton
Email: braxtons@mail.nih.gov **Phone**: 301-451-2020

Program Official: Neeraj Agarwal
Email: agarwalnee@mail.nih.gov **Phone**: 301-451-2020 **Fax**: 301-402-0528

SPREADSHEET SUMMARY
GRANT NUMBER: 1K08EY027464-01

INSTITUTION: VANDERBILT UNIVERSITY MEDICAL CENTER

Budget	Year 1	Year 2	Year 3	Year 4	Year 5
Salaries and Wages	$138,825	$138,825	$138,825	$138,825	$138,825
Fringe Benefits	$13,883	$13,883	$13,883	$13,883	$13,883
Personnel Costs (Subtotal)	$152,708	$152,708	$152,708	$152,708	$152,708
Other	$25,000	$25,000	$25,000	$25,000	$25,000
TOTAL FEDERAL DC	$177,708	$177,708	$177,708	$177,708	$177,708
TOTAL FEDERAL F&A	$14,217	$14,217	$14,217	$14,217	$14,217
TOTAL COST	$191,925	$191,925	$191,925	$191,925	$191,925

Facilities and Administrative Costs	Year 1	Year 2	Year 3	Year 4	Year 5
F&A Cost Rate 1	8%	8%	8%	8%	8%
F&A Cost Base 1	$177,708	$177,708	$177,708	$177,708	$177,708
F&A Costs 1	$14,217	$14,217	$14,217	$14,217	$14,217

PI: **Daniels, Anthony Brent**	Title: Developing alternative approaches to reduce retinal toxicity and prevent vision loss in the treatment of intraocular retinoblastoma	
Received: 02/12/2016	FOA: PA14-046	Council: 10/2016
Competition ID: FORMS-C	FOA Title: MENTORED CLINICAL SCIENTIST RESEARCH CAREER DEVELOPMENT AWARD (PARENT K08)	
1 K08 EY027464-01	Dual:	Accession Number: 3906933
IPF: 10040927	Organization: VANDERBILT UNIVERSITY MEDICAL CENTER	
Former Number:	Department: OPHTHALMOLOGY & VISUAL SCIENCES	
IRG/SRG: ZEY1 VSN (04)	AIDS: N	Expedited: N
Subtotal Direct Costs (excludes consortium F&A) Year 1: 177,708 Year 2: 177,708 Year 3: 177,708 Year 4: 177,708 Year 5: 177,708	Animals: Y Humans: N Clinical Trial: N Current HS Code: 10 HESC: N	New Investigator: Early Stage Investigator:

Senior/Key Personnel:	*Organization:*	*Role Category:*
Anthony Daniels	Vanderbilt University Medical Center	PD/PI
[redacted]	Vanderbilt University Medical Center	Other (Specify)-Mentor
	Vanderbilt University Medical Center	Other (Specify)-co-Mentor
	Vanderbilt University Medical Center	Other (Specify)-co-Mentor
	Vanderbilt University Medical Center	Other (Specify)-co-Mentor
	Vanderbilt University Medical Center	Other (Specify)-Collaborator
	Vanderbilt University Medical Center	Other (Specify)-Collaborator
	Vanderbilt University Medical Center	Other (Specify)-Collaborator
	Vanderbilt University Medical Center	Other (Specify)-Collaborator
	Memorial Sloan-Kettering Cancer Center	Other (Specify)-Collaborator

Reference Letters

[redacted]	02/12/2016
	02/12/2016
	02/12/2016

OMB Number: 4040-0001
Expiration Date: 06/30/2016

APPLICATION FOR FEDERAL ASSISTANCE
SF 424 (R&R)

3. DATE RECEIVED BY STATE	State Application Identifier TN: Tennessee

1. TYPE OF SUBMISSION*

❍ Pre-application ❍ Application ● Changed/Corrected Application

4.a. Federal Identifier

b. Agency Routing Number

c. Previous Grants.gov Tracking Number GRANT12093549

2. DATE SUBMITTED 2016-02-12

Application Identifier 00040871

5. APPLICANT INFORMATION

Organizational DUNS*: 004413456

Legal Name*: Vanderbilt University Medical Center
Department: Ophthalmology
Division: School of Medicine
Street1*: 1400 18th Avenue South
Street2:
City*: Nashville
County: Davidson
State*: TN: Tennessee
Province:
Country*: USA: UNITED STATES
ZIP / Postal Code*: 37212-2809

Person to be contacted on matters involving this application

Prefix: First Name*: Donald Middle Name: Clinton Last Name*: Brown Suffix:

Position/Title: Director, Office of Sponsored Programs
Street1*: 1400 18th Avenue
Street2:
City*: Nashville
County: Davidson
State*: TN: Tennessee
Province:
Country*: USA: UNITED STATES
ZIP / Postal Code*: 37212-2809

Phone Number*: 615-875-6070 Fax Number: 615-343-2447 Email: sponsoredprograms@vanderbilt.edu

6. EMPLOYER IDENTIFICATION NUMBER *(EIN) or (TIN)** 1620476822A2

7. TYPE OF APPLICANT* O: Private Institution of Higher Education

Other (Specify):
Small Business Organization Type ❍ Women Owned ❍ Socially and Economically Disadvantaged

8. TYPE OF APPLICATION*

● New ❍ Resubmission
❍ Renewal ❍ Continuation ❍ Revision

If Revision, mark appropriate box(es).

❍ A. Increase Award ❍ B. Decrease Award ❍ C. Increase Duration
❍ D. Decrease Duration ❍ E. Other *(specify)* :

Is this application being submitted to other agencies?* ❍Yes ●No What other Agencies?

9. NAME OF FEDERAL AGENCY*
National Institutes of Health/Unknown

10. CATALOG OF FEDERAL DOMESTIC ASSISTANCE NUMBER
TITLE: Mentored Clinical Scientist Research Career Development Award (Parent K08)

11. DESCRIPTIVE TITLE OF APPLICANT'S PROJECT*
Developing alternative approaches to reduce retinal toxicity and prevent vision loss in the treatment of intraocular retinoblastoma

12. PROPOSED PROJECT

Start Date* 09/01/2016
Ending Date* 08/31/2021

13. CONGRESSIONAL DISTRICTS OF APPLICANT
TN-005

SF 424 (R&R) APPLICATION FOR FEDERAL ASSISTANCE

14. PROJECT DIRECTOR/PRINCIPAL INVESTIGATOR CONTACT INFORMATION

Prefix: First Name*: Anthony Middle Name: B Last Name*: Daniels Suffix:
Position/Title: Asst Professor
Organization Name*: Vanderbilt University Medical Center
Department: Ophthalmology
Division: School of Medicine
Street1*: campus zip 8808
Street2:
City*: Nashville
County: Davidson
State*: TN: Tennessee
Province:
Country*: USA: UNITED STATES
ZIP / Postal Code*: 37235-0002
Phone Number*: 615-936-2483 Fax Number: 615-936-1540 Email*: anthony.b.daniels@vanderbilt.edu

15. ESTIMATED PROJECT FUNDING

a. Total Federal Funds Requested*	$959,620.50
b. Total Non-Federal Funds*	$0.00
c. Total Federal & Non-Federal Funds*	$959,620.50
d. Estimated Program Income*	$0.00

16.IS APPLICATION SUBJECT TO REVIEW BY STATE EXECUTIVE ORDER 12372 PROCESS?*

a. YES ○ THIS PREAPPLICATION/APPLICATION WAS MADE AVAILABLE TO THE STATE EXECUTIVE ORDER 12372 PROCESS FOR REVIEW ON:
DATE:
b. NO ● PROGRAM IS NOT COVERED BY E.O. 12372; OR
○ PROGRAM HAS NOT BEEN SELECTED BY STATE FOR REVIEW

17. By signing this application, I certify (1) to the statements contained in the list of certifications* and (2) that the statements herein are true, complete and accurate to the best of my knowledge. I also provide the required assurances * and agree to comply with any resulting terms if I accept an award. I am aware that any false, fictitious, or fraudulent statements or claims may subject me to criminal, civil, or administrative penalties. (U.S. Code, Title 18, Section 1001)

● I agree*

** The list of certifications and assurances, or an Internet site where you may obtain this list, is contained in the announcement or agency specific instructions.*

18. SFLLL or OTHER EXPLANATORY DOCUMENTATION File Name:

19. AUTHORIZED REPRESENTATIVE

Prefix: First Name*: Donald Middle Name: Clinton Last Name*: Brown Suffix:
Position/Title*: Director, Office of Sponsored Programs
Organization Name*: Vanderbilt University Medical Center
Department: Office of Sponsored Programs
Division:
Street1*: 1400 18th Avenue
Street2:
City*: Nashville
County:
State*: TN: Tennessee
Province:
Country*: USA: UNITED STATES
ZIP / Postal Code*: 37212-2809
Phone Number*: 615-875-6070 Fax Number: 615-343-2447 Email*: sponsoredprograms@vanderbilt.edu

Signature of Authorized Representative*
Brown,Donald Clinton

Date Signed*
02/12/2016

20. PRE-APPLICATION File Name:

21. COVER LETTER ATTACHMENT File Name:M-22_RRSF424_Cover_Letter.pdf

424 R&R and PHS-398 Specific Table Of Contents

OMB Number: 4040-0010
Expiration Date: 06/30/2016

Project/Performance Site Location(s)

Project/Performance Site Primary Location

❍ I am submitting an application as an individual, and not on behalf of a company, state, local or tribal government, academia, or other type of organization.

Organization Name:	Vanderbilt University Medical Center
Duns Number:	004413456
Street1*:	1400 18th Avenue South
Street2:	
City*:	Nashville
County:	Davidson
State*:	TN: Tennessee
Province:	
Country*:	USA: UNITED STATES
Zip / Postal Code*:	37212-2809
Project/Performance Site Congressional District*:	TN-005

File Name

Additional Location(s)

OMB Number: 4040-0001
Expiration Date: 06/30/2016

RESEARCH & RELATED Other Project Information

1. Are Human Subjects Involved?* ○ **Yes** ● **No**

1.a. If YES to Human Subjects

Is the Project Exempt from Federal regulations? ○ Yes ○ No

If YES, check appropriate exemption number: _ 1 _ 2 _ 3 _ 4 _ 5 _ 6

If NO, is the IRB review Pending? ○ **Yes** ○ **No**

IRB Approval Date:

Human Subject Assurance Number

2. Are Vertebrate Animals Used?* ● **Yes** ○ **No**

2.a. If YES to Vertebrate Animals

Is the IACUC review Pending? ● Yes ○ No

IACUC Approval Date:

Animal Welfare Assurance Number A3227-01

3. Is proprietary/privileged information included in the application?* ○ **Yes** ● **No**

4.a. Does this project have an actual or potential impact - positive or negative - on the environment?* ○ **Yes** ● **No**

4.b. If yes, please explain:

4.c. If this project has an actual or potential impact on the environment, has an exemption been authorized or an environmental assessment (EA) or environmental impact statement (EIS) been performed? ○ Yes ○ No

4.d. If yes, please explain:

5. Is the research performance site designated, or eligible to be designated, as a historic place?* ○ **Yes** ● **No**

5.a. If yes, please explain:

6. Does this project involve activities outside the United States or partnership with international collaborators?* ○ **Yes** ● **No**

6.a. If yes, identify countries:

6.b. Optional Explanation:

	Filename
7. Project Summary/Abstract*	M-25_Project_Summary.pdf
8. Project Narrative*	M-1_Narrative.pdf
9. Bibliography & References Cited	M-28_Bibliography.pdf
10.Facilities & Other Resources	M-2_Facilities.pdf
11.Equipment	M-29_Equipment.pdf
12. Other Attachments	List of Referees .pdf Authentication of key Resources Plan.pdf

Retinoblastoma (RB) is the most common intraocular malignancy in children. The recent introduction of intra-arterial chemotherapy (IAC) and intravitreal injections, both using melphalan-based regimens, has improved globe survival for eyes with advanced RB, while avoiding the systemic toxicities of intravenous chemotherapy. However, melphalan is toxic to the retina and retinal vasculature, and both treatments are associated with many ocular side effects, potentially resulting in life-long loss of vision. The goal of this research is to identify alternative drugs that are less toxic to the ocular structures but remain effective against intraocular RB, for use via IAC and intravitreal injection. In rabbits, we have developed the only small animal model of IAC, and have demonstrated excellent ocular penetration and reproducible pharmacokinetics (PK) for melphalan. We have also developed a rabbit model of RB that develops both retinal tumors and vitreous seeds grown from human RB cell lines, recapitulating features found in patients with advanced RB. Using these novel models, we plan to determine the PK, retinal/retinal vascular toxicity profiles, and efficacy of a selected group of chemotherapeutic agents that already have FDA-approval. Focusing on FDA-approved drugs will allow rapid translation of our findings into clinical practice. Both vitreous and intra-retinal drug concentrations and PK curves will be calculated for each drug following both IAC or intravitreal injection, using traditional mass spectrometry and a novel *in situ* imaging mass spectrometry technique that was developed at Vanderbilt. Once the PK curves have been calculated, the maximum tolerable dose (MTD) for each drug will be calculated for both IAC and for repeated intravitreal injections. Functional and structural ocular toxicity will be measured using a comprehensive panel of techniques. A robust Bayesian Continual Reassessment Method clinical study design will be employed. The MTD will then be used in studies of the efficacy of each drug, given by either IAC or intravitreal injection, for the treatment of RB in the above rabbit models. Following identification of safe and effective target doses *in vivo* in our rabbit model, we will confirm the absence of retinal toxicity using a cutting edge technology that allows drug toxicity to be monitored over time in *ex vivo* human retinal tissues that can be kept alive in a supportive perfusate for functional evaluation. This ensures that these drugs are safe for human eyes (not just rabbits). Dr. Daniels brings experience with ocular tumor biology and genetics, as well as clinical expertise with IAC for RB. The long-term goal of his research is to develop safer, pathway-targeted agents for the treatment of RB. This Mentored Clinical Scientist Research Career Development (K08) Award will allow Dr. Daniels to be mentored by a world-class team of mentors, who have extensive expertise in retinal biology, tumor biology and therapeutics, and in the preclinical assessment of antineoplastic agents and intraocular drugs. Through a combination of hands-on experimentation and didactics, Dr. Daniels will develop necessary skills in the use of animal models for preclinical studies, *in vivo* assessment of retinal toxicity and efficacy, and biostatistics, while developing a comprehensive drug-testing platform that can be used to assess novel compounds in the future.

PROJECT NARRATIVE

Retinoblastoma is the most common eye cancer in children, with a devastating and long-lasting influence on quality of life. While new treatments allow clinicians to save most eyes affected by this cancer, the treatments themselves are often damaging to the eye and can lead to lifelong vision loss. To address this pressing problem, our proposal leverages new experimental models that will enable us to test more thoroughly the effectiveness, safety and long-term effect of novel treatments, thus allowing us to identify new drugs that are safer to use in the eyes of children fighting retinoblastoma.

BIBLIOGRAPHY AND REFERENCES CITED

1. Murphree AL, Villablanca JG, Deegan WF, 3rd, et al. Chemotherapy plus local treatment in the management of intraocular retinoblastoma. Archives of ophthalmology 1996;114:1348-56.
2. Singh G DA. Disparities in retinoblastoma presentation, treatment, and outcomes in developed and less-developed countries. Seminars in Ophthalmology 2016;31.
3. Kunkele A, Jurklies C, Wieland R, et al. Chemoreduction improves eye retention in patients with retinoblastoma: a report from the German Retinoblastoma Reference Centre. The British journal of ophthalmology 2013;97:1277-83.
4. Chung SE, Sa HS, Koo HH, Yoo KH, Sung KW, Ham DI. Clinical manifestations and treatment of retinoblastoma in Korea. The British journal of ophthalmology 2008;92:1180-4.
5. Berry JL, Jubran R, Kim JW, et al. Long-term outcomes of Group D eyes in bilateral retinoblastoma patients treated with chemoreduction and low-dose IMRT salvage. Pediatric blood & cancer 2013;60:688-93.
6. Bartuma K, Pal N, Kosek S, Holm S, All-Ericsson C. A 10-year experience of outcome in chemotherapy-treated hereditary retinoblastoma. Acta ophthalmologica 2014;92:404-11.
7. Rodriguez-Galindo C, Wilson MW, Haik BG, et al. Treatment of intraocular retinoblastoma with vincristine and carboplatin. Journal of clinical oncology : official journal of the American Society of Clinical Oncology 2003;21:2019-25.
8. Jehanne M, Mercier G, Doz F. Monitoring of ototoxicity in young children receiving carboplatin for retinoblastoma. Pediatric blood & cancer 2009;53:1162.
9. Jehanne M, Lumbroso-Le Rouic L, Savignoni A, et al. Analysis of ototoxicity in young children receiving carboplatin in the context of conservative management of unilateral or bilateral retinoblastoma. Pediatric blood & cancer 2009;52:637-43.
10. Shin JY, Kim JH, Yu YS, et al. Eye-preserving therapy in retinoblastoma: prolonged primary chemotherapy alone or combined with local therapy. Korean journal of ophthalmology : KJO 2010;24:219-24.
11. Gombos DS, Hungerford J, Abramson DH, et al. Secondary acute myelogenous leukemia in patients with retinoblastoma: is chemotherapy a factor? Ophthalmology 2007;114:1378-83.
12. Zage PE, Reitman AJ, Seshadri R, et al. Outcomes of a two-drug chemotherapy regimen for intraocular retinoblastoma. Pediatric blood & cancer 2008;50:567-72.
13. Lee V, Hungerford JL, Bunce C, Ahmed F, Kingston JE, Plowman PN. Globe conserving treatment of the only eye in bilateral retinoblastoma. The British journal of ophthalmology 2003;87:1374-80.
14. Turaka K, Shields CL, Meadows AT, Leahey A. Second malignant neoplasms following chemoreduction with carboplatin, etoposide, and vincristine in 245 patients with intraocular retinoblastoma. Pediatric blood & cancer 2012;59:121-5.
15. Abramson DH, Daniels AB, Marr BP, et al. Intra-Arterial Chemotherapy (Ophthalmic Artery Chemosurgery) for Group D Retinoblastoma. PLoS One 2016;11:e0146582.
16. Shields CL, Mashayekhi A, Au AK, et al. The International Classification of Retinoblastoma predicts chemoreduction success. Ophthalmology 2006;113:2276-80.
17. Daniels AB, Patel S, Koyama T, Alvarez J, Friedman DL. Success rates of intravenous chemotherapy for intraocular retinoblastoma: A meta-analysis. Investigative ophthalmology & visual science 2015;56:6059.
18. Shields CL, Honavar SG, Meadows AT, et al. Chemoreduction plus focal therapy for retinoblastoma: factors predictive of need for treatment with external beam radiotherapy or enucleation. American journal of ophthalmology 2002;133:657-64.
19. Abramson DH, Dunkel IJ, Brodie SE, Kim JW, Gobin YP. A phase I/II study of direct intraarterial (ophthalmic artery) chemotherapy with melphalan for intraocular retinoblastoma initial results. Ophthalmology 2008;115:1398-404, 404 e1.
20. Munier FL, Gaillard MC, Balmer A, et al. Intravitreal chemotherapy for vitreous disease in retinoblastoma revisited: from prohibition to conditional indications. The British journal of ophthalmology 2012;96:1078-83.
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FACILITIES AND OTHER RESOURCES

Laboratory:

Dr. Daniels' mentor, [redacted] has a laboratory of approximately 1,700 square feet, including tissue culture, with all the appropriate equipment for cell and molecular studies, including PCR machines, centrifuges, laminar flow hoods, incubators, spectroscopy, electrophoresis, fluorescence microscopy, software for image analysis, and microfuges. Within the [redacted] lab space described above, Dr. Daniels and his ocular tumor research team have two dedicated lab benches, in addition to access to all of the above-mentioned common lab facilities.

Office:

Dr. Daniels has approximately 120 square feet of office space at the Vanderbilt Eye Institute. Dr. Daniels' office is [redacted] and Dr. Richmond's office, and he maintains a second office desk [redacted]. Dr. Daniels also has a shared secretary.

Computer:

[redacted] ab facilities include six IBM compatible computers. The computers are networked into the library, proteomics, cell imaging analysis, microarray core, DNA sequence and microscope facilities. Software programs are available for graphics, word processing, and DNA sequence analysis. PubMed access is available through the Eskind Medical Library website. Dr. Daniels' ocular tumor lab research team also has another shared Mac laptop (with Parallels Windows capability), which can connect to the electroretinography, fundus imaging, and optical coherence tomography equipment. Dr. Daniels' own office at the Vanderbilt Eye Institute has both PC and Mac desktop computers, and he has a personal Mac laptop.

Animal Housing Facilities:

The Division of Animal Care provides space and support for investigators using animals in their research. The Division is under the Direction of J. Wallace, DVM, and is staffed with 5 full time veterinarians in addition to the Director. The facilities and institution is AALAC accredited. The studies will be performed in Medical Center North. The animals will be housed in the large animal vivarium that is located [redacted]

S.R. Light Animal Surgical Facilities:

This laboratory provides space, equipment and personnel to assist investigators in the surgical preparation and experimental activities involving animals, particularly large animals such as rabbits. This AALAC-accredited laboratory is staffed with 2 full-time, licensed veterinary technicians and 1 full time laboratory assistant. The facility occupies approximately 1900 square feet and consists of an operating theater (1120 square feet) with a full suite for interventional endovascular procedures under fluoroscopic guidance, a dark room with a Faraday cage in which electroretinography can be performed, an instrument room, a preoperative room, a post-operative/recovery room, and storage space. The facilities maintain equipment for survival surgery, including: 9 anesthesia machines (isoflurane and sevoflurane) with mechanical ventilators, 6 electrosurgical units, 7 pulse oximeters, 2 hemodynamic monitoring systems, 1 blood gas analysis machine, 1 coulter counter and all routine surgical instrumentation (10 sets). Stock pharmaceuticals are maintained for general surgical use and linen and surgical supplies are provided. This facility is maintained by the Section of Surgical Sciences and operates as the University's Survival Surgical Suite. **Note that [redacted] a collaborator on this grant, is the Director of the S. R. Light Surgical Research Facility.**

Translational Pathology Shared Resource (TPSR):

TPSR is a 3500 square foot state-of-the-art, full-service pathology laboratory with the mission of supporting basic research in animal models and human tissue. The facility is located [redacted]

The laboratory utilizes an automated Order Entry and Database system based in CORES, the Vanderbilt Office of Research data management system. This database allows for precise collection and long-term storage of data generated by TPSR. Investigators have access to query his/her experimental data through any connection to the World Wide Web. TPSR faculty includes 2 M.D. Pathologists and 1 Veterinary Pathologist. The staff consists of 7 histologists, 2 comparative pathology technicians, 1 research assistant and a laboratory supervisor, all with expertise in the biomedical research setting. The **Research Histology** division of TPSR provides high-throughput routine histology and immunohistochemistry for animal and human tissue. The laboratory is fully automated and equipped with 6 automated microtomes (Leica), 2 cryostats (Leica), a Gemini H&E stainer (Thermo-Shandon), 6 Slidemate (Thermo-Fisher) slide writers, 1 automated coverslipper (Sakura), an Artisan (Dako) special stainer, 2 Bond Max (Leica) automated immunostainers, 2 laser capture microdissection microscopes (Olympus and Arcturis)

and a Beecher tissue microarrayer. The immunohistochemistry service last year validated 170 new antibodies in addition to maintaining the standard service requests. The laboratory is equipped with a necropsy suite with 3 downdraft tables. Consultation with a Veterinary Pathologist is also available through this service division.

Small Animal Eye Imaging and Retinal Functional Assessment:

Within the S. R. Light surgery area, Dr. Daniels maintains equipment for electroretinography (ERG), fundus photography and optical coherence tomography (OCT). **ERG** is performed using a state-of-the-art OcuScience HMsERG system, which is validated for use on all types of mammals, fish, birds, and reptiles, and has been especially programmed for rabbits. It has the ability to use "protocols" to conduct standardized series of tests as defined by the International Society for Electrophysiology of Vision (ISCEV), or customizable protocols. The calibrated mini-Ganzfeld stimulator provides illumination of the whole retina, and can produce up to 100 cd.s/m2. A custom designed rabbit Faraday cage was created for our experiments, which take place in a special "dark room" with red lights within the animal surgery suite. **Fundus Photography** is performed with the iVivo® Funduscope for small animals and likewise has been specially customized for our experiments and validated for rabbit use. It has wide brightfield fundus capabilities as well as fluorescein angiography capabilities, with the ability to capture high-resolution still images or video streams. The iVivo VET **Fundus and OCT** system which allows high resolution spectral domain (sd-)OCT and has been validated for rabbits. The technology captures fundus images and shows alignment of OCT scans on real-time retina images to accurately collect tissue data. **Ultrasound** is performed using the VisualSonics 770 High-Resolution Imaging System, which enables visualization, assessment, and measurement of anatomical structures in the B-mode (2D and 3D). Contrast enhanced techniques are also available that allow for quantification of blood flow. The high frequency at which the 770 operates (20-55MHz) allows for achieving spatial resolution down to 30 microns – currently the highest spatial resolution available in real time imaging. This ultrasound equipment is housed [redacted] and the ultrasound equipment can be transported to the MCN animal surgery when needed (<5 minute transport time). **Note that the fundus photography, ERG, and OCT equipment described above is owned by Dr. Daniels' lab, and he therefore has unlimited access to these. Nobody else uses this equipment.**

Mass Spectrometry Resource Center (MSRC):

The MSRC houses 40 mass spectrometers. All of the instruments are located in 13,000 square feet of custom-designed space [redacted] The Proteomics Core and the MS Core occupy 3,000 sq. ft. and 3,200 sq. ft. of lab space, respectively. These facilities include equipment for liquid chromatography mass spectrometry as well as histology-directed *in situ* imaging mass spectrometry, with the capability to perform directed drug tissue level assessments. **Note that [redacted] a collaborator on this grant, is the MSRC's co-director for research and he directs the mass spectrometry and proteomics core facilities.**

Vanderbilt Vision Research Center (VVRC):

The VVRC is supported by a P30 core grant from the National Eye Institute, This P30 contributes funding for several Vanderbilt cores, including Vantage (genomics), Vanguard (bioinformatics), mass spectrometry and proteomics, cell imaging (including confocal and electron microscopy), and basic histology/sectioning. VVRC members (including Dr. Daniels, co-mentor [redacted] and collaborator [redacted] have subsidized access to support their research programs through core use, and Dr. Daniels has been a recipient of VVRC Core Scholarships each year. The VVRC also maintains its own sets of equipment for animal fundus photography, electroretinography, and optical coherence tomography. **The Vice Chair for Research of the Department of Ophthalmology, Professor [redacted] is director of the VVRC and controls allocation of VVRC scholarship funds.**

Vanderbilt-Ingram Cancer Center (VICC)

Established in 1993, VICC is a matrix center within VUMC and integrates the cancer-related expertise and resources of the School of Medicine, School of Nursing, School of Arts and Sciences, School of Engineering, Peabody School of Education and the Veterans Hospital. The Center earned its initial NCI designation as a clinical cancer center in 1995, two years after its creation, and NCI-designated Comprehensive Cancer status in 2001. Research is facilitated by eight well-developed research programs within VICC and Shared Resources. As a member of VICC, Dr. Daniels has access to all of VICC's subsidized core facilities, as well as a phenomenal lecture series. [redacted] is Associate Director for Research Education at VICC, and co-mentor [redacted] is the Director of Cancer Survivorship and Program Co-leader for Cancer Health Outcomes. Co-mentor [redacted] is Director of the Division of Cancer Biostatistics. The VICC infrastructure will also be crucial in the future, as we move to clinical trials of new agents based on our findings.

Statistical Support:

The **Vanderbilt Center for Quantitative Sciences** (CQS) plays an integral role in the research enterprise of the university. The mission of the CQS is to coordinate and integrate the work of quantitative scientists across the disciplines of biostatistics, bioinformatics, biomathematics, computational biology, biomedical engineering, and other related fields, with the end goal of building bridges and streamlining quantitative collaboration for improved biomedical research. CQS members have expertise in basic science as well as clinical/translational studies, with a particular interest in high-dimensional data, including proteomic, genomic, lipidomic, and metabolomic research. The center is available to all university and medical center investigators, offering collaborative support spanning traditional statistical inputs (e.g., experimental design, sample size determination, power analysis, conventional data analysis and results interpretation), to novel statistical and bioinformatic approaches for modern technologies (e.g., advanced sample size determination for next-generation sequencing, multivariate modeling for high-dimensional data), to systems and computational biology approaches for asking questions and modeling results.

The **Division of Cancer Biostatistics** is a subunit within the Department of Biostatistics with a clearly defined group of biostatisticians with a focus on providing comprehensive biostatistical inputs for cancer research, in a collaborative model that promotes better science, from experimental design through results interpretation. The Division of Cancer Biostatistics has played a key role in the success of cancer research at the Vanderbilt-Ingram Cancer Center, and especially the success of large-scale NCI-funded initiatives by pioneering a model of the biostatistician as an integral collaborative member of the research team, rather than a fee-for-service technician. **Professor [redacted] Dr. Daniels' co-mentor on this grant, is the Chief of the Division of Cancer Biostatistics and also the Director of the Center for Quantitative Sciences, and controls allocation of biostatistical support.**

OTHER:

Access to: Flow cytometry core, with ability to sort based on fluorescent-conjugated antibody binding of surface markers in live or fixed cells, the Cell Imaging Shared Resource Core, the Human Immunology Core, and the Epithelial Biology Center Imaging Resource with Ariol equipment for scanning and quantitative analysis of stained tissue slides. Cell Imaging Shared Resource, with 5 upright and inverted fully digital confocal microscopes, among other state-of-the-art digital microscopy solutions. The specific equipment and services available through these cores can be found at: http://medschool.mc.vanderbilt.edu/oor/irc/php_corefacilities.php

EQUIPMENT

Most common lab equipment is present in the lab of mentor [redacted] and her adjacent 240 square foot tissue culture room. This includes six water-jacketed incubators, two laminar flow hoods, water baths, refrigeration, freezers (-20° and -80°), Beckman table top centrifuge, ultracentrifuge, microfuges, Nikon inverted phase microscope, Nikon light microscope and camera, electrophoresis equipment for DNA and RNA gels, gel dryer, bacterial incubators, pH meter, balances, power packs, vacuum oven, and 3 MJ Research thermocyclers. A common equipment room is available with ultracentrifuges, gel dryers, scintillation counters, gamma counters, phospho-image analyzer and lyophilizer. There is also a dark room with an X-O mat and a large cold room. A common dishwashing and autoclave room staffed by the Cancer Biology department is also available.

Rabbit surgery facilities are located [redacted] and include a full suite for interventional endovascular procedures under fluoroscopic guidance, a dark room with a Faraday cage in which electroretinography can be performed, and equipment for survival surgery, including 9 anesthesia machines (isoflurane and sevoflurane) with mechanical ventilators, 6 electrosurgical units, 7 pulse oximeters, 2 hemodynamic monitoring systems, 1 blood gas analysis machine, 1 coulter counter and all routine surgical instrumentation (10 sets). This is also where Dr. Daniels houses his lab's equipment for rabbit eye imaging and functional assessment, including an OcuScience HMsERG system for electroretinography, an OcuScience iVivo® Funduscope with video and fluorescein angiography attachments, and an OcuScience iVivo VET Fundus and Optical Coherence Tomography system. A VisualSonics 770 High-Resolution Imaging Ultrasound System is available through the [redacted] and is transported from there to the adjacent animal surgery area when needed.

The Translational Pathology Shared Resource has six automated microtomes (Leica), two cryostats (Leica), a Gemini H&E stainer (Thermo-Shandon), six Slidemate (Thermo-Fisher) slide writers, one automated coverslipper (Sakura), an Artisan (Dako) special stainer, two Bond Max (Leica) automated immunostainers, two laser capture microdissection microscopes (Olympus and Arturis) and a Beecher tissue microarrayer, as well as necropsy tables, and an extensive array of human and mouse antibodies on-hand for immunohistochemistry.

The Mass Spectrometry Resource Center has forty (40) mass spectrometers, in addition to cryostats and matrix application sprayers. The 40 mass spectrometers include a Thermo-Finnigan TSQ Quantum Ultra mass spectrometer interfaced to a Waters Acquity UPLC system (used primarily for the described liquid chromatography tandem mass spectrometry experiments), a ThermoFisher linear ion trap equipped with a MALDI source-LTQ XL (for the imaging mass spectrometry to achieve the highest sensitivity), and a Bruker MALDI TOF-Rapiflex (for the imaging mass spectrometry to achieve the greatest special resolution).

LIST OF REFEREES

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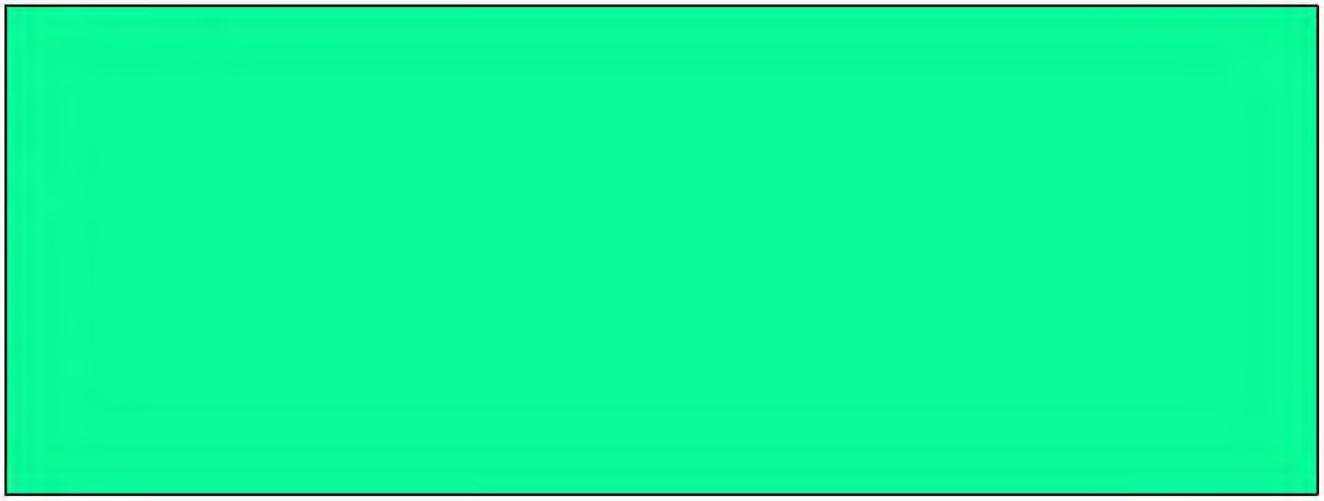


AUTHENTICATION OF KEY RESOURCES

The WERI-Rb1 human retinoblastoma cell line was purchased directly from American Type Culture Collection (ATCC), which has authenticated this cell line.

All drugs used in this research, whether used for *in vitro* cytotoxicity experiments or for rabbit experiments, are pharmaceutical grade, purchased from vendors that also supply hospitals for patient use. Therefore, we are confident in their authenticity. This is true for all the chemotherapeutic agents, immunosuppressive agents, medications used for the intra-arterial chemotherapy procedures or other procedures, and anesthetics.

OMB Number: 4040-0001
Expiration Date: 06/30/2016

RESEARCH & RELATED Senior/Key Person Profile (Expanded)

PROFILE - Project Director/Principal Investigator

Prefix: First Name*: Anthony Middle Name B Last Name*: Daniels Suffix:

Position/Title*: Asst Professor
Organization Name*: Vanderbilt University Medical Center
Department: Ophthalmology
Division:
Street1*: campus zip 8808
Street2:
City*: Nashville
County: Davidson
State*: TN: Tennessee
Province:
Country*: USA: UNITED STATES
Zip / Postal Code*: 37235-0002

Phone Number*: 615-936-2483 Fax Number: 615-936-1540 E-Mail*: anthony.b.daniels@vanderbilt.edu

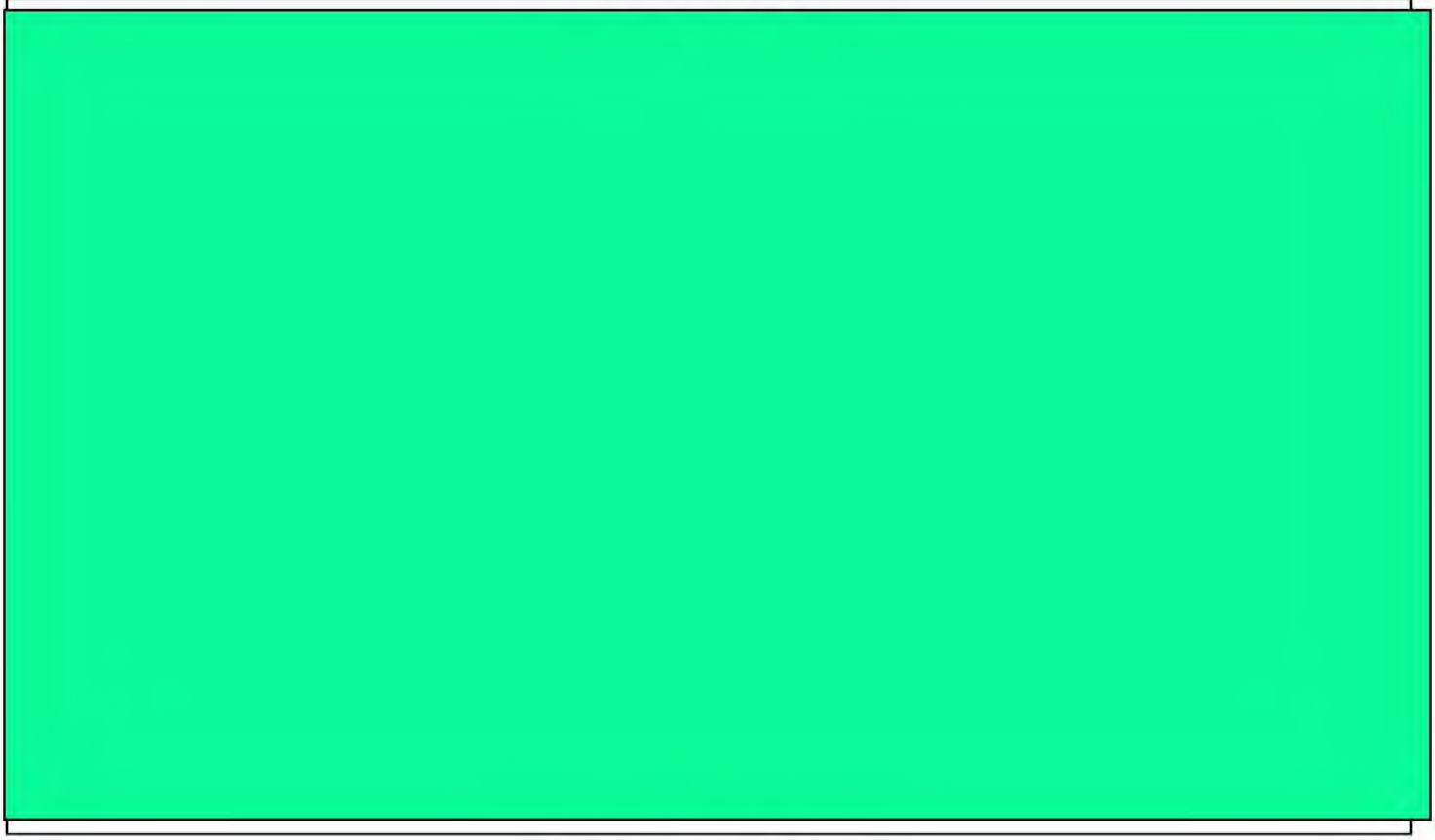
Credential, e.g., agency login:

Project Role*: PD/PI Other Project Role Category:

Degree Type: Medical Doctor Degree Year: 2007

	File Name
Attach Biographical Sketch*:	ID-0117216_BN-1_BIOSKETCH.pdf
Attach Current & Pending Support:	

PROFILE - Senior/Key Person



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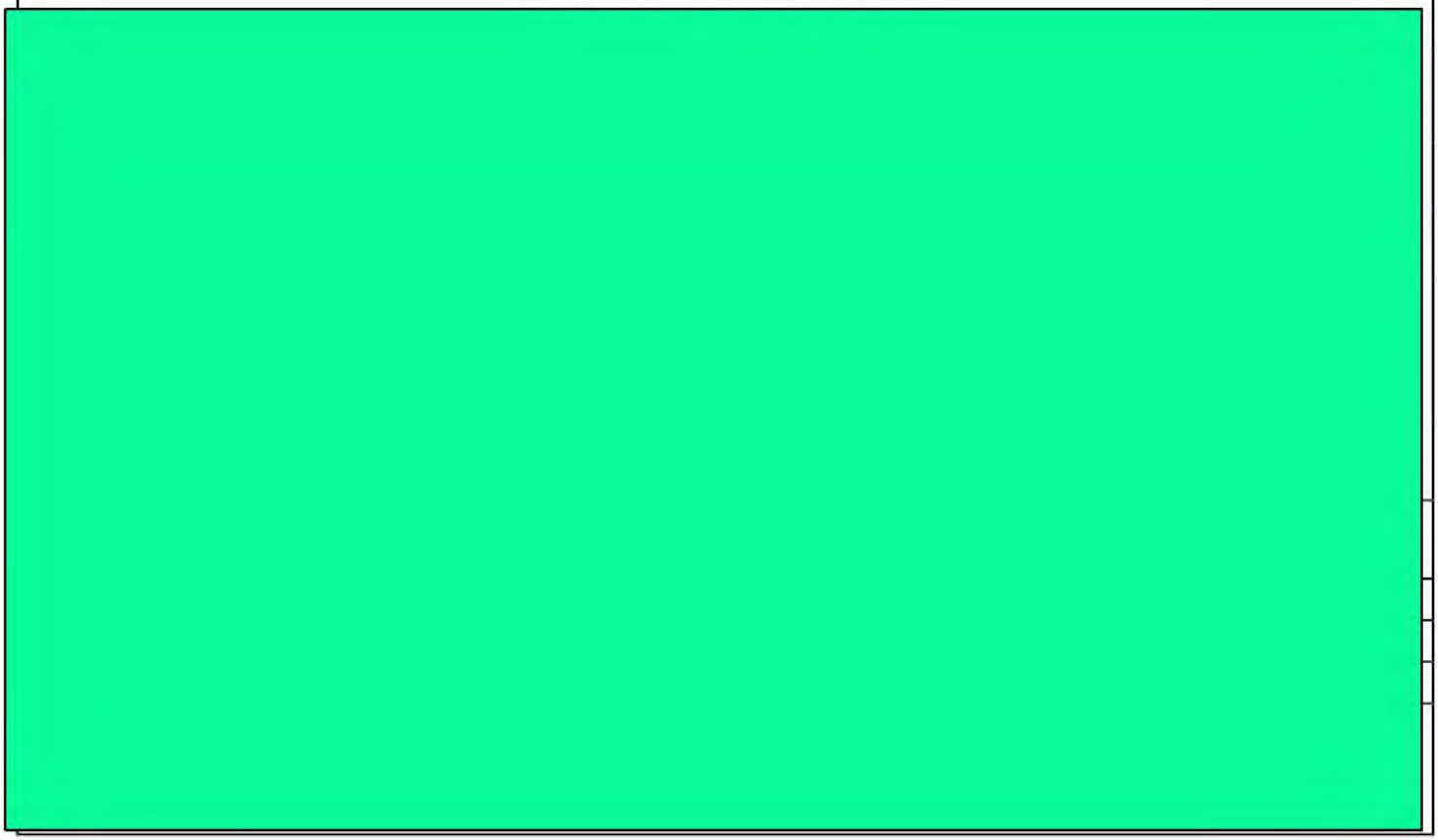

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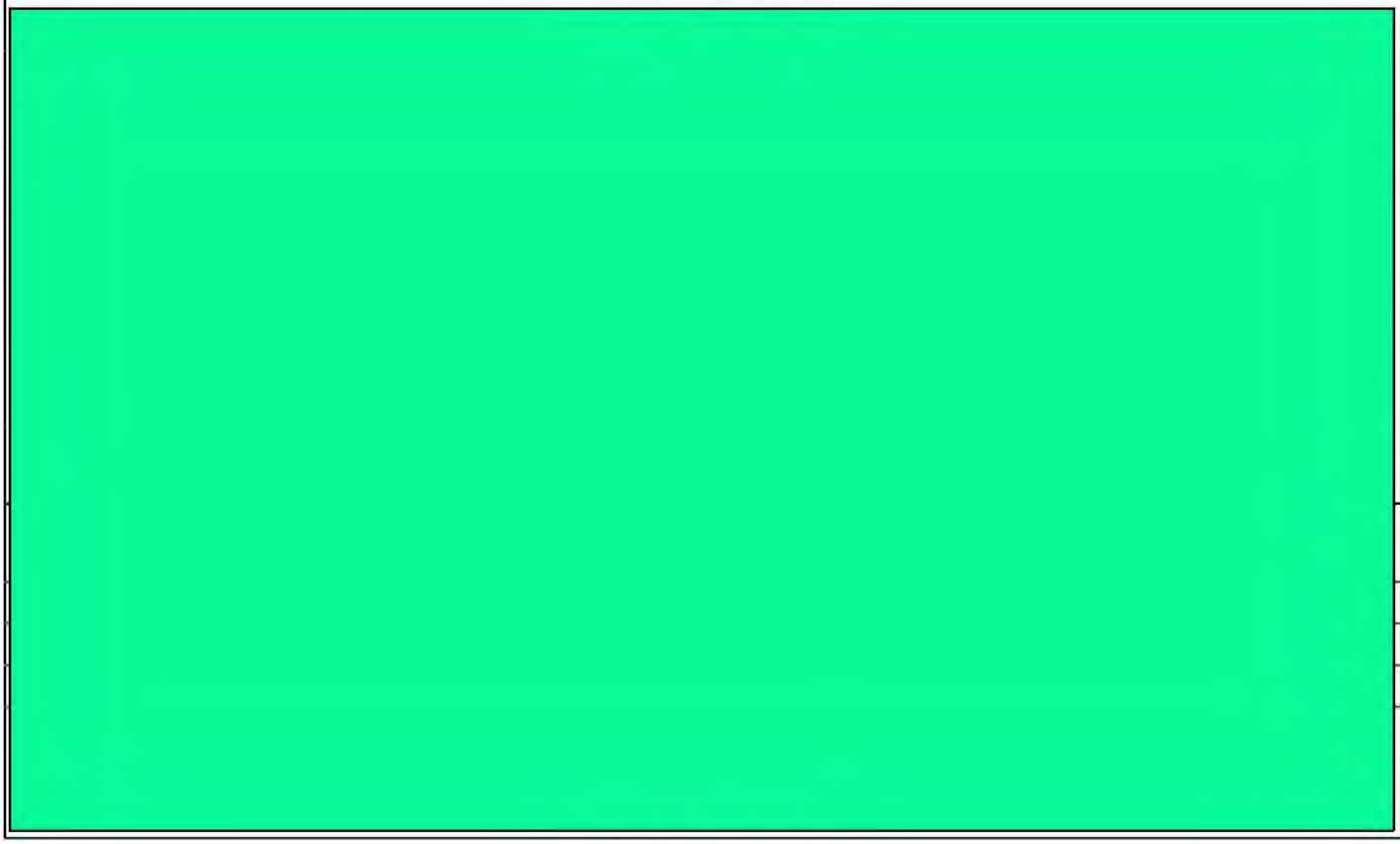
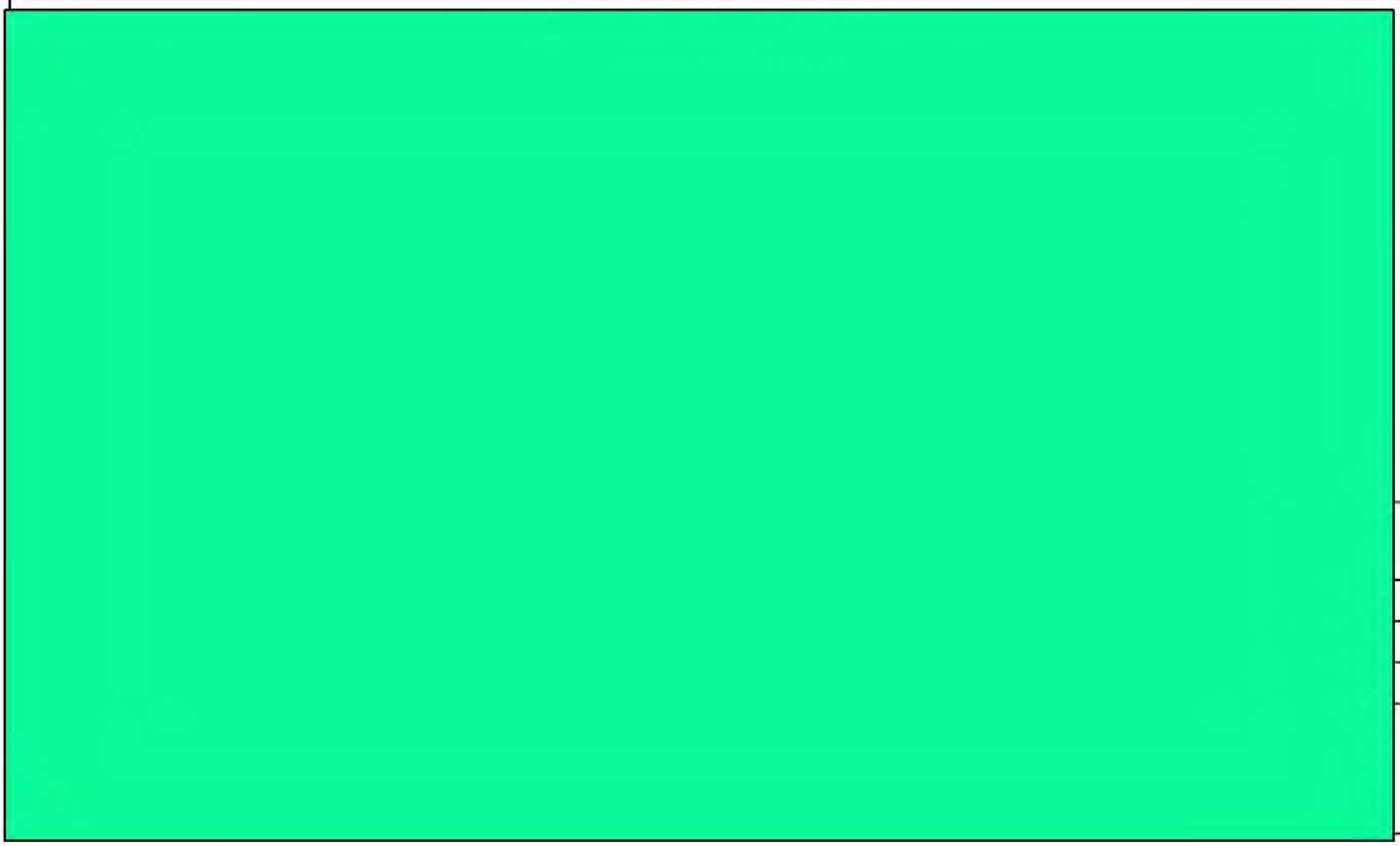


PROFILE - Senior/Key Person



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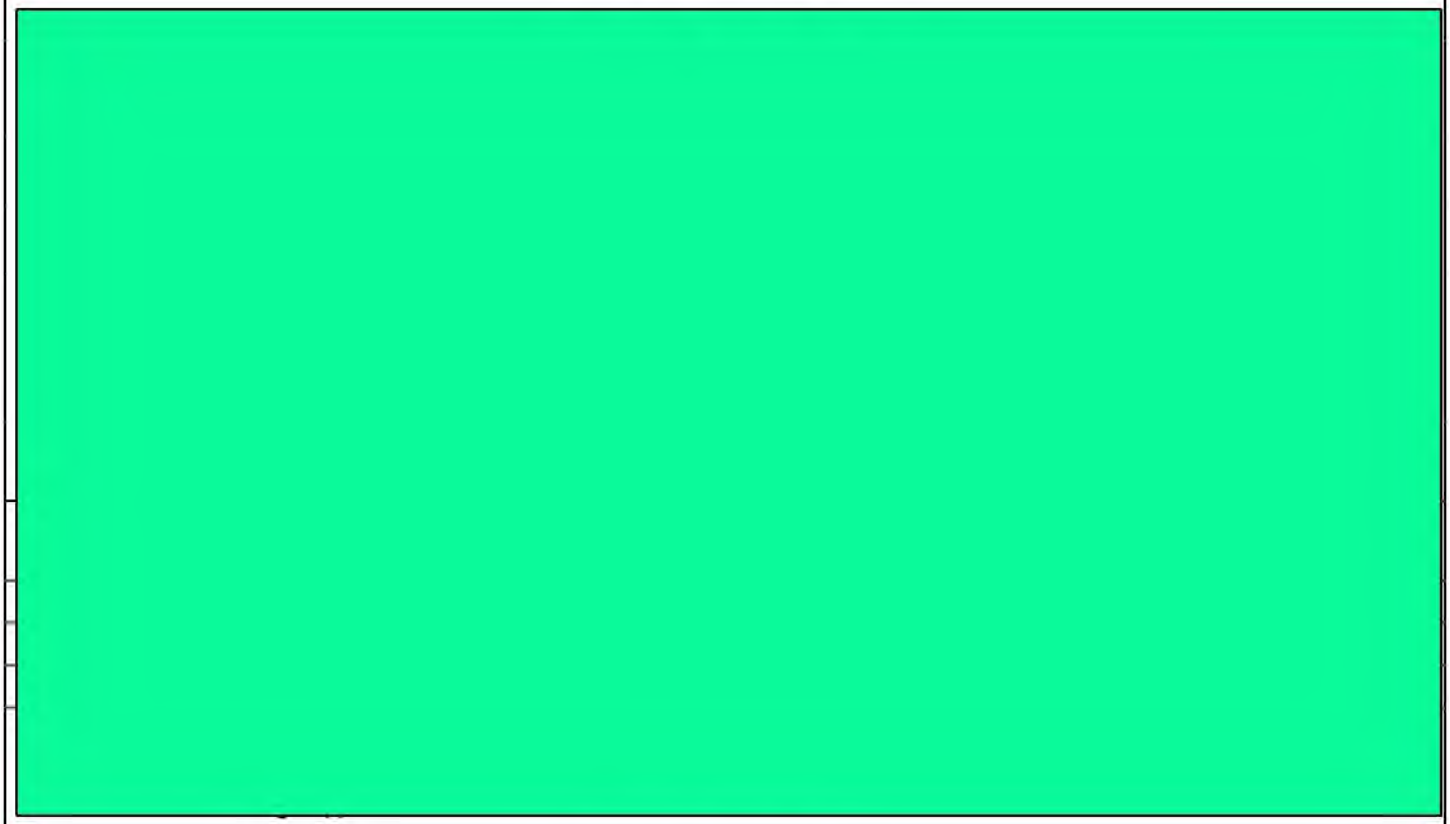

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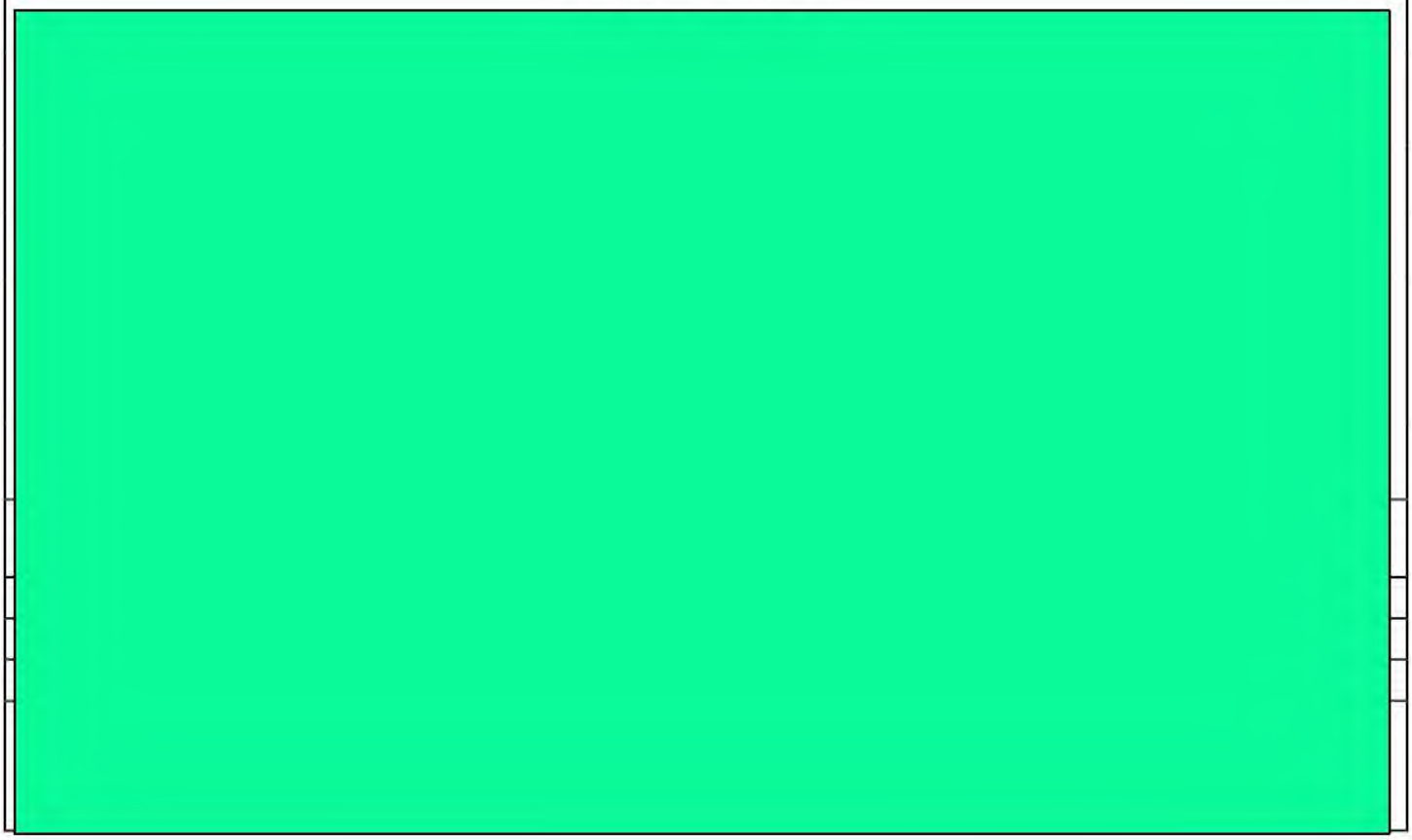

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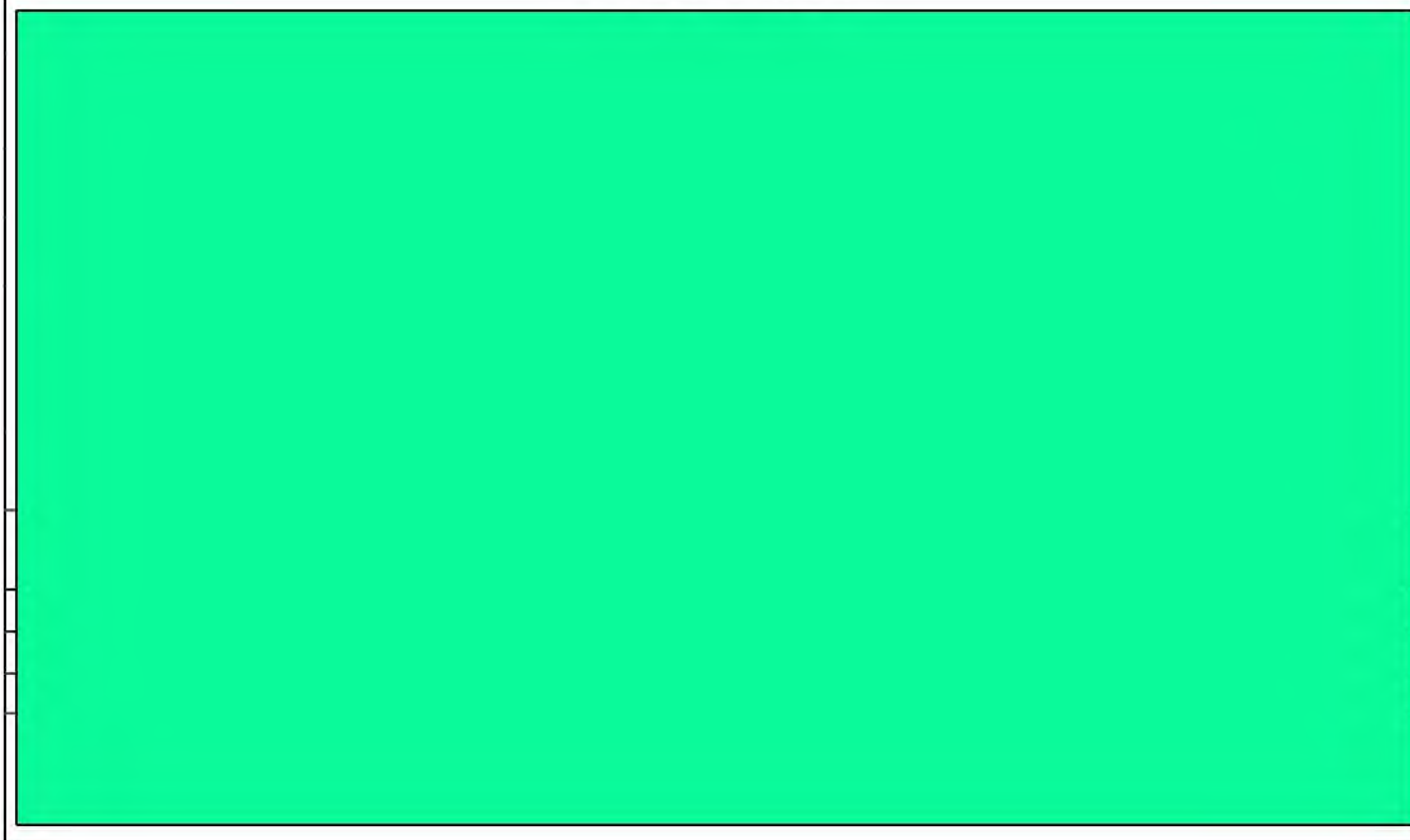
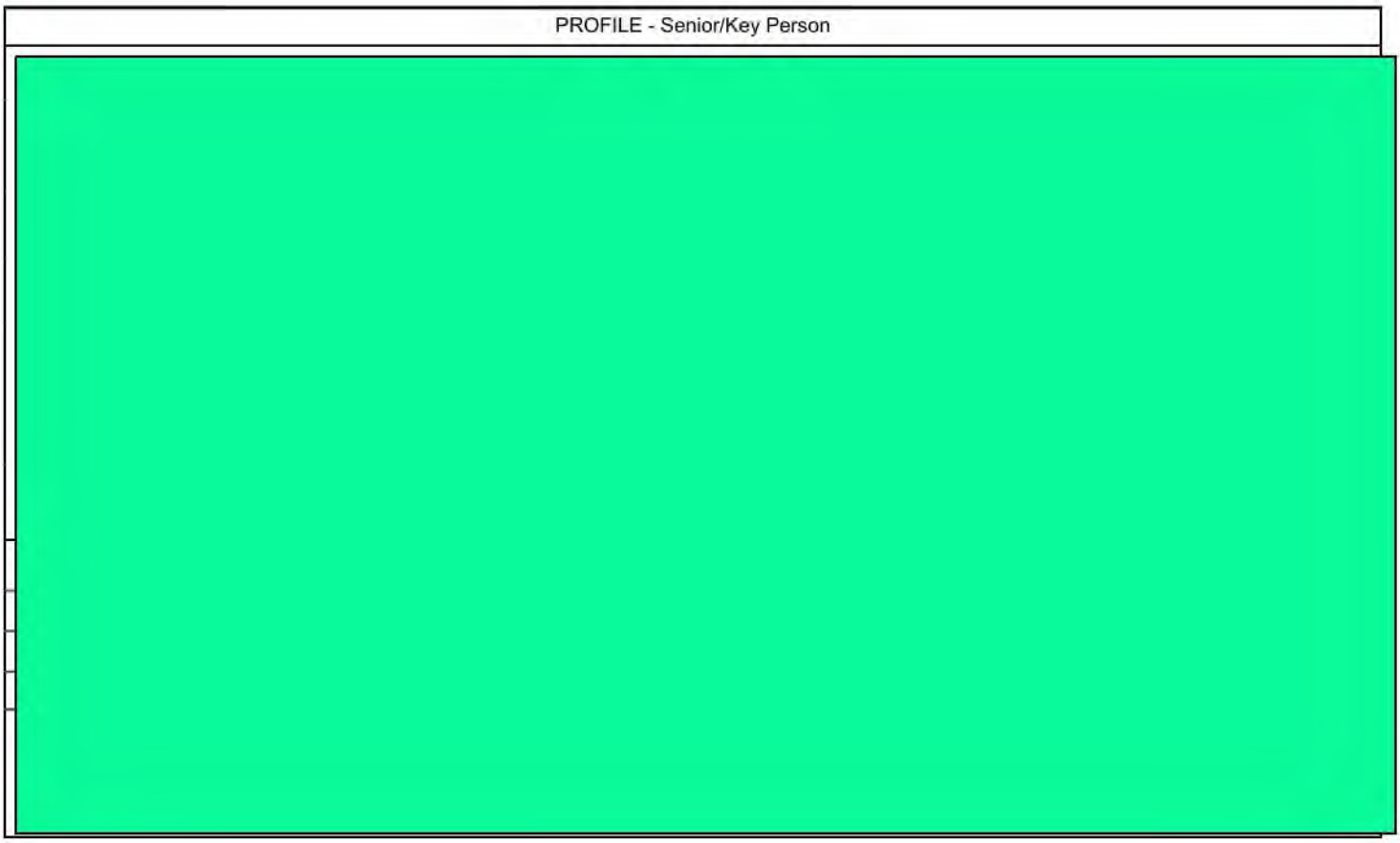


PROFILE - Senior/Key Person



OMB No. 0925-0001/0002 (Rev. 08/12 Approved Through 8/31/2015)

BIOGRAPHICAL SKETCH

Provide the following information for the Senior/key personnel and other significant contributors.

Follow this format for each person. DO NOT EXCEED FIVE PAGES.

NAME: Daniels, Anthony Brent

eRA COMMONS USER NAME (agency login): [redacted]

POSITION TITLE: Assistant Professor of Ophthalmology & Visual Sciences, Cancer Biology, Radiation Oncology

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, include postdoctoral training and residency training if applicable.)*

INSTITUTION AND LOCATION	DEGREE (if applicable)	Completion Date MM/YYYY	FIELD OF STUDY
Princeton University, Princeton, NJ	AB	06/2002	Molecular Biology (summa cum laude)
London School of Economics and Political Science, London	MSc	11/2003	Health, Population & Society (Honors)
University of Pennsylvania School of Medicine, Philadelphia, PA	MD	05/2007	Medicine
Memorial Sloan Kettering Cancer Center, New York, NY		06/2008	Transitional Year Internship
Harvard Medical School / Massachusetts Eye and Ear Infirmary, Boston, MA		06/2011	Ophthalmology Residency
Harvard Medical School / Massachusetts Eye and Ear Infirmary, Boston, MA		07/2013	Vitreoretinal Surgery Fellowship

A. Personal Statement

I am a physician-scientist and Director of Ocular Oncology at Vanderbilt. Since completing my fellowship training in 2013, my clinical practice specializes in the treatment of children with retinoblastoma using intra-arterial and intravitreal chemotherapy techniques, and the treatment of adults with other ocular malignancies. My research focuses on using novel animal models to develop treatments with reduced ocular toxicity. Specifically, we have developed a tumor model of retinoblastoma that develops intra-retinal tumors and diffuse vireous seeds similar to those seen in humans with advanced intraocular retinoblastoma. In clinical practice, we have demonstrated that intra-arterial chemotherapy achieves rates of globe salvage much higher than that achieved with traditional systemic chemotherapy. However, we also found significant rates of local adverse events, often related to the retinal and retinal vascular toxicity of currently available agents. Our rabbit model allows for intra-arterial chemotherapy delivery with reproducible pharmacokinetics to facilitate preclinical studies of toxicity and efficacy. Our goal is to develop novel compounds that target the retinoblastoma molecular pathways for translation into clinical practice, and we will use these models to evaluate these drugs. The hope is that these new drugs will become the future of retinoblastoma patient care.

We are also developing an orthotopic patient-derived xenograft murine model of uveal melanoma that forms liver metastases in order to study micrometastatic tumor dormancy and the role of the liver microenvironment. Immune surveillance appears to be a crucial mediator of micrometastatic dormancy in uveal melanoma. Some of my prior work has focused on cellular signaling pathways that mediate immune responses such as serotonin secretion and phagocytosis. We were able to identify the exact branch points in the signaling networks that could be targeted with protein tyrosine kinase inhibitors to abrogate cellular function. We discovered areas of signaling redundancy, above which function could not be abrogated using single selective inhibitors. In addition, I have previously studied the genetics of primary uveal melanoma and mutational drivers of uveal melanoma oncogenesis. Using massively parallel high-throughput analysis, we demonstrated that none of the oncogenic mutations commonly found in other human cancers are present in uveal melanoma. This is important because it means that currently available targeted inhibitors, developed for other human

cancers, are unlikely to be useful for uveal melanoma. We also showed the prevalence of specific mutations in GNAQ and GNA11 in uveal melanoma and demonstrated their mutual exclusivity in primary tumors.

The common thread among these research endeavors has been the use of targeted inhibitors of cellular signaling pathways in cancer biology. It is important to understand the tumor-specific mechanisms of oncogenesis and the conditions under which targeted inhibition of oncogenes might succeed. A previous paper of mine spelled out the molecular rationale and the framework in which clinical trials of protein tyrosine kinase inhibitors would likely succeed, using c-KIT as a case study to identify potential pitfalls. My ultimate translational goal is to discover new targeted inhibitors for use in retinoblastoma and uveal melanoma and to help bring these into clinical practice.

1. Abramson DH, **Daniels AB (co-first author and corresponding author)**, Marr BP, Francis JH, Brodie SE, Dunkel IJ, Gobin Y. Intra-arterial Chemotherapy (Ophthalmic Artery Chemosurgery) for Group D Retinoblastoma. PloS One. 2016;11(1):e0146582. doi: 10.1371/journal.pone.0146582. eCollection 2016. PubMed PMID: 26756643; PubMed Central PMCID: PMC4710506.
2. **Daniels AB**, Lee JE, MacConaill LE, Palescandolo E, Van Hummelen P, Adams SM, DeAngelis MM, Hahn WC, Gragoudas ES, Harbour JW, Garraway LA, Kim IK. High throughput mass spectrometry-based mutation profiling of primary uveal melanoma. Invest Ophthalmol Vis Sci. 2012 Oct 9;53(11):6991-6. PubMed PMID: 22977135; PubMed Central PMCID: PMC3471553.
3. **Daniels AB**, Worth RG, Dickstein RJ, Dickstein JS, Kim-Han TH, Kim MK, Schreiber AD. Analysis of FcgammaRIIA cytoplasmic tail requirements in signaling for serotonin secretion: evidence for an ITAM-dependent, PI3K-dependent pathway. Scand J Immunol. 2010 Apr;71(4):232-9. PubMed PMID: 20384866; PubMed Central PMCID: PMC2855141.
4. **Daniels AB**, Abramson DH. c-KIT in uveal melanoma: big fish or red herring?. Arch Ophthalmol. 2009 May;127(5):695-7. PubMed PMID: 19433723.

B. Positions and Honors

Positions and Employment

2007 - 2008	Transitional Year Intern, Memorial Sloan Kettering Cancer Center, Department of Medicine, New York, NY
2008 - 2013	Assistant in Ophthalmology, Active Staff, Massachusetts Eye and Ear Infirmary, Department of Ophthalmology, Boston, MA
2008 - 2013	Clinical Fellow in Ophthalmology, Harvard Medical School, Department of Ophthalmology, Boston, MA
2013 -	Attending Surgeon, Vanderbilt Eye Institute, Retina Division, Nashville, TN
2013 -	Assistant Professor of Ophthalmology and Visual Sciences, Vanderbilt University School of Medicine, Nashville, TN
2014 -	Director of Ocular Oncology, Vanderbilt Eye Institute, Vanderbilt University, Nashville, TN
2014 -	Member, Vanderbilt-Ingram Cancer Center, Host-Tumor Interactions Program, Nashville, TN
2014 -	Assistant Professor of Cancer Biology, Vanderbilt University School of Medicine, Nashville, TN
2015 -	Assistant Professor of Radiation Oncology, Vanderbilt University School of Medicine, Nashville, TN

Other Experience and Professional Memberships

2006 -	Member, Association for Research in Vision and Ophthalmology
2008 -	Member, American Academy of Ophthalmology
2008 - 2013	Member, Massachusetts Medical Society
2012 -	Guest Editor, Special Issue "Advances in Ocular Imaging", Seminars in Ophthalmology
2013 -	Member, International Society of Ocular Oncology
2013 -	Member, Nashville Academy of Ophthalmology
2013 -	Member, Tennessee Academy of Ophthalmology
2015 -	Guest Editor, Special Issue "Disparities and Eye Health and Disease", Semin in Ophthalmology

Honors

2002	Graduated Summa Cum Laude, Princeton University
2002	Thesis Prize, given to "most scholarly and significant" senior thesis, Princeton University
2002	Graduate Merit Scholarship, London School of Economics and Political Science
2003	Gamble Scholarship (University of Pennsylvania School of Medicine's highest 4-year full scholarship), University of Pennsylvania School of Medicine
2006	Jeffrey W. Berger Medical Student Research in Ophthalmology Award, University of Pennsylvania School of Medicine
2007	Max Kade Fellowship, American-Austrian Foundation
2007	Research in Epidemiology Award, University of Pennsylvania Center for Clinical Epidemiology and Biostatistics
2010	"Best Clinical Research Abstract" at Annual Meeting, Harvard Medical School Department of Ophthalmology
2010	Selected to attend the Residents Retreat, Heed Ophthalmic Foundation
2012	Gragoudas Prize for best paper in Retina by a trainee, Harvard Medical School Department of Ophthalmology
2012	Raymond R. Margherio Award, for best research by a fellow, Retina Society
2012	Ronald G. Michels Fellowship Foundation Award, given to the top retina surgery fellow nationally, Ronald G. Michels Foundation

C. Contribution to Science

1. **Genetics of Uveal Melanoma and Implications for Targeted Therapies**
Metastases develop in 50% of patients with uveal melanoma, and are rapidly and uniformly fatal. Given the paucity of effective treatments for metastatic uveal melanoma, we performed high-throughput mass spectrometry-based profiling of >100 primary uveal melanomas to determine the presence of >1000 activating mutations that might render these tumors susceptible to targeted therapeutics or protein tyrosine kinase inhibitors. We determined the prevalence of GNAQ and GNA11 mutations in human uveal melanoma. We also demonstrated that human uveal melanoma has a unique pattern of oncogenic mutations distinct from other human cancers. Prior to our use of a high-throughput screening system to identify mutations, we performed genetic analysis of primary human uveal melanomas to determine the status of the c-KIT and EGFR genes. At the time, there was in vitro evidence from cultured cell lines that mutated c-KIT may play a role in melanomgenesis. We demonstrated that there is neither mutation nor gene amplification of either gene locus in primary human tumors. This led to several platform presentations at various conferences at which I presented these findings. I also authored a paper that spelled out the molecular rationale and the framework in which clinical trials of protein tyrosine kinase inhibitors would likely succeed in the treatment of cancer, using c-KIT as a case study to identify potential pitfalls, as well as articles on patient and tumor disparities in uveal melanoma prognosis.

   a. **Daniels AB**, Lee JE, MacConaill LE, Palescandolo E, Van Hummelen P, Adams SM, DeAngelis MM, Hahn WC, Gragoudas ES, Harbour JW, Garraway LA, Kim IK. High throughput mass spectrometry-based mutation profiling of primary uveal melanoma. Invest Ophthalmol Vis Sci. 2012 Oct 9;53(11):6991-6. PubMed PMID: 22977135; PubMed Central PMCID: PMC3471553.
   b. **Daniels AB**, Abramson DH. c-KIT in uveal melanoma: big fish or red herring?. Arch Ophthalmol. 2009 May;127(5):695-7. PubMed PMID: 19433723.
   c. Nichols EE, Richmond A, **Daniels AB**. Disparities in Uveal Melanoma: Patient Characteristics. Seminars in Ophthalmology. 2016 January; 31(1).
   d. Nichols EE, Richmond A, **Daniels AB**. Tumor Characteristics, Genetics, Management, and the Risk of Metastasis in Uveal Melanoma. Seminars in Ophthalmology. 2016 January; 31(1).

2. **Efficacy and Adverse Consequences of Intra-arterial Chemotherapy for Intraocular Retinoblastoma**
While systemic chemotherapy has been a cornerstone for conservative management of intraocular retinoblastoma for the past two decades, it is associated with significant systemic toxicity and fails to cure the majority of eyes with advanced disease. Recently, intra-arterial chemotherapy has been introduced at a

handful of institutions across the United States. We have shown that rates of globe salvage in advanced (International Classification of Retinoblastoma Group D) eyes are dramatically higher with the intra-arterial technique than all published success rates using systemic chemotherapy. In addition, we demonstrate that intra-arterial chemotherapy is able to save eyes that have previously failed treatment with multiple other treatment modalities. However, we also found that all current intra-arterial regimens, which predominant rely on melphalan-based chemotherapy combinations, are associated with significant rates of local toxicity to the ocular structures, which manifest as local/regional adverse events. This study, serves as the largest analysis of its kind of the success and toxicities of intra-arterial chemotherapy for advanced (group D) retinoblastoma. A second publication reviews treatment options available around the world, and the role that access and treatment have on prognosis disparities worldwide.

a. Abramson DH, **Daniels AB (co-first author and corresponding author)**, Marr BP, Francis JH, Brodie SE, Dunkel IJ, Gobin Y. Intra-arterial Chemotherapy (Ophthalmic Artery Chemosurgery) for Group D Retinoblastoma. PloS One. 2016;11(1):e0146582. doi: 10.1371/journal.pone.0146582. eCollection 2016. PubMed PMID: 26756643; PubMed Central PMCID: PMC4710506.

b. Singh G, **Daniels AB**. Disparities in Retinoblastoma Presentation, Treatment and Outcomes in Developed and Less-developed Countries. Seminars in ophthalmology. 2016 January; 31(1).

3. **Risk Factors and Quality of Life Measures in Neuro-Ophthalmic Conditions**
In the late 2000s, there was little quantitative evidence for the risk factors for various neuro-ophthalmic conditions and how these conditions affected patients' quality of life. I performed a large case-control study of patients with idiopathic intracranial hypertension (IIH). We demonstrated a dose dependent risk associated with obesity or recent weight gain. We were the first to demonstrate that tetracycline use is a statistically significant risk factor for IIH and that patients with IIH experience significant reductions in vision-specific and overall health-related quality of life. Separately, we examined the relationship between optic neuritis and multiple sclerosis in pediatric patients. We found that pediatric patients with idiopathic optic neuritis who have brain lesions on MRI are at significant risk for developing MS, while those without MRI lesions remain at low risk. Also, in patients with MS, we found that reduction in OCT-measured retinal nerve fiber layer thickness was predictive of decreased vision-specific and non-vision-related quality of life.

a. **Daniels AB**, Liu GT, Volpe NJ, Galetta SL, Moster ML, Newman NJ, Biousse V, Lee AG, Wall M, Kardon R, Acierno MD, Corbett JJ, Maguire MG, Balcer LJ. Profiles of obesity, weight gain, and quality of life in idiopathic intracranial hypertension (pseudotumor cerebri). Am J Ophthalmol. 2007 Apr;143(4):635-41. PubMed PMID: 17386271.

b. Bonhomme GR, Waldman AT, Balcer LJ, **Daniels AB**, Tennekoon GI, Forman S, Galetta SL, Liu GT. Pediatric optic neuritis: brain MRI abnormalities and risk of multiple sclerosis. Neurology. 2009 Mar 10;72(10):881-5. PubMed PMID: 19273821.

c. Mowry EM, Loguidice MJ, **Daniels AB**, Jacobs DA, Markowitz CE, Galetta SL, Nano-Schiavi ML, Cutter GR, Maguire MG, Balcer LJ. Vision related quality of life in multiple sclerosis: correlation with new measures of low and high contrast letter acuity. J Neurol Neurosurg Psychiatry. 2009 Jul;80(7):767-72. PubMed PMID: 19240050.

4. **Determination of Genotype-Phenotype Correlations and Prognosis in Bardet-Biedl Syndrome**
Bardet-Biedl Syndrome (BBS) is a polygenic multi-organ ciliopathy that leads to blindness from a pigmentary retinopathy. Although a dozen *BBS* genes had been discovered, there was very little known about the phenotypes of each. In addition, it was known that prognosis was variable between individuals, but no genetic association with prognosis had yet been determined. As the *BBS* genes are very large genes, sequencing them in a routine clinical laboratory setting is not feasible. We demonstrated that the presence of a mutation in the most common gene, *BBS1*, had a significantly better prognosis and could be predicted by the 30-Hz flicker ERG amplitude at the time of presentation. We created receiver operator characteristic curves, which allowed for accurate prediction of the genotype, and therefore prognosis.

a. **Daniels AB**, Sandberg MA, Chen J, Weigel-DiFranco C, Fielding Hejtmancic J, Berson EL. Genotype-phenotype correlations in Bardet-Biedl syndrome. Arch Ophthalmol. 2012 Jul;130(7):901-7. PubMed PMID: 22410627.

5. **Novel Radiotherapy Techniques for Intraocular Malignancies**
We have been attempting to push the limits of the use of radiotherapy-based techniques (such as plaque brachytherapy, hyperfractionated external beam radiotherapy, and stereotactic radiosurgery) in non-traditional settings for the treatment of various intraocular malignant and benign tumors.

   a. Taubenslag KJ, Kim SJ, Attia A, Abel TW, Nichols HH, Ancell KK, **Daniels AB**. Retinal metastasis from unknown primary: diagnosis, management, and clinicopathologic correlation. Digital Journal of Ophthalmology. 2015 October 05; 21(4).

   b. [redacted]

A complete list of all publications (24 total) can be found at My Bibliography: http://www.ncbi.nlm.nih.gov/sites/myncbi/anthony.daniels.1/bibliography/47916528/public/?sort=date&direction=ascending.

D. Research Support

Ongoing Research Support

[redacted] Daniels, Anthony (PI) Role: PI
05/15/15-05/14/18
"Role of the Liver Microenvironment in Uveal Melanoma Micrometastatic Tumor Dormancy"
The goal of this project is to evaluate the role of the liver microenvironment in maintaining micrometastatic dormancy in this orthotopic patient-derived xenograft model of uveal melanoma.

[redacted] Daniels, Anthony (PI) Role: PI
07/01/15-06/30/16
"Novel Model of Multi-modality Treatment of Retinoblastoma"
The goal of this project is to create a rabbit xenograft model of retinoblastoma and to develop the techniques for direct intra-ophthalmic artery endovascular drug delivery.

Pending Research Support

[redacted]

Completed Research Support

[redacted] Daniels, Anthony (PI) Role: PI
07/01/11-06/01/12
"Isolation and Characterization of Uveal Melanoma Cells"
The goal of this project was to isolate uveal melanoma cells and characterize genetic differences in various stages of metastasis progression.

[redacted] Daniels, Anthony (PI) Role: PI
Medical Student Research Fellowship
06/01/04-08/01/04
"FcgRIIA-mediated Serotonin Secretion"
The goal of this project was to dissect out the signaling requirements for FcgRIIA-mediated serotonin secretion, and how they differ from FcgRIIA-mediated phagocytosis. This identified the exact points in the signaling cascade that could be targeted with protein tyrosine kinase inhibitors to abrogate cellular function.

Page 029 of 138 to Page 078 of 138

Personnel

OMB Number: 4040-0001
Expiration Date: 06/30/2016

RESEARCH & RELATED BUDGET - SECTION A & B, Budget Period 1

ORGANIZATIONAL DUNS*: 004413456

Budget Type*: ● Project ○ Subaward/Consortium

Enter name of Organization: Vanderbilt University Medical Center

Start Date*: 09-01-2016 **End Date*:** 08-31-2017 **Budget Period: 1**

A. Senior/Key Person

	Prefix	First Name*	Middle Name	Last Name*	Suffix	Project Role*	Base Salary ($)	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits ($)*	Funds Requested ($)*
1.		Anthony	B	Daniels		PD/PI			0	0	138,825.00	13,882.50	152,707.50

Total Funds Requested for all Senior Key Persons in the attached file

Additional Senior Key Persons: File Name: **Total Senior/Key Person** **152,707.50**

B. Other Personnel

Number of Personnel*	Project Role*	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits*	Funds Requested ($)*
0	Post Doctoral Associates	0	0	0	0.00	0.00	0.00
0	Graduate Students	0	0	0	0.00	0.00	0.00
0	Undergraduate Students	0	0	0	0.00	0.00	0.00
0	Secretarial/Clerical	0	0	0	0.00	0.00	0.00
0	Other	0	0	0	0.00	0.00	0.00
0	Other Professionals	0	0	0	0.00	0.00	0.00
0	Allocated Admin Support	0	0	0	0.00	0.00	0.00
0	**Total Number Other Personnel**					**Total Other Personnel**	**0.00**

Total Salary, Wages and Fringe Benefits (A+B) **152,707.50**

RESEARCH & RELATED Budget {A-B} (Funds Requested)

Funding Opportunity Number: PA-14-046 . Received Date: 2016-02-12T17:40:51.000-05:00

RESEARCH & RELATED BUDGET - SECTION C, D, & E, Budget Period 1

ORGANIZATIONAL DUNS*: 004413456
Budget Type*: ● Project ○ Subaward/Consortium
Organization: Vanderbilt University Medical Center

Start Date*: 09-01-2016 **End Date*:** 08-31-2017 **Budget Period: 1**

C. Equipment Description	
List items and dollar amount for each item exceeding $5,000	
Equipment Item	**Funds Requested ($)***
Total funds requested for all equipment listed in the attached file	
Total Equipment	

Additional Equipment: File Name:

D. Travel	**Funds Requested ($)***
1. Domestic Travel Costs (Incl. Canada, Mexico, and U.S. Possessions)	0.00
2. Foreign Travel Costs	0.00
Total Travel Cost	**0.00**

E. Participant/Trainee Support Costs		**Funds Requested ($)***
1. Tuition/Fees/Health Insurance		0.00
2. Stipends		0.00
3. Travel		0.00
4. Subsistence		0.00
5. Other: Other		0.00
0 Number of Participants/Trainees	**Total Participant Trainee Support Costs**	**0.00**

RESEARCH & RELATED Budget {C-E} (Funds Requested)

Tracking Number: GRANT12093630
Funding Opportunity Number: PA-14-046 . Received Date: 2016-02-12T17:40:51.000-05:00

RESEARCH & RELATED BUDGET - SECTIONS F-K, Budget Period 1

ORGANIZATIONAL DUNS*: 004413456
Budget Type*: ● Project ○ Subaward/Consortium
Organization: Vanderbilt University Medical Center

Start Date*: 09-01-2016 **End Date*:** 08-31-2017 **Budget Period:** 1

F. Other Direct Costs	Funds Requested ($)*
1. Materials and Supplies	0.00
2. Publication Costs	0.00
3. Consultant Services	0.00
4. ADP/Computer Services	0.00
5. Subawards/Consortium/Contractual Costs	0.00
6. Equipment or Facility Rental/User Fees	0.00
7. Alterations and Renovations	0.00
8 . Other Direct Costs	25,000.00
9 . All Other Costs	0.00
Total Other Direct Costs	**25,000.00**

G. Direct Costs	Funds Requested ($)*
Total Direct Costs (A thru F)	**177,707.50**

H. Indirect Costs

Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base ($)	Funds Requested ($)*
1 . MTDC, Research On/ Off campus-Remote	8	177,707.50	14,216.60
		Total Indirect Costs	**14,216.60**

Cognizant Federal Agency (Agency Name, POC Name, and POC Phone Number): Department of Health and Human Services , Steven Zuraf (301) 492-4855

I. Total Direct and Indirect Costs	Funds Requested ($)*
Total Direct and Indirect Institutional Costs (G + H)	**191,924.10**

J. Fee	Funds Requested ($)*
	0.00

K. Budget Justification* File Name: M-27_S2S_Budget_Justification.pdf (Only attach one file.)

RESEARCH & RELATED Budget {F-K} (Funds Requested)

OMB Number: 4040-0001
Expiration Date: 06/30/2016

RESEARCH & RELATED BUDGET - SECTION A & B, Budget Period 2

ORGANIZATIONAL DUNS*: 004413456

Budget Type*: ● Project ○ Subaward/Consortium

Enter name of Organization: Vanderbilt University Medical Center

Start Date*: 09-01-2017 **End Date*:** 08-31-2018 **Budget Period: 2**

A. Senior/Key Person

	Prefix	First Name*	Middle Name	Last Name*	Suffix	Project Role*	Base Salary ($)	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits ($)*	Funds Requested ($)*
1 .		Anthony	B	Daniels		PD/PI			0	0	138,825.00	13,882.50	152,707.50

Total Funds Requested for all Senior Key Persons in the attached file

Additional Senior Key Persons: File Name:

Total Senior/Key Person **152,707.50**

B. Other Personnel

Number of Personnel*	Project Role*	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits*	Funds Requested ($)*
0	Post Doctoral Associates	0	0	0	0.00	0.00	0.00
0	Graduate Students	0	0	0	0.00	0.00	0.00
0	Undergraduate Students	0	0	0	0.00	0.00	0.00
0	Secretarial/Clerical	0	0	0	0.00	0.00	0.00
0	Other	0	0	0	0.00	0.00	0.00
0	Other Professionals	0	0	0	0.00	0.00	0.00
0	Allocated Admin Support	0	0	0	0.00	0.00	0.00
0	**Total Number Other Personnel**					**Total Other Personnel**	**0.00**

Total Salary, Wages and Fringe Benefits (A+B) **152,707.50**

RESEARCH & RELATED Budget {A-B} (Funds Requested)

Funding Opportunity Number: PA-14-046 . Received Date: 2016-02-12T17:40:51.000-05:00

RESEARCH & RELATED BUDGET - SECTION C, D, & E, Budget Period 2

ORGANIZATIONAL DUNS*: 004413456
Budget Type*: ● Project ○ Subaward/Consortium
Organization: Vanderbilt University Medical Center

Start Date*: 09-01-2017 **End Date*:** 08-31-2018 **Budget Period:** 2

C. Equipment Description	
List items and dollar amount for each item exceeding $5,000	
Equipment Item	**Funds Requested ($)***
Total funds requested for all equipment listed in the attached file	
Total Equipment	
Additional Equipment: File Name:	

D. Travel	Funds Requested ($)*
1. Domestic Travel Costs (Incl. Canada, Mexico, and U.S. Possessions)	0.00
2. Foreign Travel Costs	0.00
Total Travel Cost	**0.00**

E. Participant/Trainee Support Costs		Funds Requested ($)*
1. Tuition/Fees/Health Insurance		0.00
2. Stipends		0.00
3. Travel		0.00
4. Subsistence		0.00
5. Other: Other		0.00
0 Number of Participants/Trainees	**Total Participant Trainee Support Costs**	**0.00**

RESEARCH & RELATED Budget {C-E} (Funds Requested)

RESEARCH & RELATED BUDGET - SECTIONS F-K, Budget Period 2

ORGANIZATIONAL DUNS*: 004413456
Budget Type*: ● Project ○ Subaward/Consortium
Organization: Vanderbilt University Medical Center

Start Date*: 09-01-2017 **End Date*:** 08-31-2018 **Budget Period:** 2

F. Other Direct Costs	Funds Requested ($)*
1. Materials and Supplies	0.00
2. Publication Costs	0.00
3. Consultant Services	0.00
4. ADP/Computer Services	0.00
5. Subawards/Consortium/Contractual Costs	0.00
6. Equipment or Facility Rental/User Fees	0.00
7. Alterations and Renovations	0.00
8 . Other Direct Costs	25,000.00
9 . All Other Costs	0.00
Total Other Direct Costs	**25,000.00**

G. Direct Costs	Funds Requested ($)*
Total Direct Costs (A thru F)	**177,707.50**

H. Indirect Costs

Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base ($)	Funds Requested ($)*
1 . MTDC, Research On/ Off campus-Remote	8	177,707.50	14,216.60
		Total Indirect Costs	**14,216.60**

Cognizant Federal Agency (Agency Name, POC Name, and POC Phone Number): Department of Health and Human Services , Steven Zuraf (301) 492-4855

I. Total Direct and Indirect Costs	Funds Requested ($)*
Total Direct and Indirect Institutional Costs (G + H)	**191,924.10**

J. Fee	Funds Requested ($)*
	0.00

K. Budget Justification* File Name: M-27_S2S_Budget_Justification.pdf (Only attach one file.)

RESEARCH & RELATED Budget {F-K} (Funds Requested)

Tracking Number: GRANT12093630
Funding Opportunity Number: PA-14-046 . Received Date: 2016-02-12T17:40:51.000-05:00

OMB Number: 4040-0001
Expiration Date: 06/30/2016

RESEARCH & RELATED BUDGET - SECTION A & B, Budget Period 3

ORGANIZATIONAL DUNS*: 004413456

Budget Type*: ● Project ○ Subaward/Consortium

Enter name of Organization: Vanderbilt University Medical Center

Start Date*: 09-01-2018 **End Date*:** 08-31-2019 **Budget Period: 3**

A. Senior/Key Person

	Prefix	First Name*	Middle Name	Last Name*	Suffix	Project Role*	Base Salary ($)	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits ($)*	Funds Requested ($)*
1 .		Anthony	B	Daniels		PD/PI			0	0	138,825.00	13,882.50	152,707.50

Total Funds Requested for all Senior Key Persons in the attached file

Additional Senior Key Persons: File Name: **Total Senior/Key Person** **152,707.50**

B. Other Personnel

Number of Personnel*	Project Role*	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits*	Funds Requested ($)*
0	Post Doctoral Associates	0	0	0	0.00	0.00	0.00
0	Graduate Students	0	0	0	0.00	0.00	0.00
0	Undergraduate Students	0	0	0	0.00	0.00	0.00
0	Secretarial/Clerical	0	0	0	0.00	0.00	0.00
0	Other	0	0	0	0.00	0.00	0.00
0	Other Professionals	0	0	0	0.00	0.00	0.00
0	Allocated Admin Support	0	0	0	0.00	0.00	0.00
0	**Total Number Other Personnel**					**Total Other Personnel**	**0.00**
						Total Salary, Wages and Fringe Benefits (A+B)	**152,707.50**

RESEARCH & RELATED Budget {A-B} (Funds Requested)

Funding Opportunity Number: PA-14-046 . Received Date: 2016-02-12T17:40:51.000-05:00

RESEARCH & RELATED BUDGET - SECTION C, D, & E, Budget Period 3

ORGANIZATIONAL DUNS*: 004413456
Budget Type*: ● Project ○ Subaward/Consortium
Organization: Vanderbilt University Medical Center

Start Date*: 09-01-2018 **End Date*:** 08-31-2019 **Budget Period: 3**

C. Equipment Description	
List items and dollar amount for each item exceeding $5,000	
Equipment Item	**Funds Requested ($)***
Total funds requested for all equipment listed in the attached file	
Total Equipment	
Additional Equipment: File Name:	

D. Travel	**Funds Requested ($)***
1. Domestic Travel Costs (Incl. Canada, Mexico, and U.S. Possessions)	0.00
2. Foreign Travel Costs	0.00
Total Travel Cost	**0.00**

E. Participant/Trainee Support Costs		**Funds Requested ($)***
1. Tuition/Fees/Health Insurance		0.00
2. Stipends		0.00
3. Travel		0.00
4. Subsistence		0.00
5. Other: Other		0.00
0 Number of Participants/Trainees	**Total Participant Trainee Support Costs**	**0.00**

RESEARCH & RELATED Budget {C-E} (Funds Requested)

Tracking Number: GRANT12093630
Funding Opportunity Number: PA-14-046 . Received Date: 2016-02-12T17:40:51.000-05:00

RESEARCH & RELATED BUDGET - SECTIONS F-K, Budget Period 3

ORGANIZATIONAL DUNS*: 004413456
Budget Type*: ● Project ○ Subaward/Consortium
Organization: Vanderbilt University Medical Center

Start Date*: 09-01-2018 **End Date*:** 08-31-2019 **Budget Period:** 3

F. Other Direct Costs	Funds Requested ($)*
1. Materials and Supplies	0.00
2. Publication Costs	0.00
3. Consultant Services	0.00
4. ADP/Computer Services	0.00
5. Subawards/Consortium/Contractual Costs	0.00
6. Equipment or Facility Rental/User Fees	0.00
7. Alterations and Renovations	0.00
8 . Other Direct Costs	25,000.00
9 . All Other Costs	0.00
Total Other Direct Costs	**25,000.00**

G. Direct Costs	Funds Requested ($)*
Total Direct Costs (A thru F)	**177,707.50**

H. Indirect Costs

Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base ($)	Funds Requested ($)*
1 . MTDC, Research On/ Off campus-Remote	8	177,707.50	14,216.60
		Total Indirect Costs	**14,216.60**

Cognizant Federal Agency (Agency Name, POC Name, and POC Phone Number)	Department of Health and Human Services , Steven Zuraf (301) 492-4855

I. Total Direct and Indirect Costs	Funds Requested ($)*
Total Direct and Indirect Institutional Costs (G + H)	**191,924.10**

J. Fee	Funds Requested ($)*
	0.00

K. Budget Justification*	File Name: M-27_S2S_Budget_Justification.pdf (Only attach one file.)

RESEARCH & RELATED Budget {F-K} (Funds Requested)

OMB Number: 4040-0001
Expiration Date: 06/30/2016

RESEARCH & RELATED BUDGET - SECTION A & B, Budget Period 4

ORGANIZATIONAL DUNS*: 004413456

Budget Type*: ● Project ○ Subaward/Consortium

Enter name of Organization: Vanderbilt University Medical Center

Start Date*: 09-01-2019 **End Date*:** 08-31-2020 **Budget Period: 4**

A. Senior/Key Person

Prefix	First Name*	Middle Name	Last Name*	Suffix	Project Role*	Base Salary ($)	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits ($)*	Funds Requested ($)*
1.	Anthony	B	Daniels		PD/PI			0	0	138,825.00	13,882.50	152,707.50

Total Funds Requested for all Senior Key Persons in the attached file

Additional Senior Key Persons: File Name:

Total Senior/Key Person **152,707.50**

B. Other Personnel

Number of Personnel*	Project Role*	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits*	Funds Requested ($)*
0	Post Doctoral Associates	0	0	0	0.00	0.00	0.00
0	Graduate Students	0	0	0	0.00	0.00	0.00
0	Undergraduate Students	0	0	0	0.00	0.00	0.00
0	Secretarial/Clerical	0	0	0	0.00	0.00	0.00
0	Other	0	0	0	0.00	0.00	0.00
0	Other Professionals	0	0	0	0.00	0.00	0.00
0	Allocated Admin Support	0	0	0	0.00	0.00	0.00
0	**Total Number Other Personnel**					**Total Other Personnel**	**0.00**

Total Salary, Wages and Fringe Benefits (A+B) **152,707.50**

RESEARCH & RELATED Budget {A-B} (Funds Requested)

Funding Opportunity Number: PA-14-046 . Received Date: 2016-02-12T17:40:51.000-05:00

RESEARCH & RELATED BUDGET - SECTION C, D, & E, Budget Period 4

ORGANIZATIONAL DUNS*: 004413456
Budget Type*: ● Project ○ Subaward/Consortium
Organization: Vanderbilt University Medical Center

Start Date*: 09-01-2019 **End Date*:** 08-31-2020 **Budget Period:** 4

C. Equipment Description	
List items and dollar amount for each item exceeding $5,000	
Equipment Item	**Funds Requested ($)***
Total funds requested for all equipment listed in the attached file	
Total Equipment	

Additional Equipment: File Name:

D. Travel	Funds Requested ($)*
1. Domestic Travel Costs (Incl. Canada, Mexico, and U.S. Possessions)	0.00
2. Foreign Travel Costs	0.00
Total Travel Cost	**0.00**

E. Participant/Trainee Support Costs		Funds Requested ($)*
1. Tuition/Fees/Health Insurance		0.00
2. Stipends		0.00
3. Travel		0.00
4. Subsistence		0.00
5. Other: Other		0.00
0 Number of Participants/Trainees	**Total Participant Trainee Support Costs**	**0.00**

RESEARCH & RELATED Budget {C-E} (Funds Requested)

RESEARCH & RELATED BUDGET - SECTIONS F-K, Budget Period 4

ORGANIZATIONAL DUNS*: 004413456
Budget Type*: ● Project ○ Subaward/Consortium
Organization: Vanderbilt University Medical Center

Start Date*: 09-01-2019 **End Date*:** 08-31-2020 **Budget Period:** 4

F. Other Direct Costs	Funds Requested ($)*
1. Materials and Supplies	0.00
2. Publication Costs	0.00
3. Consultant Services	0.00
4. ADP/Computer Services	0.00
5. Subawards/Consortium/Contractual Costs	0.00
6. Equipment or Facility Rental/User Fees	0.00
7. Alterations and Renovations	0.00
8 . Other Direct Costs	25,000.00
9 . All Other Costs	0.00
Total Other Direct Costs	**25,000.00**

G. Direct Costs	Funds Requested ($)*
Total Direct Costs (A thru F)	**177,707.50**

H. Indirect Costs

Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base ($)	Funds Requested ($)*
1 . MTDC, Research On/ Off campus-Remote	8	177,707.50	14,216.60
		Total Indirect Costs	**14,216.60**

Cognizant Federal Agency (Agency Name, POC Name, and POC Phone Number)	Department of Health and Human Services , Steven Zuraf (301) 492-4855

I. Total Direct and Indirect Costs	Funds Requested ($)*
Total Direct and Indirect Institutional Costs (G + H)	**191,924.10**

J. Fee	Funds Requested ($)*
	0.00

K. Budget Justification*	File Name: M-27_S2S_Budget_Justification.pdf (Only attach one file.)

RESEARCH & RELATED Budget {F-K} (Funds Requested)

OMB Number: 4040-0001
Expiration Date: 06/30/2016

RESEARCH & RELATED BUDGET - SECTION A & B, Budget Period 5

ORGANIZATIONAL DUNS*: 004413456

Budget Type*: ● Project ○ Subaward/Consortium

Enter name of Organization: Vanderbilt University Medical Center

Start Date*: 09-01-2020 **End Date*:** 08-31-2021 **Budget Period: 5**

A. Senior/Key Person

	Prefix	First Name*	Middle Name	Last Name*	Suffix	Project Role*	Base Salary ($)	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits ($)*	Funds Requested ($)*
1 .		Anthony	B	Daniels		PD/PI			0	0	138,825.00	13,882.50	152,707.50

Total Funds Requested for all Senior Key Persons in the attached file

Additional Senior Key Persons: File Name:

Total Senior/Key Person **152,707.50**

B. Other Personnel

Number of Personnel*	Project Role*	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits*	Funds Requested ($)*
0	Post Doctoral Associates	0	0	0	0.00	0.00	0.00
0	Graduate Students	0	0	0	0.00	0.00	0.00
0	Undergraduate Students	0	0	0	0.00	0.00	0.00
0	Secretarial/Clerical	0	0	0	0.00	0.00	0.00
0	Other	0	0	0	0.00	0.00	0.00
0	Other Professionals	0	0	0	0.00	0.00	0.00
0	Allocated Admin Support	0	0	0	0.00	0.00	0.00
0	**Total Number Other Personnel**					**Total Other Personnel**	**0.00**

Total Salary, Wages and Fringe Benefits (A+B) **152,707.50**

RESEARCH & RELATED Budget {A-B} (Funds Requested)

Funding Opportunity Number: PA-14-046 . Received Date: 2016-02-12T17:40:51.000-05:00

RESEARCH & RELATED BUDGET - SECTION C, D, & E, Budget Period 5

ORGANIZATIONAL DUNS*: 004413456
Budget Type*: ● Project ○ Subaward/Consortium
Organization: Vanderbilt University Medical Center

Start Date*: 09-01-2020 **End Date*:** 08-31-2021 **Budget Period:** 5

C. Equipment Description	
List items and dollar amount for each item exceeding $5,000	
Equipment Item	**Funds Requested ($)***
Total funds requested for all equipment listed in the attached file	
Total Equipment	
Additional Equipment: File Name:	

D. Travel	**Funds Requested ($)***
1. Domestic Travel Costs (Incl. Canada, Mexico, and U.S. Possessions)	0.00
2. Foreign Travel Costs	0.00
Total Travel Cost	**0.00**

E. Participant/Trainee Support Costs		**Funds Requested ($)***
1. Tuition/Fees/Health Insurance		0.00
2. Stipends		0.00
3. Travel		0.00
4. Subsistence		0.00
5. Other: Other		0.00
0 Number of Participants/Trainees	**Total Participant Trainee Support Costs**	**0.00**

RESEARCH & RELATED Budget {C-E} (Funds Requested)

Tracking Number: GRANT12093630
Funding Opportunity Number: PA-14-046 . Received Date: 2016-02-12T17:40:51.000-05:00

RESEARCH & RELATED BUDGET - SECTIONS F-K, Budget Period 5

ORGANIZATIONAL DUNS*: 004413456
Budget Type*: ● Project ○ Subaward/Consortium
Organization: Vanderbilt University Medical Center

Start Date*: 09-01-2020 **End Date*:** 08-31-2021 **Budget Period:** 5

F. Other Direct Costs	Funds Requested ($)*
1. Materials and Supplies	0.00
2. Publication Costs	0.00
3. Consultant Services	0.00
4. ADP/Computer Services	0.00
5. Subawards/Consortium/Contractual Costs	0.00
6. Equipment or Facility Rental/User Fees	0.00
7. Alterations and Renovations	0.00
8 . Other Direct Costs	25,000.00
9 . All Other Costs	0.00
Total Other Direct Costs	**25,000.00**

G. Direct Costs	Funds Requested ($)*
Total Direct Costs (A thru F)	**177,707.50**

H. Indirect Costs

Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base ($)	Funds Requested ($)*
1 . MTDC, Research On/ Off campus-Remote	8	177,707.50	14,216.60
		Total Indirect Costs	**14,216.60**

Cognizant Federal Agency (Agency Name, POC Name, and POC Phone Number)	Department of Health and Human Services , Steven Zuraf (301) 492-4855

I. Total Direct and Indirect Costs	Funds Requested ($)*
Total Direct and Indirect Institutional Costs (G + H)	**191,924.10**

J. Fee	Funds Requested ($)*
	0.00

K. Budget Justification*	File Name: M-27_S2S_Budget_Justification.pdf (Only attach one file.)

RESEARCH & RELATED Budget {F-K} (Funds Requested)

BUDGET WITH JUSTIFICATION

Statement of Budgetary Overlap:
There is no budgetary overlap between this proposal and any of the investigators' current or pending support.

Budget:
Salary and Fringe for Dr. Daniels (actual [redacted]
Requested: $138,825 salary plus $13,882.50 fringe per year

TOTAL Salary and Fringe requested for Dr. Daniels per year at [redacted] effort: $152,707.50 / year

Additional Research Support Requested:

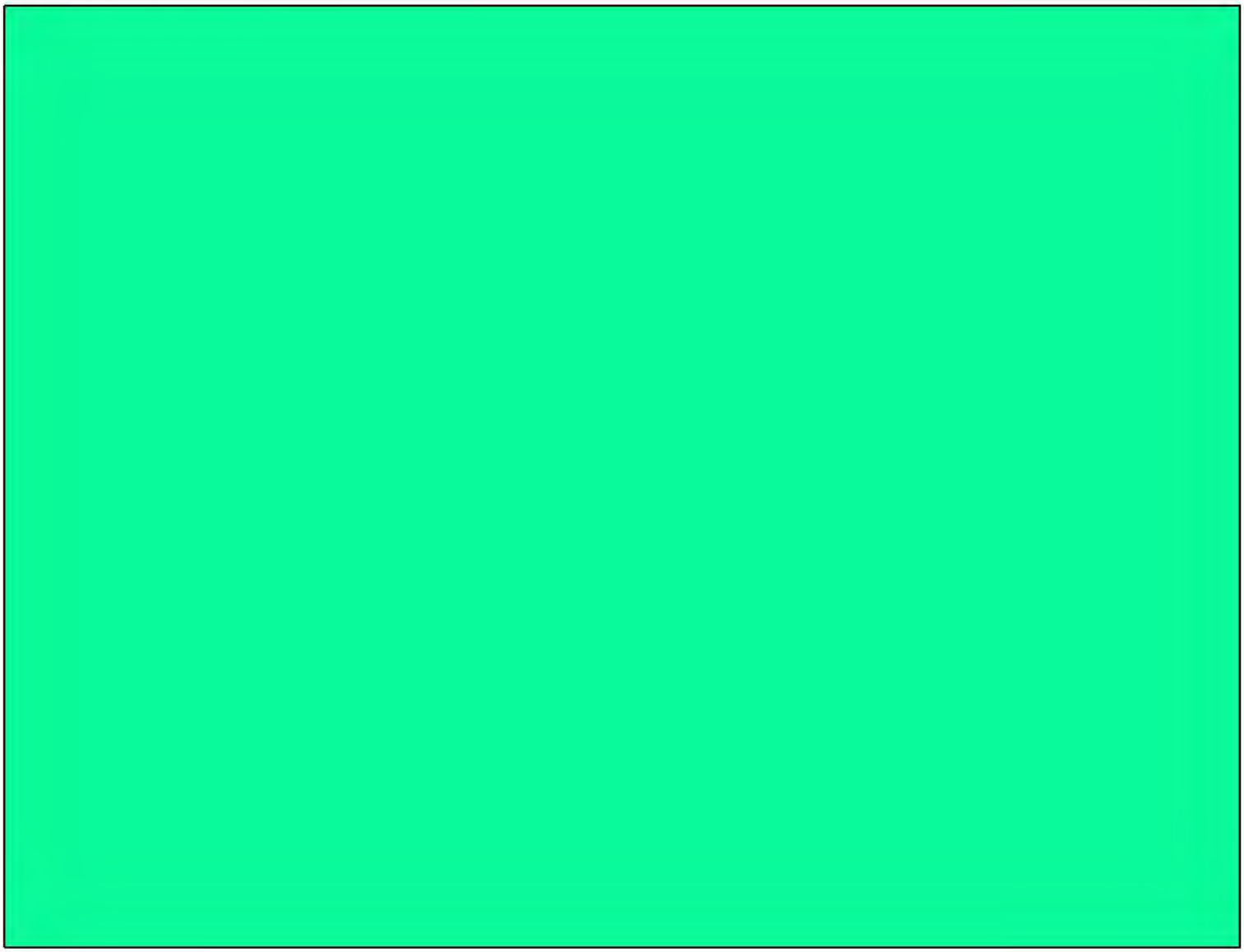
	Year 1	Year 2	Year 3	Year 4	Year 5
Rabbits	15150	12120	12120	8080	8080
Rabbit per diems	5350	10794	12880	16920	16920
Mass spectrometry core	4500				
Tissue histology core		2086			
TOTAL (excluding Dr. Daniels' salary)	**$25,000**	**$25,000**	**$25,000**	**$25,000**	**$25,000**

TOTAL REQUESTED PER YEAR (Dr. Daniels' Salary/Fringe plus research supplies): $177,707.50 / year

Personnel:

Anthony Daniels, MD, MSc (Principal Investigator, [redacted] calendar months salary and fringe) will direct the research being supported by this grant and will actively perform most of the techniques described. He has extensive experience with examination of both human patients and animals with intraocular tumors, with electroretinography and with intravitreal injections, and he currently directs retinoblastoma treatment here at Vanderbilt. He also has extensive experience with cellular signaling, targeted inhibitors of oncogenesis, retinal and tumor cell biology, and genetics. He will be responsible for the conduct of the research, the interpretation of data, manuscript publication and grant submission. He will supervise the project staff daily. [redacted] percent of his time will be protected by this grant for these research activities.

[redacted]



RESEARCH & RELATED BUDGET - Cumulative Budget

	Totals ($)	
Section A, Senior/Key Person		763,537.50
Section B, Other Personnel		0.00
Total Number Other Personnel	0	
Total Salary, Wages and Fringe Benefits (A+B)		763,537.50
Section C, Equipment		0.00
Section D, Travel		0.00
1. Domestic	0.00	
2. Foreign	0.00	
Section E, Participant/Trainee Support Costs		0.00
1. Tuition/Fees/Health Insurance	0.00	
2. Stipends	0.00	
3. Travel	0.00	
4. Subsistence	0.00	
5. Other	0.00	
6. Number of Participants/Trainees	0	
Section F, Other Direct Costs		125,000.00
1. Materials and Supplies	0.00	
2. Publication Costs	0.00	
3. Consultant Services	0.00	
4. ADP/Computer Services	0.00	
5. Subawards/Consortium/Contractual Costs	0.00	
6. Equipment or Facility Rental/User Fees	0.00	
7. Alterations and Renovations	0.00	
8. Other 1	125,000.00	
9. Other 2		
10. Other 3		
Section G, Direct Costs (A thru F)		888,537.50
Section H, Indirect Costs		71,083.00
Section I, Total Direct and Indirect Costs (G + H)		959,620.50
Section J, Fee		0.00

PHS 398 Cover Page Supplement

OMB Number: 0925-0001

1. Project Director / Principal Investigator (PD/PI)

Prefix:
First Name*: Anthony
Middle Name: B
Last Name*: Daniels
Suffix:

2. Human Subjects

Clinical Trial? ● No ○ Yes
Agency-Defined Phase III Clinical Trial?* ○ No ○ Yes

3. Permission Statement*

If this application does not result in an award, is the Government permitted to disclose the title of your proposed project, and the name, address, telephone number and e-mail address of the official signing for the applicant organization, to organizations that may be interested in contacting you for further information (e.g., possible collaborations, investment)?

○ Yes ● No

4. Program Income*

Is program income anticipated during the periods for which the grant support is requested? ○ Yes ● No

If you checked "yes" above (indicating that program income is anticipated), then use the format below to reflect the amount and source(s). Otherwise, leave this section blank.

Budget Period*	Anticipated Amount ($)*	Source(s)*

PHS 398 Cover Page Supplement

5. Human Embryonic Stem Cells

Does the proposed project involve human embryonic stem cells?* ● No ○ Yes

If the proposed project involves human embryonic stem cells, list below the registration number of the specific cell line(s) from the following list: http://grants.nih.gov/stem_cells/registry/current.htm. Or, if a specific stem cell line cannot be referenced at this time, please check the box indicating that one from the registry will be used:

Cell Line(s): Specific stem cell line cannot be referenced at this time. One from the registry will be used.

6. Inventions and Patents (For renewal applications only)

Inventions and Patents*: ○ Yes ○ No

If the answer is "Yes" then please answer the following:

Previously Reported*: ○ Yes ○ No

7. Change of Investigator / Change of Institution Questions

❑ Change of principal investigator / program director

Name of former principal investigator / program director:

Prefix:

First Name*:

Middle Name:

Last Name*:

Suffix:

❑ Change of Grantee Institution

Name of former institution*:

PHS 398 Career Development Award Supplemental Form

OMB Number: 0925-0001

Introduction (if applicable)	
1. Introduction to Application (for RESUBMISSION applications only)	
Candidate Information	
2. Candidate's Background	M-6_PHS_Career_Candidate_Background.pdf
3. Career Goals and Objectives	M-7_PHS_Career_Goals_Objectives.pdf
4. Career Development/Training Activities During Award Period	M-8_PHS_Career_Dev_Training.pdf
5. Training in the Responsible Conduct of Research	M-9_PHS_Career_Training_Resp_Conduct_Research.pdf
6.Candidate's Plan to Provide Mentoring (as applicable)	
Statements of Support	
7. Plans and Statements of Mentor and Co-Mentor(s)	M-24_PHS_Career_Mentor_Statements_Letters.pdf
8. Letters of Support from Collaborators, Contributors, and Consultants	M-12_PHS_CAREER_SupportLtrs.pdf
Environment and Institutional Commitment to Candidate	
9. Description of Institutional Environment	M-13_PHS_Career_Inst_Environment.pdf
10. Institutional Commitment to Candidate's Research Career Development	M-14_PHS_Career_Inst_Commitment.pdf
Research Plan	
11. Specific Aims	M-15_PHS_Career_SpecificAims.pdf
12. Research Strategy*	M-16_PHS_Career_Res_Strategy.pdf
13. Progress Report Publication List (for RENEWAL applications only)	
Human Subject Sections	
14. Protection of Human Subjects	
15. Inclusion of Women and Minorities	
16. Inclusion of Children	
Other Research Plan Sections	
17. Vertebrate Animals	M-18_PHS_Career_VertebrateAnimals.pdf
18. Select Agent Research	M-19_PHS_Career_SelectAgentResearch.pdf
19. Consortium/Contractual Arrangements	
20. Resource Sharing Plan(s)	M-20_PHS_Career_Resource_Sharing_Plan.pdf
Appendix (if applicable)	
21. Appendix	

Citizenship*:

- U.S. Citizen or noncitizen national
- Non-U.S. Citizen with temporary U.S. visa
- ● Permanent Resident of U.S. (If a permanent resident of the U.S., a notarized statement must be provided by the time of award)
- Permanent Resident of U.S. Pending

CANDIDATE BACKGROUND

My interests lie in identifying and implementing novel treatments for retinoblastoma (RB) that allow visual function to be preserved in affected children. As a practicing ocular oncologist, this sits at the natural crossroads of my clinical expertise in the management of children with retinoblastoma, and my research background in retinal diseases, ocular tumor biology, and targeted inhibitors of signaling pathways. I am committed to a life-long research career aimed at developing these novel vision- (and life-) saving treatments.

Clinical Training Background and Relationship to Subject Matter of Proposed Research

Upon graduating medical school at the University of Pennsylvania, I performed my medical internship year at Memorial Sloan Kettering Cancer Center. There, I was introduced to the field of ocular oncology. This was an exciting time, as [redacted] had just introduced a new intra-arterial chemotherapy technique to treat RB. I saw the power of this technique performed on the very first patients. I retained my focus on ocular tumors throughout ophthalmology residency at Harvard Medical School/Mass. Eye & Ear Infirmary. However, I was equally fascinated by the impact of treatment on surrounding ocular structures, and spent time studying retinal degenerations with [redacted] I subsequently completed a vitreoretinal surgery fellowship at Harvard, then completed additional ocular oncology training at Memorial Sloan Kettering with [redacted]

Returning to Memorial six years later, I saw first-hand how intra-arterial chemotherapy and intravitreal chemotherapy had propelled the field forward. However, the ocular toxicities associated with these treatments were also becoming apparent. Many "successfully-treated" eyes were left without usable vision, often bilaterally. In fact, [redacted] and I have recently authored a paper (Abramson, Daniels, et al., 2016) that described the prevalence of visually-significant adverse effects of current intra-arterial drug regimens.

Since joining the Vanderbilt faculty in September 2013, I personally manage all of the children with RB. We have assembled a team of ophthalmologists, neuro-interventionalists, and pediatric oncologists dedicated to moving the clinical care of these patients forward. We are one of a handful of centers in the US that provides intra-arterial and intravitreal chemotherapy, building on what I learned from [redacted]. My goal in treating these children is not only to save their lives and retain their eyes, but also to preserve life-long useful vision.

Research Background and Relationship to the Skills Necessary for the Proposed Research

In college at Princeton, I studied signaling pathways and tumor suppressors in cancer, and in medical school, I studied signaling cascades in immune response, using tyrosine kinase inhibitors of pathway proteins. Between college and medical school, I pursued a Master's degree at the London School of Economics. There, I studied population health and gained skills in biostatistics and epidemiology. Subsequently, I leveraged these epidemiologic skills in medical school in studies related to identifying risk factors for vision threatening conditions. With mentors [redacted] I first-authored the publication that quantified the relationship between obesity, weight gain, and idiopathic intracranial hypertension (Daniels et al., 2007).

In residency, I drew on these epidemiologic skills and my prior background in lab research and genetics. I extended these skills to genotype-phenotype correlations, studying Bardet-Biedl Syndrome with [redacted] I was the lead author on a massive study of this rare condition that identified the prognostic value of specific gene mutations for predicting retention of long-term vision function (Daniels et al., 2012). Our algorithm allowed clinicians to predict genotype and visual prognosis based on initial electroretinographic findings. This project involved a large group of collaborators at Harvard and the NIH, due to the complex genetics of this syndrome.

In fellowship, I studied the genetics of uveal melanoma with [redacted] capitalizing on skills I learned studying the genetics of retinal degenerations and my oncology lab experience. Again working with a large collaborative group around the US, I lead-authored a paper using a high-throughput technique to identify common mutations in uveal melanoma that might make them susceptible to protein tyrosine kinase inhibitors (Daniels et al., 2012). This leveraged my previous lab experience with these types of inhibitors as they related to immune signaling and oncology (Daniels et al., 2009; Daniels et al., 2010a; Daniels et al., 2010b). We demonstrated the high prevalence and mutual exclusivity of mutations in *GNAQ* and *GNA11*. Because of the rarity of uveal melanoma, we focused initially on those activating mutations for which inhibitors had already been developed for other cancers. I am utilizing a similar approach in my current retinoblastoma research.

I joined the laboratory of [redacted] approximately one year ago. [redacted]s world-renowned in cancer biology and in the preclinical assessment of toxicity and efficacy of antineoplastic agents in animal models. This mentor-mentee relationship has been very productive – in the last few months, I have published 3 manuscripts, with 6 more currently in press and several others under review, all in the field of ocular oncology. In addition, I have successfully competed for an internal Vanderbilt grant that supports my protected research time, and I received a one-year Career Starter Grant from the [redacted] These allowed me to generate the rabbit models used in the current research proposal.

CAREER GOALS AND OBJECTIVES

My career goal is to become a physician-scientist, independently-funded with NIH support. I plan to run my own laboratory at Vanderbilt, training graduate students and postdoctoral fellows. I envision my research program being thematically linked to my clinical ocular oncology practice, thus facilitating bedside-to-bench-to-bedside translations. As Director of Ocular Oncology, I have assembled a multispecialty team for retinoblastoma. We incorporate cutting edge techniques such as intra-arterial chemotherapy and intravitreal chemotherapy that are only available at a handful of centers across the country. However, seeing the clinical success of these new treatments also allows me to see the side effects of the currently available drugs. Thus, a patient can have complete oncologic response, with tumor regression and resolution of vitreous seeds, but at the cost of vascular occlusions, pigmentary retinopathy, and falling electroretinography amplitudes – an eye that looks good, but doesn't see well.

My specific research focus is the discovery and development of novel drugs for retinoblastoma. I plan to make use of the powerful intra-arterial and intravitreal routes, but with a focus on minimizing ocular toxicity. My current research is therefore at the perfect nexus of my area of clinical expertise in the management of retinoblastoma, and my research background in targeted therapeutics and in tumor biology and genetics. Initially, I plan to focus on drugs that are currently approved for use in humans, to allow rapid translation to clinical use. I then hope to develop novel inhibitors that target protein pathways involved in retinoblastoma. Having developed a comprehensive system to assess efficacy and ocular toxicity through the research proposed in this grant, I will be well-positioned to assess these novel agents in the preclinical setting. This focus on developing agents that target molecular pathways in retinoblastoma comes as a natural extension of my previous work, which focused on the biology of ocular cancers. In addition, my work on intracellular signaling pathways used protein tyrosine kinase inhibitors to tease out signaling networks and to identify potentially-useful antineoplastic agents.

I will be uniquely positioned to ultimately take these discoveries, based on initial clinical observations regarding ocular toxicity, back to the bedside. As a practicing ocular oncologist, I will be able to introduce these new drugs in future clinical trials to hopefully prevent life-long visual morbidity for these children. This will be facilitated by the significant resources of the Vanderbilt-Ingram Cancer Center and my active clinical practice.

In order to best achieve these career goals, there are specific objectives that I will need to accomplish during my early career. To be able to be make a real impact in my chosen research area, I will need to learn:

- to use complex animal models
- to use cutting-edge applications of mass spectrometry
- to calculate pharmacokinetic curves
- to quantitatively assess toxicity and efficacy of treatments
- to employ statistically-rigorous study design

In parallel, from a career development point of view, I will need:

- to learn lab management skills
- to establish myself as an authority in this specific research area, by publishing and presenting extensively
- to produce the data necessary to drive future hypotheses and to successfully compete for R01 funding

This K08 grant will allow me to develop these skills while being mentored by experts in each area. The protected research time will let me maximize my development and progress during these critical early years.

My specific **research objectives** for this K08 grant time period are:

Objective	Plan
Gain experience in eye cancer animal models and in *in vivo* assessment of ocular toxicity	• Conduct studies of drug efficacy and toxicity in rabbit RB model **[AR, JP, DF]** • Learn to use appropriate measures and techniques for retinal toxicity **[JP, DF]** • Gain comfort with rabbit survival surgeries and best practices **[PW]**
Acquire expertise in preclinical study design and biostatistics	• Develop and troubleshoot the preclinical study design **[YS, AR]** • Learn advanced techniques for biostatistical analysis **[YS]** • Take Dr. Shyr's advanced biostatistics of clinical trial study design course
Comfort with ocular PK assessment of drugs and cutting edge MS techniques	• Learn how to use traditional mass spectrometry and in situ imaging MS to assess drug levels in retina and vitreous **[KS]** • Develop models for intraocular PK of drugs using advanced software **[CL, YS]**

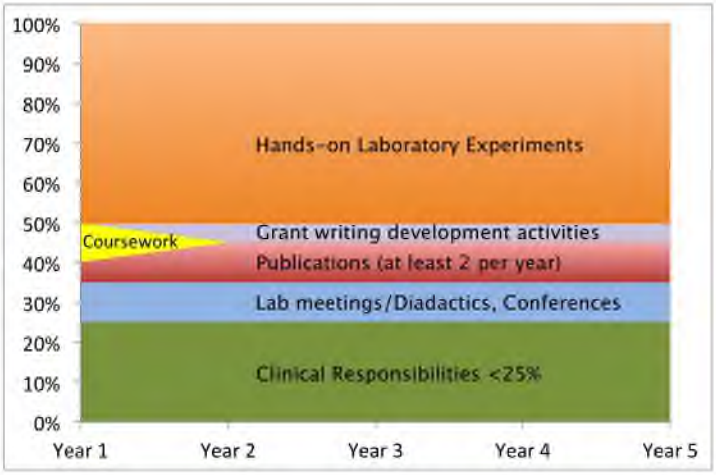
(Key: AR=Richmond, JP=Penn, DF=Friedman, YS=Shyr, PW=Williams, KS=Schey, CL=Lindsley)

My specific **career development objectives** for this K08 grant time period are as follows:

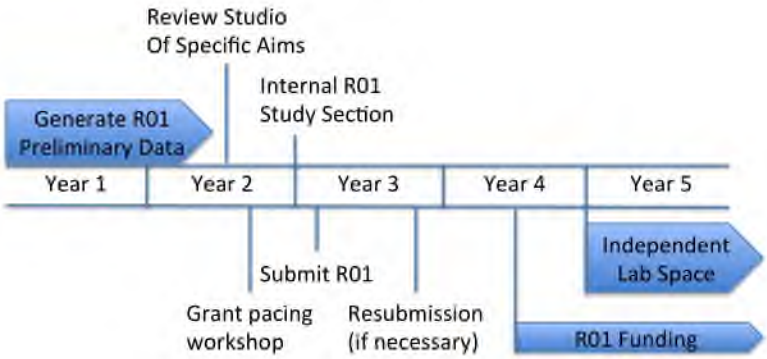
Objective	Plan
Enhance written and verbal communication **[AR, JP, DF, YS]**	• Attend and present yearly at ARVO and AACR meetings as well as at the International Society of Ocular Oncology Meeting, which is every two years • Publish at least two papers in high impact journals in this specific research area per year (as first author or senior author)
Develop lab management skills **[AR, JP]**	• Directly learn from my mentors, Drs. Richmond and Penn, who have decades of experience between them running successful translational labs • Attend Cold Spring Harbor Laboratory Workshop on Leadership in Bioscience (March 2017)
Become an effective grant writer **[AR, JP, DF, YS]**	• Participate in Vanderbilt R01 Grant Pacing Workshop • Make use of Vanderbilt EDGE Review Studios & Internal R01 Study Section • Apply for Foundation Grants, both to increase available funds and to hone my grant writing skills
Secure R01 Funding	• Apply for R01 grant early in Year 3 of the K08 award period

Effort Timeline

Descriptions of each activity listed in the table below can be found in "Candidate Plan for Career Development".



Timeline for Transition to Independence



CANDIDATE'S PLAN FOR CAREER DEVELOPMENT & TRAINING ACTIVITIES DURING AWARD PERIOD

MENTORSHIP

I am fortunate to have a very strong mentorship team here at Vanderbilt, with rock-solid departmental support. Each mentor, co-mentor, or collaborator is a world-leading expert in a specific area that applies to this mentored research proposal. Their roles in the proposed research are summarized in Table 1, below. In addition, these individuals were selected for their long histories of successfully mentoring junior faculty to independence, and their own histories of continuous funding in the fields of ophthalmology and cancer biology. All are eager to study the proposed research question, and all are invested in my success. Their specific roles in my career development and in my learning objectives are summarized in tables in the "Career Goals and Objectives" portion of this application.

[redacted]

Table 1: Role of various mentors and collaborators in the specific proposed research activities.

		Mentor	Co-Mentors			Collaborators				
	Expertise	[redacted]								
		Tumor Biology & Models	Retinal Biology & Toxicity	RB & Chemo	Biostats & Study Design	Rabbit Surgery	IAC	Mass Spec	PK	IAC & Intra-vitreals
Specific Aim	1A	X	X	X		X	X	X	X	
	1B	X		X						
	1C	X	X	X	X	X				X
	1D	X	X	X	X	X	X			X
	2A	X	X	X	X	X				X
	2B	X	X	X	X	X	X			X
	2C	X	X		X					

[redacted]

[redacted]

I present my research program, plan, and career development, and they make sure I remain on track to succeed.

SPECIFIC CAREER DEVELOPMENT ACTIVITIES

1) **Hands-on work in laboratory**. The research skills I will obtain in the laboratory setting are described in detail in the "Research Training Objectives" table in the "Career Goals and Objectives" portion of this application.

2) **Interactive Didactics**: Attend [redacted] lab meetings (attend weekly, present myself 3-4 times/year)
Attend [redacted] lab meetings (attend once per month, present myself once per year)
Attend Center for Quantitative Sciences monthly seminar series, directed by [redacted]

3) **Formal Coursework:** Over the first year of this grant, I plan to take the following semester-long courses:
1) Biostatistics 6321: Clinical Trials and Experimental Design (taught by co-mentor [redacted]
2) Biochemistry 8352: Analytical Proteomics. Focuses on mass spectrometry & bioinformatics (taught by [redacted]

4) **Hands-on Workshops:** Attend Cold Spring Harbor Laboratory Workshop on Leadership in Bioscience (3/2017)

5) **Grant Writing Activities:**

Vanderbilt R01 Grant Pacing Workshop – This workshop, which begins approximately 4 months prior to each grant cycle deadline, begins with two days of didactics and small group sessions on issues such as writing strategies, clear communication, and time management. There are also "peer groups" which extend throughout the time leading up to the submission date, to keep participants on track and to provide constructive criticism. I will participate in this during year 2 of the K08 grant period, in preparation for R01 submission early in year 3.

Vanderbilt EDGE Review Studios and Internal R01 Study Section – Vanderbilt assembles review committees composed of senior scientists with experience sitting on NIH study sections to critique grant submissions by junior faculty members. A written critique and video of the actual committee discussion is provided, with adequate time for revisions. I will utilize this at the end of year 2, prior to submitting my R01.

Foundation Grants – I will continue to submit additional grants to various research foundations, both to increase my pool of available funds and hone my own grant writing skills in preparation for my R01 submission.

6) **Conferences:** Attend the Association for Research in Vision and Ophthalmology and an American Association for Cancer Research meeting (yearly) and International Society of Ocular Oncology meeting (every other year).

7) **Research Presentation Skills:** I will present at each of the above meetings to gain comfort with audiences consisting of both ophthalmologists and oncologists. I will present at [redacted] lab meetings.

8) **Publications:** I will publish at least 2 first- or senior-author publications per year in high impact journals based on the bench research proposed in this grant submission.

9) **Additional Career Development Activities:**

Vanderbilt Elliot Newman Society – I am a member of Vanderbilt's Newman Society, which is composed of young physician-scientists in the process of transitioning to research independence. This includes a monthly lecture series on topics such as scientific communication, career planning, mentorship, leadership, and RCR.

10) **Responsible Conduct of Research:** Newman Society lecture series; Biomedical Sciences 8004 (Advanced RCR); Biomedical Research and Training full-day RCR symposium. See separate section on RCR for details.

Table 2. How the above activities relate to my training objectives (see Career Goals and Objectives Section)

Activity (see above) Number		Objective (See Career Goals and Objectives Section)			
		Development of Research Skills	Written/Verbal Communication	Lab Management	Grant Writing / Secure R01
	Laboratory Work (1)	X		X	
	Didactics, Workshops, Courses (2-4)	X	X	X	
	Grant Writing Activities (5)				X
	Presentations at Conferences (6-7)		X		
	Publications (8)		X		
	Newman Society / RCR (9-10)		X	X	X

TRAINING IN THE RESPONSIBLE CONDUCT OF RESEARCH

In medical school, we had required courses on research ethics that focused on human subjects, collaboration, data management and acquisition, misconduct and responsible publication, as well as other areas. At each institution that I have attended for my post-medical training (medical internship, residency, and fellowship), I have completed the appropriate **Collaborative Institutional Training Initiative (CITI) training**, and have remained up to date on all these modules, including those that focus on protection of human subjects and the CITI Responsible Conduct of Research (RCR) modules. This included renewing all of my CITI training since having joined the faculty at Vanderbilt in September 2013, and I have remained up to date on all re-certifications.

I plan to **attend the 9-hour symposium on RCR** offered through the Vanderbilt University Medical Center Office of Biomedical Research and Training (BRET), which will be held on Monday, May 16, 2016. This symposium covers all nine of the major topics identified by the NIH Office of Research Integrity: 1) conflict of interest, 2) policies regarding human and vertebrate animal subjects, and safe laboratory practices, 3) mentor/mentee responsibilities and relationships, 4) collaborative research, 5) peer review, 6) data acquisition and management, 7) handling research misconduct, 8) responsible authorship and publication, and 9) the scientist as a responsible member of society. In addition, during my K08 grant period, I will be taking the semester-long **advanced RCR course (Biomedical Sciences IGP 8004)**. This course covers additional topics related to the 9 key focus areas identified by the NIH Office of Research Integrity.

Furthermore, I will attend the **RCR seminar series for Mentors and Mentees,** which is held quarterly and sponsored by the Biomedical Research, Education and Training office. [redacted] and I will attend these together, along with other members of the laboratory. In addition, at our regular laboratory meeting with the [redacted] lab group, we have in-depth discussions on RCR, publication ethics, appropriate use of biostatistical methods, appropriate vertebrate animal protocols, proper handling of research involving human subjects, misrepresentation of data, appropriate documentation and record keeping in research, fraud, conflict of interest, and responsible authorship.

Twice a year, my mentor [redacted] hosts 2-hour **RCR discussion groups** with all laboratory members. In informal settings we discuss research misconduct cases. Discussion subjects also include data manipulation, data sharing, ethical standards of animal studies, IACUC rules and requirements, patient rights, informed consent, and plagiarism. We also watch RCR-related training videos and interactive tutorials. In addition, in each lab meeting/paper discussion, we discuss key RCR topics.

The Vanderbilt **Elliott Newman Society** is composed of all faculty and trainees on training or mentored grants, whether internal or external. Since successfully competing for an internal Vanderbilt Mentored Career Development Grant in 2015, I have been a member of this Society. As part of this Society, I attend **monthly RCR lectures** that focus on the various nine RCR components, and which accrue RCR hours.

On a more general note, as a Vanderbilt University Medical Center faculty member and researcher, I am required to complete ongoing training modules online related to the legal and ethical aspects of healthcare delivery and research. I have completed all training modules for the Institutional Animal Care and Use Committee (IACUC), including those related to the care of rodents, and to rabbits and other United States Department of Agriculture (USDA)-regulated species. I have also completed the online training modules required by the Institutional Review Board (IRB) through the CITI training website described above, which focus on the history, evolution and need of human subject protections, the ethical principles on which they are founded, the principles and practice of informed consent, ethical principles, and protection of vulnerable subjects/populations.

Page 106 of 138 to Page 118 of 138

Letters of Support

SPECIFIC AIMS

Retinoblastoma (RB) is the most common primary intraocular tumor in children. The introduction of systemic chemotherapy has led to 99% patient survival rates in high-income countries. However, systemic chemotherapy is associated with side effects such as neutropenia, and concern about future fertility and secondary leukemia. Eyes with advanced tumors are rarely saved by systemic chemotherapy, and often require enucleation if difficult-to-treat subretinal or vitreous seeds are present. The recent introduction of microcatheter-based intra-arterial chemotherapy (IAC) and direct intravitreal chemotherapy has led to dramatic improvements in globe salvage rates for advanced eyes. However, the current melphalan-based regimens have significant ocular, retinal and retinal vascular toxicity, when delivered intravitreally or via IAC, thus severely impacting lifelong visual prognosis for these children.

Newer agents are needed to achieve tumor control without the ocular toxicity and visual morbidity associated with currently-used drugs. Thus far, drug discovery for IAC has been hampered by the absence of a small animal model that allowed IAC drug delivery in order to test safety, toxicity, and efficacy of alternative drugs. Instead, testing of new drugs and determination of maximum tolerated dose has had to occur in children with RB, titrating efficacy with toxicity. In order to overcome this obstacle facing the RB field, we have recently developed the **first-ever small animal (rabbit) model of IAC**. We have demonstrated excellent vitreous and retinal penetration of drug and consistent pharmacokinetics in this model. We have also developed a rabbit model of RB that develops retinal tumors and diffuse vitreous seeds, along with a novel method for quantifying tumor burden and drug efficacy *in vivo*.

Our **long-term goal** is to develop novel, pathway-specific, targeted drugs with minimal ocular toxicity for use via IAC or intravitreal injection in RB, and to translate these discoveries into clinical practice. For the current proposal, we plan to focus on a small set of Food and Drug Administration (FDA)-approved drugs. Our **research objective** for this proposal is to identify drugs that demonstrate efficacy with minimal ocular toxicity. Our **overall hypothesis** is that the chemotherapy compounds we have selected for study have minimal ocular, retinal and retinal vascular toxicity, while remaining effective against human RB cell lines *in vivo* in our animal model. Focusing on FDA-approved drugs will allow **rapid and impactful translation** to clinical practice to help maximize vision for these children. This research will also allow us to develop a comprehensive drug-testing platform in which to assess ocular toxicity and efficacy of novel RB pathway-targeted drugs that we will develop in the future.

The **training objective** is for Dr. Daniels to gain expertise in the use of ocular cancer animal models, in the preclinical assessment of drug toxicity and efficacy, in pharmacokinetic analysis, and biostatistics. Throughout, he will be mentored by recognized world leaders in each related field (animal models of cancer, retinal biology, RB chemotherapy, and preclinical study design and biostatistics). This will allow him to establish himself as an independent researcher in this field. This will be accomplished via the following specific aims:

Aim 1: Assess efficacy of select chemotherapy drugs against RB, when delivered via intra-arterial and intravitreal routes, using our novel rabbit model of RB with vitreous seeds and the only rabbit model of intra-arterial chemotherapy (IAC) delivery. *We hypothesize that the drugs selected for study will remain effective against human RB cells in vivo in our rabbit model when delivered either by IAC or intravitreally.* To confirm efficacy *in vivo*, we will first **A)** determine vitreous pharmacokinetics of each drug injected intravitreally and intra-arterially, as well as the intra-retinal tissue drug concentration, using traditional mass spectrometry as well as cutting-edge *in situ* imaging mass spectrometry, then **B)** determine the cytotoxicity profiles of each drug *in vitro* in human RB cell lines, based on the empiric vitreous exposure time. We will then **C)** assess *in vivo* efficacy against RB of these intravitreally-injected drugs using an RB rabbit model of vitreous seeds with quantifiable disease burden, and **D)** assess *in vivo* efficacy of intra-arterial chemotherapies against RB using the only small animal model of IAC.

Aim 2: Assess safety and ocular toxicity of select RB chemotherapies delivered intravitreally and intra-arterially. *We hypothesize that the selected drugs will have minimal retinal and retinal vascular toxicity.* We will **A)** evaluate functional and structural ocular toxicity profiles of repeated intravitreal chemotherapy injections *in vivo* in rabbits, using a combination of electroretinography, fundus photography, optical coherence tomography, and histopathology. Similarly, we will **B)** evaluate the ocular toxicity profile of intra-arterially delivered chemotherapies in our rabbit model of IAC, using the above-mentioned modalities, in addition to fluorescein angiography. Once ocular toxicity has been studied in our rabbit model, we will then **C)** assess retinal toxicity in human retinal tissue using novel *ex vivo* human retina electrophysiologic assessment methods.

Currently, most children with retinoblastoma retain their eyes, but lifelong vision is often lost in one or both eyes. To change this outcome and develop new chemotherapies that can be delivered directly to the eye, the proposed research leverages novel animal models of IAC and RB for drug discovery. Furthermore, the research makes use of cutting edge techniques to assess tissue drug levels, as well as the first use of electrophysiologic assessment of *ex vivo* human retinal function to measure toxicity of candidate drugs. With Dr. Daniels' unique background, and mentored by a team of established experts, the goal is to move beyond simply saving eyes, to actually saving sight.

RESEARCH STRATEGY

SIGNIFICANCE

Background

Retinoblastoma (RB) is the most common primary intraocular malignancy in children. The mainstay of globe-conserving therapy has been intravenous (IV) chemotherapy.[1] In high-income countries, this has allowed patient survival rates near 99%.[2] However, systemic chemotherapy is associated with acute toxicity such as cytopenias and infection,[1,3-7] as well as long-term potential for organ dysfunction potential[8-10] and increased risk for subsequent leukemia.[3-5,11-14] We and others have shown that eyes with advanced tumors (Reese-Ellsworth [R-E] group V and International Classification of RB [ICRB] group D and E) are rarely saved by IV chemotherapy,[15-17] and often require enucleation (Figure 1A-B). IV chemotherapy has particularly poor success against subretinal or vitreous seeds.[18]

Recently, alternative local approaches for delivering chemotherapy to the eye have been developed.[19,20] These approaches not only minimize systemic absorption of drug and minimize systemic toxicity,[21,22] but also allow much higher intraocular drug concentrations to be achieved.[23] Intra-arterial chemotherapy (IAC, via a microcatheter inserted endovascularly up to the ophthalmic artery),[19] using melphalan-based regimens,[24] has led to dramatic improvements in globe salvage rates for these advanced eyes (Figure 2).[15,25] In addition, direct intravitreal injections of chemotherapy (again, predominantly melphalan) have allowed eyes with extensive vitreous seeds to be saved, whereas previously eyes with vitreous seeds (ICRB Group D or R-E Group Vb) were rarely salvageable.[20,26-28]

Figure 1. Percent globe salvage success rates without radiation (95% confidence interval) for traditional intravenous chemotherapy by Reese-Ellsworth (left) and ICRB (right) group.

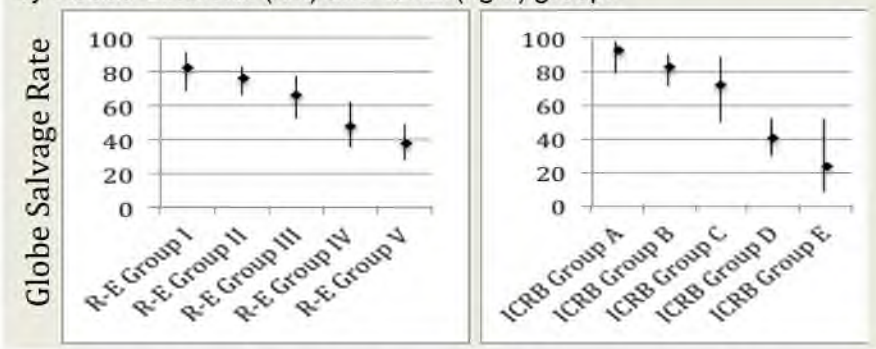
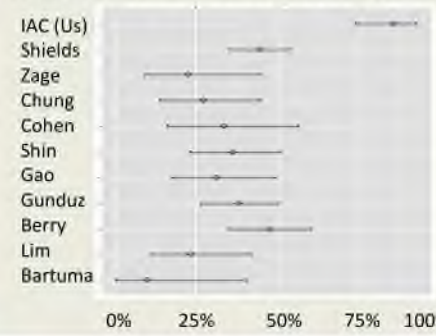
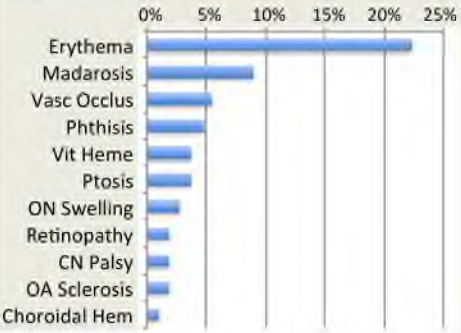


Figure 2. Our published success rate for group D eyes using IAC (top line) vs. published IV chemo success rates.



While new techniques like IAC and intravitreal chemotherapy have dramatically improved globe salvage for eyes with advanced RB,[15,25] the current melphalan-based regimens for both delivery routes have significant ocular, neuroretinal, and retinal vascular toxicity,[15,29-31] and so oftentimes the globe is preserved but at the expense of vision (Figure 3).[15] Newer agents might be able to achieve tumor control without the ocular toxicity and visual morbidity associated with currently-used drugs. However, thus far, there has not been a small-animal model that allows IAC drug delivery to test the safety and toxicity profiles and the efficacy of alternative drugs. Many drugs were first tested in the clinical setting, titrating until toxicity was reached in human infants with RB, due to the inability to perform equivalent preclinical studies in animal models. Most recent supporting animal work was performed *after* the initial trials in humans.[32,33]

Figure 3. Ocular complications from IAC melphalan combinations (from our published data).



Scientific Premise, Research Question, and Hypothesis

The scientific premise is that melphalan-containing regimens cause high ocular morbidity due to direct toxicity to the retina and retinal vascular. Thus, we would expect that alternative agents that are less toxic to the retina and retinal vasculature would be less likely to cause ocular morbidity and vision loss when used in humans.

The research question we address is whether select chemotherapies, delivered via the intra-arterial or intravitreal routes, are less-toxic alternatives to melphalan in the treatment of RB in our rabbit model. Given the high ocular morbidity associated with melphalan-containing regimens (Figure 3),[34] we will evaluate these drugs for evidence of reduced ocular, retinal, and retinal vascular toxicity, while still maintaining *in vivo* efficacy against human RB cell lines. We are initially testing FDA-approved and well-known chemotherapy drugs to maximize the speed that these findings can translate into clinical use, by serving as alternatives to the current toxic regimens.

Our hypothesis is that the alternative chemotherapy compounds we selected for study have minimal ocular, retinal and retinal vascular toxicity, while remaining effective against human RB cell lines *in vivo* in our rabbit model.

INNOVATION

There are several innovations in the proposed research:

- First/only small-animal model of intra-arterial chemotherapy
- Rabbit model of diffuse vitreous seeds recapitulating patient histology, with quantifiable tumor burden
- First use of histology-directed *in situ* imaging mass spectrometry to assess intra-retinal drug levels for IAC
- Novel technique for assessment of drug toxicity and functional studies in *ex vivo* human retinal tissue

Deliverables:

Over the course of this 5-year grant period, the following will be accomplished:

- Demonstrate safety and efficacy of at least one drug by each route (intra-arterial and intravitreal) and develop a strategy to translate this into clinical practice by means of a clinical trial
- Publish two first- or senior-author manuscripts each year
- Apply for R01 funding in Year 3 to continue the proposed RB research and revise as indicated to secure funding

APPROACH

Our Rabbit Models

Recently, IAC was performed in large animals such as pigs[21] and non-human primates (NHPs).[35] However, the techniques used in NHPs caused severe vascular damage to the eyes, much greater than that usually seen in humans.[30,33] In addition, there is currently no porcine or NHP model of RB in which to be able to test the efficacy of IAC, and certainly the development cost of such a model in these large animals would be exorbitant.

There are several advantages to using rabbit models for these purposes: 1) Rabbits can be immunosuppressed with cyclosporine to permit xeno-engraftment,[36] 2) The eye is large enough to safely inject intravitreally into a tumor-bearing eye, 3) Prior pharmacokinetic (PK) studies of melphalan have used rabbit eyes,[31] 4) Electroretinography standards are well-known for rabbits,[37] and 5) This is an established model organism for many other endovascular neurological procedures, having been used in dozens of prior studies in the neuro-interventional field.[38,39]

Our Rabbit Intra-Arterial Chemotherapy (IAC) Model

Using rabbits, we have developed the first small-animal model of IAC. With our technique, we can efficiently and selectively deliver chemotherapy (initially, using melphalan) into the ophthalmic artery and to the retina of New Zealand white rabbits, regardless of the vascular variations present (Figure 4). We have demonstrated excellent vitreous and retinal penetration and drug accumulation, with highly reproducible pharmacokinetics (again, starting with melphalan, Figures 5 and 9). Neither small vessel diameter nor dual internal and external ophthalmic arteries precludes efficient catheterization. We have found that there is significant vascular variation to the retina between rabbits, with the entire retinal vasculature deriving from the internal ophthalmic artery (off the internal carotid artery), or deriving entirely from the external ophthalmic artery (off the external carotid artery), or with half the retinal vasculature being supplied from each (Figure 4A-C). This is a key biological variable. However, regardless of the anatomy, in all cases we have been able to selectively catheterize the feeding ophthalmic artery, without issues with wedge flow fluidics, and have demonstrated consistent pharmacokinetics for melphalan regardless of the vascular variation.

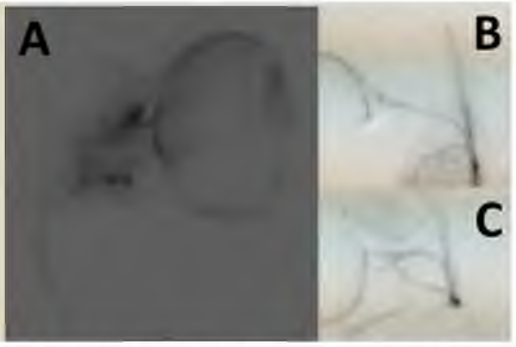


Figure 4. Selective angiograms demonstrating variations in vascular anatomy, including complete ophthalmic supply arising from the external ophthalmic artery (**A**), or with dual supply arising from a combination of the internal (**B**) and external (**C**) ophthalmic arteries.

Another key biological variable is that rabbits are merangiotic (their retinal vessels lie immediately above the surface of the retina, rather than under the internal limiting membrane as is the case in humans).[40] However, we found that this does not affect consistency of pharmacokinetics and drug penetration for intra-arterially delivered drugs. Rather, we have demonstrated highly consistent vitreous and retinal penetration and pharmaco-kinetics of melphalan delivered via the intra-arterial route, with minimal drug entering the contralateral eye (via systemic circulation; Figures 5 and 9).

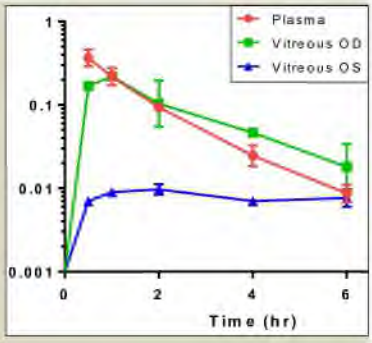


Figure 5. Pharmacokinetic curve following right-sided selective intra-ophthalmic artery infusion of 1.2mg (0.4mg/kg) melphalan. (Red: plasma, Green: right [treated] eye vitreous, Blue: left [untreated] eye vitreous). Y axis is concentration (micromolar).

Our rabbit Retinoblastoma (RB) Tumor Model

We have also developed a rabbit model of intraocular RB [redacted] Cells were purchased directly from ATCC, which has authenticated this cell line. We show that direct intravitreal injection of WERI cells leads to growth of vitreous clusters of cells akin to the vitreous seeds seen in humans with advanced RB (Figure 6). The number of tumor cells in the vitreous can be quantified by automated cell counting after harvesting the vitreous. To optimize our ability to count only these human RB xenograft cells, we transduced this cell line to express green fluorescent protein (GFP) to allow for fluorescence-assisted cell sorting and counting. We can use this model of vitreous seeds, and our ability to count the GFP-expressing RB xenografted cells, to quantify the cells remaining after intravitreal injection of an antineoplastic drug (see Aim 1C). We also show that subretinal injection of WERI cells leads to both intra-retinal and subretinal tumors akin to tumors seen in humans with RB (Figure 6).

[redacted]

Selection of Drugs to be Studied

The following chemotherapy drugs were selected for study: [redacted] All drugs are pharmaceutical grade, purchased from vendors that also supply hospitals for patient use, so we are confident in their authenticity. We applied several principles in selecting drugs to use in the proposed research plan:

1) Dose-dependent cytotoxicity in both available human RB cell lines (WERI-1-Rb and Y79).

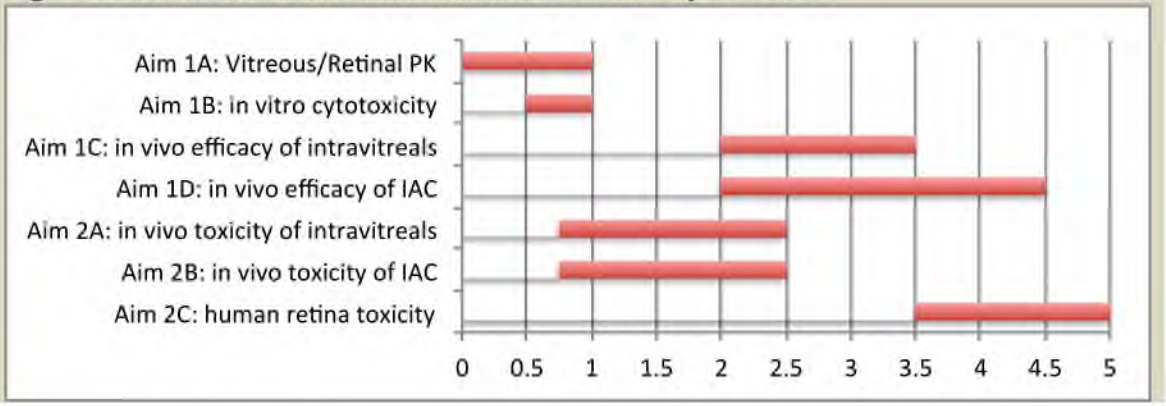
2) FDA-approved for humans. Within a given class [redacted] we gave preference to FDA-approved drugs, to allow rapid translation to clinical practice (in the future, we hope to develop novel molecular pathway-specific inhibitors [see Future Directions section]).

3) Ability to accurately measure drug concentrations by mass spectrometry (MS) at concentrations that are biologically plausible *in vivo* and consistent with the IC90 we found *in vitro*. For all selected drugs, the lower limit of detection using our MS technique was in the femtomolar range, far below the effective dose for each drug.

Detailed Plan of Proposed Research

Our goal is to identify chemotherapeutic drugs that have minimal ocular toxicity when delivered via the intra-arterial and intravitreal routes, as alternatives to currently-used regimens. We hope to replace current melphalan-based regimens, which have known retinal and retinal vascular toxicity.[29-31] While our primary interest is in establishing the (lack of) retinal toxicity of these alternative agents, we must also confirm that these non-retinotoxic agents remain effective against human RB cells *in vivo*. Figure 7 shows the timeline for the proposed aims.

Figure 7. Timeline for described research, on an aim-by-aim basis.

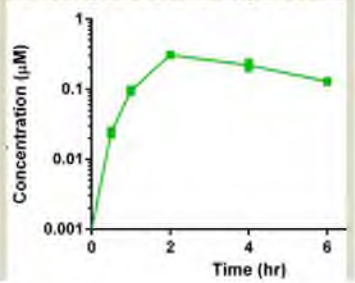


Specific Aim 1: Assess *in vivo* efficacy of select chemotherapy drugs against RB, when delivered via intra-arterial and intravitreal routes, using our rabbit models of intra-arterial chemotherapy (IAC) and RB

Aim 1A: Determine vitreous and intra-retinal pharmacokinetics of each drug injected intra-arterially or intravitreally

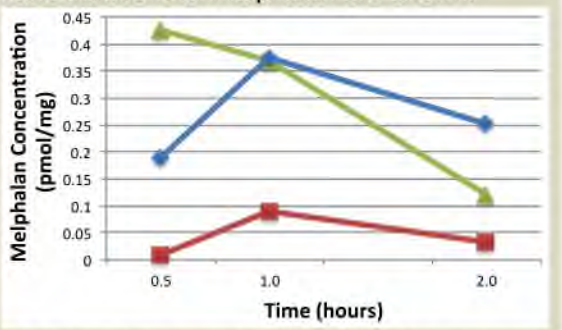
In order to determine the efficacy of each drug against RB for the treatment of vitreous seeds, it is critical to know how long the vitreous seeds in our model will be exposed to each drug. For drugs delivered via IAC, this depends on penetration into the vitreous and the rate of subsequent elimination.[21] To assess this for each drug, one ophthalmic artery of each rabbit (n=5 rabbits per drug) will be catheterized, the drug will be infused, and the vitreous will be serially sampled from each eye and from plasma at the 0.5, 1, 2, 4, and 6 hour time points. We have already shown the ability to reproducibly measure vitreous pharmacokinetics (PK) curves for intra-arterially infused melphalan (Figure 5), and this will be determined for each of the study drugs. For intravitreal delivery, the amount of time that the vitreous seeds are exposed to drug is a function of the rate of diffusion of the drug across the vitreous, and well as the rate of elimination.[41] To assess this, 1µg of each drug will be injected into one eye per rabbit (n=5 rabbits per drug). The injected eye, the uninjected eye, and plasma will be serially sampled to determine PK curves for each (Figure 7). The same time points will be used as above. For the injected eye, sampling will be obtained from the opposite side of the globe, to ensure that all points across the eye have received the drug concentration measured in the sample, as vitreous concentrations are likely to be greatest near the original injections site.

Figure 8. [redacted] PK curve following intravitreal injection.



The above experiments assess the vitreous concentration of each drug. It is also important to assess the retinal tissue concentration as well, given that the majority of disease burden resides within the main retinal tumor mass. This will be accomplished in two complementary ways. First, following IAC treatment with a given drug, rabbits will be euthanized at serial time points (0.5, 1, 2, 4, and 6 hours; 5 rabbits/time point/drug). Retinas will be dissected, harvested, snap frozen, and homogenized. Traditional liquid chromatography tandem mass spectrometry (LC-MS-MS) will then be performed to determine tissue concentration of drug normalized to total weight of retinal tissue. The second set of rabbits (n=5 rabbits/time point/drug) will likewise receive IAC and be euthanized at corresponding time points. Intact eyes will be harvested then snap frozen and sectioned. Retinal drug levels will be determined in intact eye sections using a technique for *in situ* histology-directed imaging mass spectrometry (IMS) that was pioneered at Vanderbilt.[42-45] Our longtime collaborator, Professor Kevin Schey, will assist with these LC-MS-MS and IMS aspects of Aim 1A. He is Co-director for Research for Vanderbilt's Mass Spectrometry Resource Center (MSRC), which is the NIH national resource for IMS and is the largest academic center dedicated to MS and IMS. Our other collaborator, Professor Craig Lindsley, is Co-Director of the Vanderbilt Center for Neuroscience Drug Discovery (CNDD), Director of the Medicinal Chemistry Group, and an expert in PK. He will train me in the techniques of PK analysis and assist with this aspect of Aim 1A.

Figure 9. Melphalan PK curves. Retina accumulates higher melphalan levels than vitreous following IAC. Intra-retinal (blue), vitreous (red) and plasma (green) concentrations of melphalan are shown.



Quality Control in Sample Preparation for Aim 1A: For LC-MS-MS on vitreous and retinal tissue samples: Vitreous and retinal homogenates are spiked with internal standard (carbamazepine), diluted with blank plasma, and deproteinized with acetonitrile. Calibration samples are prepared in parallel by spiking blank plasma with internal standard and known concentrations of drug (50nM-1µM final). A least-squares regression linear calibration curve is constructed for each drug by plotting peak area ratios (analyte/internal standard) vs. theoretical analyte concentrations; a weighting factor of $1/(\text{concentration})^2$ is applied to maintain homogeneity of error at lower drug levels. In the initial experiments, assay precision was 5.7% and 9.2% at the lowest and highest concentration levels respectively. For Imaging Mass Spectrometry: Intact globes are cut on a cryostat into 12 µm thick sections, which are thaw-mounted on gold-coated stainless steel MALDI target plates. MALDI matrix is homogenously applied to the sections. Mass spectra are acquired in an ordered array over the tissue section, with spectra taken every ~50-200 µm. The intensity of the drug at every spot can be plotted as a two-dimensional ion image, showing the localization and relative intensity of the drug over the eye tissue. A drug standard is always run along with the tissue section in order to quality check the MALDI signal.

Potential Pitfalls/Alternative Strategies: It is theoretically possible that we will not be able to determine the intravitreal and retinal tissue doses by IMS. The lower limit of detection (LLOD) for IMS is not as low as that for LC-MS-MS. If we are not able to determine retinal tissue distribution or concentration by IMS for a particular

drug, we will rely solely on the LC-MS-MS concentration for that drug. We have already demonstrated that, with LC-MS-MS, we are able to identify adequately low LLODs for each selected drug in the femtomolar range.

Aim 1B: Determine the cytotoxicity profiles of these drugs against RB cells *in vitro*, based on empiric exposure time

Once PK curves have been calculated for each drug in Aim 1A, the *in vitro* efficacy against the WERI human RB cell line will be determined. WERI cells will be exposed to each study drug at various concentrations *in vitro*, but only for the equivalent of 5 vitreous half-lives. The drug will then be removed and replaced with untreated media, and cell survival measured 7 days later. Seven days was selected as the endpoint since cell death might be delayed and intravitreal injections are given on a weekly basis in clinical practice. Each drug is cleared over time from the vitreous following intravitreal injection based on the above calculated PK curves. Therefore, it does not make sense to determine the *in vitro* cytotoxicity profiles for these drugs based on continuous exposure over the full week. The method we are employing will determine what minimum drug concentration is required in the vitreous to kill the WERI cells if the exposure time at that concentration is only 5 half-lives. We can then back-calculate how much drug must be injected to achieve that concentration for the full 5 half lives, based on the above PK curves. For example, we measured the half life of [redacted] in vitreous to be 1.6 hours (Figure 8). Thus, 5 half-lives is 8 hours. If WERI cells are exposed to [redacted] for 8 hours in vitro, the IC90 is achieved when the [redacted] concentration is [redacted] (Figure 10). Working backwards using the above PK curve, accounting for diffusion as well as the rate of elimination, this corresponds to an initial intravitreal injection of [redacted] This would be the logical starting dose for the intravitreal injection toxicity experiments described in Aim 2A.

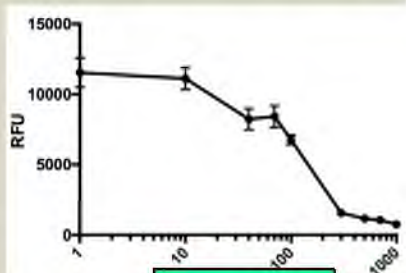


Figure 10. Cytotoxicity profile for [redacted] WERI cells were exposed to topotecan for 5 intravitreal half-lives (8 hours total), then drug was removed and replaced with untreated media. Cells were harvested at 7 days and live cells counted using CellTiter blue assay. [redacted]

Aim 1C: Using a quantitative vitreous seeds rabbit model, assess *in vivo* efficacy of drugs injected intravitreally

In our rabbit model of diffuse vitreous seeds, the exact number of RB cells present in the vitreous can be quantified by *ex vivo* vitreous harvesting, followed by collagenase treatment and flow-assisted cell counting using the green fluorescent protein that we have lentivirally transduced into these cells. After the maximum non-toxic dose has been determined for each drug (see Aim 2A), we will confirm that this non-toxic dose remains effective *in vivo* in our rabbit model. For intravitreous chemotherapy experiments, rabbits will be immunosuppressed with daily subcutaneous cyclosporine injections to permit xenoengraftment, and then the human RB cell line (WERI-Rb1) will be injected into the vitreous and allowed to grow into seeds. [redacted] eyes and saline controls. We have found that daily subcutaneous cyclosporine administration achieves negligible vitreous cyclosporine levels (<2.5 nM), and this low concentration does not contribute to tumor cell killing.

Aim 1D: Using our rabbit model of IAC, assess *in vivo* efficacy of intra-arterial chemotherapies against RB

We have demonstrated that we have a viable and reproducible rabbit model of IAC (see Figures 4,5,9) that achieves consistent vitreous and retinal penetration and pharmacokinetics with the initial drug that we studied, melphalan. In this aim, intraretinal/subretinal RB xenograft tumors will be created in our cyclosporine-immunosuppressed rabbits, as described above (Figure 6). We will determine the *in vivo* efficacy of the study drugs delivered via IAC. [redacted] Each rabbit will receive a total of two monthly IAC treatments of either study drug or saline. One month after the final treatment, tumor size will be measured by ultrasonography and fundus photography. The rabbits will then be euthanized and eyes submitted for histopathology to confirm the proportion of live cells in the tumor mass.

Biostatistical Considerations to Ensure Rigorous Study Design for Aims 1C and 1D:

In Aim 1C, the efficacy of a drug is measured by the mean difference (between the experimental and control groups) in the number of live tumor cells in the vitreous following treatment. In Aim 1D, the efficacy of the drug is measured by the mean difference in tumor size *reduction* between the study drug group and the control (saline) group. Tumor size reduction is defined as [1- ratio of tumor size at the end of treatment to the tumor size before the treatment]. To determine the efficacy (and 95% confidence interval) of **one**

Table 1. Sample size estimation for different effect sizes using t-test (power=0.8, 2-sided type I error=0.05).

Effect size (ratio of mean difference to standard deviation)	N per group	Total N (5 drugs, plus controls)
1.0	17	102
1.5	8	48
2.0	5	30
2.5	4	24
3.0	3	18

drug, using 2-sample t-test with equal variance, the required number of rabbits for each group at different effect sizes are estimated (Table 1) with 80% power and two-sided type I error rate of 0.05. If we expect the mean difference between the two groups to be 0.5 relative to the standard deviation, based on Table 1, we will need 5 rabbits per group for each study drug. Since 5 drugs will be studied, the total number of rabbits may reach to 30 if we use one common control group (5 rabbits/drug group + 1 saline control group).

Potential Pitfalls/Alternative Strategies: It is possible that, for a particular drug, the MTD will be below the effective dose either for IAC or intravitreal delivery. If the dose-limiting toxicity is vascular with IAC, we will reduce the rate of infusion and intersperse pulsatile saline boluses, which will reduce the drug concentration to the local vascular endothelium. This has proven effective in clinical practice, and we have recapitulated this procedure in our rabbit model as well. If there is unavoidable retinal toxicity at the minimum effective dose, we will attempt to treat with a lower dose, and extend the number of treatment sessions beyond the number described above. Using more treatments at a lower dose is similarly effective in patients, to avoid toxicity.

Specific Aim 2: Assess ocular toxicity of select chemotherapies delivered intravitreally and intra-arterially

Aim 2A: Evaluate functional & structural ocular toxicity profiles of repeat intravitreal drug injections *in vivo* in rabbits

(see Biostatistical Considerations – Experimental Design section, below). For example, our preliminary data for suggests that the IC90-equivalent dose of can be injected multiple times without causing ocular toxicity or suppression of ERG amplitudes (Figure 11). The details of how we define toxicity on a rabbit-by-rabbit basis are discussed below (see Biostatistical Considerations – Defining Functional Toxicity). For each drug, once the maximum non-toxic dose has been determined, this dose will then be used in the *in vivo* efficacy experiments described in Aim 1C, above.

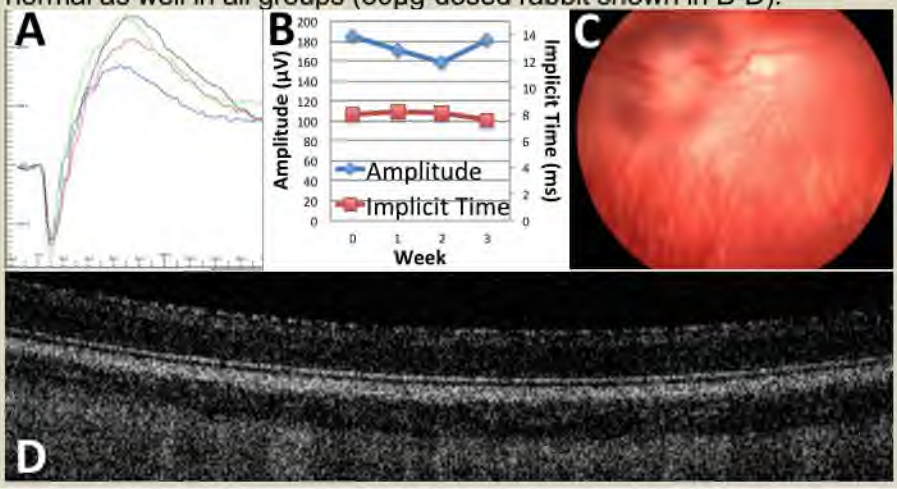


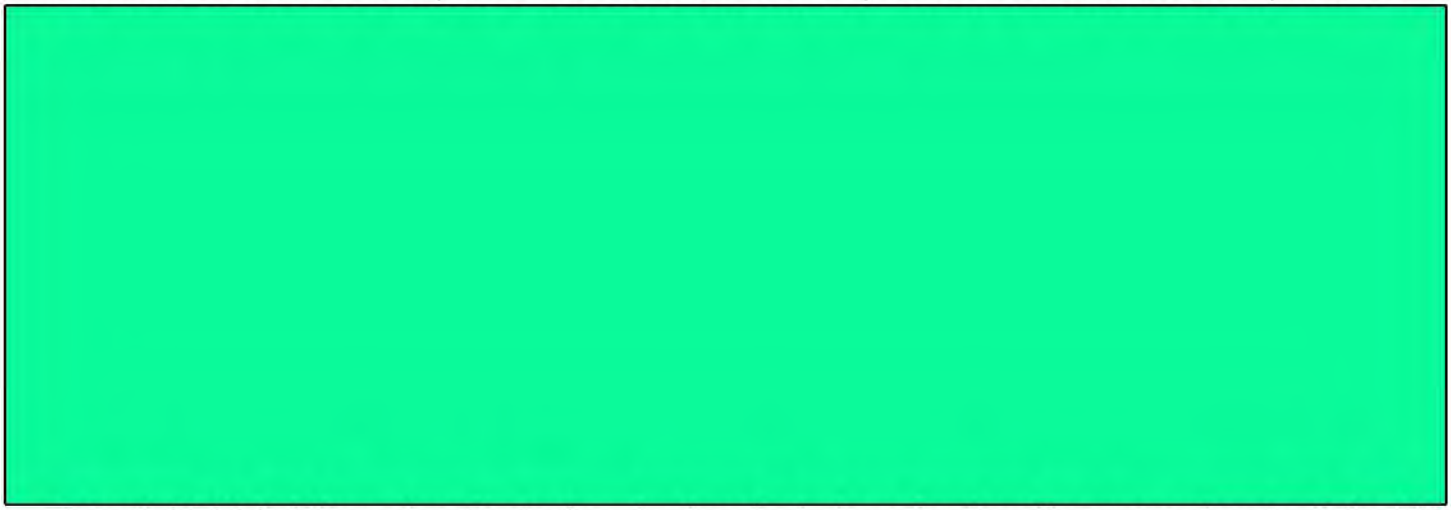
Figure 11. ERG responses to dark-adapted 10cd scotopic white flash, following 3 weekly injections of topotecan 5μg, 15μg, 30μg, or saline control. **A)** ERG responses for each concentration at 4-week time-point. Even at 30μg (equivalent to IC90 of WERI cells), there was no reduction in ERG amplitude compared to baseline. **B)** Graph of 25cd A wave amplitudes and implicit times each week for 30μg topotecan-treated rabbit. Fundus appearance **(C)** and OCT **(D)** both remained normal as well in all groups (30μg-dosed rabbit shown in B-D).

Aim 2B: Evaluate the ocular toxicity profile of intra-arterially delivered chemotherapies in our rabbit IAC model

Biostatistical Considerations to Ensure Rigorous Study Design for Aims 2A and 2B:

Defining Functional Toxicity: Scotopic and photopic recordings will be obtained for each rabbit at each time point. For the intravitreal injections, this will be at baseline, then prior to each subsequent injection (total of 3 injections), and one week following the final injection. In IAC toxicity experiments, ERG recordings will be obtained at baseline prior to the IAC procedure, and then one month later. A wave amplitude, A wave implicit time, B wave amplitude, and B wave implicit time will all be recorded for each flash intensity. Rather than

simply using the average amplitude or implicit time for each, we will use the individual flash level data. For each test, a linear mixed-effects model (time points as the cluster) will be applied to the ERG response data (from the time points listed above) for each parameter for the rabbit being studied. A linear curve against time will be fitted as the fixed-effect. For the fitted curve, we will compare the fitted value at the final time point to the fitted value at the baseline time point. We assume that ERG amplitude will decrease and/or implicit time will

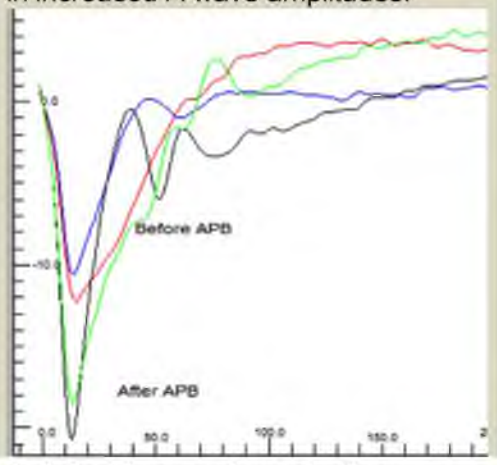


Potential Pitfalls/Alternative Strategies: As described above for the efficacy experiments, if the IAC toxicity appears predominantly vascular in nature, we will reduce the rate of infusion and intersperse pulsatile saline boluses to reduce the dose to local vascular endothelium. This is effective in clinical practice, and we have recapitulated this technique in our rabbit model. If there is unavoidable severe retinal toxicity by either route even at the IC50, we will not pursue that particular drug further in the efficacy experiments (Aims 1C-1D).

Aim 2C: Assess drug toxicity in human retinas using novel *ex vivo* human retina physiologic assessment methods

We have an ongoing collaboration with the scientists at OcuScience, LLC, who have developed a technology to maintain function in *post mortem* human retinas from donor eyes for an extended period of time by sustaining the harvested retina in supportive perfusate. Repeat ERG measurements can then be obtained over time. They have demonstrated the ability to obtain serial ERG measurements from *ex vivo* human retinas and to measure toxicity related to drugs included in the perfusate (Figure 12). Thus, toxicity studies can be performed using human retinal tissue, kept alive after death, from donor eyes. Using the maximum non-retinotoxic (but still effective) intravitreal drug concentrations determined in our *in vivo* rabbit experiments, we will assess toxicity in human retinas using this *ex vivo* technology. The scientists at OcuScience have collaborated with us to develop a technique allowing drug concentration in the perfusate to be adjusted over time to match the PK curves determined in our rabbit experiments (Aim 1A). The current technology allows up to 12 retinal samples to be tested in parallel from each donor eye, thus allowing multiple concentrations and multiple drugs to be tested against matched control samples from the same donor's retina. Both macula and peripheral retinal areas can be tested. This will help control for expected biological variability between donors. ERG responses from the human retinas will be recorded at baseline and at regular intervals over the subsequent week and compared to untreated control tissue from the same donor retina. Each drug/dose will be repeated across at least 3 distinct human donors' retinas.

Figure 12. *Ex vivo* ERG responses before and after introduction of a toxic drug (2-amino-4phosphonobutyric acid [APB], an ON bipolar cell channel blocker) into the perfusate. Note that, as an ON bipolar cell channel blocker, APB toxicity results in *increased* A wave amplitudes.



Future Directions: Once alternative, less-toxic drugs are identified through this proposed research program, they will then be moved to a clinical trial of off-label use in children with advanced intraocular RB. Co-mentor Yu Shyr and the resources of the Vanderbilt-Ingram Cancer Center will be instrumental. The goal is to ultimately proceed to developing novel, RB pathway-targeted inhibitors with minimal ocular toxicity for IAC and intravitreal delivery. With our collaborator, Craig Lindsley, who has expertise in synthesizing targeted inhibitors of pathway proteins, we will be able to assess toxicity and efficacy of candidate compounds using techniques perfected in the above studies.

VERTEBRATE ANIMALS

1. DESCRIPTION OF PROCEDURES

The proposed experiments will evaluate the ocular toxicity, safety, and efficacy of selected chemotherapies against retinoblastoma when delivered by the intra-arterial or intravitreal route. We have developed the first small animal model (in rabbits) of intra-arterial chemotherapy and a rabbit xenograft model of retinoblastoma in which to test these drugs. The tumor models are generated in New Zealand white rabbits immunosuppressed with daily subcutaneous cyclosporine injections (15 mg/kg/day), by injecting the human retinoblastoma cell line, WERI-Rb1, into the subretinal space (to generate retinal tumors) or into the vitreous (to generate vitreous seeds), or both. Note that we have lentivirally transduced the WERI-Rb1 cells to express green fluorescent protein.

Specific procedures on an aim-by-aim basis are described below. The size/age/weight and number of rabbits per experiment are detailed in Table 1.

NOTE: Aims 1B and 2C do not involve the use of animals.

Aim 1A: Determine vitreous and intra-retinal pharmacokinetics of each drug injected intra-arterially or intravitreally

Procedure 1A-1: Vitreous pharmacokinetics after intravitreal injection: All procedures are conducted under general anesthesia and are terminal. Rabbits will be intubated to ensure delivery of inhaled anesthetic, and will be monitored throughout. Animals will receive a single intravitreal injection (<50ul) of each chemotherapy agent (one agent per individual rabbit). Injections will be performed in only one eye per rabbit. In addition, blood sampling from ear vein will be performed to determine the pharmacokinetics of the administered compounds. A standard small-gauge valved vitrectomy cannula will be placed 2mm behind the limbus in each eye, and vitreous humor will be collected by serial taps with a hypodermic needle. Blood will be collected via a percutaneous catheter. Blood and vitreous will be collected at Baseline, 0.5, 1, 2, 4, and 6 hours following intravitreal injection, after which time the animal will be euthanized. Five previously in vitro tested chemotherapeutic compounds will be assessed.

Procedure 1A-2: Vitreous pharmacokinetics after intra-arterial delivery: Under general anesthesia as above, femoral artery access will be obtained following cut down and the artery bathed in verapamil. A microcatheter will be inserted and advanced to the ophthalmic artery under fluoroscopic guidance. During navigation, vasospasm may be encountered and will be treated with intra-arterial nitroglycerin. The internal and external ophthalmic arteries will be identified, and the dominant vascular supply to the eye identified by angiography. Once the microcatheter is in position proximal to or within an ophthalmic artery, a selective angiogram will be performed to assess flow to the eye and retina. A continuous or pulsatile injection of a given study drug will be administered intra-arterially through the microcatheter, with interspersed infusions of saline to improve fluidics. Vitreous will be collected from both eyes by serial taps, as described above. Blood and vitreous will be collected at baseline, 0.5, 1, 2, 4, and 6 hours following intra-arterial injection, at which time the animal will be euthanized. Five previously in vitro tested chemotherapeutic compounds will be assessed.

Procedure 1A-3: Retinal pharmacokinetics following intra-arterial chemotherapy delivery: A single intra-arterial injection of a given drug will be given per rabbit, as described above. Each rabbit will be euthanized at a given time point (0.5, 1, 2, 4, and 6 hours following treatment). A vitreous sample and blood sample will be obtained at that time. Retinal drug concentration will be determined in one group of rabbits by liquid chromatography tandem mass spectrometry, and in the other group of rabbits by imaging mass spectrometry. For the first group (1A-3a in table), following euthanasia, the eye will be removed and the retina harvested and snap frozen prior to analysis. For the second group (1A-3b in table), the eye will be removed following euthanasia and the entire globe will be snap frozen prior to analysis.

Aim 1C: Using a quantitative vitreous seeds rabbit model, assess *in vivo* efficacy of drugs injected intravitreally

Vitreous seed tumors will be generated by direct intravitreal injection of GFP-transduced WERI-Rb1 human RB cell lines into one eye of cyclosporine-immunosuppressed New Zealand white rabbits, as described above. The eye will be monitored weekly by clinical examination and fundus photography to monitor growth of vitreous seeds. Once vitreous seeds are visible clinically, three weekly injections of one of the selected chemotherapy agents will be given. One week following the final injection, the rabbit will be euthanized and the eye removed. The vitreous will be harvested and the number of cells quantified by flow analysis.

Aim 1D: Using our rabbit model of IAC, assess *in vivo* efficacy of intra-arterial chemotherapies against RB

Retinal tumors will be generated by subretinal injection of WERI-Rb1 human RB cell lines into one eye of cyclosporine-immunosuppressed New Zealand white rabbits, as described above. The eye will be monitored weekly by clinical examination, fundus photography, and B scan ultrasonography to monitor growth of the retinal tumor. After 1 month of growth, two intra-arterial treatments will be performed, one month apart, using the procedure described above under Aim 1A. Each rabbit will receive a single study drug or saline control, and will receive the same drug for both treatments. Prior to each treatment, and again one month following the final treatment, the tumor will be measured again by clinical examination, fundus photography, and B scan ultrasonography. The rabbit will be euthanized and the eye removed and submitted for histopathologic analysis.

Aim 2A: Evaluate functional & structural ocular toxicity profiles of repeat intravitreal drug injections *in vivo* in rabbits

All examinations and subsequent injections will be performed under general anesthesia and preemptive analgesia will be administered. The rabbit's pupils will be dilated with tropicamide and cyclopentolate. Each animal will undergo dark adaptation for 1 hour in its cage. Anesthesia will then be induced with ketamine and xylazine. Following the attainment of an adequate anesthetic plane, scotopic and photopic electroretinography recordings will be obtained for the study eye, followed by clinical examination, fundus photography, and optical coherence tomography. An intravitreal injection of drug will be performed The details of dosing and procedure for dose escalation over the course of the study are described in the "Description of Proposed Research" section. Each week, the clinical assessments and imaging studies will be performed prior to intravitreal injection, for a total of three weeks (total of three injections). One week following the third injection, repeat clinical assessments and imaging studies will be performed, then the rabbit will be euthanized and the eye removed and submitted for histopathology.

Aim 2B: Evaluate the ocular toxicity profile of intra-arterially delivered chemotherapies in our rabbit IAC model

Baseline assessments will be performed as described above for Aim 2A under general anesthesia with ketamine and xylazine. However, in addition, an intravenous fluorescein angiogram will be performed and complete blood count (CBC) obtained. The following day, an intra-arterial treatment will be performed. For this, anesthesia will be induced and maintained with isoflurane via intubation, and femoral and ophthalmic artery access obtained with a microcatheter, as described above (see Aim 1A). A single intra-arterial treatment will be performed with a given study drug (or saline control) for each rabbit, as described above (see Aim 1A). CBC will be obtained weekly. After 1 month, repeat clinical assessments, imaging studies, and CBC will be performed, as above. The rabbit will then be euthanized, and the eye will be removed and submitted for histopathology. See "Description of Proposed Research" section for details of dosing and procedure for dose escalation over the course of the study.

Table 1. Number of animals per procedure

Aim	Procedure Number (see above numbering)	Procedure brief description	Rabbit weight at start of procedure	Number of drugs studied	Number of rabbits per drug	**Total number of rabbits**
1A	1A-1	Vitreous PK after intravitreal injection	2.8-3.0 kg	5	5	**25**
1A	1A-2	Vitreous PK after intra-arterial delivery	3.0-3.2 kg	5	5	**25**
1A	1A-3a	Retinal PK after intra-arterial delivery by LC-MS-MS	3.0-3.2 kg	5	25 (5 at each time point)	**125**
1A	1A-3b	Retinal PK after intra-arterial delivery by IMS	3.0-3.2 kg	5	25 (5 at each time point)	**125**
1B	NO ANIMALS USED IN THIS AIM					
1C		Efficacy of intravitreal drugs	2.8-3.0 kg	5 + control	5	**30**
1D		Efficacy of intra-arterial drugs	3.0-3.2 kg	5 + control	5	**30**
2A		Toxicity of intravitreal drugs	2.8-3.0 kg	5 + control	10	**60**
2B		Toxicity of intra-arterial drugs	3.0-3.2 kg	5 + control	10	**60**
2C	NO ANIMALS USED IN THIS AIM					
					TOTAL NUMBER OF RABBITS:	**480**

2. JUSTIFICATION FOR USE OF ANIMALS AND CHOICE OF SPECIES

In order to assess the *in vivo* efficacy and ocular toxicity of the selected chemotherapeutic agents, it is necessary to conduct these experiments in live animals.

While mice and rats have been used previously to test intravenous chemotherapy, it is not technically possible to place an endovascular catheter into the ophthalmic artery of these smaller animals. In addition, one cannot inject chemotherapy directly into their eyes and be certain not to disrupt a growing tumor or vitreous seeds. At the other end of the spectrum, porcine and non-human primates have been used to develop intra-arterial chemotherapy models. However, we did not choose these existing models for two main reasons: 1) There is no current model of retinoblastoma in either pigs or non-human primates, and therefore efficacy of drugs could not be assessed, and 2) non-human primates and pigs would represent higher sentient vertebrates, and would thus not be favored. In contrast, rabbits were selected for several reasons: 1) Rabbits can be immunosuppressed with cyclosporine to permit xeno-engraftment, 2) The rabbit eye is large enough to safely inject drug intravitreally into a tumor-bearing eye, 3) Prior pharmacokinetic studies of melphalan have been performed in rabbit eyes, 4) Electroretinography standards are well-known for rabbits, and 5) The larger-sized New Zealand White Rabbit is already an established model organism for many other endovascular procedures, having been used in dozens of prior studies in the interventional neuroradiology field. One disadvantage of rabbits is that they are merangiotic, with the retinal vessels lying immediately above the internal limiting membrane (as compared to humans where the vessels lie below the internal limiting membrane). However, our preliminary data demonstrates that intra-arterial chemotherapy delivery in our rabbit model remains consistent and reproducible despite this fact.

3. Minimization of Pain and Distress

Analgesics (Buprenex, ketoprofen, and/or meloxicam) and anesthetics (isoflurane and/or ketamine/xylazine) will be used throughout all rabbit experimental procedures for the alleviation of potential distress, discomfort, pain, and injury that may occur in the experimental and/or surgical settings. In addition, rabbits will be monitored for any signs of distress or pain by both Dr. Daniels and his laboratory personnel, as well as by staff veterinarians and animal care personnel, throughout the post-operative and treatment protocols. All rabbits will be allowed ~1 week to acclimate after shipment from the vendor prior to being used in any experiments.

4. Methods of Euthanasia

Euthanasia will be performed by a barbiturate overdose followed by thoracotomy, which is an acceptable AMVA method of euthanasia.

SELECT AGENT RESEARCH

Select agent research is not applicable to this proposal

RESOURCE SHARING PLAN

This research will comply with both the letter and spirit of the NIH Statement on Sharing Research Data issued on February 26, 2003 while complying with local, state and federal laws and regulations. It is the intention to make available final research datasets gathered during this research performed in a timely manner after publishing said data. The PI supports sharing of the research data and resources to promote rapid advances in vision and cancer research. Should this project develop into any scientific advancement, the PI will be more than willing to share this with the scientific community.