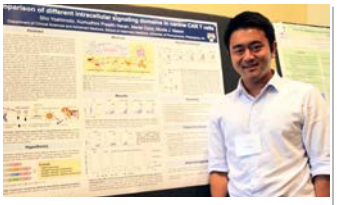

ANNUAL FACULTY RESEARCH RETREAT

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WEAR WHITE FOR CANCER RESEARCH

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GENE THERAPY PROGRAM IN OPHTHALMOLOGY

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SCIENCE WITH STUDENTS

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NEWSLETTER

What about regenerative medicine?

Dr. **Andrew Vaughan** is an assistant professor (tenure track) in the Department of Biomedical Sciences. Dr. Vaughan received a B.S. in Biochemistry from the University of Nebraska before earning his Ph.D. in molecular and cellular biology from the University of Washington. In Seattle, he worked with gene therapy

Little known cells may explain how influenza leads to Asthma

Continued on page 2

pioneer Dr. Dusty Miller at the Fred Hutchinson Cancer Research Center. Having gained an interest in pulmonary regeneration due to his work with the Jaagsiekte retrovirus, a causative agent of lung cancer in sheep, Dr. Vaughan pursued a postdoctoral fellowship with Dr. Hal Chapman at the University of California San Francisco, studying somatic stem cells and lung injury repair.

How do we think about regenerative medicine?

In a 2001 episode of South Park, the ever-scheming Eric Cartman plots to obtain large quantities of embryonic stem cells through nefarious means. His ultimate goal—to clone his favorite restaurant, Shakey's Pizza. While an obviously absurdist take on the potential of stem cell therapy, the biomedical community has been dogged by similarly simplistic notions of how cell therapy might work. Countless studies have claimed that injecting ill-defined "stem cells" into a damaged tissue will regenerate that organ or otherwise provide therapeutic benefits. The existence of clandestine "stem cell clinics" further cements these unrealistic expectations of stem cell-based therapies.

While stem cells are often considered essential for regenerative medicine, many of our fully developed somatic tissues already possess great capacity for regeneration. Indeed, the liver has been recognized as especially regenerative since Classical Greece, and similar reparative properties of the intestine and epidermis are well known. Based on his interest in lung injury repair, the Vaughan laboratory set out to determine if they could capitalize on these inherent capabilities, harnessing and directing the preexisting capacity for adaptive repair.

Influenza presents a particular challenge for lung repair, given that large regions of epithelium are completely destroyed by infection[1]. Nonetheless, many (but not all[2-4]) people recover completely, highlighting a previously under-appreciated capacity of the lung for repair. However, lung regeneration turns out to be quite complex at the cellular level and involves multiple players. While still a postdoc, Dr. Vaughan identified a rare progenitor cell type, similar to basal cells in the upper airway, that expands dramatically to re-cover the denuded alveoli in heavily injured areas [5, 6]. While highly migratory and proliferative, these cells (marked by cytokeratin 5 expression [krt5]) do not restore function to gas-exchanging alveoli. Current models recognize these dysplastic cells as an effective emergency response[7], but their long-term persistence is maladaptive, and is likely

Continued on page 4

Publications

S Singh, E Elenio, N A Leu, R-A Romano, A E **Vaughan**, J DeRiso, Kameswaran S and **Rumela Chakrabarti** (2019) A new Elf5Cre ERT 2- GFP BAC transgenic mouse model for tracing Elf5 cell lineages in adult tissues. *FEBS Lett.* 593(10): 1030-1039

Tait Wojno, Elia D; **Hunter, Christopher A**; Stumhofer, Jason S (2019) The Immunobiology of the Interleukin-12 Family: Room for Discovery. *Immunity* 50 (4): 851-870

Casal ML, Engiles JB, Zakošek Pipan M, Berkowitz A, Porat-Mosenco Y, Mai W, Wurzburg K, Xu MQ, Allen R, ODonnell PA, **Henthorn PS**, Thompson K, Shore EM (2019) Identification of the Identical Human Mutation in ACVR1 in two Cats With *Fibrodysplasia Ossificans Progressiva*. *Vet Pathol.* 56(4):614-618

Annual Faculty Research Retreat 2019—This year's 25th faculty research retreat entitled "Social Entrepreneurship in Research" was held at the Inn at Swarthmore on June 14 with 150 in attendance. Vice Dean for Research, Phillip Scott opened the symposium by reviewing the history of the annual retreat. Bruce Freedman, chaired the faculty organizing committee consisting of Phillip Scott, Nicola Mason, Giacomo Gianotti, Anna Kashina, Andrew Vaughan, Patricia Mundy, Barbara Dallap-Schaer, Raimon Duran-Struuck, and Molly Church. Dan Rader, Seymour Gray Professor of Molecular Medicine, Perelman School of Medicine, delivered the **Marshak Lecture** entitled "A Genome-first Approach to Understanding Biology, Disease, and Therapeutic Targets". Speakers included Keiko Miyadera (Clinical Sciences and Advanced Medicine, Kotaro Sasaki, Kyla Ortved, Daniel Beiting, Nicola Mason, Boris Striepen, and Ron Harty. A new event was added to the program—the ***Poster Blitz.*** Twenty-four poster participants gave a scintillating one-minute pitch on the greatness of their poster. Winners of the poster competition were as follows: Snahlata Singh 1st Place (Rumela Chakrabarti laboratory), Christina Cho 2nd Place (Serge Fuchs laboratory), and a tie for 3rd place — Sho Yoshimoto (Nicola Mason laboratory) and Ratnesh Srivastava (Rumela Chakrabarti laboratory). Biomedical Sciences Chairperson, Dr. Ellen Puré awarded the **Zoetis Prize** for Veterinary Research Excellence to Dr. Serge Fuchs.

Ellen Puré with Serge Fuchs

Bruce Freedman, Michael May and Ron Harty at the retreat

Sabina Hlavaty in the *Poster Blitz*

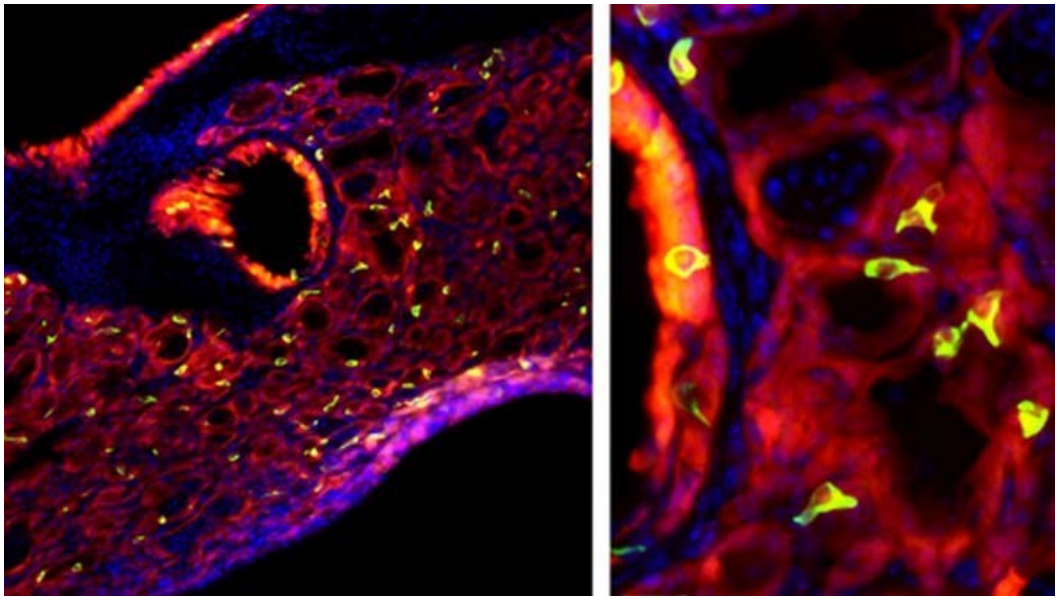
Kyla Ortved delivers her talk

Poster winners

continued from page 2

the source of so-called "bronchiolization and "honeycomb cysts" in patients with interstitial lung diseases / fibrosis[6].

Notably, other progenitor cells in the lung are much more effective at reconstituting function in regenerating alveolar epithelium. Alveolar type 2 cells (AT2s) are abundant in normal lung, where they produce the surfactant necessary to keep alveolar surface tension low, reducing the work of breathing. AT2s have been long recognized as resident progenitor cells, capable of self-renewal and differentiation into the gas-exchanging type 1 cells[8]. These cells are also lineage restricted, as Dr. Vaughan's fate mapping analysis showed that they are unable to give rise to the aforementioned dysplastic cell types. Therefore, "euplastic" regenerative responses from AT2 progenitors are generally beneficial for recovery. Unfortunately, given that AT2 cells are a direct target of many lung injuries, their regenerative capacity can be overwhelmed.



Tuft cells expressing DCLK1 (green) arise in injured alveoli after influenza infection. These cells are initially derived from progenitor cells present in the airways (p63 lineage trace, red).

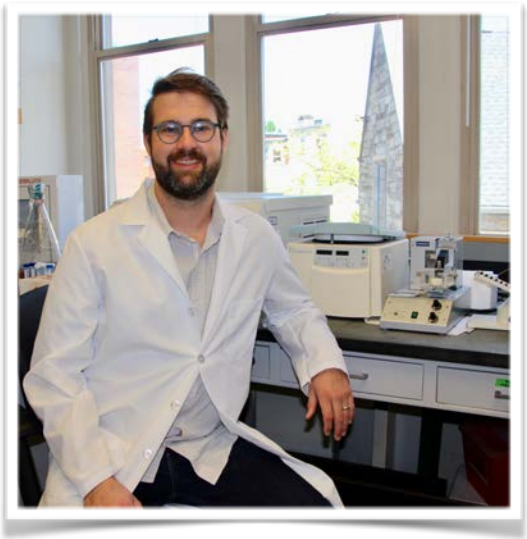
Recognizing the limitations of endogenous AT2s, Dr. Vaughan's group proceeded to artificially supplement this key progenitor population after H1N1 influenza infection. Utilizing an orthotopic lung transplantation assay, they showed that transplanted healthy AT2 cells proliferated dramatically and retained their AT2 identity, exhibiting efficient euplastic differentiation. This even resulted in improved gas exchange capacity, as measured by pulse oximetry, despite the low engraftment rate of transplanted cells. Dr. Vaughan is currently determining the transcriptional repertoire of these transplanted cells to identify key genes involved in their successful engraftment. By deciphering this "engraftome", the Vaughan lab hopes to identify target genes that could be modulated to enhance the progenitor cell potential of native AT2s to promote more effective regeneration.

Dr. Vaughan's laboratory is also interested in the dysplastic "emergency" regeneration that occurs after influenza. While examining the persistence of the Krt5+ cells, they noticed that many of these cells differentiate into solitary chemosensory cells (SCCs), AKA tuft cells. While SCCs were first observed nearly 100 years ago, they have only been recently recognized as the sole source of an important cytokine, IL-25, required for type 2 inflammation, the immune response associated with parasite infections and allergic asthma. Curiously, they are essentially identical to type 2 taste bud cells, utilizing taste sensing "gustatory" pathways to detect molecules associated with various pathogens.

SCCs are absent from the lung in naïve mice, but are normally present in the nasal passages and small intestine, where they are central mediators of both immunity and tissue remodeling / differentiation. Using compounds that exclusively activate tuft cells, Dr. Vaughan showed that injured lungs containing SCCs exhibit acute inflammation upon stimulation. Moreover, administration of these ligands promoted the appearance of even more tuft cells, indicating the presence of a feed-forward loop. Based on tuft cell-mediated metaplasia in the small intestine, the Vaughan lab predicts that these ectopic tuft cells perpetuate the surrounding dysplastic epithelium. These initial studies were recently published in the *American Journal of Physiology – Lung Cellular and Molecular Physiology*, and were highlighted on the NIH Director's Blog, https://directorsblog.nih.gov/tag/tuft-cells/

Taken together, Dr. Vaughan's work reveals that the contribution of competing subtypes of stem/progenitor cells result in qualitatively and functionally disparate outcomes of regeneration. To return to the South Park anecdote, instead of crudely injecting cells into diseased tissues, Dr. Vaughan hopes to identify environmental cues and paracrine signals that empower the regenerative properties the lung already possess to therapeutically minimize dysplastic responses while maximizing adaptive regenerative pathways that restore function. Cloning a pizza parlor in the process will just be considered an extra perk!

Dr. Vaughan's research is funded by the NIH/NHLBI (R00HL131817) and the Landenberger Research Foundation. His laboratory is located in the Old Vet Building 366E and his office in 370E.



Kwaku Quansah and Nandy Costa of the Vaughan Laboratory

Dr. Vaughan—continued from Page 5

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4. Chen, J. *et al.* Long term outcomes in survivors of epidemic Influenza A (H7N9) virus infection. *Sci Rep* **7**, 17275 (2017).
5. Xi, Y. *et al.* Local lung hypoxia determines epithelial fate decisions during alveolar regeneration. *Nat Cell Biol* **19**, 904-914 (2017).
6. Vaughan, A.E. *et al.* Lineage-negative progenitors mobilize to regenerate lung epithelium after major injury. *Nature* **517**, 621-625 (2015).
7. Zuo, W. *et al.* p63(+)Krt5(+) distal airway stem cells are essential for lung regeneration. *Nature* **517**, 616-620 (2015).
8. Evans, M.J., Cabral, L.J., Stephens, R.J. & Freeman, G. Transformation of alveolar Type 2 cells to Type 1 cells following exposure toNO2. *Experimental and Molecular Pathology* **22**, 142-150 (1975)

Recent Awards (direct costs)

Wm Beltran
FDN for Fighting Blindness—Canada.
Preclinical evaluation of an iPSC-derived photoreceptor therapeutic in canine model of retinitis pigmentosa $380,918
1/9/19—12/31/20

Wm Beltran
FDN for Fighting Blindness—Canada
RV-20/20- Development of a Neogenin Based-Strategy to Prevent Photo-receptor Degener-ation $250,000
7/1/19-- 7/31/19

Andres Blanco
Leukemia Research FDN—Dual targeting of LSD1 and KAT6A to induce therapeutic differentiation in AML
$100,000 7/1/19—6/30/20

Kotaro Sasaki
Open Philanthropy
Human germ cell development and its *in vitro* reconstitution. $600,000 4/1/19–3/31/22

Bruce Freedman
NIH/AI R21 Novel Mechanisms of c-Rel Dependent Thymic Regulatory T Cell Development $275,000
6/21/19—5/31/21

Karina Guziewicz
Iveric bio Inc.
Gene Therapy for BEST1-associated macular dystrophies- Projects 1-3
$556,715 1/30/19–1/28/21

John Wolfe
NIH/NS —Translational studies on cerebrospinal fluid (CSF)-directed gene therapy for global neurometabolic brain
$1,004,205 4/1/19–3/31/24

James Marx
ASLAP Foundation Summer Fellowship Program
$2,500 6/1/19—8/31/19

Lisa Murphy/Julie Ellis
PA Game Commission—Comprehensive Wildlife Health Program for the State of Pennsylvania
$6,773,595 5/1/19—4/30/24

Igor Brodsky
Mark Foundation—Rational design of novel combination immunotherapies: enhancing the cytotoxic activity of chimeric antigen receptor T cells using the SMAC mimetic birinapant.
$10,089.00 2/4/19—8/31/20

Boris Striepen
NIH/AI R01 Genetic Analysis of *Cryptosporidium.*
$ 1,250,000 5/1/19—4/30/24

Daljit Vudathala
Pa Commonwealth—Influence of Hemolysis on Nutritional Mineral Analysis of Bovine and Equine Serum $10,940 1/1/19—9/30/19

Andres Blanco
NIH/NCI K22 The role of the histone chaperone Chaf1b in sustaining the Hoxa9-driven AML differentiation block.
$ 573,844 9/1/18—8/31/21

Rumela Chakrabarti
Amer. Cancer Society: DLL1 Mediated Notch Signaling in Tamoxifen Resistance of Breast Cancer
$660,000 7/1/19-6/30/23

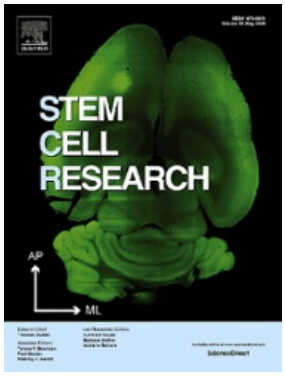
Publications

Sateriale A, Šlapeta J, Baptista R, **Engiles JB**, Gullicksrud JA, Herbert GT, Brooks CF, Kugler EM, Kissinger JC, **Hunter** CA, **Striepen B**. (2019) A Genetically Tractable, Natural Mouse Model of *Cryptosporidiosis* Offers Insights into Host Protective Immunity. *Cell Host Microbe* S1931-3128 (19)30251-3.

Sorenmo KU, Durham AC, Kristiansen V, Pena L, Goldschmidt MH, and **Stefanovski D** (2019) Developing and testing prognostic bio-scoring systems for canine mammary gland carcinomas. *Vet Comp Oncol.* 2019 May. [Epub ahead of print]

Predina JD, **Runge** J, Newton A, Mison M, Xia L, Corbett C, Shin M, Sulyok LF, **Durham** A, Nie S, Singhal S, and **Holt D** (2019) Evaluation of Aminolevulinic Acid-Derived Tumor Fluor-escence Yields Disparate Results in Murine and Spontaneous Large Animal Models of Lung Cancer. *Sci Rep* 9(1):7629

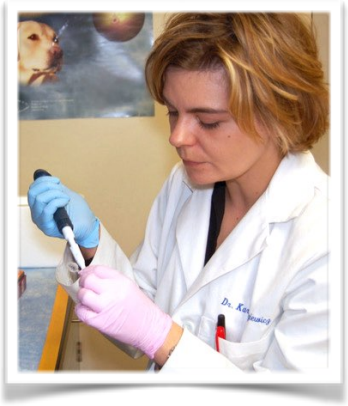
Wear white for cancer research was celebrated on **June 14th** at the Annual Faculty Research Retreat— a group photo commemorating the Penn Vet Cancer Research Center's support of a future immune to cancer. The Wear White event was promoted by the Cancer Research Institute in an effort to raise awareness about the lifesaving potential of immunotherapy. By wearing white, the group stood together for science and the search for immune-based cures.

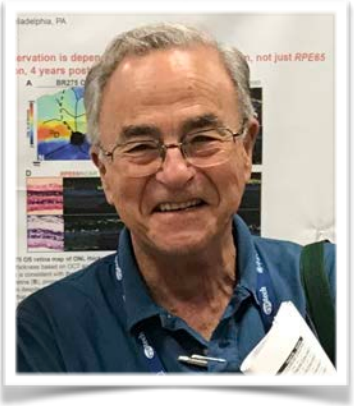


Yang, Feikun, Zhang A, and **Richardson, DW** (2019) Regulation of the tenogenic gene expression in equine tenocyte-derived induced pluripotent stem cells by mechanical loading and Mohawk. *Stem Cell Res* 39: 101489 https://doi.org/10.1016/j.scr.2019.101489

Lieberman A, Barrett R, Kim J, Zhang KL, Avery D, Monslow J, Kim H, Kim BJ, **Puré E** and Ryeom S. (2019) Deletion of calcineurin promotes a pro-tumorigenic fibroblast phenotype. *Cancer Res* canres. 0056.2019, Epub ahead of print

DeLaney AA, Berry CT, Christian DA, Hart A, Bjanes E, Wynosky-Dolfi MA, Li X, Tummers B, Udalova IA, Chen YH, Hershberg U, **Freedman BD, Hunter CA, Brodsky** IE (2019) Caspase-8 promotes c-Rel-dependent inflammatory cytokine expression and resistance against Toxoplasma gondii. *Proc Natl Acad Sci U S A.* 116(24):11926-11935.





An exclusive license agreement was issued with Ophthotech (*now **Iveric bio**)* for Drs. Karina Guziewicz and Gustavo Aguirre's global BEST1 gene therapy program. Proof-of-concept studies demonstrating that the AAV-*BEST1* gene therapy product candidate restored the anatomy between photoreceptors and retinal pigment epithelial (RPE) cells in the naturally occurring canine disease model with distinct phenotypic similarities to human bestrophinopathies were published in March 2018 in the journal *Proceedings of the National Academy of Sciences*(PNAS), titled: "*BEST1 gene therapy corrects a diffuse retina-wide micro-detachment modulated by light exposure*." Drs. Guziewicz and Aguirre are members of Penn Vet's Department of Clinical Sciences and Advanced Medicine.

CANINE—Involving students in research, Penn Vet's Jennifer Punt, VMD, PhD, recruited students from high schools and undergraduate programs to pair them with Penn veterinary students in the field of canine immunology studies. They call the program CANINE—for canine immunity in education. See more about this program in Katherine Unger Baillie's article: https://penntoday.upenn.edu/news/canine-cancer-immunity-education

5 Steps to Cure Sarcoma—Drs. Nicola Mason and Kathryn McGonigle reported on the Penn Vet team who participated in the 5K run (and walk) and fundraiser on May 31. Collectively $135,000 was raised for **sarcoma research** and the day brought 1700 people together to celebrate loved ones—both people and dogs who have been affected by sarcoma!

Osteosarcoma is an aggressive cancer that frequently arises in the long bones of large-breed dogs. Approximately 90-95% of dogs with osteosarcoma have undetectable metastatic disease at presentation. Targeted immunotherapy has demonstrated remarkable therapeutic effects in a phase 1 trial of canine osteosarcoma.

The PennVet team included staff as well as pet parents, all excitedly supporting Dr. Mason's influential research

The **Penn Vet Research Newsletter** is distributed quarterly. Suggestions, comments, requests and story ideas may be directed to: resnews@vet.upenn.edu

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