OVERVIEW AND SYLLABUS (REVISED 12/2/21)
CAMB 633 – Advanced Seminar in Gene Therapy
Spring 2022
Weekday (Jan 20 – May 5); Thursday 3.30 PM – 5.00 PM

COURSE GOALS:

The course will provide students with a conceptual framework for the critical appraisal of the literature and seminar presentations. There are several specific goals for this course. One is to introduce students to current approaches in the field of gene therapy and ask them to clearly and critically review select articles from the literature for rigorous evaluation of a specific hypothesis through experimental design and data interpretation, with emphasis on scientific rigor and reproducibility in this field. A second goal is to challenge students' understanding of the spectrum of potential applications of gene and cell therapy technologies, but also to critically consider the ethical boundaries of these novel approaches. A third goal is to discuss how, some of these novel therapies, are utilized to create partnerships with industry or launch a novel biotech company. A fourth goal is to investigate the requirements to bring a novel drug to the FDA for its approval. These goals will be achieved through paper reviews, lectures and class discussions.

COURSE DESCRIPTION:

Prerequisites: CAMB 633 does not attempt to cover all aspects of gene therapy, and is therefore not appropriate for students seeking a lecture course that provides a comprehensive survey of the field. For students who already have notions of cell manipulation, immunotherapy and vector biology. Suggested courses are: CAMB 610 (Molecular Basis of Gene Therapy); at least one course in immunology.

Structure of the course: Each student will be responsible to lead discussion of several scientific manuscripts. The number of papers will be defined by the number of students enrolled in this class.

This class will give priority to GTV students, followed by CB, and MVP students, but also accept other students with the required prerequisites. Enrollment is capped at 12 students, but can be increased based on student interest.

Class: Each class will involve a review of a manuscript in the field of gene therapy selected by course faculty (Drs. Kurre, Pardi, Melenhorst or Rivella). At the beginning of the course students and faculty will assign primary research papers and potentially review articles that provide relevant background to the students. The assigned student presenter will prepare slides covering background and paper figures and mis encouraged to meet with the faculty lead ahead of time. Two faculty will be present for each class. The student leader will introduce the paper and ask the group to each cover a portion of the result section in order to promote discussion, interaction and participation. Emphasis will be placed on technical rigor and reproducibility as well as the broader scientific context. Each session will last 1 hour, including presentation of the manuscripts and Q&A. Each session will cover one manuscript on a weekly base, alternating these classes with lectures (see below). Each presentation will be utilized to grade the students (50%).

Lectures: during each lecture, a faculty or external speakers will lecture for 40 minutes followed by a 15 minutes breakout discussion. The student will attend and ask questions during or at the

end of the lecture. These lectures will happen independently from the paper discussion. Dr. Kurre, or a substitute, will also be present at each lecture.

COURSE GRADE:

Grades will be based on the quality of the seminar and participation in discussion. The course grade will be based on: 50% on paper presentation and 50% on participation. Participation will be evaluated by attendance, engagement in prompted and/or unprompted discussions. The faculty will guide and coordinate the conversation during the presentation. The presentation will last ~45 minutes. During the last ten minutes, the students will be encouraged to discuss if and how this paper will impact the field of gene therapy, including potential future outcomes. The presentations will be graded, and the participating faculty and/or course director should provide feedback at the end of class.

COURSE DIRECTOR: Peter Kurre
CO-DIRECTORS: Stefano Rivella, Norbert Pardi, Joseph Melenhorst

Faculties:
Peter Kurre (Perelman SOM, Department of Pediatrics, Division of Hematology)
Dr. Kurre will focus on aspects of Gene Therapy that relate to the unique biology of the Hematopoietic Stem Cells (HSC) commonly targeted for genetic correction in monogenic diseases. In depth discussion of these studies will aid our understanding of key HSC traits and clonal dynamics within the compartment. The papers explore the importance of disease specific phenotypes that are often central to the overall translational strategy and successful clinical outcomes. Foundational aspects of HSC biology, access, targeting, conditioning, *in vivo* selection and stem cell clonality will be covered.

The number of papers that will presented will depend on the number of students attending the course.

PROPOSED ARTICLES FOR DISCUSSION (Title / link/ • rationale)

1) Hematopoietic Stem Cells Count and Remember Self-Renewal Divisions.
https://www-ncbi-nlm-nih-gov.proxy.library.upenn.edu/pubmed/27839867
- This article examines divisional history and function of human HSC, providing a context for our understanding of HSC targeted for gene transfer.

2) Rapid mobilization of murine and human hematopoietic stem and progenitor cells with AMD3100, a CXCR4 antagonist
https://www-ncbi-nlm-nih-gov.proxy.library.upenn.edu/pubmed/15837815
- This article discusses strategies for HSC mobilization that are central to ex vivo HSC directed Gene Therapy.

3) Structural basis for the recognition of LDL-receptor family members by VSV glycoprotein
https://www-ncbi-nlm-nih-gov.proxy.library.upenn.edu/pubmed/29531262
Mystery solved: VSV-G-LVs do not allow efficient gene transfer into unstimulated T cells, B cells, and HSCs because they lack the LDL receptor
https://www-ncbi-nlm-nih-gov.proxy.library.upenn.edu/pubmed/?term=verhoyen+ldl
- These companion papers examine core principles of vector surface engineering for HSC targeting

4) Transfusion independence and HMGA2 activation after gene therapy of human □-thalassaemia
https://www-ncbi-nlm-nih-gov.proxy.library.upenn.edu/pubmed/20844535

- This article examines clinical outcomes and evidence for lineage specific transcriptional deregulation of HMGA2 after gene transfer to human HSPC from b-thalassemia patients.

5) Lentiviral Gene Therapy Combined with Low-Dose Busulfan in Infants with SCID-X1
https://www-ncbi-nlm-nih-gov.proxy.library.upenn.edu/pubmed/30995372

- This paper presents clinical outcomes of a small cohort of patients with immunodeficiency who received conditioning prior to infusion of gene corrected HSC.

6) Successful engraftment of gene-corrected hematopoietic stem cells in non-conditioned patients with Fanconi anemia
https://www-ncbi-nlm-nih-gov.proxy.library.upenn.edu/pubmed/31501599

- This article reports outcomes of a pilot trial for HSPC directed gene transfer to Fanconi Anemia patients and provides a good basis on which to discuss questions of stem cell clonality for restored hematopoietic function. Additionally, it touches on the notion of inherent selection for genetically modified cells.

Norbert Pardi (Perelman SOM, Department of Medicine, Division of Infectious Diseases)
Dr. Pardi will focus on the use of antibodies and RNA molecules for immunological but also gene editing therapies.

1) mRNA vaccines for infectious diseases
https://www.ncbi.nlm.nih.gov/pubmed/23159882

- This article provides proof-of-concept for the protective efficacy of mRNA vaccines using non-replicating in vitro transcribed mRNA.

2) Monoclonal antibody therapy using mRNA
https://www.ncbi.nlm.nih.gov/pubmed/28794134

- This article discusses applications of mRNA for antibody gene transfer.

3) mRNA-based therapy for protein replacement
https://www.ncbi.nlm.nih.gov/pubmed/28202722

- The authors demonstrated that therapeutic levels of factor IX can be achieved by mRNA delivery in a preclinical mouse model of hemophilia B.

4) Gene editing with mRNA therapeutics
https://www.ncbi.nlm.nih.gov/pubmed/29499956

- This article provides proof-of-concept for the applicability of mRNA therapeutics for in vivo gene editing.

Joseph Melenhorst (Perelman SOM, Department: Pathology and Laboratory Medicine)
Dr. Melenhorst will focus on current challenges and progress in immunogene therapy of cancer.

CARs: Mechanisms of response

1) Fry, T.J., Shah, N.N., Orentas, R.J., Stetler-Stevenson, M., Yuan, C.M., Ramakrishna, S., Wolters, P., Martin, S., Delbrook, C., Yates, B., et al. (2018). CD22-targeted CAR T cells induce remission in B-ALL that is naive or resistant to CD19-targeted CAR immunotherapy. Nat. Med. 24, 20–28.
https://www.ncbi.nlm.nih.gov/pubmed/29155426

2) Finney, O.C., Brakke, H., Rawlings-Rhea, S., Hicks, R., Doolittle, D., Lopez, M., Futrell, B., Orentas, R.J., Li, D., Gardner, R., et al. (2019). CD19 CAR T cell product and disease attributes predict leukemia remission durability. J. Clin. Invest. 129, 2123–2132.
https://www.ncbi.nlm.nih.gov/pubmed/30860496

- These two papers contribute to our growing understanding of clinical responses to immunogene therapies wherein the malignancy is targeted using autologous chimeric antigen receptor (CAR)-engineered T cells.

Immunogenicity

3) Cassotta, A., Mikol, V., Bertrand, T., Pouzieux, S., Le Parc, J., Ferrari, P., Dumas, J., Auer, M., Deisenhammer, F., Gastaldi, M., et al. (2019). A single T cell epitope drives the neutralizing anti-drug antibody response to natalizumab in multiple sclerosis patients. Nat. Med. 25, 1402–1407.
https://www.ncbi.nlm.nih.gov/pubmed/?term=31501610

4) Xu, J., Chen, L.-J., Yang, S.-S., Sun, Y., Wu, W., Liu, Y.-F., Xu, J., Zhuang, Y., Zhang, W., Weng, X.-Q., et al. (2019). Exploratory trial of a biepitopic CAR T-targeting B cell maturation antigen in relapsed/refractory multiple myeloma. Proc. Natl. Acad. Sci. 201819745.
https://www.ncbi.nlm.nih.gov/pubmed/?term=30988175

- Immunogenicity is still an issue with various gene therapies and both papers here nicely illustrate the dramatic impact of a single T cell epitope in a neutralizing, humanized antibody in determining efficacy in a non-cancer setting and the immunogenicity of non-human species CAR T cells.

Clonal expansion due to insertional mutagenesis

5) Shah, N.N., Qin, H., Yates, B., Su, L., Shalabi, H., Raffeld, M., Ahlman, M.A., Stetler-Stevenson, M., Yuan, C., Guo, S., et al. (2019). Clonal expansion of CAR T cells harboring lentivector integration in the CBL gene following anti-CD22 CAR T-cell therapy. Blood Adv. 3, 2317–2322.
https://www.ncbi.nlm.nih.gov/pubmed/?term=31387880

6) Espinoza, D.A., Fan, X., Yang, D., Cordes, S.F., Truitt, L.L., Calvo, K.R., Yabe, I.M., Demirci, S., Hope, K.J., Hong, S.G., et al. (2019). Aberrant Clonal Hematopoiesis following Lentiviral Vector Transduction of HSPCs in a Rhesus Macaque. Mol. Ther.
https://www.ncbi.nlm.nih.gov/pubmed/?term=31023523

- We have demonstrated the profound impact of a lentiviral vector insertion in the kinetics and therapeutic efficacy in a leukemia patient. The above papers similarly identify insertional mutagenesis in a human CAR T cell trial (Shah et al.) and the clonal tracking in a non-human primate model (Espinoza). No mechanism, but sufficient material to start the discussion around vector-related oncogenicity – concern or not.

Augmenting CAR T cell efficacy

7) Choi, B.D., Yu, X., Castano, A.P., Bouffard, A.A., Schmidts, A., Larson, R.C., Bailey, S.R., Boroughs, A.C., Frigault, M.J., Leick, M.B., et al. (2019). CAR-T cells secreting BiTEs circumvent antigen escape without detectable toxicity. Nat. Biotechnol. 1–10.
https://www.ncbi.nlm.nih.gov/pubmed/?term=31332324

8) Xu, Y., Lv, L., Liu, Y., Smith, M.D., Li, W.-C., Tan, X., Cheng, M., Li, Z., Bovino, M., Aubé, J., et al. (2019). Tumor suppressor TET2 promotes cancer immunity and immunotherapy efficacy. J. Clin. Invest. 129, 4316–4331.
https://www.ncbi.nlm.nih.gov/pubmed/?term=31310587

- Current immunogenicity trials show plenty of efficacy but also resistance to immunotherapies. These two papers explore means to boost the anti-tumor response.

LECTURES

Approaching industry
Speaker: Dana Hammill
The purpose of conducting research is to seek truth and expand knowledge for the benefit of social development. Oftentimes, new technologies can be patented and licensed to an outside party for commercialization purposes, which allows the public access to the new technology on the market. This lecture explores the options available to researchers at an academic institution to disclose inventions, patent and license with the intention of forming start-up or a strategic alliance with a start-up, biotech, or pharma company. Types of strategic alliances will be explored with examples of each. The lecture will close with deep dive into the largest, most successful academic-pharma alliance between UPenn and Novartis for the commercialization of Kymriah™, the first FDA-approved gene therapy in the United States.

Industry prospective-1 Spark
Speakers: Katherine High, Chief Scientific Officer of Spark
Dr. High will discuss how do you create an integrated gene therapy company, from discoveries in an academic environment to production of gene therapy drugs.

Industry prospective-2 Spark
Speaker: Jeff Marrazzo, Chief Executive Officer of Spark
Dr. Marazzo will discuss how do you create an integrated gene therapy company, from concept to reality. He will walk through the history of Spark and the modules that made it a reality**.**

Industry prospective-Tmunity
Speaker: Usman "Oz" Azam, President & CEO Tmunity Therapeutics
Dr. Azam will discuss how do you create an integrated cell and gene therapy company, from concept to reality. He will walk through the history of Tmunity and the modules that made it a reality**.**

GT and Ethical Issues
Speaker: Kiran Musunuru
Recently, the birth of twin girls whose genomes were altered before birth using CRISPR gene-editing techniques was announced. The feat wasn't necessarily a technical breakthrough, but raised ethics and scientific concerns about the application of this technology. Dr. Musunuru will discuss the potential applications of this technology, but also the potential abuses in absences of clear guidelines.

Going to the FDA: From Bench to Bedside: Regulatory Pathway to IND Submission for Cell and Gene Therapy products.

Speaker: Nancy Robinson Garvin

The lecture will discuss the FDA governing body for cell and gene therapy products (CBER), the various types of FDA meetings available to researchers (INTERACT, Type A, Type B (Pre-IND), or Type C) and how to request each meeting type, data needed to support the request, and timeline from submission to approval/clinical trial. The lecture will also review the various types of FDA applications (IND, NDA, ANDA, OTC, BLA, DMF, EUA) focusing primarily on the IND submission. Providing a framework for the preclinical, pharm/tox studies, and CMC data needed to support an IND submission as well as distinguish the various types of IND (standard, Emergency Use, and Treatment IND) and when each is applicable as well as the difference requirements for commercial vs research IND. The lecture will conclude with a discussion of the new four distinct FDA approaches (Priority Review, Breakthrough Therapy, Accelerated Approval, & Fast Track) for new breakthrough/first in human therapies and how this can apply to novel cell and gene therapy drug products and the timelines for each, etc.