

peptide as well as an understanding of structural characteristics that may contribute to TIL13831's co-receptor independence.

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### Synthesis and application of cyanuric chloride lipids for peptide presentation

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**OBJECTIVES/GOALS:** My long-term career objective is to become an established independent researcher focused on understanding and modulating immune responses to biologics in order to enhance their efficacy and understand the underlying mechanisms by which these interact with the immune system. **METHODS/STUDY POPULATION:** In this study we will evaluate the utility of cyanuric chloride based synthetic lipids in the presentation of peptide epitopes of the gene delivery vector, adeno-associated virus (AAV). The lipopeptide conjugates will be administered to mice via liposomal formulations to assess their ability to induce immune responses by ELISA as compared to mice treated with the AAV. The three-dimensional conformation of the peptides will be evaluated using nuclear magnetic resonance to determine their similarity with the natural conformation that these peptides adapt on the viral surface. Additionally, to assess the translatability of this conjugation strategy, we will test the ability of our lipopeptides to bind to serum antibodies from human subjects. **RESULTS/ANTICIPATED RESULTS:** We anticipate that peptide presentation using cyanuric chloride lipids will achieve a robust response in mice following immunization. Immunizations with our lipids should induce the production of antibodies targeting AAV, albeit a milder response than the virus itself, given the complexity of viral epitopes. Nuclear magnetic resonance will inform us on how to improve the synthetic lipids to optimize peptide presentation by altering the characteristics of the lipid anchors. Finally, by using human serum to test for the ability of our lipopeptides to bind to antibodies in serum from patients positive for AAV antibodies, we can become informed on whether our strategy has utility in human studies or whether our method is limited to animal models of human disease. **DISCUSSION/SIGNIFICANCE OF IMPACT:** The current work seeks to develop a strategy to present peptides from a well characterized biologic, AAV, on a liposome surface. Our ultimate purpose is to employ liposomal formulations as decoys that target AAV-specific lymphocytes to improve the *in vivo* efficacy of AAV.

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### Targeting ERG Through Toll-Like Receptor 4 in Prostate Cancer

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**OBJECTIVES/GOALS:** The objective of this research was to learn how the oncogenic transcription factor, ERG, is regulated in prostate cancer. If we could learn how ERG is regulated and which genes are important for its oncogenic phenotype in prostate cells, we could design new therapeutic strategies against ERG, which has proven to be difficult to target. **METHODS/STUDY POPULATION:** We conducted an shRNA screen in prostate cells to determine candidate genes and pathways that are important for ERG function. To validate the findings of the screen, we performed a variety of cell-based

functional assays, including trans-well migration, wound healing, and clonogenic survival assays. To further investigate the mechanism between ERG and the genes revealed by the screen, we performed biochemical and molecular biology experiments such as Western blotting and qRT-PCR for protein and mRNA expression, co-immunoprecipitation assays to determine protein-protein interactions, and chromatin immunoprecipitation (ChIP-qPCR) to determine transcription factor binding to DNA sites. **RESULTS/ANTICIPATED RESULTS:** The screen revealed that genes involved in the toll-like receptor 4 (TLR4) pathway are important for ERG-mediated migration. We tested the effect of a TLR4 inhibitor on ERG function and observed decreased migration and clonogenic survival exclusively in ERG-positive cells. Expression of pMEK and pERG was reduced when TLR4 was inhibited, which suggests a mechanism in which TLR4 upregulates pMEK, leading to the phosphorylation and activation of ERG. This is supported by functional assays in which cells expressing a phosphomimetic ERG are resistant to the TLR4 inhibitor. We demonstrated that ERG drives the transcription of *TLR4* and its endogenous ligands *HSPA8* and *BGN*. Therefore, ERG can sensitize the cell to TLR4 activation by increasing the number of receptors as well as providing the ligands needed for stimulation. **DISCUSSION/SIGNIFICANCE OF IMPACT:** This research provides a new therapeutic pathway for treating ERG-positive patients through TLR4 inhibition. This can be beneficial because many patients become resistant to the standard therapy, leaving very few treatment options. TLR4-based therapies could provide an alternative for patients who have developed resistance.

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### The cardioprotective effects of ramipril during the course of experimental hypercholesterolemia in rabbits

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**OBJECTIVES/GOALS:** High cholesterol is among the major causes of cardiometabolic complications. People with high cholesterol have about twice the risk of heart disease as people with lower levels. Approximately every 40 seconds, an American will have a heart attack. Costs related to Heart Attack exceed 60 Billion USA dollars per year. Renin-Angiotensin-Aldosterone System (RAAS) is implicated in the genesis of coronary heart disease and in the perpetuation of heart failure. Angiotensin-Converting Enzyme inhibitors (ACE-I) have emerged as the treatment of choice for patients with all degrees of heart failure. Many clinical trials (Consensus, 1987; Save 1990) provide the evidence that ACE-I preserves cardiac function, prevents cardiovascular death, myocardial infarction & stroke and limit remodeling after myocardial infarction. However, there are still controversies in cardiology and a debate over cardioprotection is continuing:

- Do ACE Inhibitors have unique properties, beyond their antihypertensive effect?
- Can we protect the heart during hypercholesterolemia?
- In which way hypercholesterolemia affects mitochondria bioenergetics?
- How does ramipril affect mitochondrial bioenergetics during the course of experimental hypercholesterolemia?

**Objectives/Goals were:** To evaluate the mitochondrial actions of chronically administered ramipril (non-SH-containing ACE inhibitor) in cholesterol-fed rabbits by determining the influence of ramipril on: