Update on the Research of

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More than a quarter century ago, Dr. Shah established the Oppenheimer Atherosclerosis Research Center at Cedars-Sinai Medical Center. Today, Dr. Shah and a team of 20 collaborators continue forging new ground in their efforts to identify the mechanisms leading to atherosclerosis (plaque buildup in the arteries), a fatal illness at the root of most heart attacks, strokes and sudden deaths.

Thanks to the support of generous donors who share our passion to save lives from heart disease, Dr. Shah and his colleagues continue to expand our understanding of atherosclerosis and develop more powerful and effective approaches to heart disease treatment and prevention. Their discoveries will ultimately improve the lives of patients nationwide who are battling not only heart disease but also other illnesses. Following are some key developments that have taken place in the laboratory this past year.

1. **APOA-I MILANO GENE TRANSFER & THERAPY**: Dr. Shah's research of the mutant gene ApoA-I Milano continues to make great progress. ApoA-I Milano is a naturally occurring mutant gene that encodes the ApoA-I Milano protein and produces HDL, or *high-density lipoprotein.* Also called "good" cholesterol, HDL provides greater protection against atherosclerosis and vascular inflammation — conditions that result in clogged arteries, heart attack, and stroke. In previous investigations, Dr. Shah and his team demonstrated that intravenous injection of this protein (manufactured by genetic engineering technology using bacteria as a factory) dramatically reduces plaque buildup and shrinks pre-existing plaque in animal models. Most recently, Dr. Shah and his team have successfully transferred the ApoA-1 Milano gene into animals using an innocuous virus. Unlike the intravenous injection that requires regular repetition to maintain the protective effect of the HDL, the gene transfer allows the body to create its own supply of HDL. Dr Shah's lab has demonstrated that this approach shrinks existing plaque in animal models. The next step is to find an industry partner to bring this concept into human studies.

2. **APOA-I MILANO AS A TREATMENT FOR ALZHEIMER'S DISEASE:** Dr. Shah and his team continue to make steady progress in their work to prevent Alzheimer's disease using gene-based therapy and ApoA-1 Milano gene transfer to the brain of mice genetically engineered to have Alzheimer-like pathology. A pilot study has shown favorable effects on the brain and larger and longer studies are planned to confirm the preliminary results.

3. **ACTIVE ATHEROSCLEROSIS VACCINE:** Dr. Shah and his colleagues continue their work to develop an active vaccination strategy against cholesterol plaque buildup by identifying specific antigens

within cholesterol particles that provoke a protective immune response. This active vaccine contains an antigen that when injected stimulates the immune system to produce antibodies against cholesterol particles. This vaccine against plaque build-up may also reduce high blood pressure and rupture of aortic aneurysm. In the past year, Dr. Shah and his colleagues have created a new formulation of the active vaccine using nanoparticles as a carrier. Preliminary experiments suggest that this nanovaccine reduces plaque build-up in animal models. However, a lot more work is needed to fully develop this type of vaccine before human trials can be contemplated. *(For more on the active vaccine, see the update below from Dr. Xiaoning Zhao, Recipient of The Heart Foundation's Steven S. Cohen Endowed Fellowship in Atherosclerosis Research.)*

4. **PASSIVE CHOLESTEROL VACCINE:** In addition, Dr. Shah and his team have also pioneered the development of a passive cholesterol vaccine that involves the injection of a pre-formed antibody named Orticumab to reduce cholesterol plaque buildup inside arteries. There is reason to believe that this antibody may also help Psoriasis, a skin disease associated with chronic inflammation and increased risk of atherosclerosis. This passive cholesterol vaccine has been tested and shown to be safe in a Phase 1 human trial. Plans are now underway to initiate a Phase 2 efficacy human trial within the next few months after a delay caused by Covid-19 related restrictions.

5. **NEW NON-STATIN CHOLESTEROL LOWERING DRUG:** As mentioned in the previous update, Dr. Shah and his team were participating in clinical trials of a new non-statin cholesterol-lowering drug, Evanicumab, in patients with severe genetic defects causing them to have statin-resistant, very high cholesterol levels and a grave risk of premature death from heart disease. This study is now complete, and there is every reason to believe that the company manufacturing this medication will seek FDA approval in 2021. Availability of this novel drug will be of great help for patients with genetic causes of very high cholesterol levels and high risk of premature heart disease.

6. **IDENTIFICATION OF NEW GENE WHICH MAY HELP IN THE TREATMENT OF OBESITY, HIGH CHOLESTEROL & CHRONIC INFLAMMATION:** In addition to the novel genes tenascin C, pleotrophin (PTN) and KLF14, Dr. Shah and his team have also identified a gene called GATA 3 that appears to regulate body metabolism, body weight, and heart-scar formation after a heart attack in mouse models. They have now submitted applications for two NIH grants to further study this gene that may offer a new target for the treatment of obesity, high cholesterol and chronic inflammation.

Project Update from Xiaoning Zhao, PhD
Recipient of the Steven S. Cohen Endowed Fellowship in Atherosclerosis Research

Through our Steven S. Cohen Endowed Fellowship in Atherosclerosis Research, under the direction of Dr. P.K. Shah, The Heart Foundation is training the next generation of extraordinary physician-researchers in cardiac medicine and paving the way for revolutionary advances in atherosclerosis treatment and prevention.

The past year has been very productive for the vaccine research project but also challenging due to the pandemic that has afflicted the world. The focus of the studies has been on testing the use of a nanoparticle-based vaccine formulation for dose and efficacy against atherosclerosis in the mouse model. The nanoparticle vaccine construct was developed in collaboration with Dr. Eun Ji Chung of USC. We have identified 2 potentially efficacious doses and have opted to continue with mechanistic studies with the higher of the 2 doses. The properties of a good vaccine formula include the ability to provoke an immune response and the persistence of the formula to assure prolonged immune responses. Our mechanistic studies demonstrated that the nanoparticle vaccine provoked immune responses characterized by calming of the inflammatory signals that exacerbate atherosclerosis. We also demonstrated persistence of the nanoparticle vaccine formula using imaging of its bio-distribution in the body. The successful reduction in atherosclerosis in the mouse model further validated these properties of the nanoparticle formulation. The use of the nanoparticle vaccine formulation is advantageous because nanoparticle-based therapeutics are already being tested in humans for other diseases and have been found to be safe. The potential clinical application of the nanoparticle vaccine is now being tested in the mouse model with a humanized immune response that was developed by our lab. The humanized mouse model enabled us to develop a tool, called the pentamer, to assess the persistence of the immune response to the nanoparticle vaccine. The preliminary results demonstrate very encouraging data that support the persistence of the immune response to the vaccine over a long period of time. Studies continue in this regard as we slowly pivot towards potential clinical application.

While this pandemic, which has highlighted the importance of the immune system in health and disease, has been very challenging to the research team, we continue to move forward, assured by the steady leadership of Dr. Shah. The team has employed safety protocols developed in constant dialogue with the institution and the team members. Although our work has been scaled down to match these safety adaptations, it most definitely continues.